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Optimizing Productivity of Food Crop Genotypes in Low Nutrient Soils

Prepared by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

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FOREWORD

Global climate change is likely to exacerbate plant abiotic stress in the coming decades by increasing water stress and by accelerating soil fertility degradation. To respond to this set of challenges, there is a need to develop agricultural systems with significantly greater productivity and resilience that at the same time use limited natural resources more efficiently. Low phosphorus (N) and nitrogen (P) availabilities are primary limitations to productivity in low input agriculture, and fertilizers are primary resource inputs in intensive agriculture. A critical feature of future agricultural systems will be new crop varieties with improved conversion of soil resources to yields. These new cultivars would have improved productivity in low input systems and decreased input requirements in high input systems.

Many scientists are currently turning their attention to roots, the hidden half of the plant, as central to their efforts to produce crops with better yields without causing environmental damage. Several root traits are known to be associated with P and N acquisition efficiency in low N and P soils. These root traits include root hairs, root length, root branching and root density. The identification of root traits for enhanced P and N acquisition is enabling crop breeders to develop new genotypes with better yields in low fertility soils of Africa, Asia and Latin America. However, in order to use a trait as a selection criterion for crop improvement, either direct phenotypic selection or through marker assisted selection, it is necessary to develop protocols to measure accurately the root traits that enhance N and P acquisition in the glasshouse and in the field, which can provide robust and rapid evaluation of many root systems' architectural traits in targeted production environments using different crops.

The objective of the Coordinated Research Project on Optimizing Productivity of Food Crop Genotypes in Low Nutrient Soils was to develop integrated crop, soil and nutrient management practices that help increase crop production in marginal lands by identifying and promoting the development of food crop genotypes (cereals and legumes) with enhanced N and P usage efficiency.

This CRP was implemented following the recommendations of a consultants meeting of international experts. The research network included ten contract holders from Brazil, Burkina Faso, Cameroon, China, Cuba, Ghana, Malaysia, Mexico, Mozambique and the United States of America and six agreement holders from Australia, Benin, France, Germany, Kenya and Nigeria.

The CRP was conducted in collaboration with national agricultural research systems in Africa, Asia and Latin America, and with three centres of the Consultative Groups on International Agricultural Research (CGIAR): The Africa Rice Center (WARDA), the International Institute of Tropical Agriculture (IITA) and International Center for Tropical Agriculture (CIAT). The CRP was supported by in-house research and the provision of $^{15}N/^{14}N$ isotope ratio analysis of ^{15}N enriched plant samples at the FAO/IAEA Agriculture & Biotechnology Laboratories, Seibersdorf, Austria. Upstream research on ^{15}N and ^{32}P methodologies, protocols for evaluation of plant root traits that enhance N and P acquisition and utilization efficiencies were carried out at the IAEA prior to the commencement of the CRP and through an individual research contract.

The IAEA wishes to acknowledge P.M. Chalk and all of the CRP participants for their valuable contributions. The IAEA officer responsible for this publication were J.J. Adu-Gyamfi and L.K. Heng of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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CONTENTS

SUMMARY

This 5-year Coordinated Research Project (CRP) entitled 'Selection and evaluation of food (cereal and legume) crop genotypes tolerant to low nitrogen and phosphorus soils through the use of isotopic and nuclear-related techniques' established a research network and supported the efforts of teams of scientists in sixteen Member States (Australia, Benin, Burkina Faso, Brazil, Cameroon, China, Cuba, France, Germany, Ghana, Kenya Malaysia, Mexico, Mozambique, Nigeria and the United States of America The aim of this CRP was the development of integrated crop, soil and nutrient management practices to increase crop production in marginal lands by identifying and promoting the development of food crop genotypes (cereal and legume) with enhanced nitrogen (N) and phosphorus (P) use efficiency and greater productivity in marginal lands. The research teams adopted an integrated approach to crop, soil, and nutrient management practices in predominant cropping systems during the project implementation. Studies were conducted along four main areas of investigation to (1) Develop and validate screening protocols for plant traits that enhance N and P acquisition and utilization in major food cereal and legume crops grown in low fertility soils (2) Employ validated screening protocols to identify genotypes with superior N and P acquisition and / or utilization. (3) Identify mechanisms for adaptation and high productivity of selected legumes and cereals to low N and P soils using of isotopic techniques (stable ^{15}N and radioactive ^{32}P / ^{33}P) and (4) Assess the selected genotypes with traits for enhanced nutrient acquisition and/or utilization in selected cropping systems, including yield and productivity.

The studies conducted within this CRP concern two major food security cereal crops namely upland rice (*Oryza sativa* L.) and maize (*Zea mays* L.) and three legumes namely common bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* L.) and cowpea (*Vigna unguiculata* L.). Studies were conducted across a wide geographical area in both the northern and southern hemispheres under a wide range of environmental and edaphic conditions. Experiments were conducted in the laboratory or in the glasshouse for rapid screening at the early seeding stage using the paper-roll cigar method, while the final evaluation and selection of the genotypes were carried out under field conditions. The main specific research results and recommendations arising from this CRP are summarized based on the following four outputs below:

1. PROTOCOLS FOR EVALUATION OF ROOT TRAITS (ARCHITECTURE AND MORPHOLOGY) CONTRIBUTING TO ENHANCED N AND P ACQUISITION

Root architectural phenes influence P and water acquisition from the soil. Crop genotypes with shallow roots, many basal root whorls, adventitious roots and basal roots have advantages in acquiring P from low P soils, while genotypes with deeper basal roots and longer primary roots will acquire water from deeper soil horizons. Developing protocols to accurately measure root traits that enhances N and P acquisition in the glasshouse and in the field, which can provide robust and rapid evaluation of many RSA traits in targeted production environments using different crops is vital. A simple visual method to evaluate root phenes of crops at the early seedling stage using the paper-roll cigar method or at the late growth stage in the field phenotyping using shovelomics were developed and validated. This methodology is available in 3 different languages at a website http://roots.psu.edu or http://www.naweb.iaea.org/nafa/swmn/news-swmcn.hmtl. The field phenotyping using shovelomics should have utility for evaluating food crop genotypes for low P and drought tolerance in developing countries of Africa, Asia and Latin America. During the 5-year period of the CRP, a new version of the SIMROOT model, capable of simulating a large diversity of root systems, was created. In addition, protocols for fractionation of soil P using ^{32}P to

elucidate mechanisms of P acquisition from different soil P pools were developed and fine-tuned in the Seibersdorf Laboratories to support the CRP.

2. VALIDATION OF SCREENING PROTOCOLS TO SELECT GENOTYPES WITH SUPERIOR N AND P ACQUISITION AND / OR UTILIZATION

Germplasm of maize, upland rice, common bean, cowpea and soybean were exchanged among the participants or were acquired from four CGIAR Institutes (soybean and cowpea lines from IITA, maize from CIMMYT, common bean from CIAT, upland rice from The African Rice Center (formerly WARDA) and other advanced research institutes. One hundred and fifty to two hundred genotypes of maize, common bean, soybean, rice and cowpea were rapidly screened at the early seedling stage for enhanced N and P acquisition using the paper-roll cigar method (Fig. 1a) or PVC tubes of length 15.0 cm and 3.4 cm inner diameter (Fig. 1c). Twenty five genotypes with different abilities to grow under low P and N conditions selected from the different crops at the seedling stages were further evaluated under field conditions (Fig. 1b) at two or more sites under diverse agro ecological environments. Data on environmental variables (latitude, longitude, altitude, rainfall, and temperature), soil classification, soil physico-chemical characteristics and systems studied were recorded. Root characteristics evaluated included Basal Root Whorls Number (BRWN), Root Hairs Length Density (RHLD), Basal Root Growth Angle (BRGA), Root Length (RL), Root Length Density(RLD), Root Angle (RA), Root Branching (RB), Adventitious Root Length (ARL), Adventitious Roots Number (ARN) Adventitious Root Branching (ARB), Basal Root Length (BRL), Basal Root Number (BRN), Basal Root Branching (BRB), Basal Root Depth (BRD), Primary Root Depth (PRD), Primary Root Branching (PRB), Seminal Root Length (SRL), Lateral Root Length (LRL), Lateral Root Number (LRN), Seminal Root Elongation (SRE), Root Arbuscular Mycorrhiza Colonization (RAMC) and Root Biomass (RB). In addition, data on Shoot Biomass (SHB), Grain Yield (GY), Stem Diameter (STDIA), Leaf Area Index (LAI), Plant Height (PLHT), Leaf Chlorophyll (LCHL) and Nodule Weight (NODWT) for legumes were recorded (see Fig. 2).

The results from the 16 countries for the five crops (upland rice, maize, soybean, common bean, soybean and cowpea showed that (i) Branching angle interval and seminal root length were identified as suitable root selection parameters for soil N use efficiency, while adventitious rooting and root hair formation were identified as suitable plant parameters for selecting P use efficiency (ii) P efficiency strongly correlated with genotypic differences in root hair length, root hair plasticity, lateral root number (iii) Genotypes with more root cortical aerenchyma (RCA) had deeper roots and produced 2 times shoot biomass in low N soils than genotypes with less (RCA). For beans, RHLD, BRGA, BRWN, ARL and BRN were identified as the most suitable traits (Fig. 3 and Table 1). We concluded that (1) seedling screening tools demonstrated significant genotypic variation for root traits, including root length, angle, number of axial roots and branching as well as root hair parameters (length and density), (2) Cultivars identified with some of these traits proved superior for uptake of P and N under conditions of nutrient stress, and (3) Cultivars with superior growth, nutrient acquisition and use efficiency obtained good yields of grain under conditions of nutrient stress (Fig. 3).

(a) (b) (c)

FIG. 1. (a) A growth chamber cigar roll method (b) field sampling and (c) transparent glass tube for evaluating genotypic variations in roots (root architecture and morphology) and plant growth traits associated with N and P acquisition efficiency in maize at the vegetative growth stage.

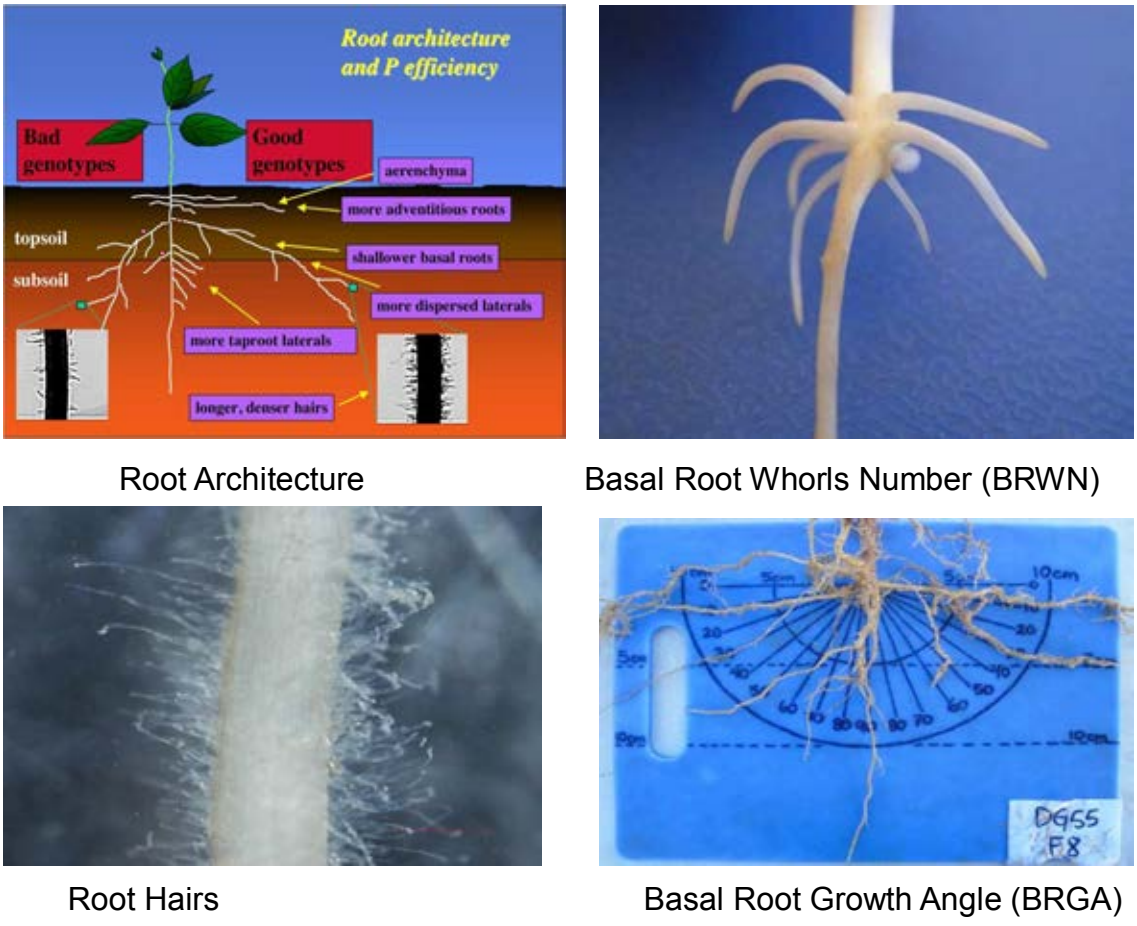

FIG. 2. Root phenes associated with genotypic differences in adaptation to low nitrogen and phosphorus.

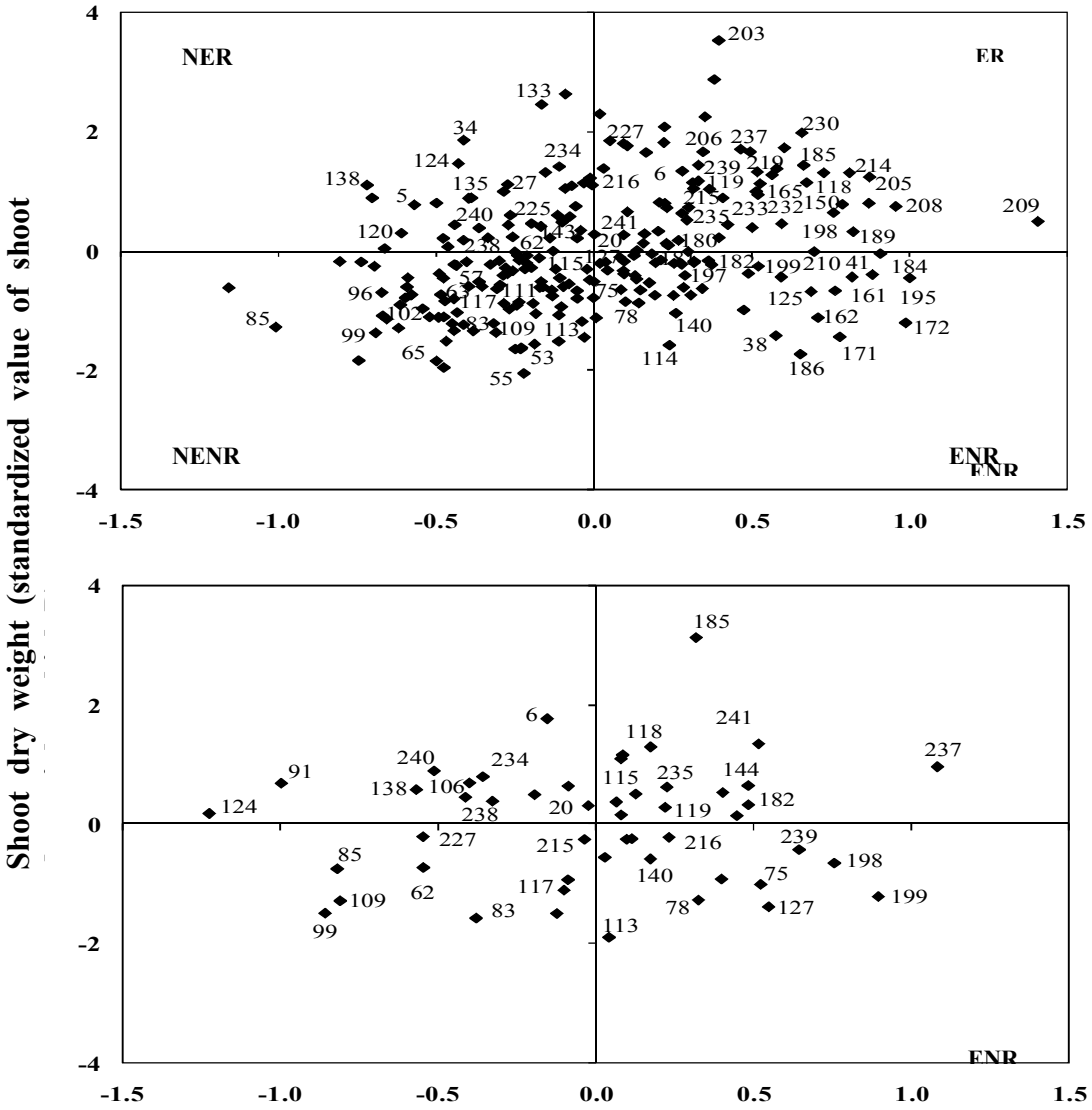

FIG. 3. Division of charts of 242 and 50 common accessions according to P efficiency and standardized value of shoot dry weight under high P conditions. Phosphorus efficiency is expressed as PEI, which is an assessment index calculated from principal component analysis. Standardized values of shoot dry weight are estimated as the following function: $Xs = (X - \bar{X})/SD$ *. Categories represented by efficient and responsive (ER), non-efficient and responsive (NER), non-efficient and non-responsive (NENR), and efficient and non-responsive (ENR). Accession numbers are indicated* [1].

3. ASSESS THE EFFECT OF SELECTED GENOTYPES ON CROPPING SYSTEMS PERFORMANCE

The assessment and selection of selected crop genotypes with enhanced different root characteristics that explore nutrients from different soil depths under field conditions is relevant for enhancing food security and long-term sustainability of soil fertility. Five to ten genotypes were further accessed in regard to their performance for improved productivity in low-input systems. Five rice and five maize genotypes were selected that had the highest N use efficiency (66-80%) and the highest P use efficiency (6-8%), and also provided a 15–30% yield increase over the other genotypes with low N and P use efficiency. For common bean,

soybean and cowpea, genotypes with deep root systems that produced 20–40% better yield, 45% increase in BNF and 40% less soil erosion in low P soil were identified. P–efficient legumes contributed to soil fertility by enhanced BNF, which is quite sensitive to P supply. Economically, the greater productivity of N efficient genotypes would permit third world farmers greater flexibility in soil management options, purchasing fertility inputs, etc., in addition to greater food security and household income.

TABLE 1. PHENOTYPIC CORRELATIONS AMONG NODAL ROOT TRAITS AND SHOOT BIOMASS, NUMBER OF NODAL ROOTS (NODAL_NO), NODAL ROOT LENGTH (NODAL_RL), NODAL BRANCHING (NODAL_BR), NODAL ROOT ANGLE (NODAL_RA), SHOOT DRY WEIGHT (SHDW), AND GRAIN YIELD (GY)

HP/LP [a]	Nodal_No	Nodal_RL	Nodal_Br	Nodal_Ra	ShDW	Gy
Experiment 1 (n = 242)						
Nodal_No	*0.49***	0.25***	0.39***	0.37***	0.38***	0.10
Nodal_RL	0.42***	*0.39***	0.50***	0.48***	–0.09	0.24**
Nodal_Br	0.55***	0.51***	*0.49***	0.51***	0.10	0.21**
Nodal_Ra	0.33***	0.31***	0.46***	*0.32***	–0.02	0.07
ShDW	0.49***	–0.03	0.21**	–0.02	*0.50***	0.09
Gy	0.18*	0.07	0.28**	0.10	0.16	*0.68****
Experiments 1 + 2 (n = 50)						
Nodal_No	*0.32***	0.36**	0.32**	0.13	0.41**	0.09
Nodal_RL	0.19	*0.39****	0.52***	0.31**	0.00	0.21*
Nodal_Br	0.25*	0.54***	*0.26**	0.24	–0.03	0.18
Nodal_Ra	0.05	0.21	0.45**	*0.32***	0.04	0.25*
ShDW	0.48***	–0.01	–0.01	–0.24*	*0.36***	0.05
Gy	0.01	0.38***	0.35**	–0.06	0.02	0.54***

[a]For each environment, values below the diagonal represent correlations within the low P treatment; values above the diagonal represent correlations within the high P treatment; values on the diagonal (*italic*) correspond to across-P treatment correlations.

*, **, *** denote significance at $P<0.05$, $P<0.01$, $P<0.001$, respectively.

4. IDENTIFY MECHANISMS FOR ADAPTATION AND HIGH PRODUCTIVITY TO LOW N AND P SOILS USING OF ISOTOPIC TECHNIQUES

Nuclear, isotopic and related conventional techniques were employed to obtain quantitative estimates on optimization of N and P uptake and utilization from fertilizers and soils. For instance, stable ^{15}N and radioactive ^{32}P) techniques were employed to obtain quantitative estimates for identifying N– and P–efficient crop genotypes in low N and P environments. In order to understand the mechanisms of the genotypic tolerance to low–P soil to utilize P from the sparingly soluble P forms, 5 maize genotypes selected out of 116 inbred lines, were used as the criteria in a ^{32}P isotope tracer experiment to follow the recovery of ^{32}P in soil P fractions. The L–value and P availability of soil was also assessed. After the addition of ^{32}P–Pi to the soil with no P fertilizer applied for 25 d, 29.0% of ^{32}P was quickly transformed into Ca_2–P (rapidly available P), and 66.1% of ^{32}P was transformed into Al–P, Fe–P and Ca_8–P (slowly available P). Only 5.0% of ^{32}P was transformed into O–P and Ca_{10}–P (plant–unavailable P). Moreover, in the soil with P fertilizer applied, ^{32}P transformation into Ca_2–P increased, and the transformation into Ca_8–P + Fe–P + Al–P and O–P, Ca_{10}–P significantly decreased compared to the soil with no P fertilizer applied ($P<0.05$). This result suggested a higher rate for water–soluble P transformation to slowly available and plant–unavailable P in P deficient soil than in soil with sufficient P. Low–P tolerant cultivar DSY–32 regulated soil P–use efficiency and plant P content according to exogenous P fertilizer application. However, another low–P tolerant cultivar, DSY–2, used

soil P more efficiently, regardless of the application of exogenous P [2]. It was therefore concluded that the ^{32}P tracer technique proved to be a valuable tool that sought physiological explanations for superior genotype performance (Table 2).

TABLE 2. L-VALUES OF MAIZE GENOTYPES WITHOUT EXTERNAL P FERTILIZER

Variety no.	Specific activity of		L-value
	Plant (Bq μg^{-1})	Soil (Bq g^{-1} soil)	(μg P g^{-1} soil)
DSY–30	22.4 ± 2.5†	4216 ± 144	190 ± 28
DSY–2	20.0 ± 0.1	4484 ± 154	225 ± 7
DSY–32	7.8 ± 1.0	3965 ± 136	513 ± 45
DSY–79	11.4 ± 0.8	3572 ± 122	315 ± 32
DSY–48	64.9 ± 2.9	3623 ± 124	56 ± 1

†Values following means are ± standard errors

A similar result was reported in a low–P soil for maize that was more efficient than soybean in taking up soil P [3]. The available P (Bray II) and the Ca–P were the fractions most depleted by plants followed by the Fe–P fractions. For common bean, efficient genotypes with long root hairs had lower specific activity values compared to inefficient genotypes, since these were able to take up P from two different pools with a greater total P accumulation (Fig. 4). For upland rice, ^{15}N and ^{32}P were employed to obtain quantitative estimates for optimization of plant N and P by N– and P–efficient crop genotypes in low N and P environments. The ^{15}N enrichment in plants ranged between 0.629 to 0.753 atom % ^{15}N excess, while the ^{15}N enrichment in upland rice seeds ranged from 0.553 to 0.757 atom % ^{15}N excess. Variety Merah showed the highest N use efficiency in upland rice with 80.7% and the lowestN use efficiency in upland rice was obtained from variety Tenom with 40.7%. N use efficiency by these upland rice genotypes showed that high N is utilized (40–80% of applied N), with good grain yield (Table 3) and P use efficiency is similar to other crops (2.4–8%).

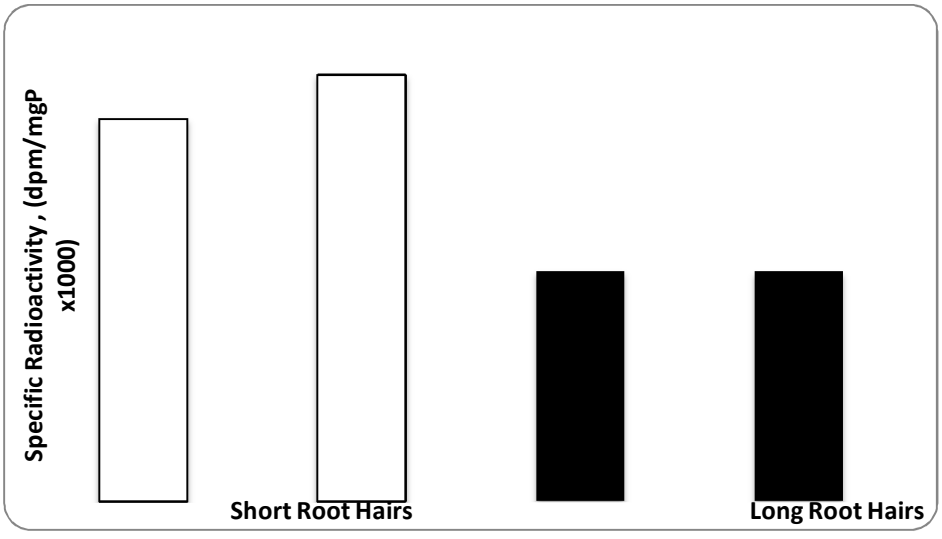

FIG. 4. Specific radioactivity in plant tissue of 4 genotypes contrasting for root hairs: Genotypes with short root hairs (open bars) and genotypes with long root hairs (full bars). Genotypes GxG 41 and DxG 53 have long root hairs, while genotypes GxG 23 and DxG 11 have short root hairs. Genotype DxG 53 (a RIL from DOR364xG19833).

TABLE 3. ^{15}N ENRICHMENT, AMOUNT OF NITROGEN DERIVED FROM FERTILIZER AND PERCENTAGE NITROGEN USE EFFICIENCY OF UPLAND RICE

Variety	^{15}N (atom % excess)		N derived from fertilizer (kg ha^{-1})			% N use efficiency
	Plant	Grain	Plant	Grain	Total	
Nabawan	0.671 a	0.553 a	40.2 de	23.0 bc	63.2 bc	42.1 bc
Tenom	0.721 a	0.614 a	31.3 e	29.7 abc	61.0 c	40.7 c
WRDA 20	0.629 a	0.684 a	27.7 e	41.5 ab	69.2 bc	46.2 bc
WRDA 99	0.731 a	0.696 a	48.3 cde	51.8 a	100.1 abc	66.7 abc
Sintok	0.711 a	0.654 a	64.0 bcd	38.8 ab	102.7 ab	68.5 ab
Pulut Petai	0.770 a	0.758 a	70.6 bc	43.0 ab	113.6 a	75.7 a
Merah	0.729 a	0.714 a	109.3 a	11.7 c	121.0 a	80.7 a
Kuku Belang	0.753 a	0.685 a	78.6 b	21.1 bc	99.7 abc	66.4 abc

Data within a column followed by the same lower case letter are not significantly different ($P<0.05$)

5. CONCLUSIONS

The main conclusions from the CRP are summarized as (i) Seedling screening tools demonstrated significant genotypic variation for root traits. These included root length, angle, number of axial roots and branching as well as root hair parameters (length and density) (ii) Cultivars identified with some of these traits proved superior for uptake of P and N under conditions of nutrient stress (iii) Cultivars with superior growth, nutrient acquisition and efficiency obtained good yields of grain under conditions of nutrient stress (iv) In some cases positive agro-ecological outcomes were identified that are related to the performance of cultivars selected for favourable root traits (vi) Nuclear tools, specifically the use of ^{15}N and ^{32}P as tracers proved valuable in studies that sought physiological explanations for superior genotype performance (v) The genotypes of rice, common bean, maize, soybean and cowpea identified in a number of cases provide valuable resources for plant breeding programmes aimed at enhancing P and N use efficiency in crops.

The CRP created a database on how cereals and legumes can acquire N and P in low nutrient soils, and this database could be further expanded and interpreted using multivariate analysis on how cereal and legume crops can acquire N and P in low nutrient soils. The studies carried out within the framework of the CRP fall within the major FAO / IAEA programme on crop improvement in harsh environments, and clearly show the value and strength of an interdisciplinary research approach. The expertise in agronomy and soil science of the majority of participants was complemented by expertise in plant breeding and genetics contributed by other participants and IAEA staff at Seibersdorf and Headquarters in Vienna.

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ROOT TRAITS FOR BETTER PHOSPHORUS ACQUSITION AND USE IN LOW–P SOILS

DIFFERENTIAL ABILITY OF MAIZE AND SOYBEAN TO ACQUIRE AND UTILIZE PHOSPHORUS FROM SPARINGLY SOLUBLE FORMS IN LOW- AND MEDIUM–P SOILS USING ^{32}P

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Abstract

A glasshouse pot experiment was conducted to evaluate the differential ability of maize (*Zea mays*) and soybean (*Glycine max*) to utilize soil phosphorus (P) for plant growth from total–P, available–P and inorganic (Ca–P, Al–P and Fe–P) soil P pools using a carrier-free ^{32}P solution. A maize variety (DK 315) and a soybean variety (TGX 1910–4F) were grown in pots containing 1 kg of a low available P soil (Hungarian) or a medium available P (Waldviertel) soil labelled with ^{32}P for 42 days or without ^{32}P (unlabelled) for 42 and 60 days. The shoot and root biomass of maize and soybean were significantly greater when grown on the Waldviertel than on the Hungarian soils. The shoot P concentrations were higher for soybean (1.7–2.2 g kg^{-1}) than for maize (1.1–1.4 g kg^{-1}). The total radioactivity (dpm × 10^6) was higher in plants grown in Waldviertel than in Hungarian soil and the values reflected plant P uptake and shoot biomass of soybean and maize. The L–values (µg P g $soil^{-1}$) of maize and soybean were higher in Waldviertel (72–78) than in Hungarian (9.6–20) soil. No significant differences in L–values were observed for maize and soybean grown on the Waldviertel soil, but for the Hungarian soil, the L–values were higher for maize (20.0) than for soybean (9.6) suggesting that in this low–P soil, maize was more efficient than soybean in taking up soil P. The available P (Bray II) and the Ca–P were the fractions most depleted by plants followed by the Fe–P fractions in the two soils, but differences between the crops were not significant. When soil P is limited, maize and soybean are able to access P mainly from the available P (Bray II), Fe– and Ca–P sparingly soluble fractions and not Al–P from the soil.

1. INTRODUCTION

Soils characterized by poor phosphorus (P) availability are widespread globally [1] and for these soils to be agriculturally productive, they require regular application of water-soluble superphosphate or ammonium phosphate fertilizers to either maintain the soil P status of fertile soils or increase that of soils with inherently low P fertility. Soluble phosphate applied to P deficient soils is retained by iron (Fe), aluminum (Al) and calcium (Ca) ions and are virtually unavailable to most plant species. It is reported that P fertilizer recovery efficiencies commonly range from 5 to 20% (sometimes are as low as 1% in high P–fixing soils) for the first crop and thereafter 1–5% for subsequent crops, indicating there is scope for improving P use by crops [2].

Plants differ greatly in their ability to grow on low P soils because they have developed specific physico-chemical mechanisms/processes to utilize P compounds in these low P fertility soils [3]. These mechanisms include (i) alterations (morphological and physiological) to root systems, i.e. mycorhizal plants have better water uptake and Al tolerance in acid soils [4] (ii) secretion of low-molecular-weight organic compounds

(exudates production), i.e. malonic, oxalic, citric, malic and piscidic acids secreted by roots of pigeon pea help to release low-soluble P compounds in soils [5] (iii) secretion of enzymatic compounds, i.e. phosphatases, and (iv) molecular changes such as enhanced expression of P transporters.

Studies using crop species having more efficient acquisition of P is a strategy to evaluate and identify crop plants with genotypic variation in their ability to access and utilize sparingly soluble forms of soil P (Ca-P, Al-P and Fe-P). This idea has been proposed as a possible means for overcoming P deficiency stress in soils and as a means to optimize P fertiliser use in cropping systems where P is poorly available [6]. Intra-specific variations in a crop's ability to use sparingly soluble forms (P associated with Al, Fe and Ca) in low-P available soils have been well documented for lupin [6], pigeonpea [7], soybean, cowpea and maize [8].

The amount of plant available P in a soil can be defined as the amount of orthophosphate that leaves the solid phase of the soil and arrives in the soil solution at a time when the plant is growing and can take it up. Radio-isotopic P techniques, using the principle of isotopic exchange, allow measurement of the amount of orthophosphate that can be transferred from the soil solid to the solution over a given time [9], and can thus provide a powerful alternative means for characterizing soil P availability and the sources of P, with minimum modifications of soil P forms compared with conventional extraction methods. The technique has been used to measure the quantity of available P in soils in determining the E-value or exchangeable P [10], the L-value or labile P [11] using plants grown in a soil labelled with carrier-free ^{32}P or ^{33}P-orthophosphate, and the A-value or available P [12]. Studies in soils labelled with ^{32}P to distinguish between different forms of P accessed by plants have shown that white lupin can access up to six times more P compared with a range of crop plants [13]. Cluster roots may represent more than 60% of the total root mass. Since P isotopes provide the unique advantages of being able to identify P sources and quantify their impacts [14], greater use of ^{32}P and ^{33}P isotopes in P flux and balance measurements offers considerable advantages over conventional techniques.

The study aimed to evaluate the differential ability of maize and soybean to access and utilize P from different soil pools using two soils, one low- and one medium-P. The experiment aimed to test the hypothesis that the efficient P uptake from sparingly soluble P forms (Al-, Fe- and Ca-P) by different crops can be used as criteria to evaluate crop plants tolerant to low available P soils.

2. MATERIALS AND METHODS

2.1. Plant growth conditions

Two experiments were simultaneously set up to include two treatments consisting of non-labelled (without ^{32}P) and labelled (with ^{32}P) imposed on soils with low and medium available P with maize (cereal) and soybean (legume) as test crops in a factorial design with four replications. In all there were 20 pots for the radioisotope (including four pots without plants as control) and 36 pots (including four pots without plants and two sampling periods) for the unlabelled treatment.

A low-P soil from Hungary (total P, 302; available P (Bray P2), 21; Olsen P, 13.3 mg kg^{-1}; pH_{KCl}, 5.6; classified as Dystric Eutrocrepts), and a medium-P soil (Waldviertel) from Austria (total P, 502; available P (Bray P2), 44; Olsen P, 12.8 mg kg^{-1}; pH_{KCl}, 5.6) were used. More detailed physical and chemical characteristics of the two soils are given in Table 1.

TABLE 1. PHYSICAL AND CHEMICAL PROPERTIES OF THE SOILS

Properties†	Hungarian soil	Waldviertel soil
Sand (g kg^{-1})	830	273
Silt (g kg^{-1})	88	582
Clay (g kg^{-1})	82	145
Bulk density (g cm^{-3})	Not determined	1.29
Particle density (g cm^{-3})	Not determined	2.65
Pore volume (%)	Not determined	0.51
Saturated water content (%)	Not determined	47
pH (H_20/KCl)	5.5/4.6	6.5/6.0
Total P (mg kg^{-1})	302	502
Available P (Bray/Olsen) (mg kg^{-1})	21/13.3	44/12.8
Inorganic P (Ca/Al/Fe) (mg kg^{-1})	36/85/65	56/144/68
EC (25°C) (µS cm^{-1})	Not determined	166
Total N (g kg^{-1})	0.83	1.21
Organic C (g kg^{-1})	7.91	20
Ca (cobalthexamine) (cmol k g^{-1})	1.82	13.69
Mg (cobalthexamine) (cmol kg^{-1})	0.61	3.13
K (cobalthexamine) (cmol kg^{-1})	0.09	0.15
Na (cobalthexamine) (cmol kg^{-1})	0.04	0.06
CEC (cobalthexamine) (cmol kg^{-1})	2.66	23.59

†EC, electrical conductivity; CEC, cation exchange capacity

A maize variety (DK 315) from Austria and a soybean variety (TGX 1910–4F) from IITA, Nigeria, were grown in plastic pots (1 plant per pot) containing 1 kg of soil in a naturally lit glasshouse with a temperature regime of 34/21°C day/night and relative humidity of 40–70%. Each pot received basal fertilizer equivalent to 200 kg–N ha^{-1} as ammonium sulphate and 50 kg K ha^{-1} as potassium chloride. Prior to planting, the weight and P concentration of soybean and maize seeds used for the experiment were determined. The amount of P in seed was 0.98 mg P kg^{-1} soil (0.35 g with 2.8 mg kg^{-1} P) for maize and 3.48 mg P kg^{-1} soil (0.59 g with 5.9 mg kg^{-1} P) for soybean. Phosphorus–32 labelled $K_2H^{32}PO_4$ (specific activity of 40.7 GBq $mmol^{-1}$) was applied to the pots.

To ensure uniform labelling of the soil five portions of dilute ^{32}P solution (50 ml for Waldviertel and 30 ml for Hungarian soils were applied to a layer of 200 g soil) to achieve 70% of the water holding capacity of each soil. A total of 250 ml for the Waldviertel and 150 ml for the Hungarian containing 12.4 MBq (335 µCi) of a $K_2H^{32}PO_4$ solution was applied to each of the 20 pots containing 1 kg soil. Pre-germinated maize and soybean seeds were sown at one per pot immediately after the addition of ^{32}P. Twenty ml of inoculum (*Bradyrhizobium japonicum*) mixture was added to all the soils. On 20th August (28 days after sowing), nitrogen was applied at 100 kg–N ha^{-1} as ammonium sulphate to the maize to ensure adequate N for the plants. The plants were watered on a pot weight basis and were supplied everyday with an amount of water equal to the evapotranspiration loss, maintaining as much as possible constant soil water content in the pots.

2.2. Plant and soil sampling and analyses

The first plant sampling for the labelled and the non-labelled treatments was done at 42 days after sowing (DAS) whereas the non-labelled treatments were allowed to grow till 60 DAS. Soil samples (10–12 g) was taken with a special soil auger (inner diam. 8 mm, outside diam. 10 mm and length 25 cm) at 0, 1, 5, 42 and 60 DAS, oven-dried at 70 °C for 18 h, milled and a portion used for analysis. Plants were harvested and separated into shoots

(radioisotope-labelled) and shoot and roots (non-radioisotope), chopped into small pieces, oven dried, weighed, and ground. The root dry weight of the radioactive plants was estimated using the root/shoot ratio of the non-radioisotope plants, in order to prevent the complications of root sampling and washing for the ^{32}P-labelled pots. Total P in soils was determined using the colorimetric method [15] after acid digestion, and available P (Bray P2 and Olsen) determined by the colorimetric method after extraction.

The inorganic soil P fractions were measured according to a fractionation scheme based on the method described by Sekiya [16]. Briefly the fractionation involved a sequential extraction of Ca-P (300 mg of soil extracted with acetic acid), Al-P (extracted with ammonium fluoride after the extraction of Ca-P) and Fe-P (soil after extraction of Al-P was washed twice with saturated sodium chloride and discarded and then extracted with and sodium hydroxide) and the P in extracts determined by a colorimetric method. The ^{32}P radioactivity in all the fractions (total-P, available-P, Ca-P, Al-P and Fe-P) was measured by liquid scintillation spectrometry (Packard 2000) using 1ml solution and 9 ml of Aquasol-2 (NEN research product). The ground plant materials were wet digested in 4 ml H_2SO_4 and 3 ml H_2O_2 for 2 min until the digest was colourless, total P was measured on diluted aliquots [17] and ^{32}P was determined by liquid scintillation counting. P in the maize and soybean seeds was determined after five seed samples, each of 100 mg, were ground and acid digested. The total radioactivity in each pot at sowing was 744×10^6 dpm.

3. RESULTS

3.1. Physico-chemical characteristics of the soils used

The Waldviertel (silty loam) was slightly acidic, medium total P, available P, and C content whereas the Hungarian (loamy sand) was acid, comparatively low total P, available P and carbon content (Table 1). The soil P fractions of the original soil indicated higher Ca-P and Al-P and exchangeable bases (Ca, Mg, K, and Na) in the Waldviertel than the Hungarian soil.

3.2. Plant growth and P uptake

The shoot and root biomass of both maize and soybean were significantly greater when grown in the Waldviertel than in the Hungarian soil and there was a significant increase in shoot dry weight per plant from 42 to 60 DAS (Fig. 1). Shoot weight of maize increased from 8.1 g at 42 DAS to 19.1 g plant^{-1} at 60 DAS in Waldviertel, and from 2.2 g at 42 DAS to 5.7 g plant^{-1} in the Hungarian soil. For soybean, there was an increase from 4.1 g at 42 DAS to 9.8 g plant^{-1} for the Waldviertel and from 1.0 g at 42 DAS to 2.3 g plant^{-1} in the Hungarian soil. Shoot weight of maize and soybean decreased by 75–80% when grown in Hungarian compared to Waldviertel soil irrespective of the two sampling dates (Fig. 1).

Soybean plants grown in Waldviertel soil were well nodulated (0.56 g plant^{-1}) on the roots whereas plants grown in the Hungarian soil produced very few nodules. There was no significant difference in shoot and root weight of labelled and unlabelled treatments confirming that the addition of carrier-free P–32 solution did not result in any change in soil chemical properties. The root/shoot ratio was generally higher at 42 (0.6–0.7) than at 60 (0.2–0.45) DAS for maize and soybean and higher for Hungarian than for Waldviertel soil except at 60 DAS.

The shoot P concentrations were higher for soybean (1.8–2.2 mg g^{-1}) than for maize (1.1–1.4 mg g^{-1}) and decreased with plant age for maize but not significantly for soybean (Fig.

2). Plant shoot P reflected the total and available P concentrations in the two soils with shoot P of plants grown on Waldviertel soil higher than those grown on the Hungarian soil (Fig. 2). Although the plant P concentrations were high in soybean compared to maize, the total P amount (mg P) was significantly higher in maize than in soybean and the values for maize increased in Waldviertel from 17.6 mg P at 42 DAS to 27.3 mg P at 60 DAS, while for soybean values decreased from 13 mg P at 42 DAS to 22 mg P at 60 DAS (Fig. 2). There was 80% or more reduction in total P amount when plants were grown in Hungarian compared to Waldviertel soil.

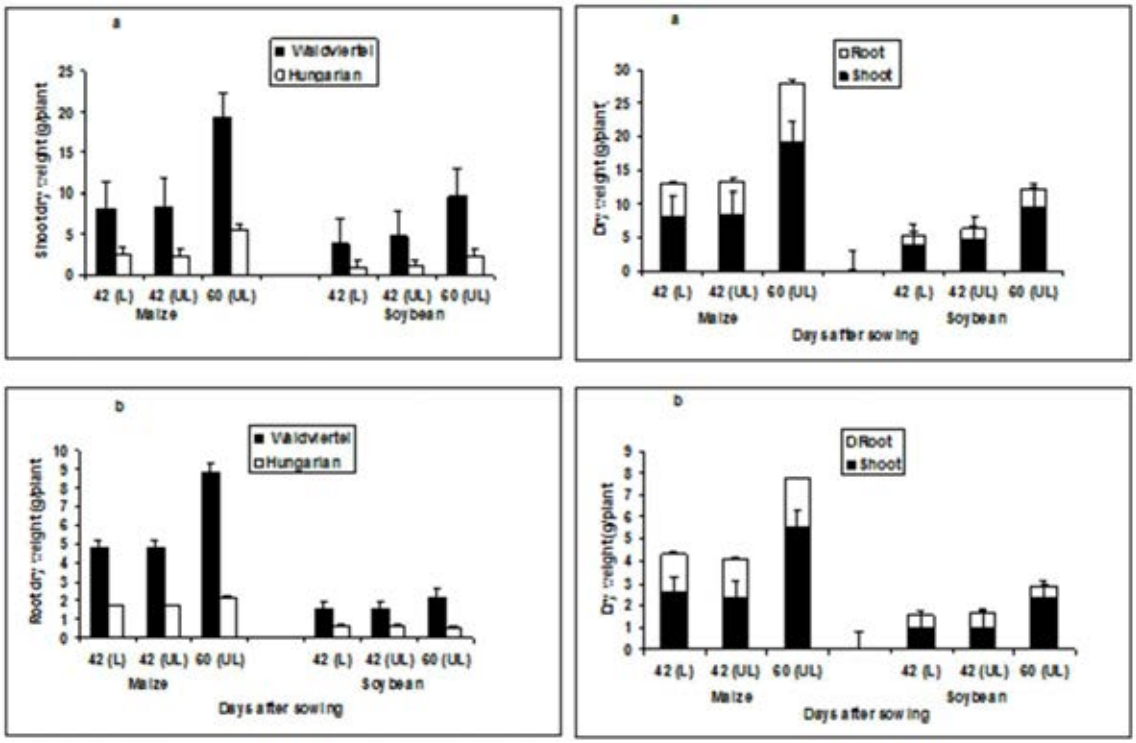

FIG. 1. Dry weight of shoot (a) and root (b) for maize and soybean grown in Waldviertel and Hungarian soils labelled (L) and unlabelled (UL) with ^{32}P.

In the Hungarian soil, there was no significant change in Ca-P but a slight decrease in Fe-P, but a substantial increase in Al-P (85–98 mg kg^{-1}) from 0 to 60 DAS (Fig. 3). In the Waldviertel soil, Ca-P decreased (from 56 to 45 mg kg^{-1}), Al-P (from 144 to 133 mg kg^{-1}) and Fe-P (from 68 to 59.5 mg kg^{-1}) at 0 to 45 DAS (Fig. 3).

The percentage distribution of the inorganic and the available P pools are shown in Fig. 4. The Bray2 and the Ca-P were the fractions depleted most by plants followed by the Fe-P fractions in the two soils, and differences observed between the crops were not significant All the soil P fractions (except Al-P that increased slightly) decreased from 0 to 60 DAS in the two soils.

3.3. Isotopic exchange parameters

3.3.1. Total radioactivity and specific activities (SA) in soils

Total radioactivity in total P decreased from 1 to 42 DAS whereas all the other fractions (available P and the inorganic fractions) increased in soils. For total P the total radioactivity (dpm × 10^6) decreased from 746 at 1 DAS to 568 (Waldviertel) and 688 (Hungarian) at 42 DAS. The total radioactivity (dpm × 10^6) in the available P decreased from 82.1 (Waldviertel) in to 388.5 (Waldviertel) and 539 (Hungarian). A similar increase was

observed for Ca-P, Al-P and Ca-P. The variation in specific activity (kBq mg^{-1} P) in the different soil P pools of the two soils at 1 and 42 DAS is shown in Fig. 5. In the Waldviertel soil there was a sharp increase (6 times) in the SA in the Bray-P fraction and an increase (2 times) in the Fe-P fraction in the soybean. The SA of the soil fractions from maize and soybean treatments was higher in the Hungarian than in the Waldviertel soil but the trend was similar to that of the Waldviertel soil.

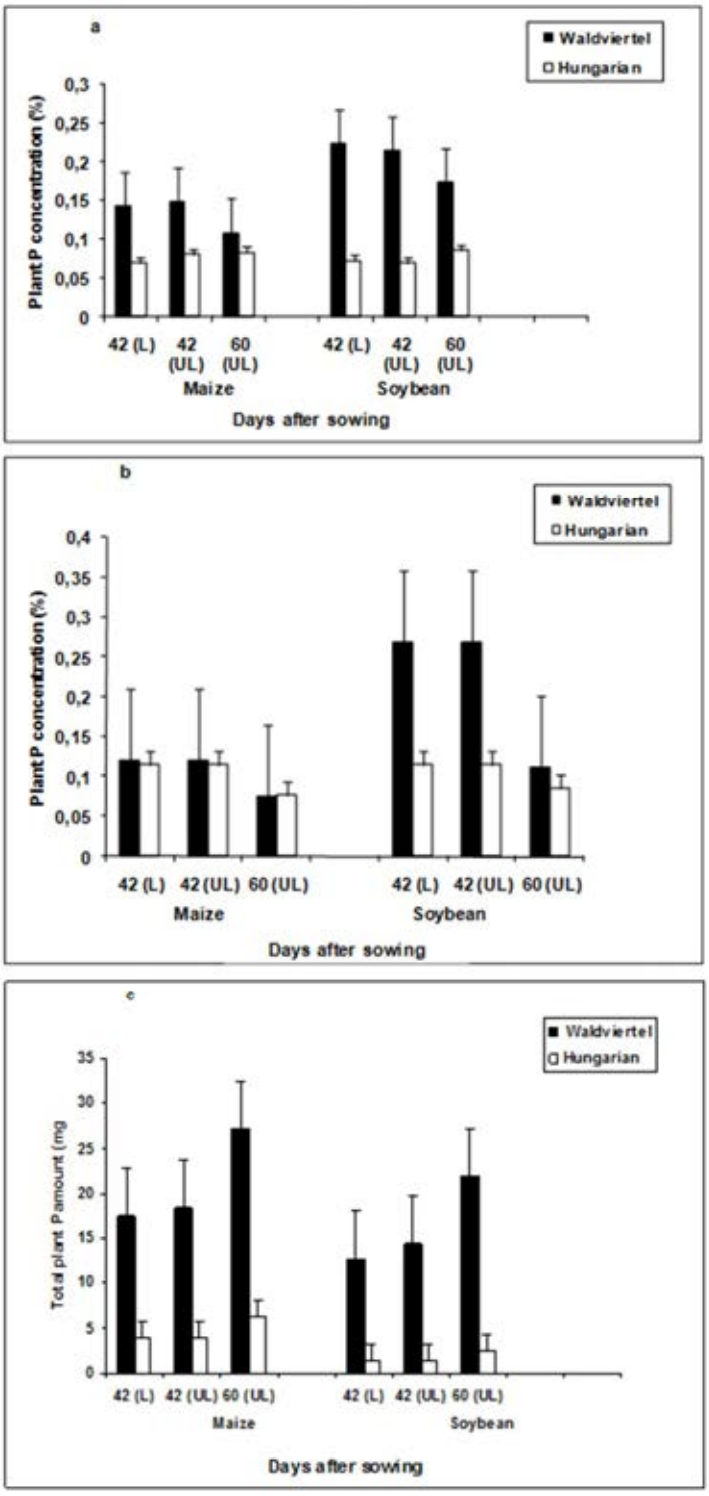

FIG. 2. Phosphorus concentration of shoot (a) and root (b) and plant P amount (c) for maize and soybean grown in two soils. For legend see Fig. 1.

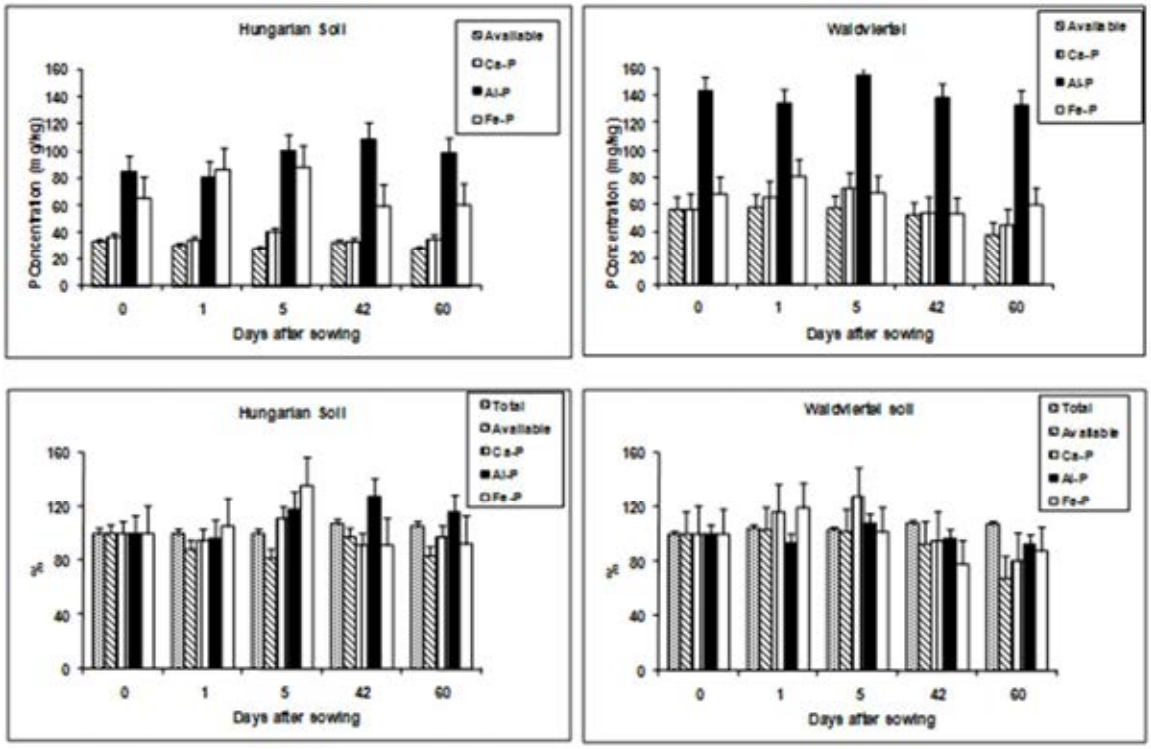

FIG. 3. Inorganic P pools (Fe-P, Al-P and Ca-P) and available-P extracted from the two soils at 0, 1, 5, 42 and 60 DAS.

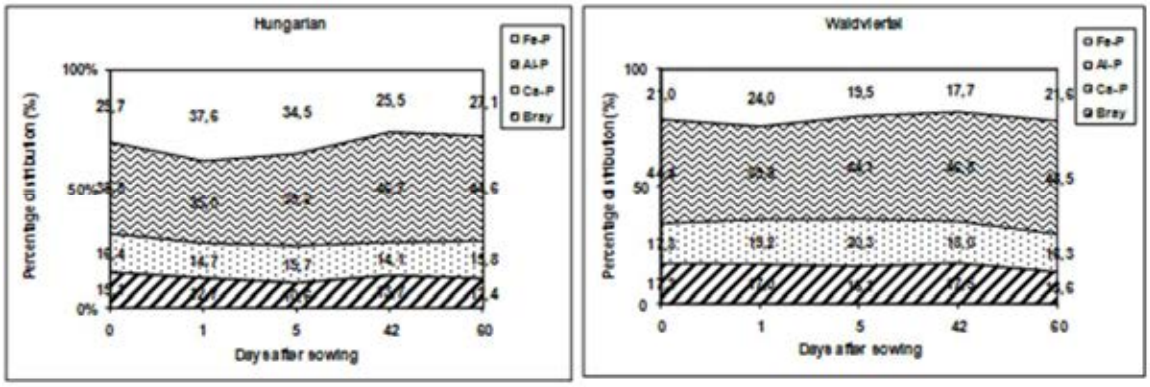

FIG. 4. Percentage distribution of inorganic P pools (Fe-P, Al-P and Ca-P) and available-P (Bray P2) extracted from the two soils at 0, 1, 5, 42 and 60 DAS.

3.3.2. Radioactivity in plants and L-values

The total radioactivity (dpm × 10^6) in shoot of maize was higher (108.0) than that in soybean (76.6) irrespective of the soil used. The total radioactivity was higher in plants grown in Waldviertel than in Hungarian soil and the values reflected the plant P uptake and shoot biomass of soybean and maize (Fig. 6). However, the specific radioactivity (dpm × 10^3 mg^{-1} P or kBq × 10^3 mg^{-1} P) showed reversed trends to the values of total radioactivity, with soybean recording higher values than maize. Percentage recovery of radioactivity in shoot was higher for maize (14.5%) than for soybean (10.3%) and was four times higher in Waldviertel (mean recovery 12.4%) than in the Hungarian soil (mean recovery 2.5%) (Fig. 6). In the roots the recovery for maize was 7.2% (Waldviertel) and 4.6% (Hungarian), and for soybean 5.0% (Waldviertel) and 1.0% (Hungarian), respectively.

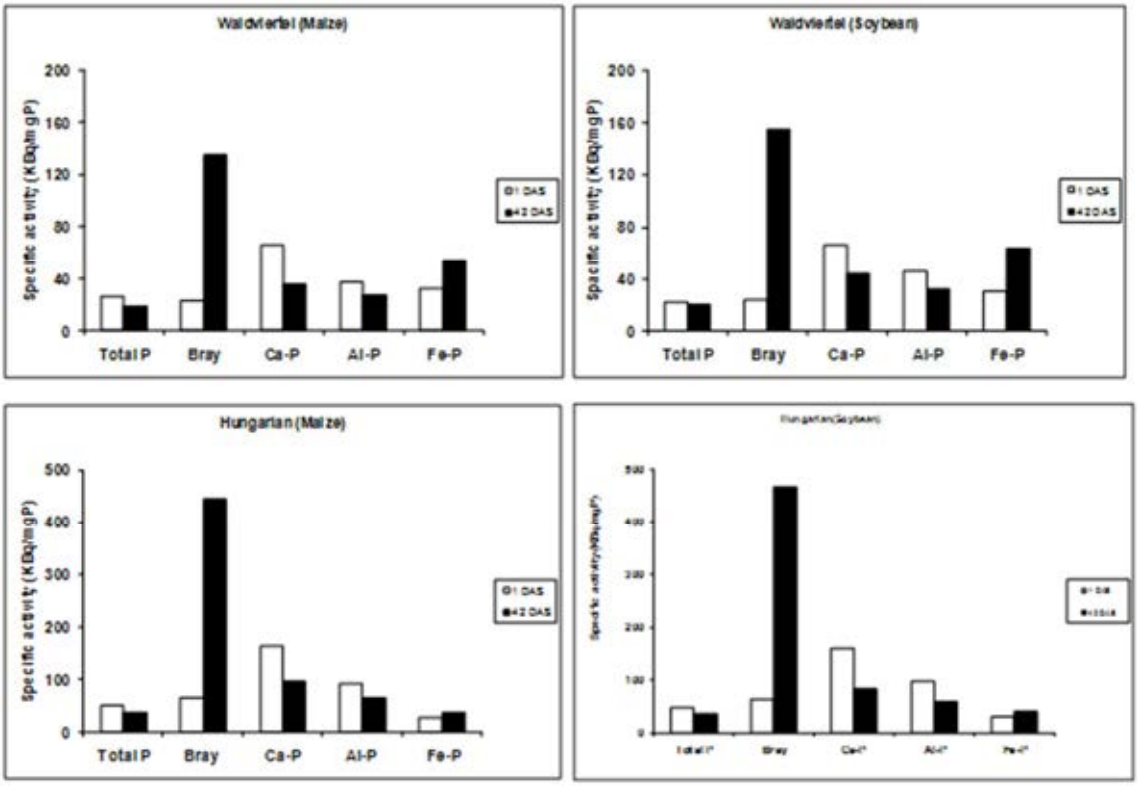

FIG. 5. Total radioactivity and specific activities of maize and soybean grown on low- (Hungarian) and medium-P (Waldviertel) soils.

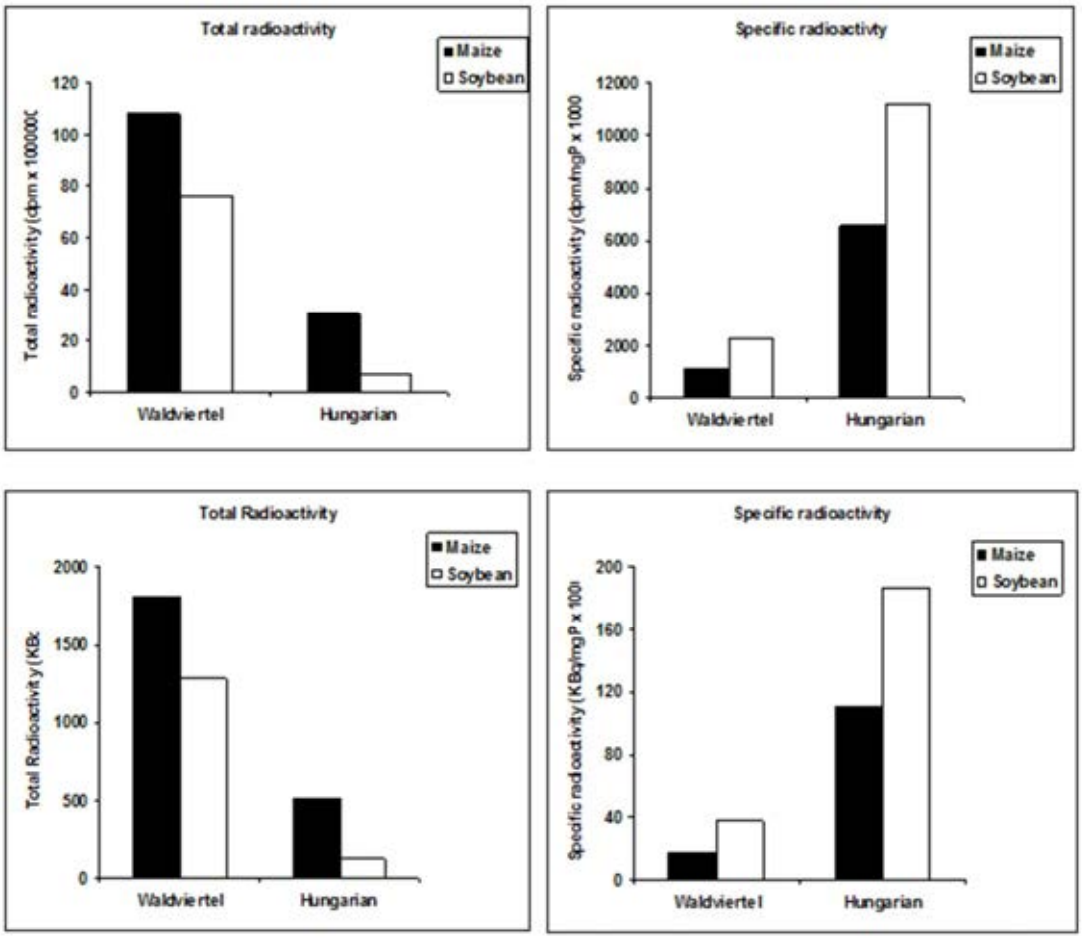

FIG. 6. Variations in specific activities in the different soil pools from 1 to 42 DAS in the two soils.

To assess the amount of isotopically exchangeable P, the L-value was estimated using the following equation:

$L = (^{31}P \text{ shoot} - ^{31}P \text{ seed}) \times ^{32}P \text{ added to soil} / (^{32}P \text{ shoot})$

where L is the L-value (µg P g^{-1} soil), the initial applied dose of ^{32}P (Bq kg^{-1} soil), ^{32}P shoot is the activity of shoot mass (Bq g^{-1} DM), ^{31}P shoot is the total amount of P in shoot biomass (mg P g^{-1} DM).

The L-values (µg P g^{-1} soil) in maize and soybean were higher in the Waldviertel (>70.0) than in Hungarian soil (<20.0). No significant differences in L-values were observed for maize and soybean grown on the Walviertel soil, but for the Hungarian soil, the L-values were higher for maize (20.0) than in soybean (9.6) suggesting that in this soil, maize was more efficient to take up P than soybean (Fig. 7).

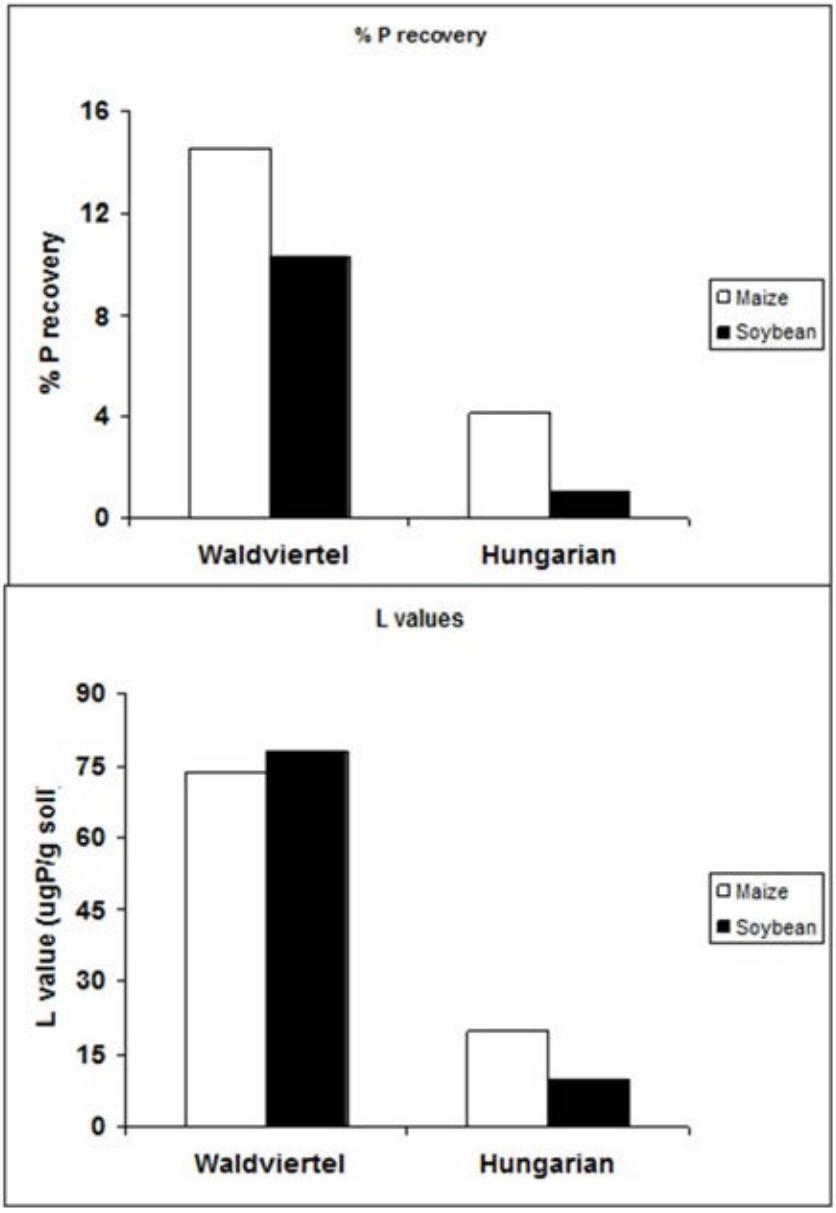

FIG. 7. Percentage P recovery in plant shoots and the L-values calculated for maize and soybean grown on two soils.

4. DISCUSSION

4.1. Plant growth and P uptake

Maize and soybean grown in the Hungarian soil had low shoot P concentrations (<1 mg g^{-1} P) suggesting a strong P limitation. Plants showed symptoms of P deficiency 42 DAS and produced three time less biomass than when grown in the Walviertel soil (Fig. 1). The P concentration in shoot tissue of maize (1.1–1.5 mg g^{-1} P) was low compared to that in soybean (1.8–2.2 mg g^{-1} P) and this is attributed to a dilution effect as biomass increased (see Fig. 2 and Fig. 3). However, in the low available P soil, total P accumulation in shoot was higher in maize that in soybean suggesting that maize has a greater ability to take more P from low available P soils than soybean. The Hungarian soil used had 21 mg kg^{-1} (Bray II) and 13.5 mg kg^{-1} Olsen) P, and for 1 kg soil, more than 12 mg P is expected to be available to the plant. This suggests that the available P extraction using Bray II may contain other P that is not easily available to maize and soybean. It is reported that Resin-P and $NaHCO_3$-Pi are labile soil fractions that are considered the most available for plant growth [18]. Other factors such low pH (4.6) may have contributed to P fixation, and the low organic C (7 mg kg^{-1}) may have also soil. In addition, the fact that no nodules were observed on soybean grown in the Hungarian soil despite the Rhizobium inoculation, while soybean grown on the Waldviertel was well nodulated (0.6 mg DM $plant^{-1}$) suggesting that nodule formation in the soybean was severely impaired by P deficiency [18] no doubt contributing to the poor growth. Under low P conditions, maize and soybean distributed more dry matter and P in roots than in shoots as shown by the higher root/shoot ratio in the Hungarian than in the Waldviertel soil indicating that, when P is limiting, the sink capacity for DM and P in roots is high compared to the shoot.

4.2. Dynamics of P fractions

The available P measured by Bray P2 was double that in the Waldviertel compared to the Hungarian soil, although no difference in Olsen P was observed. The fact that plants (maize and soybean) showed severe deficiency when grown on the Hungarian soil, and the P concentration in plant was below 1 mg g^{-1}, suggests that Bray P2 overestimated the available/labile P fractions in the soil. The relatively strong acidic reagents used in Bray P2 could dissolve a substantial amount of inorganic P during extraction of the soil [19]. Aluminium-P and Fe-P were the highest inorganic fractions extracted using the Sekiya method, and in the Waldviertel soil the Al-P fraction was more than double the amount in the Ca-P and Fe-P fractions at the beginning of the experiment. The Bray P2 and the Ca-P were the fractions depleted by plants followed by the Fe-P fractions in the two soils, and differences observed between the crops were not significant. Our results suggest that more P was released in the soil solution (labile) from the available and Ca-P fractions for plant uptake than from the Fe- and Al-P fractions. It is reported that the amount of Ca-bound P in soil is more important for crop production; if Ca-bound P is present plants may be able to take up more P to acidify the rhizosphere [20].

The principal aim of the direct chemical extraction method is to extract the labile inorganic-P (Pi) in the soil (i.e. Pi fraction that can move into soil solution). However, the available P extracted with a relatively strong acid (0.1N HCl + 0.03N NH_4F) may extract more than labile Pi and include non-labile or stable soil P, or may fail to extract labile Pi in certain soil types [21]. For example, an acid extractant such as Bray-1 cannot be used on calcareous soils because of neutralization. On the other hand, Bray-1 and Mehlich-3 extractants are designed to extract P from non-calcareous soils or soils with a pH lower than 7.4 [22, 23] while the Olsen test is preferred for calcareous soils. Available P, defined as the quantity of P

which will come into solution for plant uptake during its life cycle, is both time- and plant-specific. Therefore, there is a high probability that the depletion of P from the Bray P2-fraction may also include soil Ca- and Fe-P fractions. However, neither maize nor soybean depleted a significant amount of P from the Al-P fractions (Fig. 5), although it has been reported that some crops such as rice, groundnut and pigeon pea have the ability to take up P from Fe- and Al-P fractions, possibly because of the release of carboxylic anions from roots [8]. Al-P was the inorganic fraction least taken by cowpea genotypes [9].

Among the inorganic pools, Al-P, Fe-P and Ca-P are the three major fractions in the soil. Our results imply that when P is not supplied, maize and soybean are able to access mainly Fe- and Ca-P but not Al-P from the soil. Alternatively, the plant P uptake resulted in a considerable decrease on Bray P2 with time, which would enhance dissolution of the sparingly soluble P. This is supported by the fact that the changes over time in available P were more sensitive than changes in total P [24].

4.3. Isotopically exchangeable parameters and P uptake by maize and soybean in low- and medium-P soils

The high total radioactivity in maize compared to soybean suggests that plant P uptake from soil was greater by maize than by soybean irrespective of the soil used. In addition, whereas there was a 1.4 times increase in radioactivity in maize over soybean in the Waldviertel soil, there was 4.3 times increase by maize over soybean. These data suggest that maize could take up more P at low-available P conditions than soybean. This is supported by the fact that the L-value (with seed P uptake correction factor) of maize was double that of soybean in the low-P Hungarian soil, even though others [25] observed that determination of E and L-values is not precise enough to identify plant species or cultivars able to take up P from slowly or non-exchangeable P pools, or to quantify precisely the rate at which P in the soil organic matter is mineralized.

In the low-P soil (Hungarian) both crops depleted more P from Bray P2 followed by Fe- and Ca-P fractions. Addition of Fe-P resulted in a significant increase in shoot dry matter yield (SDMY) whereas the addition of Al-P did not lead to an increased SDMY in cowpea [11]. Wang et al. [26] showed the differential ability of cotton, wheat and white lupin to utilize P fractions in the rhizosphere soil, where P utilized by cotton was mainly from $NaHCO_3$-Pi, whereas wheat and white lupin markedly depleted the HCl-Pi pool under P deficient conditions [26]. Similar results have been reported for common bean and wheat grown in mono-cropping and intercropping systems [7].

Maize and soybean grown in medium-P (Waldviertel) soil had lower specific radioactivity (dpm $\times 10^3$ mg^{-1} P or kBq x 10^3 mg^{-1} P) in shoot than those grown on the low-P Hungarian soil, and the values were lower in maize than in soybean. Low specific radioactivity indicates that plants were using otherwise unavailable P sources. These results therefore suggest that maize was more efficient in taking up P from otherwise sparingly soluble inorganic-P sources than soybean. A high specific activity in soybean compared to maize suggests that soybean is not efficient in taking up P from the sparingly soluble inorganic pools. Furthermore, the percentage P recovery of radioactivity in shoots was higher for maize (14.5%) than for soybean (10.3%), and was four times higher in Waldviertel (mean recovery 12.4%) than in the Hungarian (mean recovery 2.5%) soil. In the roots the recovery for maize was 7.2% (Waldviertel) and 4.6% (Hungarian), and for soybean 5.0% (Waldviertel) and 1.0% (Hungarian), respectively. This confirms the results described above i.e. that maize is more efficient in taking up P from the soil than soybean.

Contrary to our expectation, the results indicated that plants could take up P more efficiently from the other sparingly soluble P fractions in the Waldviertel (medium-P) than in the Hungarian (low-P) soil. The specific radioactivities of Bray P2 and the Fe-P fractions increased significantly whereas that of the Ca- and Al-P fractions decreased from 0 to 42 DAS. However, this could not explain precisely the uptake of P from slowly or non-exchangeable P pools. Other factors such as the low N and C concentrations coupled with the low pH (4.6) could have hampered the uptake of P (especially by soybean which is susceptible to low pH) in the Hungarian soil. Other factors such as P concentration in the soil solution (P intensity) may have been the limiting factor for plant growth. Rhizosphere processes such as organic acid exudation may have caused sparingly soluble soil P to become exchangeable, but without significantly renewing the P concentration in the soil solution which is vital to raise P uptake. Thus adjusting the soil pH and raising the intensity through the application of small quantities of P fertilizer could be essential to obtain adequate yields of maize and soybean on the soils like the Hungarian example used here.

5. CONCLUSIONS

The main findings from this study is that maize was more efficient in taking up P from otherwise sparingly soluble inorganic-P sources than soybean in the medium-P (Waldviertel) soil; as indicated by the low specific radioactivity (dpm $\times 10^3$ mg^{-1} P or kBq $\times 10^3$ mg^{-1} P) in shoots. The L-values (with seed P uptake correction factor) of maize was double that of soybean in the low-P (Hungarian) soil suggesting the superiority of maize to access sparingly soluble P from soils compared with soybean. When P is not supplied, maize and soybean are able to access P mainly from the available P (Bray P2), Fe- and Ca-P sparingly soluble fractions and not Al-P from the soil. Maize and soybean showed severe deficiency when grown on the Hungarian soil and the P concentration in plant was below 1 mg g^{-1} suggesting that Bray P2 overestimated the available/labile P fractions in the soil. Contrary to our expectation, the results indicated that plants could take up P more efficiently from the other sparingly soluble P fractions in the Waldviertel (medium-P) than in the Hungarian (low-P) soil.

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CONTRIBUTION OF ROOT TRAITS TO PHOSPHORUS ACQUSITION EFFICIENCY BY MAIZE LANDRACES IN ACID SOILS OF THE HIGHLANDS IN CENTRAL MEXICO

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Abstract

Plants have a wide range of mechanisms and morphological features that increase availability and acquisition of orthophosphate from soil. Root growth, root branching, and root hair morphology are important for the efficient acquisition of phosphorus (P). The series of studies reported here was based on the hypothesis that Mexican maize landraces, which have developed mostly in environments with low P availability and have a well-developed root system, could be a source of variation for the improvement of phosphorus acquisition. Several studies were conducted to evaluate genotypic variation in both root (root architecture and morphology, including root hairs) and plant growth traits associated with P acquisition efficiency (PAE) and/or P utilization efficiency (PUE) of maize landraces in a P-deficient Andisol in the Central Mexican Highlands, and to identify genotypic differences, among both efficient and inefficient in P acquisition and responsive and non-responsive maize landraces to applied P. The results showed that accessions differed greatly in plant growth, grain yield, root morphology, total uptake of P, PAE, PUE, and P efficiency defined as growth with suboptimal P availability. Phosphorus-efficient accessions had not only greater biomass per unit of absorbed P, but also larger root systems, greater P uptake per unit root weight, more nodal roots, nodal root laterals, and greater root hair density of nodal root main axes and first-order laterals than did P-inefficient accessions under P deficiency. Root biomass allocation, as quantified by the allometric partitioning coefficient (*K*) was not altered by P availability in the efficient accessions, but inefficient accessions had less biomass partitioning to roots (i.e. a lower *K*) under low P conditions. Accessions with enhanced nodal rooting and laterals had greater P uptake and growth under low P. Dense root

hairs on nodal root main axes and first-order laterals conferred a marked benefit under low P, as evidenced by increased plant biomass and grain yield. Late maturity accessions had improved growth and yield under low P. Accessions DP x Tromba, HV313 x DEM, Macho III-04, and CIMMYT-1, were categorized as the most P efficient under low P and as the most responsive to increased P availability on a P-deficient acidic soil of this region. P-efficient accessions such as DP x Tromba accessed soil P not available to P-inefficient accessions. These results indicate that landraces of the Central Mexican highlands exhibit variation for several root traits that may be useful for genetic improvement of P acquisition efficiency in maize.

1. INTRODUCTION

Low soil fertility, especially suboptimal phosphorus availability is a principal, pervasive constraint to maize production in the Purhepecha Region [1]. Application of lime and P-containing fertilizers is usually the recommended treatment for enhancing soil P availability and stimulating crop yields. However, in this region, few maize producers can afford intensive chemical inputs. Genetic improvement of phosphorus efficiency, defined as yield ability at low phosphorus supply and better response to phosphorus inputs, is therefore an attractive prospect for the productivity of low-input systems [2]. Several genetic traits have been identified with potential for breeding phosphorus efficient crops, including root exudates, root-hair traits, cortical aerenchyma, topsoil foraging through basal and adventitious rooting [3].

Local maize germplasm have already been identified and evaluated for root traits conferring phosphorus acquisition efficiency, and specific traits responsible for P uptake efficiency among local landraces under low P environments have been characterized. Several root traits with potential in breeding phosphorus efficient maize include root hair traits and topsoil foraging through seminal, nodal, and lateral rooting. Therefore, it seems possible to screen a large number of maize accessions under field conditions for those root traits adapted to low P efficiency at different plant growth stages.

The present sequence of studies aimed to (a) assess the genetic variability of local genotypes for root traits that enhance phosphorus acquisition in a low P soil, (b) identify and validate phenotypic traits conferring P acquisition efficiency in maize, and (c) employ validated screening protocols for use of ^{32}P isotope techniques to identify genotypes selected for superior phosphorus acquisition and /or utilization.

2. ASSESS THE GENETIC VARIABILITY OF LOCAL GENOTYPES FOR ROOT TRAITS THAT ENHANCE PHOSPHORUS ACQUISITION IN A LOW P SOIL [1]

2.1. Introduction

Suboptimal soil phosphorus (P) availability is a principal yield-limiting factor for maize in many areas of the developing world, affecting at least eight million hectares of maize production globally [4]. Few unfertilized soils have adequate phosphorus availability for intensive crop production [5]. Low response to P fertilization and low P fertilizer recovery are the main obstacles to increased P efficiency in cereals, especially on P-adsorbing, acid soils, where applied P fertilizers often have poor efficacy [6].

[1] Full details of this study have been published as BAYUELO-JIMÉNEZ, J.S., et al., Genetic variation for root traits of maize (Zea mays L.) from Purhépecha Plateau, under contrasting phosphorus availability, Field Crops Res. 121 (2011) 350–362.

Plants display a variety of adaptations to low phosphorus availability, including changes in root morphology and architecture [3, 7, 8], increased production and secretion of P-mobilizing root exudates [9], increased proliferation and elongation of root hairs [10], modification of carbon metabolism and alternative respiratory pathways and enhanced expression of P_i transporters [11]. Phosphorus is relatively immobile in soil and its availability is typically greater in topsoil and declines substantially with depth [3]. Root architectural traits that enhance topsoil foraging such as shallower growth of basal roots, adventitious rooting and greater dispersion of lateral roots [3, 12, 13], and root hair formation [10, 14] may therefore enhance phosphorus acquisition in low phosphorus environments. Genotypic variation in adventitious root formation has been observed in several crops including common bean (*Phaseolus vulgaris* L.) [12] and maize (*Zea mays* L.) [15]. In common bean phosphorus availability regulates adventitious rooting [12] and two major quantitative trait loci (QTL) accounted for 19 to 61% of the total phenotypic variation for adventitious root traits under low phosphorus conditions [16].

Root hairs are particularly important for phosphorus acquisition. Considerable genetic variation in root hair length is correlated with phosphorus acquisition among genotypes of common bean (*P. vulgaris* L.) lentil (*Lens culinaris* L.) [17], wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* L.) [18].

The deployment of root architectural traits in plant breeding programs has great potential to alleviate P deficiency, a primary constraint to crop production in world agriculture [2]. Evaluation of P efficient germplasm among existing Mexican landraces is of interest in this regard since Mexico is the global centre of maize genetic diversity [19]. In particular, landraces of Michoacan State are well adapted to low P environments and may possess traits not common in elite germplasm. One of the most important traditional maize growing areas in this region is the Purhepecha Plateau [1]. In this area, it is common to find both synthetic hybrids and hybrids that have gone through creolization, a process by which improved varieties are exposed to farmer management, seed selection, and hybridization with landraces [20]. Over 60 percent of the total arable land is phosphorus-deficient in this region [1]. The objective of this study was to evaluate landraces of the Purhepecha Plateau for adaptation to low P soils, and their expression of root traits that could be important in P acquisition.

2.2. Materials and methods

2.2.1. Cigar roll culture system

A growth chamber screening- cigar roll method was conducted to identify phenotypic traits conferring phosphorus efficiency in 108 maize genotypes at the emergence stage (VE) in 2007. Maize seeds were surface sterilized for 1 min in 0.5% solution of NaOCl and then washed in deionized H_2O before germination. In each replicate, four typical seeds from the seed stock for accession were selected and wrapped in brown germination paper (Anchor Paper, St. Paul, MN, USA) as a cigar roll. Two batches of 108 cigar rolls were soaked vertically in two plastic containers filled with 5 l nutrient solution with low or high phosphorus. The low and high phosphorus treatments were amended with KH_2PO_4, at 1 µM (LP) and 1 mM (HP), respectively, in nutrient solution. Seedlings were germinated in darkness at 28 ± 1 ^oC in a growth chamber for 3 days, then grown under a photoperiod of 14 / 10 h at 28 / 22 ^oC (light/darkness) with photosynthetically active radiation (PAR) of 200 µmol photons m^{-2} s^{-1} at the soil level. The relative humidity was 65%. The nutrient solution pH was adjusted to 6.0 daily. Seedlings were grown in a growth chamber for healthy root system growth for up to 10 d. Plant seedlings were harvested 12 days after germination and the roots were preserved in 30% ethanol. At the time of the harvest, the root system consisted of a

primary root with emerged lateral roots, and 0–6 seminal roots. Seminal roots were dissected from seedlings. Seminal root length, seminal root number and root hairs were visually evaluated [21]. The experiment was a randomized complete block design with a split-plot arrangement of treatments. The main plots were low- and high-P; subplots were 108 accessions (3 maturity types and 4 breeding groups). There were four replicates staggered in time. For each replicate, there were four plants.

2.2.2. Field studies

Two experiments were conducted under low and high P fertilization and rain-fed conditions, in farmers' fields in Pontzomaran and Bonilla, in the central highlands of Michoacan, Mexico. Ponzomaran is located at 19° 24′ N, 101° 38′ W, 2400 masl; 800–1000 mm precipitation during the cropping season. Bonilla is located 19° 39′ N, 101° 01′ W, 2400 masl; 900–1100 mm precipitation. Soils of the study sites were vitrands (Table 1).

Experiments were arranged in a randomized complete block design with four replications in a split plot arrangement of treatments where P level was the main plot and accessions the sub-plots. Each experimental unit (plot) consisted of five 5-m long rows for each accession. Seeds were sown at 6 cm depth, 25 cm spacing between plants, and 60 cm spacing between rows. Experiments were conducted in P-deprived soils, because during the four previous maize growing seasons no fertilizers were applied. The low (LP) and high (HP) phosphorus treatments consisted of 23 kg P_2O_5 ha^{-1} and 97 kg P_2O_5 ha^{-1}, applied as calcium super phosphate, at seeding. All plots were additionally supplemented with 60 kg–N ha^{-1}as urea at seeding and 60 kg–N ha^{-1} at silking. Maize accessions were seeded within the optimum sowing dates (around April 17–23, after the beginning of 2007 and 2008 rainy season). Weeds were controlled with bromoxynil (3, 5-dibromo-4-hydroxy-benzonitrile) at 1 l ha^{-1} applied 20 days after emergence and also by manual cultivation.

2.2.3. Plant material

Two hundred and forty two local maize accessions were grown in 2007 (Table 2). All maize accessions are originally from the Purhepecha region, which have been recently used by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) maize breeding programme, and they differed in both yield, P responses to fertilization, stalk lodging susceptibility and grain type (floury to flint corn). The maize accessions were represented by three maturity types according to the number of days to silking: Early (E) 75–85 d, Intermediate (I) 85–95 d, and Late (L) 95–105 d, and four breeding groups within each maturity type: Landraces (C), Advanced landraces (AC), Hybrids x landraces (HxC), and Synthetic hybrids (S). Landraces in advanced generation are advanced breeding lines of landrace types collected in low P soils from Purhepecha Plateau and crossed with advanced generation of commercial hybrids. Contrasting accessions (P-efficient and P inefficient ones) were selected from the 242 maize accessions and planted in the succeeding crop cycle, Experiment 1 in Ponzomaran (2007) (n = 50 accessions) and Experiment 2 in Bonilla (2008) (n = 50), which allowed a comparison across locations (Table 2).

2.2.4. Plant measurements

Root crowns were excavated at the same growth stage by removing a cylinder of 30 to 40 cm soil depth and 50 cm from the shoot base (4 replicates) between 60 and 69 days after planting (DAP), which corresponded to the late vegetative growth stage (V8). The excavated root crowns were then shaken briefly to remove large fractions of the soil adhering to the root crown. The root crowns were immersed in water with P-free soap for about 8 min in order to

facilitate soil removal. After washing, the clean root crowns were stored in 25% ethanol at 4° C. Root architectural measurements were taken at the laboratory. The root system was characterized by four root types: the embryonic primary root, seminal roots emerging from the scutellar node, shoot-borne crown (nodal) and brace roots [22].

TABLE 1. PROPERTIES OF THE TOPSOIL (0–20 CM) OF THE ANDISOL IN PONZOMARAN, SAN JUAN TUMBIO, BONILLA, CHARAHUEN, AND LADERAS LOCATIONS, MICHOACAN, MEXICO

Parameter	Pontzomaran	Juan Tumbio	Bonilla	Charahuén	Laderas
Environmental variables					
Latitude (N)	19° 24′	19° 31′	19° 30′	19° 39′	19° 30′
Longitude (W)	101° 38′	101° 36′	101° 41′	101° 41′	101° 41′
Altitude (m.a.s.l.)	2280	2140	2240	2340	2240
Rainfall (mm)	800–1000	700–1400	900–1100	800–1100	900–1100
Temperature (°C)	5.4–24.1	14–20	14–20	14–22	15–20
Climate Temperate sub-humid	Cw	Cw	Cw	Cw	Cw
Soil classification					
Soil Survey Staff	Vitric Andisol	Vitric Andisol	Vitric Andisol	Vitric Andisol	Vitric Andisol
FAO-ISRIC-SICS	Eutric Haplaund	Eutric Haplaund	Eutric Haplaund	Eutric Haplaund	Eutric Haplaund
Soil characteristics					
Sand (%)	38.6	48.1	55.1	53.7	59.7
Clay (%)	38.5	18.9	11.6	13.6	7.6
Silt (%)	22.9	36.0	33.3	32.7	32.7
Apparent density (g cm^{-3})	0.86	1.08	0.89	1.01	0.85
pH	5.5	6.1	6.1	6.1	6.0
Organic matter (%)	7.9	6.2	4.2	3.7	4.1
Cation exchange capacity (cmol kg^{-1})	18.6	14.7	15.3	16.2	16.2
Exchangeable aluminium (cmol kg^{-1})	0.09	0.04	0.03	0.12	0.04
K (mg kg^{-1})	131	444.7	454	644	469
Ca (mg kg^{-1})	1225	1450	2254	1583	1860
Mg (mg kg^{-1})	84	248	394	375	324
Fe (mg kg^{-1})	40.8	33.5	37.6	57.1	37.1
Mn (mg kg^{-1})	26.8	23.2	20.7	65.8	9.56
Inorganic N (mg kg^{-1})	35	27	17.4	18.9	57.1
Available phosphorus Bray 1 (mg kg^{-1})	1.20	4.75	2.74	3.48	6.04
Low P	1.07	3.06	1.39	3.20	–
High P	3.84	8.69	2.24	9.7	–
System studied *Zea mays* L.					
Production cycle (Spring-Summer)/system	Rain-fed	Rain-fed	Rain-fed	Rain-fed	Pot
Fertilization: Nitrogen (Urea)†	120	120	120	120	60
Fertilization: Phosphorus ($P_2 O_5$)†	23 97	23 97	23 97	23 97	23 50

†Unit is kg ha^{-1}

TABLE 2. LANDRACES FROM THE PURHEPECHA PLATEAU, MICHOACAN, MEXICO (EXPERIMENTS 2007, 2008, 2009, 2010)

Maize types (Breeding groups, BG)	Maturity #		
	Early 78	Intermediate 102	Late 62
Landrace (C) †	32 (9) (2) (1) [c,d,e]	39 (19) (9) (3)	36 (12) (6) (3)
Advanced Landrace (AC) ‡	21 (10) (4) (2)	46 (11) (4) (1)	17 (9) (7) (3)
Hybrids x Landrace (H x C)	18 (8) (4) (2)	10 (7)	6 (2) (4) (1)
Synthetics (S) (checks)	7 (6) (6) (2)	7 (3) (3) (1)	3 (2) (1)
Racial classification	Conico, Chalqueño Purhepecha	Purhepecha Chalqueño	Purhepecha Chalqueño
Kernel	White, Purple, Yellow, Red	White, Purple, Yellow, Red	White, Yellow, Red
Days to flowering	75–85	85–95	95–105
Collected altitude (m.a.s.l.)	2500–2600 Highland	2200–2400 Western region	2200–2400 Eastern region

†Native population (landrace).
‡Advanced generation of landrace.
#Numbers in parenthesis indicate the common accessions included in Experiment 1 (Cigar rolls), Experiment 2 (2008) and Experiments 1, 2, and 3 (2009 and 2010), respectively.

Eight root traits were visually scored: seminal root length and number, seminal root branching and angle; nodal root length and number, nodal root branching and angle. A rating scale of 1–4 was used to rank crown root architecture, particularly for root branching and root angle where 1 = first order lateral branching and 4 = multiple lateral branches with up to 4 orders of branching. For nodal root angle, one indicates shallow root angles (1 = 0–22.5^{o}); 2 = 22.5–45^{o}; 3 = 45–67.5^{o}, and four indicates steep root angles (4 = 67.5–90^{o}).

Before root hair analyses, nodal roots were immersed in an ultrasound bath for 5–10 min in order to remove remaining soil particles without damaging root hairs. For root hair evaluation, root fragments were dyed in 0.05% trypan blue, and placed in a Petri dish with deionized water. Root hairs were visually evaluated using a rating scale of 1–9 to rank the density/length as follow: 1 = no root hairs; 3 = low root hair density/length; 5 = intermediate root hair density/length, 7 = between 5 and 9 rating scale; 9 = abundant root hairs [21]. Eight characters were measured for root hairs of nodal roots; root hair density and root hair length from the basal and middle region of nodal root main axis and root hair density and length from the basal and middle region of nodal root first order laterals, using a dissecting microscope (Zeiss STEMI DV4, Göttingen, Germany) at 30x magnification.

One sample per replication per maize accession were harvested between 60 and 69 DAP, which correspond to the vegetative growth stage V8. Shoot and root biomass was determined after drying at 60 ^{o}C to constant weight. Grain yield, adjusted to 10% moisture, was determined from the total harvest area. Relative biomass allocation to roots was determined by the allometric partitioning coefficient (*K*) derived from a series of paired measurements of root dry weight and shoot dry weight by linear regression of the form: $K = (\log R - \log b)/ \log S$ where *R* is root dry weight (g), *S* is shoot dry weight (g), *b* is a constant, and *K* is the allometric coefficient [23].

2.2.5. Statistical analyses

Before analyses of variance, data of mean values of each genotype for each variable were subjected to test for heterogeneous error variances using Bartlett´s Test [24]. Statistical

differences among maturity groups were ascertained from the SAS Generalized Linear Model Procedure [24]. A Protected Least Significant Difference (PLSD) was constructed when the F-tests indicated statistically significant differences among treatment effects ($P \leq 0.05$).

Phosphorus efficiency of maize accessions was determined by phosphorus efficiency index (PEI) [25] and assessed using principal component analysis (PCA) of standardized values of plant growth and grain yield parameters at low P, and relative values at low P to those obtained under high P supply. PCA was computed by means of SAS FACTOR procedure with the PRIN option and VARIMAX method for orthogonal rotation [24] on all 242 maize accessions. The relative weight of each principal component was weighted by the corresponding contribution rate accounting for variation of all growth traits. Consequently, PEI values of different accessions were calculated according to the retained principal component and their relative weigh, namely PEI = $^{242}\sum_{i=1} PC_i$ x RW_i. The criterion used for classification of maize accessions was determined by the method of cluster analysis and maize accessions were divided into 3 categories according to the P efficiency index (Fig. 1), and 4 categories according to P efficiency index in combination with growth potentials (shoot dry weight or grain yield at high P) (Fig. 2). A distance matrix was produced with the standardized data with the dissimilarity Euclidian distance coefficient and a Ward´s minimum variance clustering method was performed, rendering a dendrogram through the SYSTAT software [26].

2.3. Results and discussion

2.3.1. Seedling growth stage (VE): Phosphorus efficiency and correlation with root traits

Phosphorus efficiency was assessed using principal component analysis of 4 growth parameters of the 108 accessions. The 4 parameters at low P along with 4 indexes at low P relative to high P differed significantly among accessions. Three principal components (PCs) of each accession with eigen values greater than one were retained whose cumulative contribution was 97%. The relative weight of each principal component was weighted by the corresponding contribution rate accounting for variation of all growth traits. All accessions were grouped into 3 clusters: cluster 1 (PEI < –0.85) with 6 inefficient accessions, cluster 2 (–0.85 < PEI < –0.10) with 53 moderately efficient accessions and cluster 3 (PEI > 0.22) with 49 efficient accessions (data not shown). The responses of the parameters of the 108 accessions into three phosphorus efficient groups indicates that the root and shoot dry weights and root to shoot ratio increased with increasing P efficiency at low P (76%, 33%, 32%, respectively). The efficiency groups also differed in the pattern of seminal roots and root hair formation. Significant variation existed in all three phosphorus efficiency groups, though variation was least in the lowest efficiency group. Accessions of the phosphorus efficient group increased seminal root length under LP environments (35%), whereas root hair density (45%) and length (34%) of seminal roots were significantly greater in LP than in HP. These results suggest an adaptive benefit of increased seminal root length for the uptake of immobile nutrients such as P by increasing soil exploration [8].

The results of correlation analysis between PEI and root traits (Table 3) indicate that P efficiency was positively correlated with dry weights of roots and shoots at LP and HP, suggesting that screening for P efficiency should be conducted under P deficiency or under P deficiency along with a P-adequate control. Moreover, shoot dry weight should be used as an ideal parameter at the seedling stage for breeding P-efficient accessions of maize. PEI was also correlated with root architectural traits including seminal root length and tap root length at low P (Table 3). In addition, PEI was correlated positively with basal root hair density and

length of seminal main root and tap root axes at low P (Table 3). Therefore, it was indicated that the P-efficient accessions facilitated biomass accumulation, root growth, and P uptake under P deficiency, generally consisted with previous reports in maize [29].

TABLE 3. PEARSON CORRELATION COEFFICIENT AMONG PHOSPHORUS EFFICIENCY INDEX (PEI) AND ROOT TRAITS OF MAIZE ACCESSIONS GROWN IN A P-DEFICIENT SOLUTION WITH LOW P (1 μM, LP) OR WITH HIGH P ADDITION (1 MM, HP) AT THE SEEDLING GROWTH STAGE

Variables	P Level	Correlation coefficient (r)				
			Dry weight (mg plant^{-1})			
			Root		Shoot	
			HP	LP	HP	LP
		PEI	0.23	0.88***	0.33*	0.55***
Tap root length (cm)	HP	0.35**	0.57***	0.56***	0.59***	0.51***
	LP	0.43**	0.49***	0.55***	0.43***	0.49***
Seminal root length	HP	0.30*	0.62***	0.55***	0.52***	0.49***
(cm)	LP	0.39**	0.49***	0.53***	0.43***	0.50***
Seminal root hair	HP	–0.14	0.06	–0.06	–0.01	–0.13
density	LP	0.19	0.27*	0.26*	0.24*	0.34**
Tap root hair density	HP	0.34**	0.49**	0.51***	0.48***	0.42**
	LP	0.27*	0.31*	0.32*	0.31**	0.41**
Seminal root hair length	HP	0.26*	0.38**	0.40**	0.32**	0.25*
	LP	0.24*	0.21	0.26*	0.16	0.32**

* , **, ***, Significant at $P<0.05$, $P<0.01$, $P<0.001$.

2.3.2. Late vegetative growth stage (V8): Phosphorus efficiency and P responsiveness

We observed substantial variation among maize landraces from the Central Mexican highlands for growth in low P soil. Accessions could be grouped into 3 categories of P efficiency based on growth and grain yield parameters at low P and their relative values to those at high P (Fig. 1) and 4 categories according to P efficiency (PEI) in combination with P responsiveness (shoot dry weight or grain yield at high P) (Fig. 2). This study indicated that 41 accessions of Exp. 1 (n = 242) and 14 accessions across locations (n = 50) had the lowest growth and yield reduction and the highest levels of P efficiency (PEI > 0.69 and PEI> 0.56, respectively) under low P. Among common accessions, ZR-SGC (75), CB-CMP (78), Coangantzio (119), DP X Tromba (127), Cruz Gorda (140), San Gregorio (144), Macho III-05 (180), Macho III-05 (182), Cimmyt-1 (185), CBVA-AS-1 (198), CCHEDE (199), Macho-II 04 (237), Macho IV-03 (239) and Macho IV-05 (241) had consistently higher PEI in LP (Table 4).

When the combination of PEI with P responsiveness at high P is considered, accessions CB-GMP (57), CB-RSL (63), Santa Clara (115), Camémbaro (118), HV-313 X DE (135), Macho III-04 (181), Macho III-05 (182), Macho I-03 (233), Macho-II 04 (237) and Macho IV-05 (241) were the best accessions for P-deficient soil of this region (Fig. 2). These accessions were categorized as the most P efficient and as the most responsive to increased P availability. Applications of 97 kg P_2O_5 ha^{-1} (equivalent to 41.7 kg P ha^{-1}) increased shoot biomass and grain yield of the accessions. However, the increase was nearly equal to the difference between accessions in low P soil (23 kg P_2O_5 ha^{-1} equivalent to 9.9 kg P ha^{-1}) (Fig. 3). Thus, in low P soil, the P-efficient accessions seem to produce the same amount of dry

matter and grain yield as accessions with 97 kg P_2O_5 ha^{-1} less P fertilizer applications. These results demonstrate the added advantage of selection and breeding for P efficiency for maintaining productivity in low P soils.

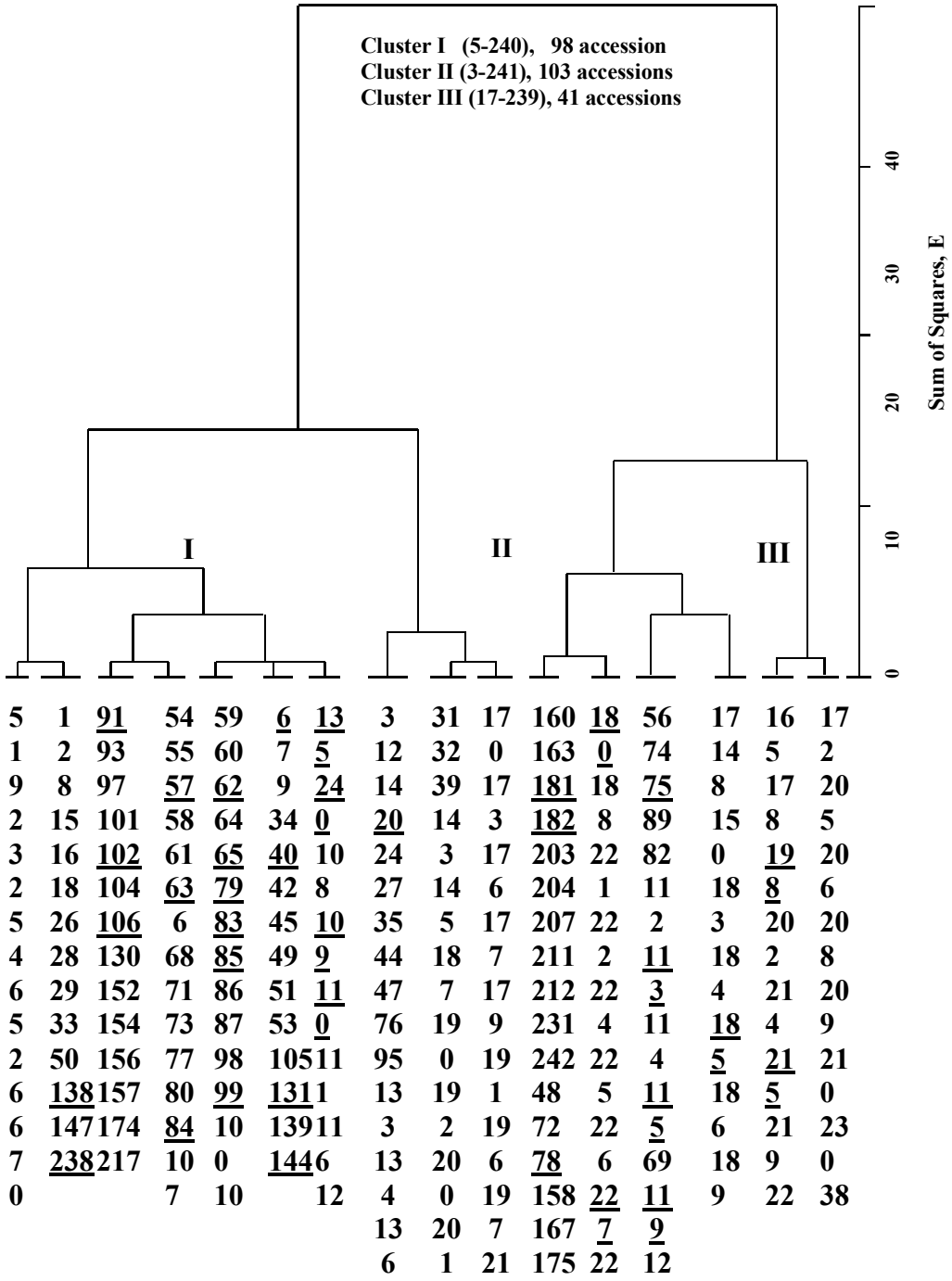

FIG. 1. Clustering of 242 accessions for P efficiency by the Ward cluster method according to PEI of each accession, where PEI is the parameter for assessing P efficiency obtained from principal component analysis. Cluster I-III represents high P efficiency, moderate P efficiency and low P efficiency, respectively. Underlined entries represent the 50 accessions selected from the 242 accessions and planted in the subsequent crop cycle.

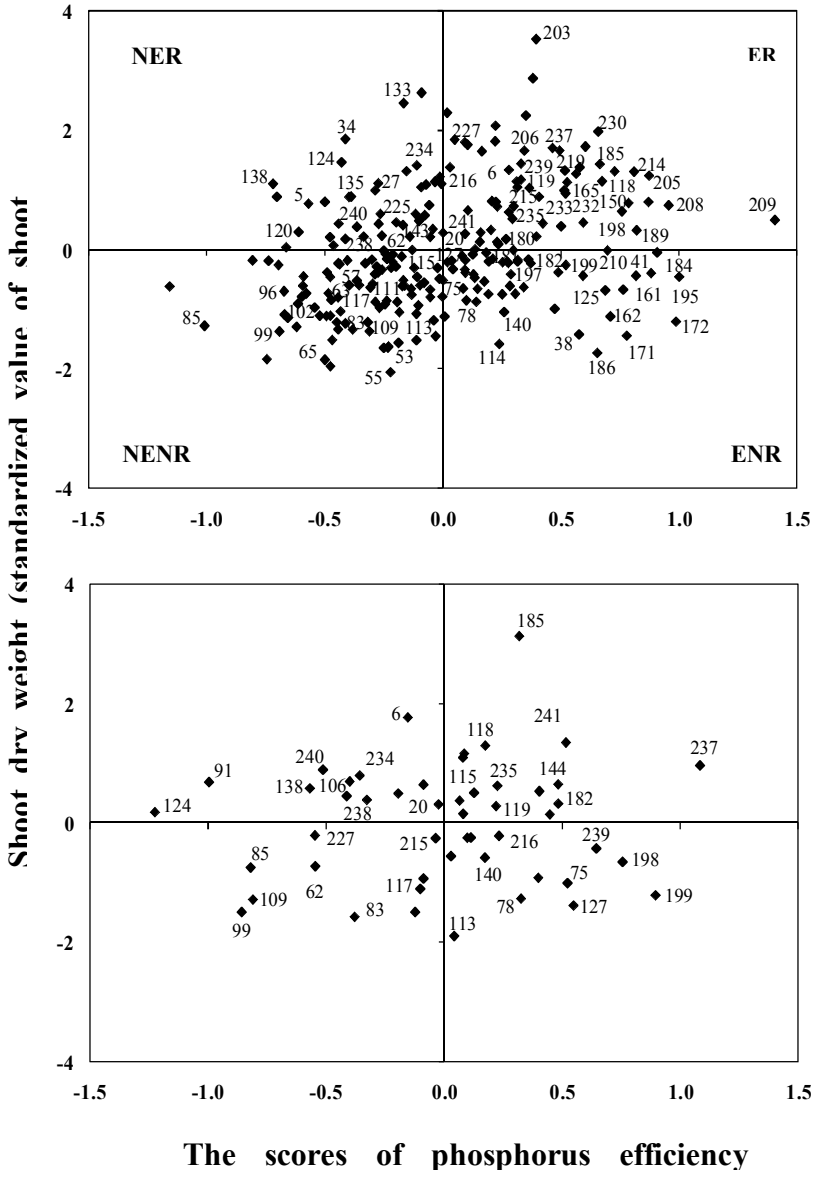

FIG. 2. Division of charts of 242 and 50 common accessions according to P efficiency and standardized value of shoot dry weight under high P conditions. Phosphorus efficiency is expressed as PEI, which is an assessment index calculated from principal component analysis. Standardized values of shoot dry weight are estimated as the following function: $Xs = (X - \bar{X})/SD$ *. Categories represented by efficient and responsive (ER), non-efficient and responsive (NER), non-efficient and non-responsive (NENR), and efficient and non-responsive (ENR). Accession numbers are indicated.*

A common finding in many screens for P efficiency is that traditional varieties or landraces have greater P-use efficiency than modern cultivars [6, 27]. This is often attributed to the impact of breeding programs being performed under nutrient replete conditions [28]. Moreover, it is considered that while such programs have produced cultivars with large harvest indexes, which are also relatively internally P-efficient, further gains in P-limited yield are most likely to be achieved by improving the external P efficiency [27]. A higher P efficiency in plants can be achieved by improving P acquisition and/or internal P utilization [28]. Late P-efficient accessions ZR-SGC (75), Coangantzio (119), DP X Tromba (127), Macho IV-03 (239), and Macho IV-05 (241) had an excellent relationship between grain yield and shoot biomass ($r^2 = 0.83$) under low P conditions, suggesting that P utilization through

better dry matter accumulation and partitioning of above-ground dry matter to grain is the most critical factor in expression of high P efficiency. Alternatively, other late P efficient accessions SC-JC-11-Mz4 (38), PM-TA-16 Mz21 (41), Camémbaro CRM-00-05 (118), H-791 99-04-05 (125), and Macho IV-03 (239) can be selected showing a significant relationship between shoot biomass and nodal root number (r^2 = 0.56) and nodal root branching (r^2 = 0.60) or between grain yield and nodal root branching (r^2 = 0.54) and nodal root length (r^2 = 0.46) suggesting that P acquisition ability of genotypes is a decisive factor in expression of high P efficiency. It seems likely that P efficiency mechanisms may be different among the accessions of a given species.

2.3.3. Mechanisms involved in P efficiency: root growth and allocation of dry matter

Genetic differences in P efficiency were associated with variation for root growth and root architecture. Accessions that were P efficient (less shoot biomass depression under low P) maintained a higher root to shoot ratio under low P. Phosphorus efficient accessions had greater biomass allocation to roots than inefficient accessions (Fig. 3), and better maintenance of biomass allocation to roots under P stress (r^2 = 0.01 to r^2 = 0.04). Increased biomass allocation to root growth is beneficial for P acquisition, since P is relatively immobile in soil, but may slow overall plant growth because of the increased respiratory burden of root tissue [7]. Plants with low rates of root respiration may be able to maintain greater root biomass than inefficient genotypes without increasing overall root carbon costs.

2.3.4. Mechanism involved in P efficiency: root architecture and morphology

The response of axile roots (seminal and nodal) and the length and number of lateral roots to P stress varies substantially among maize genotypes [29]. Genotypes with increased or sustained elongation of axile roots and lateral root development under P deficiency had superior ability to acquire P and maintain growth (r^2 = 0.50 to r^2 = 0.60). In this crop, a large proportion of the mature root system consists of nodal roots, so the maintenance of nodal root formation, when overall growth is inhibited by P-deficiency, could result in an increased proportion of root length in the adventitious root system. In this study, we confirm that enhanced nodal rooting and greater nodal branching (nodal root laterals) is indeed important for plant adaptation to low phosphorus in maize (Fig. 3).

Nodal rooting was significantly correlated with plant growth in the field among contrasting accessions and across locations (Table 5). Efficient accessions with greater nodal rooting and lateral branching at low P had greater biomass then did inefficient accessions with reduced nodal root formation and lateral branching (Fig. 3). The greater nodal rooting of P efficient accessions under low P could be explained by the greater overall biomass and the weak allometric relationship of plant biomass with root biomass (r^2 = 0.04). Therefore, if nodal roots in the topsoil are advantageous for acquiring phosphorus under limited P availability, as suggested by [1], this weak allometric relationship would facilitate the selection of efficient accessions with high nodal rooting in the field. Genotypic differences in the specific root length and P concentration of the lateral roots of maize has been associated with reduced biomass and P investment to the extension to lateral roots [29]. Therefore, these results suggest that enhanced lateral rooting under P stress may be harnessed as a useful trait for the selection and breeding of more P-efficient maize genotypes.

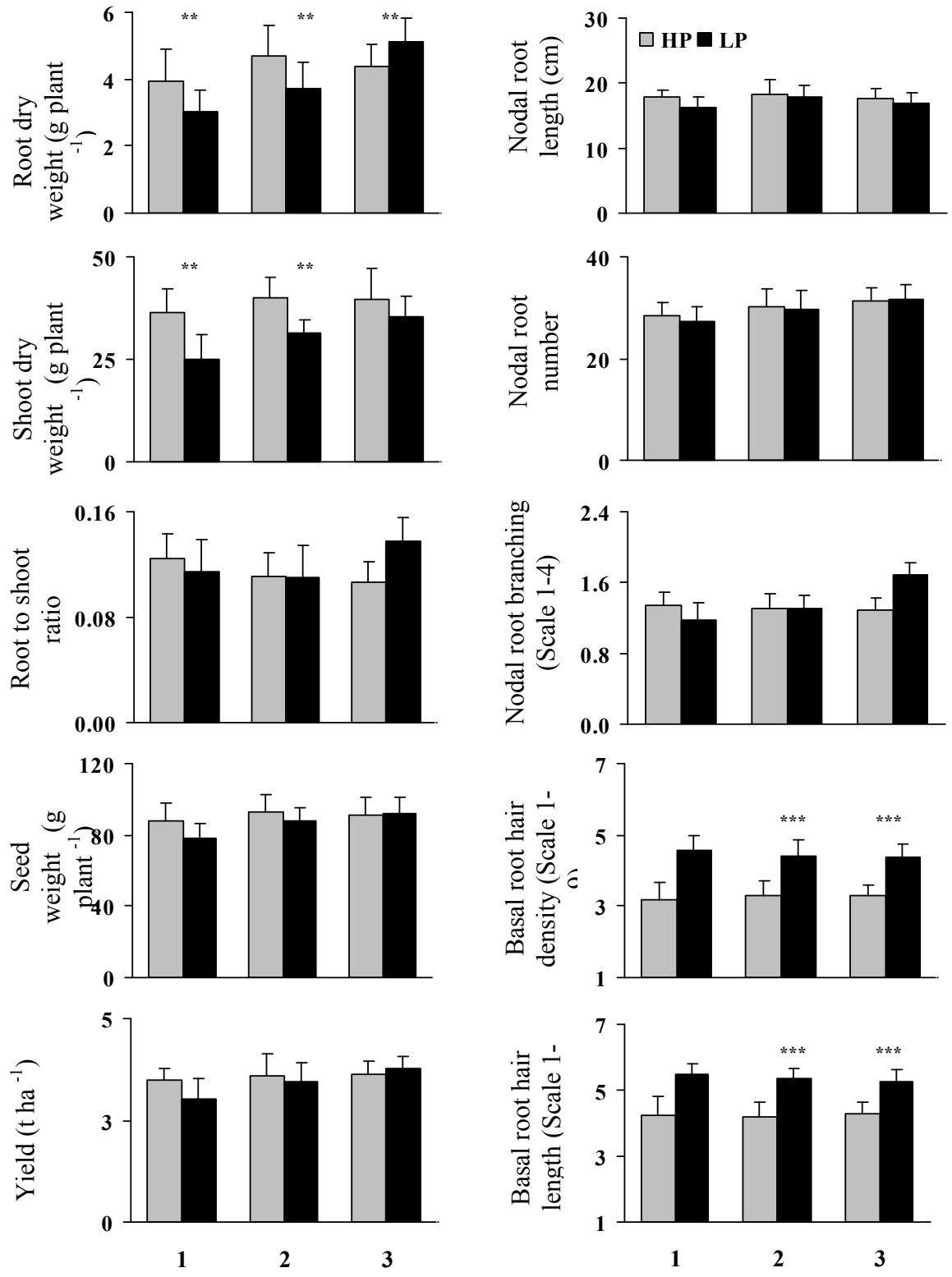

*FIG. 3. Variation of ten parameters of maize plants under low P and high P conditions with low, medium and high P efficiency. Phosphorus efficiency was ranked according to principal component analysis and cluster analysis of 50 common accessions. 1 to 3 representing P efficiency from low to high. Bars are the standard error of means of 14, 22, and 14 accessions, respectively. Within the P efficient group, bars with *, **, and *** indicate significance at P<0.05, P<0.01, P<0.001, respectively.*

TABLE 4. SELECTION OF 50 MAIZE ACCESSIONS ACCORDING TO P EFFICIENCY INDEX (PEI) IN PONZOMARAN (EXPERIMENT 1, 2007), BONILLA (EXPERIMENT, 2008), AND COMBINED EXPERIMENTS

ID	Accession	M†	BG‡	Experiment 1 (2007)			Experiment 2 (2008)			Experiments 1 + 2		
				ID	PEI#	Cluster§	ID	PEI	Cluster	ID	PEI	Cluster
118	Camémbaro CRM-00-05	L	AC	118	1.02	E	237	0.97	E	237	1.08	E
185	CIMMYT-1 M-00-04-05	E	HxC	185	0.70	E	127	0.93	E	199	0.89	E
182	Macho-III-05	I	S	182	0.68	E	198	0.88	E	198	0.76	E
119	Coangatzio CR-03-04-05	L	AC	119	0.59	E	199	0.85	E	239	0.64	E
215	Zacapu Cr-03-05	I	AC	215	0.57	E	239	0.78	E	127	0.55	E
180	Macho-III-03	I	S	180	0.56	E	115	0.54	E	75	0.52	E
239	Macho-IV-03	L	S	239	0.56	E	216	0.53	E	241	0.52	E
235	Macho-I-05	E	S	235	0.53	E	84	0.53	E	182	0.48	E
181	Macho-III-04	I	S	181	0.51	E	83	0.48	E	144	0.48	E
237	Macho-II-04	E	S	237	0.49	E	65	0.45	E	119	0.45	E
233	Macho-I-03	E	S	233	0.47	E	135	0.43	E	235	0.40	E
199	CCHEDE CM-00-04-05	E	AC	199	0.47	E	75	0.35	ME	140	0.40	E
241	Macho-IV-05	L	S	241	0.45	E	140	0.31	ME	78	0.33	E
198	CBVA-AS-1-P CM-01-04-05	E	HxC	198	0.43	E	79	0.28	ME	185	0.32	E
6	SHUI-MRC-2	L	C	6	0.27	ME	218	0.27	ME	216	0.23	ME
140	Cruz Gorda CrM-00-04-05	E	AC	140	0.20	ME	111	0.23	ME	115	0.22	ME
216	Tepetate Cr-03-05	I	AC	216	0.20	ME	106	0.22	ME	181	0.22	ME
20	UR-RP-7	E	C	20	0.14	ME	102	0.21	ME	118	0.17	ME
75	ZR-SGC-6	L	C	75	0.13	ME	63	0.18	ME	180	0.17	ME
78	CB-GMP-11	I	C	78	0.12	ME	57	0.16	ME	57	0.13	ME
227	San Isidro CM-03-04	I	AC	227	0.08	ME	180	0.10	ME	113	0.11	ME
127	DP X Tromba	L	HxC	127	0.08	ME	117	0.07	ME	65	0.10	ME
113	Coangatzio CM-00-04-05	L	AC	113	0.05	ME	241	0.06	ME	63	0.09	ME
234	Macho-I-04	E	S	234	0.04	ME	119	0.06	ME	111	0.08	ME
62	PICH-PFC-1	I	C	62	0.03	ME	235	0.05	ME	135	0.08	ME
65	PICH-MZU-12	I	C	65	–0.05	ME	144	0.02	ME	233	0.06	ME
115	Santa Clara CM–03–04-05	L	AC	115	–0.06	ME	236	–0.03	ME	102	0.04	ME
218	Turiran CM-03-05	I	AC	218	–0.08	ME	233	–0.05	ME	84	0.03	ME
238	Macho-II-05	E	S	238	–0.08	ME	113	–0.07	ME	20	–0.02	ME
240	Macho-IV-04	L	S	240	–0.13	ME	78	–0.07	ME	215	–0.04	ME

TABLE 4. SELECTION OF 50 MAIZE ACCESSIONS ACCORDING TO P EFFICIENCY INDEX (PEI) IN PONZOMARAN (EXPERIMENT 1, 2007), BONILLA (EXPERIMENT, 2008), AND COMBINED EXPERIMENTS (continued)

109	Paso del muerto CM-03-04-05	L	AC	109	–0.15	ME	99	–0.07	ME	236	–0.09	ME
117	H-7545 M-00-04-05	L	HxC	117	–0.16	ME	20	–0.08	ME	79	–0.09	ME
236	Macho-II-03	E	S	236	–0.18	ME	85	–0.12	ME	117	–0.10	ME
144	San Gregorio CM-00-04-05	E	AC	144	–0.19	ME	138	–0.17	ME	83	–0.12	ME
135	HV-313XDE M-00-04-05	E	HxC	135	–0.23	ME	182	–0.33	I	6	–0.15	ME
63	CB-RSL-2	L	C	63	–0.23	ME	238	–0.35	I	218	–0.19	ME
83	OP-4	I	C	83	–0.24	ME	40	–0.37	I	238	–0.33	I
57	CB-GMP-9	L	C	57	–0.27	ME	181	–0.41	I	234	–0.36	I
40	PA-JRG- 13- Mz 4	L	C	40	–0.28	ME	185	–0.42	I	40	–0.38	I
111	CRM-00-04-05	L	AC	111	–0.29	ME	131	–0.43	I	131	–0.40	I
79	PICH-EGM-4	I	C	79	–0.29	ME	234	–0.45	I	106	–0.41	I
84	AJ-MAM-5	L	C	84	–0.29	ME	215	–0.51	I	240	–0.51	I
131	El Tigre CM-00-04-05	E	AC	131	–0.30	ME	227	–0.56	I	62	–0.55	I
102	NPZ-GAS-2	I	C	102	–0.30	ME	124	–0.68	I	227	–0.55	I
124	Corupo CM-99-04-05	L	AC	124	–0.31	ME	62	–0.73	I	138	–0.57	I
106	UR-VR-6	I	C	106	–0.68	I	240	–0.74	I	109	–0.81	I
138	DE X HV313 M-00-04-05	E	HxC	138	–0.71	I	118	–0.77	I	85	–0.82	I
91	SANAZ-EGS-6	I	C	91	–1.10	I	91	–0.78	I	99	–0.86	I
85	PICH-MZU-17	I	C	85	–1.13	I	109	–0.79	I	91	–1.00	I
99	TZU-ATM-7	E	C	99	–1.18	I	6	–0.96	I	124	–1.22	I
					(PEI > 0.51) (–0.96 < PEI < –0.14) (PEI < –0.96)			(PEI > 0.67) (–0.58 < PEI < 0.08) (PEI < –0.58)			(PEI > 0.56) (–0.63< PEI < 0.04) (PEI < –0.63)	

†Maturity: Early (E), Intermediate (I), Late (L)
‡BG: Breeding groups: Landrace (C), Advanced Landraces (AC), Hybrids x Landraces (HXC), and Synthetics (S)
#Phosphorus efficiency was expressed as P efficiency index (PEI) gained from the principal component analysis
§Rating of maize accessions as efficient (E), moderately efficient (ME), and inefficient (I) was determined according to the order of the PEI value gained from the principal component analysis and classification analysis

Several lines of evidence show that root hairs contribute to P acquisition [14]. Low P availability increases the length and density [30] of root hairs. In this study we observed large variation for the ability to develop root hairs along the nodal root axis and nodal first-order laterals (Fig. 3). Whereas several inefficient accessions had shorter and fewer root hairs in both regions, efficient accessions developed much longer and denser hairs on nodal roots. The presence of denser root hairs of main axis ($r^2 = 0.67$ to $r^2 = 0.81$) and nodal first-order laterals ($r^2 = 0.71$), was associated with plant performance at low P. Genetic variation in root-hair length and density in maize is controlled by several major quantitative trait loci (QTL) [31], suggesting that this trait could be selected in breeding programs through marked-assisted selection.

TABLE 5. PHENOTYPIC CORRELATIONS AMONG NODAL ROOT TRAITS AND SHOOT BIOMASS: NUMBER OF NODAL ROOTS (NODAL_NO), NODAL ROOT LENGTH (NODAL_RL), NODAL BRANCHING (NODAL_BR), NODAL ROOT ANGLE (NODAL_RA), SHOOT DRY WEIGHT (SHDW), AND GRAIN YIELD (GY)

HP/LP†	Nodal_No	Nodal_RL	Nodal_Br	Nodal_Ra	ShDW	Gy
Experiment 1 (n = 242)						
Nodal_No	*0.49***	0.25***	0.39***	0.37***	0.38***	0.10
Nodal_RL	0.42***	*0.39***	0.50***	0.48***	–0.09	0.24**
Nodal_Br	0.55***	0.51***	*0.49***	0.51***	0.10	0.21**
Nodal_Ra	0.33***	0.31***	0.46***	*0.32***	–0.02	0.07
ShDW	0.49***	–0.03	0.21**	–0.02	*0.50***	0.09
Gy	0.18*	0.07	0.28**	0.10	0.16	*0.68****
Experiments 1 + 2 (n = 50)						
Nodal_No	*0.32***	0.36**	0.32**	0.13	0.41**	0.09
Nodal_RL	0.19	*0.39****	0.52***	0.31**	0.00	0.21*
Nodal_Br	0.25*	0.54***	*0.26**	0.24	–0.03	0.18
Nodal_Ra	0.05	0.21	0.45**	*0.32***	0.04	0.25*
ShDW	0.48***	–0.01	–0.01	–0.24*	*0.36***	0.05
Gy	0.01	0.38***	0.35**	–0.06	0.02	0.54***

†For each environment, values below the diagonal represent correlations within the low P treatment; values above the diagonal represent correlations within the high P treatment; values on the diagonal (*italic*) correspond to across-P treatment correlations

*, **, ***, Significant at $P<0.05$, $P<0.01$, $P<0.001$

3. IDENTIFY AND VALIDATE PHENOTYPIC TRAITS CONFERRING P ACQUISITION EFFICIENCY IN MAIZE PLANTS [2]

3.1. Introduction

Nutrient use efficiency is defined as the ability of a genotype to acquire plant nutrients from the medium and/or to incorporate or utilize them in shoot and root biomass production or economic yield [32]. Phosphorus use efficiency can be divided into P acquisition efficiency (PAE) and P utilization efficiency (PUE) [27]. PAE refers to mobilizing P from poorly soluble sources or to take up the soluble P available in the soil solution, and PUE is the ability to produce biomass or yield efficiently using the scarce acquired P. Enhancing P use efficiency by plants can be achieving through improving P acquisition and/or utilization [6, 33].

[2] Full details of this study are in preparation as BAYUELO-JIMÉNEZ, et al., Phosphorus efficiency and responsiveness in maize landraces from the Purhépecha Plateau: relationships between plant growth, root traits and phosphorus uptake.

As described in the general introduction, the main mechanisms related to increased P acquisition efficiency include root architecture, increased proliferation and elongation of root hairs, root morphology, mycorrhizal associations, high affinity transporters, and the genotypic capacity to secrete organic compounds in the rhizosphere like phosphatases and organic acids [34]. A higher P utilization efficiency is mainly attributed to efficient translocation and use of the stored P in plants [35]. Higher internal utilization efficiency has also been attributed to a higher grain yield per unit of P in the grain (quotient of utilization) and to a higher ability to transfer nutrient from shoot to grains, called P harvest index [32].

The existence of considerable variation on root architectural traits that increase topsoil foraging could enhance the feasibility of improved crops for enhancing mineral nutrient use efficiency [2]. Identification of cultivars with greater tolerance to suboptimal phosphorus nutrient levels offers considerable promise for increasing the crop production potential of marginal low fertility land throughout the world [28]. In assessing maize genotypic variation for tolerance to P deficiency, great variation was observed among existing landraces of Michoacan state in their tolerance to P deficiency [36]. Phosphorus efficient landraces of the Central Mexican Highlands can be of benefit in improving the use of native soil P and residues of P applied as fertilizer on both low P soils and on soils adequately supplied with P. To achieve any progress in this area, there is a need to first identify genotypes that differ in their ability to use P efficiently and that respond differently to applied phosphorus. The present study was undertaken to identify plant growth traits associated with P acquisition efficiency (PAE) and/or P utilization efficiency (PUE) in maize landraces growing on P-deficient Andisol in the Central Mexican Highlands.

3.2. Materials and methods

Three experiments were conducted under low and high P fertilization and rain-fed conditions, in farmers' fields in Pontzomaran, San Juan Tumbio, and Bonilla, in the central highlands of Michoacan, during the 2007 and 2008 growing season. The geographical co-ordinates, altitude, soil type and the annual rainfall for each site are given in Table I. Experimental design in all cases was randomized complete blocks with four replications in a split plot arrangement of treatments, with P level as the main plots and accessions as the sub-plots. Crop sequences and fertilizer additions were identical as described in Section 2.2.2.

Fifty and twenty-six local maize accessions were grown in 2007 in Ponzomaran (Exp. 1) and San Juan Tumbio (Exp. 2), respectively. Common accessions were planted in the succeeding crop cycle, Experiment 3 in Bonilla (2008) (n = 50), which allowed a comparison across locations (Table 2). One plant per replication per accession was harvested between 78 and 89 DAP, which correspond to the silking stage [37]. Analyses for root architecture (crown roots and root hairs) and biomass production were performed as described previously. Root and shoot dry tissue samples were ground and analyzed for P content [28]. The phosphorus acquisition efficiency (PAE) is a measure of the P absorption per unit root dry weight (DW) (mg P g^{-1} root DW), and was calculated from total P per plant divided by root DW (g) [28]. The phosphorus utilization efficiency (PUE) is a measure of DW return per unit P uptake (g DW mg^{-1} P), and was calculated as total plant DW divided by P content per plant [28]. The phosphorus efficiency of maize accessions was determined by the phosphorus efficiency index (PEI) [25] and assessed using principal component analysis [24].

3.3. Results and discussion

3.3.1. Phenotypic traits conferring P acquisition efficiency

A number of physiological traits could contribute to P efficiency, by improving P acquisition from the soil or by improving the utilization of acquired P in growth [28, 32]. Comparisons of maize accessions in three soil environments and maturity types showed that differences in P acquisition were due to a large variation for root growth, root to shoot ratio, root and shoot P content (Tables 6, 7 and 8) and root architecture (data not shown). In general, greater root and shoot dry weight and grain yield were observed in plants with late maturity under low soil P. Therefore, late P-efficient accessions were the most efficient in terms of biomass produced per unit of P absorbed (Tables 7 and 8), produced the largest root system (25.4 cm root^{-1} and 70.5 nodal roots plant^{-1}) (Table 9), had the largest root uptake per unit root weight (17 to 24.5 mg P g^{-1} root DW), and had more nodal rooting than did P-inefficient accessions (85, 99, 59, 78, 71 and 89% in nodal root length, nodal root number, nodal root angle, and root hair density and length, respectively).

TABLE 6. ROOT AND SHOOT GROWTH PARAMETERS AND GRAIN YIELD OF MAIZE ACCESSIONS OF DIFFERING MATURITY IN PONZOMARAN (EXPERIMENT 1, 2007), SAN JUAN TUMBIO (EXPERIMENT 2, 2007), AND BONILLA (EXPERIMENT 3, 2008) WITH LOW P (LP) AND HIGH P ADDITION (HP)

Experiment	P level	Root dry weight (g plant^{-1})			Shoot dry weight (g plant^{-1})			Grain yield (Mg ha^{-1})		
		Early	Middle	Late	Early	Middle	Late	Early	Middle	Late
1 (2007)	LP†	7.2a	6.4 a	6.5a	69.7a	61.0 b	65.1a	2.8a	3.0a	3.3a
	HP	9.8a	9.0 a	10.4a	83.4a	77.2 b	83.4a	3.1b	3.0b	3.6a
2 (2007)	LP	10.4b	10.5 b	11.4a	126.2b	120.9b	150.0a	3.3a	3.3b	3.8a
	HP	9.8b	10.2 b	12.9a	129.0b	122.6b	143.2a	3.1b	3.3b	3.9a
3 (2008)	LP	9.1b	10.1 b	11.7a	151.5c	167.5b	189.3a	3.9 a	3.8 a	4.5a
	HP	11.6b	12.3 b	13.9a	173.9c	190.4b	205.7a	4.2 a	4.0 a	3.9a

†Within rows, means followed by the same letter are not significantly different according to LSD (P<0.05).

When LP availability limits plant growth, the dry weight produced will depend on P uptake and the efficiency of internal P use for dry matter production [39]. Dry weight accumulation in LP level was tightly correlated with P uptake, but not with tissue P concentration (Fig. 4, Table 11). This suggests that tissue P concentration is not a reliable criterion in assessing maize genotypes for P deficiency. Therefore, tolerance to P deficiency is entirely dependent on genotypic differences in P uptake.

P uptake itself is a function of the root size and root efficiency [27], where root efficiency was defined as P uptake per unit dry weight (Table 7). Genotypic variation existed for both traits and high P acquisition can be achieved, either due to large roots (P-efficient accessions 40, 75, 109, 113, 115, 182, 199 and 234) (Table 9) or with a more efficient root system (6, 40, 75, 78, 109, 182, 199 and 234). Phosphorus-efficient accessions, particularly of late maturity, had a large root system (12.3 to 16.5 g plant^{-1}) and their P uptake (301.7 mg plant^{-1}) and PAE (23.4 mg P g^{-1} root DW) was above average under LP conditions (Table 8). As a result, the correlation between P uptake and root efficiency was high (Table 11). This indicates that a better adaptation to P deficiency as a result of a large root system may be a more reliable criterion to identify efficient accessions to LP soils.

TABLE 7. SHOOT P CONCENTRATION, ROOT AND SHOOT P CONTENT OF MAIZE ACCESSIONS OF DIFFERING MATURITY IN PONZOMARAN, SAN JUAN TUMBIO, AND BONILLA WITH LOW P (LP) AND HIGH P ADDITION (HP)

Experiment	P	Shoot P concentration (mg P g^{-1})			Root P content (mg)			Shoot P content (mg)		
		Early	Middle	Late	Early	Middle	Late	Early	Middle	Late
1 (2007)	LP†	0.98a	1.00 a	0.98a	5.8 a	5.1 a	5.7a	68.6a	60.7a	65.2a
	HP	1.01a	0.99 a	1.07a	7.8 b	7.4 b	9.4a	85.4a	76.2b	88.8a
2 (2007)	LP	1.07a	1.08 a	1.08a	5.6 b	6.8 a	7.7a	134.3b	134.9b	187.0a
	HP	1.12a	1.18 a	1.18a	5.7 b	6.9 a	7.2a	144.6b	147.7b	197.0a
3 (2008)	LP	1.51a	1.50 a	1.49a	5.8 b	7.2 a	6.7a	229.0b	252.7a	280.2a
	HP	1.56a	1.49 a	1.58a	7.2 a	7.6 a	8.2a	271.4b	284.1a	301.7a

†Within rows, means followed by the same letter are not significantly different according to LSD (P<0.05).

TABLE 8. TOTAL P UPTAKE, P ACQUISITION EFFICIENCY (PAE), AND P UTILIZATION EFFICIENCY (PUE) OF MAIZE ACCESSIONS OF DIFFERING MATURITY IN PONZOMARAN, SAN JUAN TUMBIO, AND BONILLA WITH LOW P (LP) AND HIGH P ADDITION (HP)

Experiment	P level	Total P uptake (mg $plant^{-1}$)			PAE (mg P g^{-1} root DW)			PUE (g DW mg^{-1} P)		
		Early	Middle	Late	Early	Middle	Late	Early	Middle	Late
1 (2007)	LP†	74.7a	65.7 b	70.8ab	11.1a	12.3 a	12.2a	1.08a	1.1 a	1.1a
	HP	93.9ab	83.7 b	98.0 a	9.9a	9.9 a	10.7a	1.06a	1.1 a	0.1a
2 (2007)	LP	139.9	141.8 b	194.7a	14.4b	13.3 b	19.5a	1.00a	1.0 a	0.9b
	HP	150.7	155.4 b	204.1a	15.8b	15.7 b	18.1a	0.94a	0.9 a	0.8b
3 (2008)	LP	234.8	259.9ab	287.0a	27.3a	28.0 a	26.4a	0.69a	0.7 a	0.7a
	HP	278.7	291.8ab	319.9a	24.8a	25.3 a	23.3a	0.68a	0.7 a	0.7a

†Within rows, means followed by the same letter are not significantly different according to LSD (P<0.05).

Root system architecture, morphology, and biochemistry, are key traits for optimizing P acquisition, and thus their P use efficiency and responsiveness [6]. Architectural traits associated with enhanced topsoil foraging include shallower growth angles of axial roots, enhanced adventitious rooting, a greater number of axial roots, and greater dispersion of lateral roots [2]. Maize genotypes with increased or sustained elongation of nodal roots and lateral root development under P deficiency had superior ability to acquire P and maintain growth [36]. This study confirmed that enhanced nodal rooting and greater nodal branching (85 to 99%) is indeed important for plant adaptation to LP in maize. Nodal rooting was significantly related with P acquisition and plant growth in the field among P efficient groups (Table 10). P-efficient accessions [i.e. Paramuén Cr-03-JR10 (40), ZR-6 (75), Paso del Muerto Cr-03-TA (109), and Macho III-05 (182)] with greater nodal rooting and lateral branching at LP had greater biomass and P uptake efficiency (r^2 = 0.53 to r^2 = 0.94) then did inefficient accessions with reduced nodal root formation and lateral branching (r^2 = 0.18 to r^2 = 0.23). The greater nodal rooting of P-efficient accessions under LP could be explained by the greater overall biomass and the weak allometric relationship of plant biomass with root biomass (r^2 = 0.05). Therefore, if nodal roots in the topsoil are advantageous for acquiring P under limited P availability, this weak allometric relationship would facilitate the selection of efficient accessions with high nodal rooting in the field.

The presence of root hairs conferred a marked benefit under LP conditions, as evidenced by increased plant biomass, P uptake and reproductive output (grain yield) of P efficient accessions (Table 10), and decreased those parameters on P-inefficient ones (r^2 =

0.17, r^2 = –0.34, r^2 = 0.19). The competitive advantage of root hairs is presumably due to greater P uptake [40]. Phosphorus mobility in soil is governed by diffusion rather than mass flow [14], and therefore root P uptake is limited by localized P depletion around the root. Root hairs extend the P depletion zone from the root epidermis, thereby increasing the rate of P uptake and the total amount of P accessible by the root. Several lines of evidence show that root hairs contribute to P acquisition [40]. Root hairs, particularly denser and longer root hairs of nodal first-order laterals were associated with plant performance, grain yield and root P acquisition at LP (Table 10). Late P-efficient accessions Paramuén Cr-03-JR10 (40), Paso del Muerto Cr-03-TA (109), Paso del Muerto CM-00 (113), and Santa Clara CM-03 (115) can be selected showing a significant relationship between root hairs and root P acquisition, suggesting that P acquisition ability of genotypes is a decisive factor in expression of high P efficiency.

3.3.2. Phenotypic traits conferring P utilization efficiency

Phosphorus utilization efficiency represents the amount of dry matter produced per unit of P absorbed (g DM mg^{-1} P) [41]. Any species able to maintain metabolic activities at low tissue P concentration and produce more dry matter per unit of P absorbed is considered efficient in P utilization. In this study, there were accessions in which P content in shoot had a highly significant and positive correlation with root dry weight (r = 0.54**) and shoot dry weight (r = 0.78***) suggesting that accessions with higher root dry weight accumulated higher amount of shoot P and produced higher shoot dry matter at LP. Thus, under P-stress, better P acquisition and PUE by the P-efficient accessions for biomass synthesis collectively formed the basis of higher shoot dry matter production, evidencing that P uptake and PUE are important plant traits for selecting low P-tolerant accessions (Table 8).

Thus, under P-stress, better P acquisition and PUE by the P-efficient accessions for biomass synthesis collectively formed the basis of higher shoot dry matter production, evidencing that P uptake and PUE are important plant traits for selecting low P-tolerant accessions (Table 9). In addition, PUE and whole–plant P uptake were positively correlated with each other under LP and HP conditions (Table 11), suggesting lack of any interactions between these two parameters under either of the growth conditions. This, therefore, suggests the possibility of a combination of these two parameters to improve growth responses of accessions to P. Finally, late P-efficient accessions Paramuén Cr-03-JR10 (40), ZR-6 (75), CM-754 (117), Macho IV-03 (239), and early-P efficient accessions M-I-03 (233), M-I-04 (234), and Macho-II (236) had an excellent relationship between grain yield and shoot biomass (r^2 = 0.77***) under LP conditions, indicating that P utilization through better dry matter accumulation and partitioning of above-ground dry matter to grain is the most critical factor in expression of high P efficiency of these accessions.

Phosphorus efficiency is a very complex phenomenon affected by a large number of plant mechanisms and various physiological and biochemical traits associated with P acquisition from soil and P utilization at the cellular level [28]. As presented in Table 10, accessions can be selected showing an excellent correlation between P efficiency and P content leading to a suggestion that P acquisition is a decisive factor in expression of high P efficiency. Alternatively, from Table 8 and 9, accessions can be selected which are more or less the same in P content, but differing greatly in P efficiency, leading to a suggestion that P utilization ability is the most critical factor in expression of high P efficiency. In both cases, root system architecture and morphology affected the ability of a plant to acquire P from the soil, and thus their P use efficiency and responsiveness to external P. It seems likely that P efficiency mechanisms may be different among the accessions of a given species.

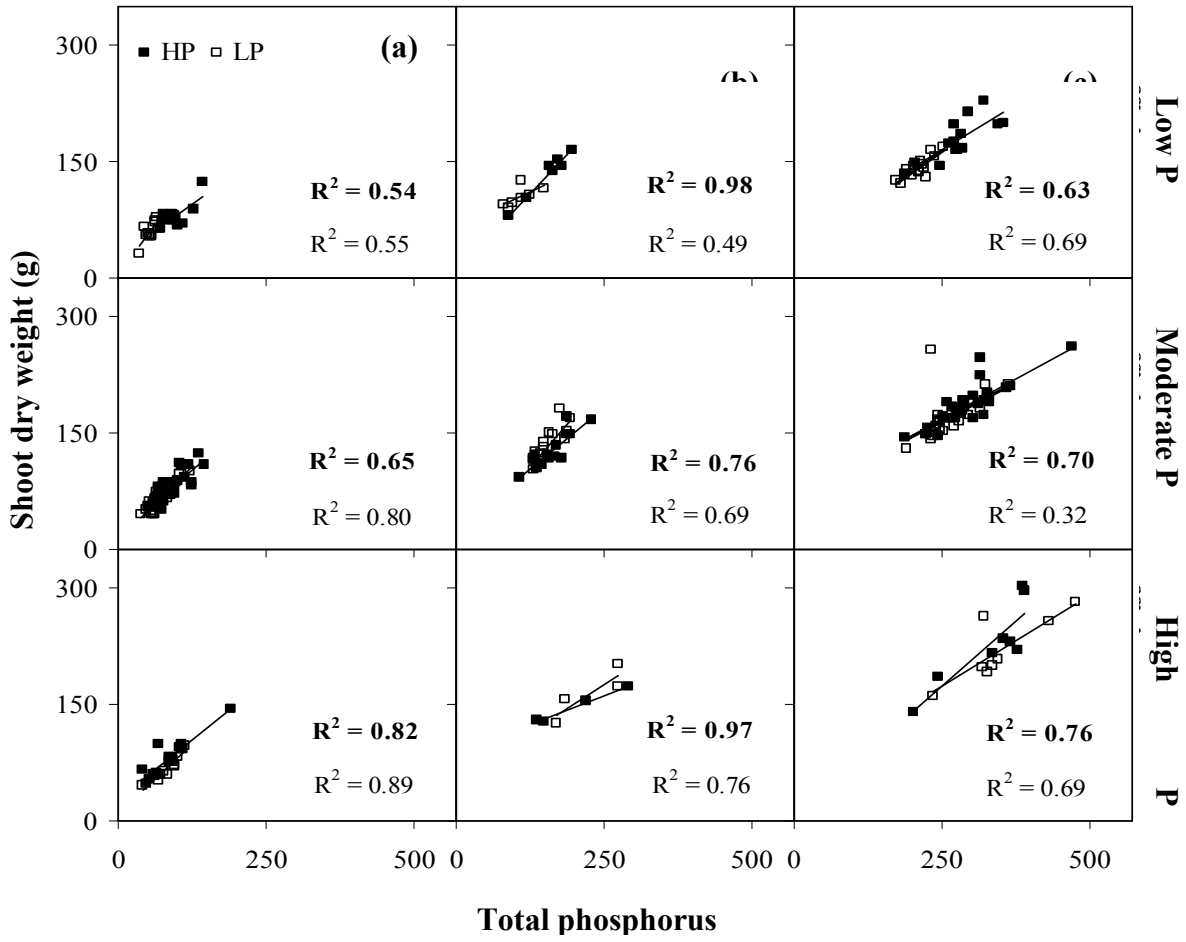

*FIG. 4. Relationship between total P uptake and shoot biomass of low, moderate and high P efficient accessions grown under high- (HP) and low- (LP) phosphorus availability measured at the silking stage in (a) Experiment 1 (2007), (b) Experiment 2 (2007), and (c) Experiment 3 (2008). Each data point represents the mean of four replicates of 12, 24, and 14 accessions; 7, 15, and 4 accessions; 14, 28, and 8 accessions, respectively. *, **, and *** indicate significance at P<0.05, P<0.01, P<0.001.*
*FIG. 4. Relationship between total P uptake and shoot biomass of low, moderate and high P efficient accessions grown under high- (HP) and low- (LP) phosphorus availability measured at the silking stage in (a) Experiment 1 (2007), (b) Experiment 2 (2007), and (c) Experiment 3 (2008). Each data point represents the mean of four replicates of 12, 24, and 14 accessions; 7, 15, and 4 accessions; 14, 28, and 8 accessions, respectively. *, **, and *** indicate significance at P<0.05, P<0.01, P<0.001.*

3.3.3. Phosphorus efficiency and P responsiveness

Large genotypic variation within maize landraces was confirmed for plant growth in LP soil. Fifty common maize accessions were grouped into 3 categories of P efficiency based on growth, grain yield, tissue P concentration and P content, PAE and PUE parameters at LP and their relative values to those at HP. Our results indicated that 12 accessions across locations had the lowest growth and yield reduction and the highest levels of P efficiency (PEI > 0.54) under low P. Among accessions, Paramuén Cr-03-JR10, PICH-1, PICH-4, PICH-17, Paso del Muerto Cr-03-TA, San Gregorio CM 00, Macho III-05, M-I-0, Macho I-05, and Macho-II 03 had consistently higher PEI in LP (Table 11). When the combination of PEI with P responsiveness at HP is considered, accessions SHUI-2 (6), Paramuén Cr-03-JR10 (40), ZR-6 (75), CB-2 (78), Paso del Muerto Cr-03-TA (109), CCHEDE CM-00 (199), Macho III-05 (182), M-I-03 (233), M-I-04 (234), and M-IV-03 (239) were the best accessions for P-

deficient acidic soil of this region (Table 11). These accessions were categorized as the most P efficient under LP and as the most responsive to increased P availability. The natural genetic variation observed among maize landraces demonstrates the potential for breeding cultivars with improved P efficiency, which will ultimately utilize applied inorganic P fertilizers more efficiently. In addition, the relationship between PEI and plant content and dry matter showed a significant correlation ($r = 0.65$ to $r = 0.93$) at LP. Such a positive correlation indicates that PEI based on dry matter and plant content uptake can be an appropriate index for P efficiency evaluation.

4. USE OF ^{32}P ISOTOPE TECHNIQUES TO IDENTIFY GENOTYPES FOR SUPERIOR PHOSPHORUS ACQUISITION AND / OR UTILIZATION

4.1. Introduction

Andisols contains considerable amounts of P but a large proportion is bound to different soil constituents, forming complexes of limited bioavailability [42]. This type of soil is commonly referred to as a P-fixing soil and the concentration of phosphate in the soil solution is suboptimal for crop production. A first line of strategy for soils with low total P content is regular amendment with small doses of P fertilizer. However, in soils with high total P content that fix most of the P, P fertilizer will be equally fixed. In this type of soil, plants do respond to P fertilizer application, but annual P applications are needed to sustain crop yields [34].

The use of isotopes has enabled tracing of the dynamics of fertilizers in the soil-plant system [43]. The use of ^{32}P isotope as a tracer applied to the soil with phosphate fertilizer permits the detection of exchangeable phosphate ions in the solution and those absorbed by the plant [44]. The method of isotopic dilution is a useful, practical and economical methodological alternative when it is not possible to mark the nutrient of interest isotopically. The method consists in marking the soil with the isotope to differentiate the origin of the nutrient: soil and fertilizer [43]. The objective of the present study was to assess the P uptake and recovery from the P fertilizer by selected maize accessions using the ^{32}P isotope dilution technique.

4.2. Materials and methods

The efficiency of P uptake was determined for five maize accessions from the Purhepecha region represented by two early (San Gregorio and synthetic M-I-04); two intermediate (PICH-4 and Zacapu), and one late (DP x Tromba) accessions and a commercial variety (cv. Leopardo) used as a check (Table 2). These genotypes differed in both P efficiency and P responsiveness at early vegetative stages [46]. This study was conducted in the National Nuclear Research Institute (ININ), Mexico, in a greenhouse with an atmosphere between 14.3 and 26.5^{o}C, 50 to 70% relative humidity, and a fall photoperiod (13h day/11h night). Artificial light for 12 hours per day from cool-white, fluorescent tubes supplemented the fall daylight. Plants were grown in pots with 6 kg of Vitric Andisol soil of low available soil P, which was obtained from a plot on Laderas, Pátzuaro, Michoacan, where maize landraces are cultivated (Table 1).

The experiment design was completely randomized with a factorial arrangement of (3 × 6) treatments: three levels of P supply and 6 genotypes. There were four replications for a total of 72 units. Each experimental unit was composed for one pot and included P additions as diammonium phosphate $(NH_4)_2HPO_4$ at rates of 0.0 g P pot^{-1}, 0.22 g P pot^{-1} and 0.44 g P pot^{-1}. These rates are equivalent to 0, 25 kg P ha^{-1} and 50 kg P ha^{-1}.

TABLE 9. SHOOT DRY WEIGHT, P ACQUISITION EFFICIENCY (PAE), AND P UTILIZATION EFFICIENCY (PUE) OF COMMON MAIZE ACCESSIONS GROWN IN A P-DEFICIENT SOIL ITH LOW P (LP) OR WITH HIGH P ADDITION (HP), IN TWO SEASONS BETWEEN 2007 AND 2008. ACCESSIONS ARE RANKED BY P EFFICIENCY INDEX (PEI)

ID	Accessions	M‡	BG#	Shoot dry weight (g plant^{-1})					PAE (mg P g^{-1} root DM)				PUE (g DM mg^{-1} P)			
				LP	HP	PEI§	SdShΨ	PRϕ	LP	HP	SdPAE	PR	LP	HP	SdPUE	PR
40	Paramuén Cr-03-JR10	L	C	153.7	195.9	0.75	3.38	ER	17.2	18.2	0.55	ER	0.83	0.87	1.24	ER
109	Paso del Muerto Cr-03	L	AC	155.6	173.9	0.66	1.70	ER	21.8	19.7	0.44	ER	0.72	0.88	–0.16	ENR
78	CB-2	I	C	106.6	133.9	0.37	0.21	ER	17.4	19.8	0.92	ER	0.78	0.92	–0.48	ENR
236	M-II-03	E	S	116.1	139.3	0.30	0.63	ER	16.2	15.4	–0.18	ENR	0.84	0.91	–0.52	ENR
233	M-I-03	E	S	105.5	146.8	0.20	0.98	ER	18.4	16.3	–0.02	ENR	0.76	0.87	0.31	ER
239	M-IV-03	L	S	112.3	131.9	0.10	0.10	ER	16.2	17.7	–0.01	ENR	0.81	0.89	0.19	ER
75	ZR-6	L	C	142.2	146.7	0.05	1.03	ER	18.7	17.7	0.27	ER	0.90	0.89	0.46	ER
234	M-I-04	E	S	141.2	137.7	0.04	0.58	ER	22.2	20.0	1.05	ER	0.93	0.95	–0.63	ENR
182	M-III-05	I	S	129.9	127.9	0.64	–0.43	ENR	20.0	19.2	0.57	ER	0.84	0.86	0.45	ER
144	San Gregorio CM-00	E	AC	101.7	106.5	0.60	–1.14	ENR	19.5	14.4	–0.62	ENR	0.85	1.18	–2.56	ENR
79	PICH-4	I	C	135.8	124.0	0.54	–0.12	ENR	17.4	14.9	–0.29	ENR	0.87	0.86	0.03	ER
235	M-I-05	E	S	123.8	127.7	0.41	–0.09	ENR	17.0	15.7	–0.28	ENR	0.83	0.83	0.61	ER
62	PICH-1	I	C	114.2	122.5	0.33	–0.39	ENR	14.6	15.6	–0.31	ENR	0.87	0.89	–0.18	ENR
199	CCHEDE CM-00	E	AC	124.5	117.5	0.19	–0.48	ENR	15.1	18.7	0.75	ER	1.00	0.96	1.35	ER
6	SHUI-2	L	C	102.5	116.8	0.15	–0.45	ENR	15.4	20.2	1.18	ER	0.92	0.89	0.99	ER
	Average			114.9	128.6				17.9	16.7			0.92	0.88		
	LSD (0.05)†			52.4	52.6				9.0	7.9			0.23	0.21		

†To compare paired values among accessions
‡Maturity: early (E), intermediate (I), late (L)
#BG: Breeding groups: landrace (C), advanced landrace (AC), hybrids x landrace (H x C), and synthetic (S)
§Phosphorus efficiency index (PEI) obtained from the principal component analysis
ΨStandardized value of shoot dry weight (SdSh), P acquisition efficiency (SdPAE), and P utilization efficiency (SdPUE) under high P conditions. Data from the 3 individual experiments were standardized by dividing relative values by the standard deviation of the trial
ϕPhosphorus responsiveness (PR) is expressed by shoot biomass or grain yield under HP level. Four categories, efficient and responsive (ER), non-efficient and responsive (NER), non-efficient and non-responsive (NENR), and efficient and non-responsive (ENR)

TABLE 10. PHENOTYPIC CORRELATIONS AMONG ROOT TRAITS: NODAL ROOT LENGTH (NOD_RL), NUMBER OF NODAL ROOTS (NOD_NO), NODAL BRANCHING (NOD_BR), NODAL ROOT ANGLE (NOD_RA), ROOT HAIR DENSITY (RHD_BNR), (RHD_MNR) AND LENGTH (RHL_BNR), (RHL_MNR) FROM THE BASAL AND MIDDLE REGION OF NODAL ROOT FIRST ORDER LATERALS AND MEASURES OF P EFFICIENCY: SHOOT DRY WEIGHT, GRAIN YIELD, P ACQUISITION EFFICIENCY, AND P UTILIZATION EFFICIENCY OF P-EFFICIENT ACCESSIONS ACROSS LOCATIONS

Parameter		Shoot dry weight (g plant^{-1})			Grain yield (Mg ha^{-1})			P acquisition efficiency (mg P g^{-1} root DW)			P utilization efficiency (g DW mg^{-1} P)		
	P	Early	Middle	Late	Early	Middle	Late	Early	Middle	Late	Early	Middle	Late
Nod_RL	HP	**–0.49**†	0.02	**–0.85**	–0.08	–0.01	–0.09	–0.04	–0.02	**–0.54**	0.04	0.00	**0.45**
	LP	**0.50**	0.01	**0.76**	**–0.72**	**0.67**	**0.88**	**–0.96**	0.25	0.11	0.13	**–0.63**	0.11
Nod_No	HP	0.01	0.13	0.19	–0.21	0.02	0.00	–0.27	**0.79**	**0.35**	0.28	**–0.36**	**–0.73**
	LP	**0.94**	**0.53**	**0.75**	–0.21	**0.32**	**0.56**	**–0.89**	0.02	**0.43**	**0.61**	**0.48**	0.01
Nod_Br	HP	0.05	**0.28**	**0.73**	**0.46**	–0.23	0.04	**0.52**	0.06	**0.42**	**–0.54**	0.13	–0.23
	LP	**0.57**	**0.82**	**0.31**	**–0.90**	0.35	0.04	**–0.82**	0.22	**0.73**	0.02	**0.52**	**–0.38**
Nod_ra	HP	**0.56**	0.01	**–0.40**	0.11	–0.09	–0.05	–0.07	0.05	**–0.49**	–0.06	–0.14	**0.89**
	LP	–0.25	–0.10	**–0.75**	**–0.54**	**0.59**	**–0.80**	0.03	**0.85**	–0.02	**0.66**	**0.75**	**0.50**
RHD_bnr	HP	**0.45**	–0.17	–0.07	0.05	0.02	**0.45**	0.02	–0.05	**–0.32**	–0.02	**0.62**	0.29
	LP	0.17	**–0.38**	–0.01	**0.63**	0.08	**–0.80**	0.02	0.00	**0.61**	**0.57**	–0.15	–0.08
RHL_bnr	HP	**–0.98**	–0.21	**0.95**	**–0.86**	0.07	0.03	**–0.81**	–0.16	**0.70**	**0.80**	**0.96**	**–0.57**
	LP	**0.44**	–0.12	**0.44**	**0.34**	–0.05	**–0.96**	–0.02	0.04	**0.50**	**0.83**	**–0.36**	–0.02
RHD_mnr	HP	0.03	–0.25	–0.26	–0.11	–0.03	**0.60**	–0.15	–0.14	**–0.34**	0.16	**0.61**	0.03
	LP	**0.31**	**–0.52**	**0.34**	**0.47**	–0.24	**–0.30**	0.02	–0.05	**0.97**	**0.72**	–0.05	**–0.67**
RHL_mnr	HP	**–0.53**	–0.12	**0.79**	**–0.94**	–0.04	0.20	**–0.97**	–0.07	**0.55**	**0.97**	**0.90**	**–0.69**
	LP	**0.74**	–0.12	**0.81**	**0.46**	–0.16	**–0.82**	0.19	–0.12	**0.69**	**0.99**	–0.20	–0.12

†Correlation coefficients in bold type are significant at $P<0.05$

TABLE 11. PHENOTYPIC CORRELATIONS AMONG ROOT P CONCENTRATION (RPCT), ROOT P CONTENT (RPC), SHOOT P CONCENTRATION (SPCT), SHOOT P CONTENT (SPC), TOTAL P CONTENT (TPC), ROOT DRY WEIGHT (RDW), SHOOT DRY WEIGHT (SHDW), TOTAL DRY WEIGHT (TDW), P ACQUISITION EFFICIENCY (PAE), P UTILIZATION EFFICIENCY (PUE), AND GRAIN YIELD (GY)

HP/LP†	RPCt	RPC	SPCt	SPC	TPC	RDW	ShDW	TDW	PAE	PUE	GY
RPCt	***0.15***	0.58***	–0.10	0.06	0.01	0.06	0.11	0.11	0.00	–0.01	–0.08
RPC	0.54***	***0.43****	0.04	0.37*	0.38*	0.82***	0.39*	0.47**	–0.38*	0.11	0.01
SPCt	0.13	0.02	***0.20***	0.61***	0.51**	0.14	0.10	0.09	0.37*	0.90***	0.27
SPC	0.18	0.28*	0.66***	***0.52*****	0.88***	0.38*	0.81***	0.81***	0.44**	0.60***	0.21
TPC	0.22	0.36*	0.65***	0.99***	***0.44****	0.37*	0.72***	0.73***	0.52**	0.68***	0.19
RDW	0.11	0.88***	–0.03	0.54**	0.32**	***0.46****	0.35*	0.45**	–0.56***	0.12	0.07
ShDW	0.13	0.31*	0.14	0.78***	0.77***	0.30*	***0.47****	0.99***	0.32**	0.16	0.04
TDW	0.14	0.41**	0.13	0.77***	0.78***	0.41*	0.99***	***0.43****	0.11	0.25	–0.13
PAE	0.06	–0.61***	0.43**	0.39*	0.42**	–0.76***	0.40*	0.16	***0.34****	0.54***	0.11
PUE	0.16	0.02	0.98***	0.66***	0.65***	–0.05	0.19	–0.22	0.48**	***0.17***	0.25
Gy	0.15	0.16	–0.01	0.22	0.19	0.07	0.26	0.25	0.05	0.00	***0.57*****

†For each environment, values below the diagonal represent correlations within the lowP treatment; values above the diagonal represent correlations within the high P treatment; values on the diagonal (*italic*) correspond to across-P treatment correlations

*, **, ***, Significant at $P<0.05$, $P<0.01$, $P<0.001$, respectively

The Andisol soil was labelled with ^{32}P by applying a high activity (10 mC_i). The tracer was added in 300 ml of water at pH 6.0 to ensure uniform labelling of soil at concentrations of 0.395 mC_i pot^{-1} and 0.791 mC_i pot^{-1}. Dilute solution was applied into the soil at a depth of 10 cm, near to the plant root zone. Plants were irrigated with distilled water applied to the soil to field capacity (33 kPa) to avoid leaching of the radioactive ^{32}P-labelled marker and other nutrients. Two seeds were planted in every pot. Five days after emergency, seedlings were thinned to one plant per pot. N was added as a solution of NH_4NO_3 at 60 kg–N ha^{-1} to the pots in which maize was grown. The N was split applied, 33% at planting, 33% at 14 days after planting (DAP), and 33% at 28 DAP.

One sample per replication per maize accession was harvested at 39 DAP; shoots were cut at 1 cm above the soil surface, and dried in an oven at 70 ^oC during 2 days. After determining the shoot dry weight, shoot were cut in pieces < 5 mm and a sample of 0.5 g of every shoot was digested in hot H_2SO_4.The P concentration in the digests were determined colorimetrically [38]. ^{32}P activity in the digest was determined by liquid scintillation counting. Samples were counted in a Beckman LS-60000LL liquid scintillation system. The content of P in plant was calculated by multiplying the P concentration by the dry weight (P concentration in plant (mg P g^{-1}) × dry matter yield (g P pot^{-1}) / 100). Based on the isotopic dilution method, the proportion of P taken up by the plants from the fertilizer and soil were calculated [43]. The percentage of P derived from the fertilizer (% Pddf) was obtained dividing the specific activity (SA) in the plant by SA in the fertilizer × 100. Thus, the percentage of P derived from the soil (% Pdds) was obtained by difference: 100 – (% Pdds). The amount of P fertilizer extracted by the crop (P-fertilizer yield) was calculated by multiplying the total P yield and the percentage of P derived from fertilizer: P-fertilizer yield (mg P pot^{-1}) = (Total P yield × % Pddf) / 100. The P-fertilizer extracted by the plant relative to the dose of P applied (efficiency P-fertilizer) is known as P use efficiency: P-fertilizer efficiency (%) = (P-fertilizer yield / dose of P applied) x 100.

4.3. Results and discussion

Due to the very low available soil P utilized in this study (6 mg P kg^{-1} soil), which is typical of Mexican volcanic soils, the response of the maize to fertilizer application was evident (Fig. 5). The dry matter (DM) production increased from 1.88 g (control) to 2.81 and 5.01 g for the rates of 25 and 50 mg P kg^{-1} soil, respectively. The increases in both DM and P content of all accessions, except Zacapu and DP x Tromba, resulted in doubling the DW accumulated by the crop, when the P rate was increased from 25 to 50 mg P kg^{-1} soil as diammonium phosphate. In DP x Tromba, total P content was comparable and responded similarly to P application (Fig. 5). The smallest P contents were obtained in LP treatment in cv. Leopardo.

The mean specific activity of P in DP x Tromba was significantly smaller than all other accessions which had the highest value under HP and LP conditions (Fig. 6). If all the species drew their P from the same pool of available P, then the specific ^{32}P activity of the P in their shoots should be comparable, although concentrations of P in the shoots and the amounts of P accumulated may differ [45]. Consequently, the lower specific radioactivity of the P taken up by DP x Tromba indicates that it was able to access a pool of soil P that was less available to all other accessions.

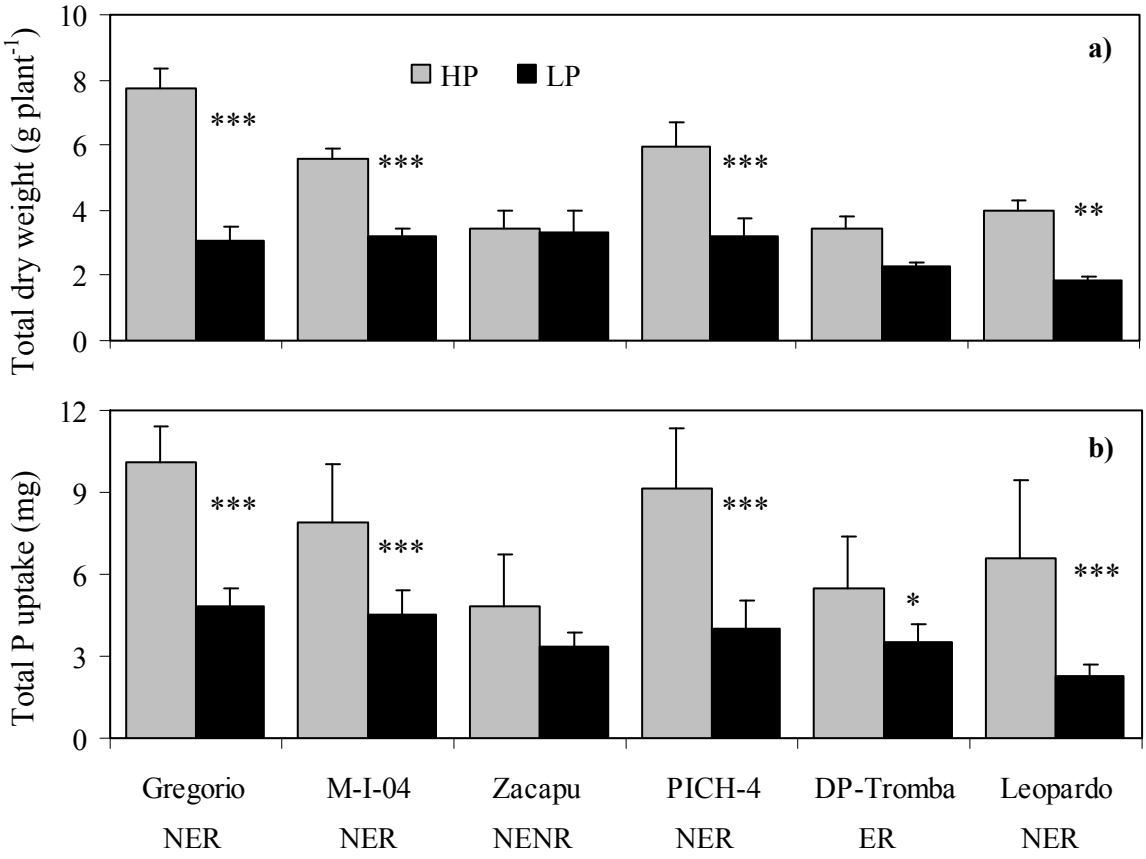

*FIG. 5. Total dry weight (a) and total P content (b) for maize accessions grown in a Vitric Andisol. The error bar represents standard deviation from the mean. Within maize accessions, bars with *, **, and *** indicate significance at P<0.05, P<0.01, P<0.001, respectively. Categories represented by efficient and responsive (ER), inefficient and responsive (NER), and inefficient and non-responsive (NENR).*

The P uptake derived from soil (% Pdds) and from fertilizer (% Pddf) was significantly affected by the P supply, accession, and interaction (P<0.0001), suggesting that phosphate fertilization improved P uptake from soil or fertilizer due to the initially low P content in the soil (Table 1). The % Pdff values for HP were, in general, larger than in LP for all accessions. However, San Gregorio and PICH-4 showed the highest uptake derived from fertilizer at LP. Under LP condition, significantly larger % Pdds and lower P fertilizer yield in the plant was observed for DP x Tromba (Fig. 6). Different % Pdds in maize accessions and cultivar indicate that chemically different pools of soil P were utilized, with DP x Tromba accessing a larger pool than all other accessions. The increase in % Pdds indicates that when P deficiency occurs, DP x Tromba can exert certain mobilization mechanisms and access non-labile P in the Andisol soil.

In comparing P-fertilizer yield and the efficiency of P fertilizer, large differences among accessions were found (Fig. 6). San Gregorio, M-1-04, and PICH-4 produced the highest values of P-fertilizer yield and % P-fertilizer use efficiency when the P-fertilizer was applied at the seedling stage. The % P-fertilizer use efficiency in these accessions was 14, 5.3 higher than cv. Leopardo at HP conditions. These accessions were the most responsive to high P rates of application indicating to be more demanding for their growth and development, as identified in previous studies in the field [46].

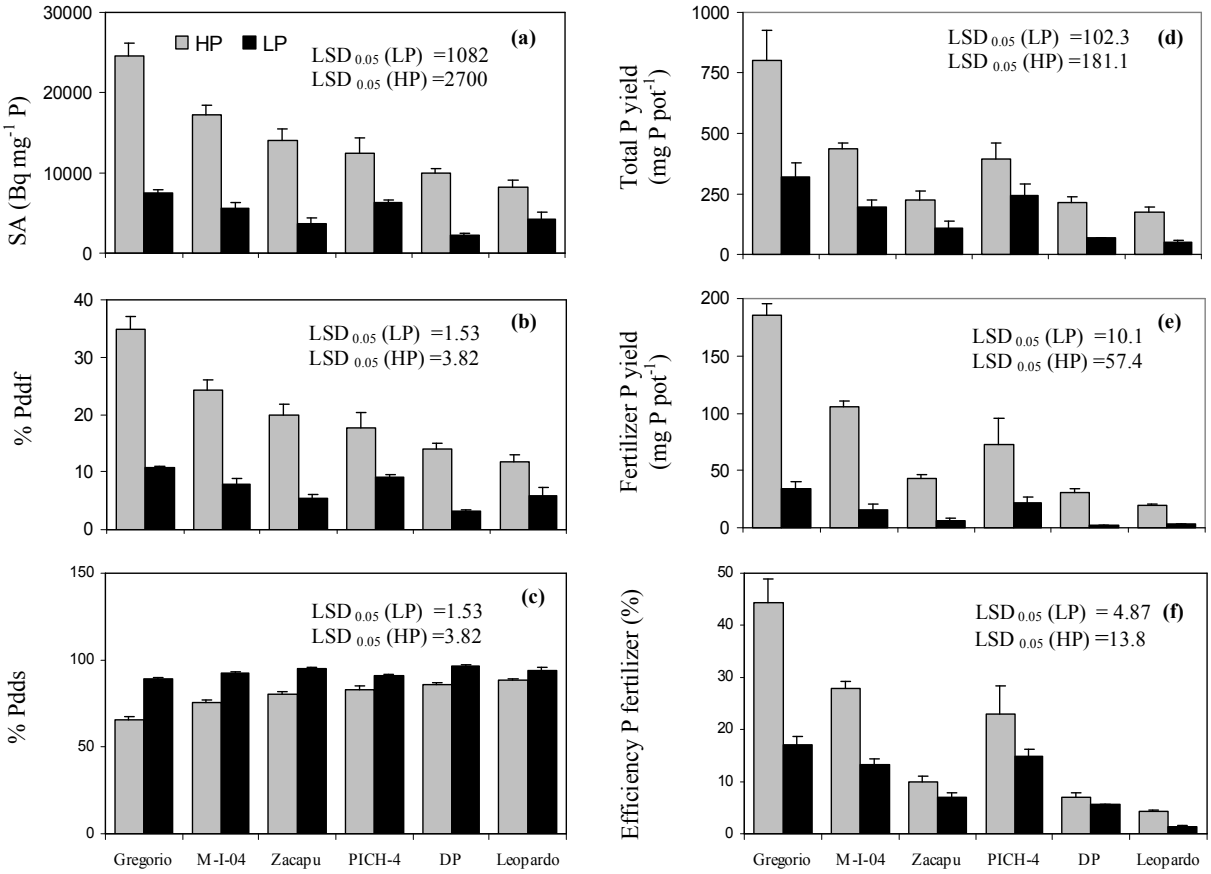

FIG. 6. Specific radioactivity of the P taken by the plant (a), %Pddf (b), %Pdds (c), total P yield (d), fertilizer P yield (e), and fertilizer P efficiency (f) for maize accessions grown in an Andisol.

We have shown that the accession DP x Tromba is able to access non-labile P. However, different varieties need to be tested for their ability to mobilize non-labile P. Recent studies have investigated genotypic differences of maize in P efficiency [36]. The authors showed that there exist differences in P efficiency under low P conditions. Particularly, they showed that the development of an extensive root system with dense root hairs was one of the main strategies of acquiring soil P for P-efficient accessions. In our study, DP x Tromba was able to access non-labile P, but this did not enable it to increase P uptake. It is therefore relevant to investigate more maize accessions for their ability to access and utilize non-labile P.

Compared to the results for P-inefficient accessions, the significantly lower values of the specific activity of P in the late P-efficient DP x Tromba and the higher values of P derived from soil by this accession clearly show for the first time that DP x Tromba utilizes soil P from a normally non-labile soil P pool that is not utilized by P-inefficient accessions and cv. Leopardo. In conclusion, these data demonstrate that P-efficient accession has a specific mechanism to mobilize and use a fraction of soil that is not utilized by other accessions of the given species.

5. CONCLUSIONS

The first two studies described above were aimed to assess the genotypic variation in root architecture and plant growth traits associated with P acquisition efficiency of maize landraces to a P-deficient Andisol in the Central Mexican Highlands. The Mexican Highlands, including the Purhepecha Plateau, with strong dominance of traditional varieties, are an important repository of genetic resources. Controlled environment and field studies confirmed that root architecture in maize landraces was responsive to low P availability and that variation in these traits within landraces contributed to differing capacities for P acquisition. P-efficient accessions adapted to low P produced more nodal and lateral roots and long and dense root hairs that greatly increase the soil volume that roots can exploit. In this Andisol, Pi was low, independent of P fertilization. The only possibility for the maize plants to absorb more P was to access more soil volume by increased root growth. Nodal rooting was best correlated with P acquisition efficiency and grain yield, followed by root hair density and length on the nodal first-order laterals. A more P uptake efficient plant would be a combination of the high P acquisition and the extended root system seen in P efficient accessions of late maturity. Hence, root traits of more P acquisition efficiency in maize landraces exist, opening the possibility to breed for more P acquisition efficient varieties.

The final study was aimed to determine the ability of maize accessions to acquire adequate amounts of P from fertilizer and soil that are very low in available P, by measuring the specific radioactivity of plant P in P-efficient and P-inefficient accessions grown on a ^{32}P-labeled soil. Lower values of the specific radioactivity of P in the late P-efficient DP x Tromba and the higher values of P derived from soil by this accession clearly show, for the first time, that P-efficient accessions utilize soil P from a normally non-labile soil P pool that is not utilized by P-inefficient accessions. These data demonstrated that a P-efficient accession has a specific mechanism to mobilize and use a fraction of soil that is not utilized by other accessions of the same species. Hence, there is a scope for identifying P mobilizing accessions through the use of isotopic dilution techniques. The results also showed that accessions San Gregorio, M-1-04, and PICH-4 were the most responsive to high P application indicated by the highest P-fertilizer use efficiency.

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SELECTION AND EVALUATION OF MAIZE GENOTYPES TOLERANCE TO LOW PHOSPHORUS SOILS

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Abstract

Maize species differ in their ability to take up phosphorus (P) from the soil, and these differences are attributed to the morphology and physiology of plants relative to their germplasm base. An effective method of increasing P efficiency in maize is to select and evaluate genotypes that can produce a high yield under P deficient conditions. In this study, 116 maize inbred lines with various genetic backgrounds collected from several Agricultural Universities and Institutes in China were evaluated in a field experiment to identify genotypic differences in P efficiency in 2007. Overall, 15 maize inbred lines were selected from the 116 inbred lines during the 5-year field experimental period based on their 100-grain weight in P-deficient soil at maturity, when compared to the characteristics exhibited in P-sufficient soil. All of the selected lines were evaluated in field experiments from 2008 to 2010 for their tolerance to low-P at the seedling and maturity stages. Inhibition (%) was used and defined as the parameter measured under P limitation compared to the parameters measured under P sufficiency to evaluate the genotypic variation in tolerance. Inhibition of root length, root surface area, volume, root: shoot ratio and P uptake efficiency could be used as indices to assess the genotypic tolerance to P limitation. Low-P tolerant genotypes could uptake more P and accumulate more dry matter at the seedling stage. A strong relationship between the total biomass and root length was exhibited. In order to understand the mechanisms of the genotypic tolerance to low-P soil to utilize P from the sparing soluble P forms, 5 maize genotypes selected out of the 15 maize inbred lines, according to the four quadrant distribution, was used as the criteria in a ^{32}P isotope tracer experiment to follow the recovery of ^{32}P in soil P fractions. The ^{32}P tracer results showed a higher rate for water-soluble P transformation to slowly available P in P deficient soil than in soil with sufficient P. The L-values showed that different genotypes had different soil P-use efficiency and low-P tolerance mechanisms. A low-P tolerant cultivar DSY-32 regulated soil P use efficiency and plant P content according to the L-value under exogenous P fertilizer application. However, another low-P tolerant cultivar, DSY-2, utilized soil P more efficiently, regardless of the L-value. In conclusion, the study exploited the physiological-biochemical mechanism on P-uptake, and P-transport of selected maize genotypes in low-P soil under field conditions, and the ^{32}P tracer technique proved to be a valuable tool that sought physiological explanations for superior genotype performance.

1. INTRODUCTION

Phosphorus (P) is one of the essential nutritional elements for all organisms. However, P deficiency in many regions of the world, especially in developing countries, limits plant growth and crop productivity [1, 2]. Plants are almost entirely dependent on P absorbed from soil [3], and while total phosphate is quite abundant in many soils, it is largely unavailable to plants (means “P-deficiency in heredity”) [4]. In fact, P utilization efficiency is only 15% and no more than 25%, even including subsequent utilization by plants [5]. To increase crop productivity, farmers often apply substantial amounts of phosphate fertilizers to compensate for the low-P use efficiency of crops [6]. However, applying fertilizers in large amounts not only exhausts limited P resources, but also leads to environmental pollution. Therefore, development of low-P tolerant cultivars may help reduce the demand for P, since numerous studies have shown that differences in tolerance to low-P stress exist among genotypes within crop species [7].

Maize is an important grain and forage crop worldwide after wheat and rice, which is also sensitive to P and subjected to P-deficiency in heredity [8]. Due to population growth and the generally low level of available P in soils, it is difficult to increase global maize production by extending the area of cultivated land. As a result, a complementary approach that has received more attention is the adaptation of crops to unfavourable soil conditions by selecting, and evaluating genotypes with enhanced nutrient use efficiency in soils with a low nutrient status, and/or requiring moderately low external inputs to induce expression of their genetic potential for adequate production. This strategy is now considered a promising, energy-efficient, eco-friendly and socio-economically feasible approach [9]. Recently, several researchers have begun screening and improving the tolerance to P deficiency in maize cultivars [3, 7, 10], but most of these were carried out under hydroponic or pot experimental conditions. Due to differences in the growing environment between greenhouse and field conditions, cultivars may vary greatly in response to low-P supply, and P efficiency may differ among maize inbred lines. Therefore, special attention should be given to screening maize genotypes that are tolerant to low-P soils under field conditions. Although the workloads are large and there are a great number of uncontrollable conditions in field experiments, the results of such studies more closely approximate the actual conditions. Plants differ greatly in their ability to grow on low-P soils, because they have developed specific physical-chemical and biological mechanisms to utilize P compounds in such soils [11]. Quantifying soil P into different pools could identify the availability of these fractions to the plant. Naruzzaman et al. [12] have shown genotype variation of crops in their ability to access and utilize sparingly soluble forms of soil P (Ca-P, Al-P, Fe-P), which has been proposed as a possible means for overcoming P deficiency stress and to optimize P fertilizer use where P is poorly available.

In a pre-screening experiment, 116 maize inbred lines with various genetic backgrounds collected from several Agricultural Universities and Institutes in China were employed in a field experiment in 2007, which resulted in selection of 15 maize inbred lines by evaluation in a two year field experiment during 2008 and 2009, to identify an index system for assessment of low-P tolerant maize genotypes. To verify that P uptake from sparingly soluble P forms by different maize genotypes can be used as a criterion to evaluate maize tolerance to low available P soils, 5 lines were selected out of the 15 maize inbred lines and used in a ^{32}P isotopic tracer experiment to determine recovery of ^{32}P in different soil P fractions in 2010.

2. METHODOLOGY

2.1. Experiment 1: Field experiment

2.1.1. Experimental site

A study was carried out in 2007, 2008 and 2009 at Langfang experimental station, located in Hebei Province (E 116°35'; N 39°36'). The frost period at this site extends to 182 days, mean annual sunshine duration is 2660 hours and mean annual evapotranspiration is 1909.6mm. Annual precipitation is 554.9 mm, 70-80% of which distributed during June to August. The annual mean temperature is 11.9^{o}C, the mean monthly maximum 26.2^{o}C, in July and minimum –4.7^{o}C, in January. Soil chemical characteristics were pH 8.5, available P 4.91 mg kg^{-1}, available N 55.8 mg kg^{-1}, available K 92.6 mg kg^{-1} and organic matter 14.38 g kg^{-1}. The soil was characterized as typically P deficiency.

2.1.2. Plant materials

A total of 116 maize inbred lines with various genetic backgrounds were collected from several Agricultural Universities and Institutes in China and employed in field Experiment 1 in 2007. Overall, 15 maize inbred lines were selected from Experiment 1 based on the difference in their 100-grain weight when grown in P-deficient soil and P-sufficient soil at the mature stage. Specifically, the low-P tolerant genotypes DSY-30, DSY-2, DSY-31, DSY-20, DSY-21, DSY-39, DSY-101, DSY-33, DSY-32, DSY-23 and DSY-93, and the low P sensitive genotypes, DSY-113, DSY-79, DSY-129 and DSY-48 were selected for experiments 2 and 3, respectively, in 2008 and 2009.

2.1.3. Field management

Maize was sown on 25th April 2007, 5th May 2008 and 13th May 2009, respectively. Row width was 50 cm, and distance between plants within a row was 30 cm, resulting in an overall planting density of 12 plants m^{-2} with pest control carried out as required. Water was applied by sprinkler irrigation as needed.

2.1.4. Experimental design

Two treatments with different P application levels of 0 and 52 kg P ha^{-1} corresponded to the treatments of P0 and P1; i e, P-deficiency and P-sufficiency, a randomized complete block design with three replication. Urea, superphosphate, and potassium chloride were applied with N (225 kg–N ha^{-1} in total, 1/2 N of which was applied basally, while the remaining 1/2 is applied as top-dressing during the bell-mouthed stage), P (applied basally) and K (87 kg K ha^{-1} applied basally). To prevent impurity, maize cover package pollination was utilized.

2.1.5. Measurement and statistical analyses

Ten representative plants (48 plants per treatment) were sampled at maturity, and the 100-grain weights of the maize inbred lines were measured in 2007. During 2008 and 2009, plants were harvested at both the seedling and maturity stages. Plants collected during the seedling stage were separated into roots and shoots to assess morphological traits, dried at 65°C in a forced-air oven for 48 h and weighed to determine the root and shoot dry weight. The collected roots were placed in a transparent water filled tray (15 × 25 cm) on a scanner (EPSON PERFECTION 4990 PHOTO, Model J1318) to facilitate root spreading. The system was used to scan all fine root fragments and the program calculated root traits such as root length, root volume and root surface area for all fine roots. The data obtained were then analysed using the digital image analysis system, WinRHIZO Pro 2007a. Plants harvested at the mature stage were divided into roots and shoots and then dried at 65°C in a forced-air oven for 48 h, weighed and the 100-grain weight determined. P nutrient efficiency methods [13] indicated that resource capture and P efficiency were highly interrelated. In this study, P uptake efficiency was measured as the amount of P in the plant materials, including the roots and shoots at the seedling stage (mg P plant^{-1}). The P content in the plant material was determined using the Mo-Sb-Vc colorimetric method [14] after digestion in sulphuric acid-perchloric acid [15].

The inhibition (%) due to P limitation was defined as follows:

$$\text{Inhibition (\%)} = \{(P1 - P0) / P1\} \times 100$$

Root: shoot ratio was calculated as (root dry weight in g plant^{-1}) / (shoot dry weight in g plant^{-1}).

2.2. Experiment 2: ^{32}P isotopic tracer experiment

2.2.1. Soil and plant materials

The soil was air-dried, ground and passed through a 2 mm sieve. Each pot contained 2 kg of the soil. Based on the results of a previous 3-year field experimental selection, 5 typical cultivars were employed: genotypes DSY-30 and DSY-2, responsive and tolerant to low-P soil (named as low-P tolerant genotype); DSY-32, non-responsive and tolerant (named as low-P tolerant genotype); DSY-48, non-responsive and sensitive and DAY-79, responsive and sensitive (named as low-P sensitive genotype).

2.2.2. ^{32}P carrier-free solution preparation

A ^{32}P carrier-free solution was prepared (100 ml) with a specific radioactivity of 185 MBq ml^{-1}. Four ml (7.4 MBq) of the solution was added individually to the soil of each pot, and the solution and soil were homogenized. The pots were covered with Al foil to avoid light, for equilibration and the analysis of P fractionations.

2.2.3. Treatments

To evaluate the P pool of low-P soil for different maize genotypes, the pots were separated into two groups: one group was planted and the other group was without plants. Each group included treatment 1 (the P0 treatment), with no P application but 200 mg N kg^{-1} and 166 mg K kg^{-1}, and treatment 2 (the P1 treatment), which included 66 mg P kg-1, 200 mg N kg^{-1} and 166 mg K kg^{-1}. Each treatment was applied in 3 replications. One week after preparing the pots, they were placed in a greenhouse. Soil samples were taken on days 0, 3, 7, 14 and 25 , and plant samples were taken only on day 25.

2.2.4. Measurements

2.2.4.1. Inorganic P fractionations

The sequential P fractionation of Ca_2-P, Ca_8-P, Al-P, Fe-P, O-P (occluded P) and Ca_{10}-P was performed according to [16].

Ca_2-P: One g of an air-dried soil sample was passed through a 100-mesh sieve and put into a 100 ml centrifugation tube. Fifty milliliters of $NaHCO_3$ (0.5 mol l^{-1}, pH = 7.5) was added. The sample was shaken for 1 h at 20-25°C and centrifuged at 3500 rev. min^{-1} for 8 min. The upper solution of the extract was collected in a 50-ml flask for Ca_2-P analysis.

Ca_8-P: The soil was washed twice with 25 ml of 95% ethanol after the extraction of Ca_2-P, and 50 ml CH_3COONH_4 (0.5 mol l^{-1}, pH =4.2) was added. The soil was dispersed homogenously and put aside for 4 h. The soil was shaken for 1 h at 20-25°C and centrifuged at 3500 rev. min^{-1} for 8 min. The upper solution of the extract was collected in a 50 ml flask for Ca_8-P analysis.

Al-P: One g of an air-dried soil was passed through a 100-mesh sieve and placed in a 100-ml centrifugation tube. Fifty ml of NH4Cl (1 mol l^{-1}] was added, and the sample was shaken for 30 min at 20-25°C. The sample was centrifuged at 3500 rev. min^{-1} for 8 min. The upper solution was discarded, and 50 ml NH_4F (0.5 mol l^{-1}, pH = 8.2) was added. The solution

was shaken for 1 h at 20-25°C, and centrifuged at 3500 rev. min^{-1} for 8 min. The upper solution of the extract was collected in a 50-ml flask for Al-P analysis.

Fe-P: The soil was washed twice with 25 ml of a saturated NaCl solution after the extraction of Al-P. The upper solution was discarded after centrifugation at 3500 rev. min^{-1} for 8 min. Fifty ml of NaOH (0.1 mol l^{-1}] was added. The sample was shaken for 2 h at 20-25°C and put aside 16 h. The sample was shaken for 2 h at 20-25°C once more, and centrifuged for 10 min at 4500 rev. min^{-1}. The upper solution was collected in a flask, and 1.5 ml H_2SO_4 was added. The solution was homogenized by shaking and set aside overnight. The solution was filtered, and the filtrate was collected in a flask for Fe-P analysis.

O-P: The soil was washed twice with 25 ml of a saturated NaCl solution after the extraction of Fe-P. The upper solution was discarded after centrifugation for 8 min at 3500 rev. min^{-1}. Forty ml of $Na_3C_6H_5O_7 \cdot 2H_2O$ (0.3 mol l^{-1}) was added, and the sample was stirred until the soil was dispersed homogenously. One g of $Na_2S_2O_4$ was added, and the solution was placed in a bain-marie at 80-90°C, until the temperature of the solution inside of the tube and the temperature of the water outside were balanced. The solution was stirred for 15 min, and 10 ml NaOH (0.5 mol l^{-1}) was added. The solution was stirred for 10 min and centrifuged for 10 min at 4500 rev. min-1. The upper solution was collected in a 100 ml volumetric flask. The soil was washed twice with 20 ml of a saturated NaCl solution and added to the solution after centrifugation. The volume of the extract was kept constant at 100 ml with distilled water. Ten ml of the extract was removed and placed into a 50 ml flask. Ten ml of a mixed acid solution (H_2SO_4: $HClO_4$: HNO_3 = 1: 2: 7, in volume) was added, and the solution was put into a small funnel. The solution was boiled until HNO_3 and $HClO_4$ totally decomposed and a back-flow of H_2SO_4 appeared. The crystalline solid was boiled and dissolved with 50 ml of distilled water after the solution was cooled. The extract was collected, and the volume was made constant at 100 ml with distilled water for O-P analysis.

Ca_{10}-P: Fifty ml of H_2SO_4 (0.5 mol l^{-1}] was added after the extraction of O-P. The solution was shaken for 1 h at 20-25°C and centrifuged for 10 min at 4500 rev. min^{-1}. The upper solution was collected in a flask for Ca_{10}-P analysis. The P content in all extracted fractions was determined using the MO-SB colorimetric method.

2.2.4.2. Measurement of ^{32}P radioactivity

^{32}P radioactivity in the fractions of Ca_2-P, Ca_8-P, Al-P, Fe-P, O-P and Ca_{10}-P was measured using a liquid scintillation counter (LS-6500, BECKMAN, USA). The maize L-value (μg P g^{-1} soil) was calculated according the followed formula:

L-value = (^{32}P Bq g^{-1} soil) / (^{32}P Bq μg^{-1} P in plant)

2.3. Data statistics

All data are presented as the means of three replicates with standard errors. Differences between treatment means were compared by the least significant difference (LSD) test at $P<0.05$.

3. RESULTS

3.1. Experiment 1: Field experiment

3.1.1. Screening of maize genotypes tolerant to low-P soil

The frequency distribution of the inhibition (%) of the 100-grain weight of 116 maize cultivars under P limitation (P0) compared with P sufficient (P1) is shown in Fig. 1. Overall, the results were found to be normally distributed (KS test, Z value = 0.928, P value = 0.356). Maize genotypes tolerant to low-P soils were defined according to the distribution of inhibition that increased under low-P limitation, and a change in the inhibition that was lower than –5%. Low-P sensitive genotypes were selected when the inhibition was higher than 10%.

Four quadrant analyses for the selected typical maize genotypes showed that tolerance to low-P was primarily distributed in the first (responsive and efficient) and fourth quadrants (non-responsive and efficient). However, low-P sensitive maize genotypes were mainly distributed in the second (responsive and inefficient) and third quadrants (non-responsive and inefficient) (Fig. 2). Based on the depression of the 100-grain weight and the four quadrant analyses, the following typical 15 inbred lines were selected out of the 116 maize varieties for further analysis: genotypes tolerant to low-P, DSY-30, DSY-2, DSY-31, DSY-20, DSY-21, DSY-39, DSY-101, DSY-33, DSY-93, DSY-23 and DSY-32; genotypes sensitive to low-P , DSY-113, DSY-79, DSY-129 and DSY-48.

Genotypes tolerant to low-P produced higher 100-grain weight than low-P sensitive genotypes under low-P limitation in the field experiments 1, 2 and 3 (Fig. 3). 100-grain weight of DSY-30 (genotype tolerant to low-P) had the highest value, 39.2 g, 37.4 g and 38.4 g in the three years of the experiment, respectively. It was significantly higher than DSY-113, DSY-79, DSY-129 and DSY-48 (genotypes sensitive to low-P) ($P<0.05$). 100-grain weight of DSY-48 was significantly lower than other maize genotypes in the three years of the experiment ($P<0.05$).

3.1.2. Root architectural traits at seedling stage in field experiments 2 and 3

3.1.2.1. Adventitious root angle

Root architectural plasticity might be an important factor in the acquisition by plants of immobile nutrients such as P. The results of two field experiments suggested that adventitious root angle correlated with adaptation to low-P. Adventitious root angles of maize genotypes under the P0 treatment were more or less pronounced than those under the P1 treatment (Fig. 4).

Effect of limiting P on the adventitious root angles of typical maize genotypes were showed in Fig. 5. Adventitious root of low-P sensitive genotypes had deeper growth angles than low-P tolerant genotypes in the field experiment 3 (Fig. 5B). Adventitious root angles DSY-30, DSY-2, DSY-21, DSY-33, DSY-93, DSY-20 and DSY-79 in low-P tolerant genotypes were lower than mean value (56.38°). Our results demonstrated that variation for adventitious root angles existed in maize, which P could modulate root shallowness independently, and that a shallow root system was beneficial for plant performance in maize at low-P limitation.

3.1.2.2. Root length, root surface area, root volume

Root morphological parameters such as decreased root length, root surface area and root volume are shown in Table 1. In 2008, increased root lengths were observed for maize varieties grown under low-P limitation. Specifically, 64% of the genotypes that were tolerant to low-P showed increased root lengths under low-P limitation, while only 25% of the genotypes that were sensitive to low-P showed increased root length under low-P treatment. The inhibition of DSY-93 was significantly higher than that of other genotypes. The total root

surface area and root volume of low-P tolerant genotypes were higher than those of low-P sensitive genotypes under low-P limitation, except for DSY-113. These findings indicated that low-P tolerant genotypes could accelerate root growth under low-P conditions, resulting in roots exploring more soil volume for the uptake of nutrients.

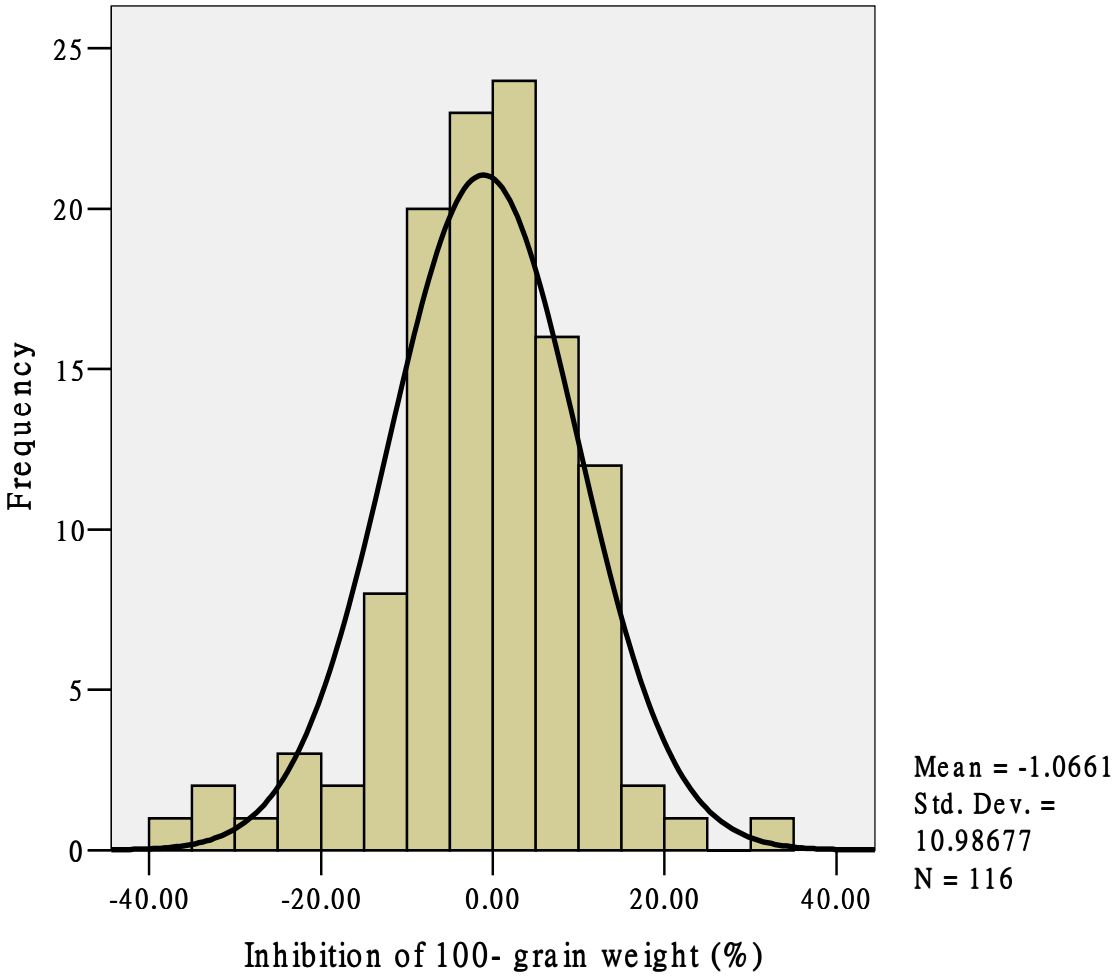

FIG. 1. Frequency distribution of the inhibition of the 100-grain weight of 116 maize inbred lines in field experiment.

Our data also showed that Inhibition of root length ranged from –44.0 to 47.7% in 2009. The total root length of low-P tolerant genotypes increased dramatically under P-deficient conditions such as DSY-30, DSY-2, DSY-31, DSY-21, DSY-39, DSY-33 and DSY-32 that developed larger root lengths than low-P sensitive genotypes. The inhibition of root length in DSY-21 (low-P tolerant genotype) was significantly lower than those of other genotypes, which indicated that low-P tolerant genotypes developed larger root length under low-P limitation. On the contrary, low-P sensitive genotypes developed smaller root lengths under P deficient conditions compared to normal P conditions. The above results indicated that root length of low-P tolerant genotypes were essential for their high ability to acquire soil P. Comparing the inhibition of total root surface area and that of total root volume between low-P tolerant and sensitive genotypes, the results showed that the inhibition of root surface area and total root volume ranged from –45.5 to 47.1%, and –49.6 to 58.3%, respectively. The inhibitions were lower in low-P tolerant genotypes than in low-P sensitive genotypes.

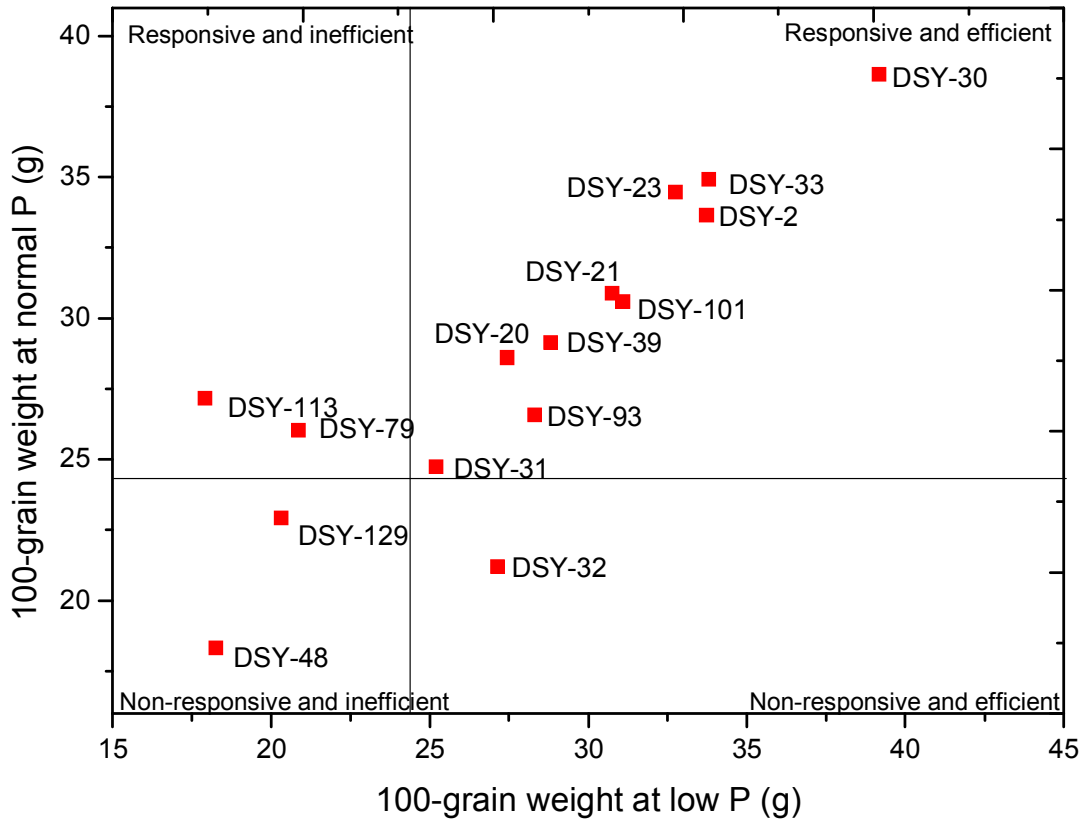

FIG. 2. 100-grain weight of typical inbred lines of maize under P0 and P1 treatments in field experiment 1.

TABLE 1. CHANGES IN ROOT MORPHOLOGICAL CHARACTERISTICS OF SEEDLINGS IN FIELD EXPERIMENTS 2 AND 3

Genotype	No.	Inhibition (%) of					
		Root length		Root surface area		Root volume	
		2008	2009	2008	2009	2008	2009
P tolerant	DSY-30	–6.9	–6.7	–16.1	–12.2	–25.6	–20.0
	DSY-2	–56.7	–41.6	–10.1	–45.5	22.1	–49.6
	DSY-31	11.0	–29.0	18.1	–28.5	25.0	–27.8
	DSY-21	–17.3	–44.0	5.5	17.3	23.8	52.5
	DSY-39	–7.4	–19.2	8.3	–14.6	21.2	–10.0
	DSY-101	–1.1	29.7	–5.4	27.9	–9.9	26.0
	DSY-33	50.3	–13.0	34.4	–10.7	13.0	–8.3
	DSY-32	34.1	1.4	40.0	–8.0	45.0	–18.0
	DSY-23	11.8	47.7	5.3	50.4	–1.0	53.0
	DSY-93	–127.4	22.0	–53.2	46.5	–3.0	54.3
	DSY-20	–45.5	14.9	–47.6	–1.1	–49.7	–20.1
P sensitive	DSY-113	–68.9	34.8	–56.4	41.0	–45.6	46.7
	DSY-79	17.6	32.9	26.7	32.2	34.5	31.5
	DSY-129	28.2	24.5	22.7	29.1	16.7	33.4
	DSY-48	2.3	32.9	3.3	47.1	4.4	58.3

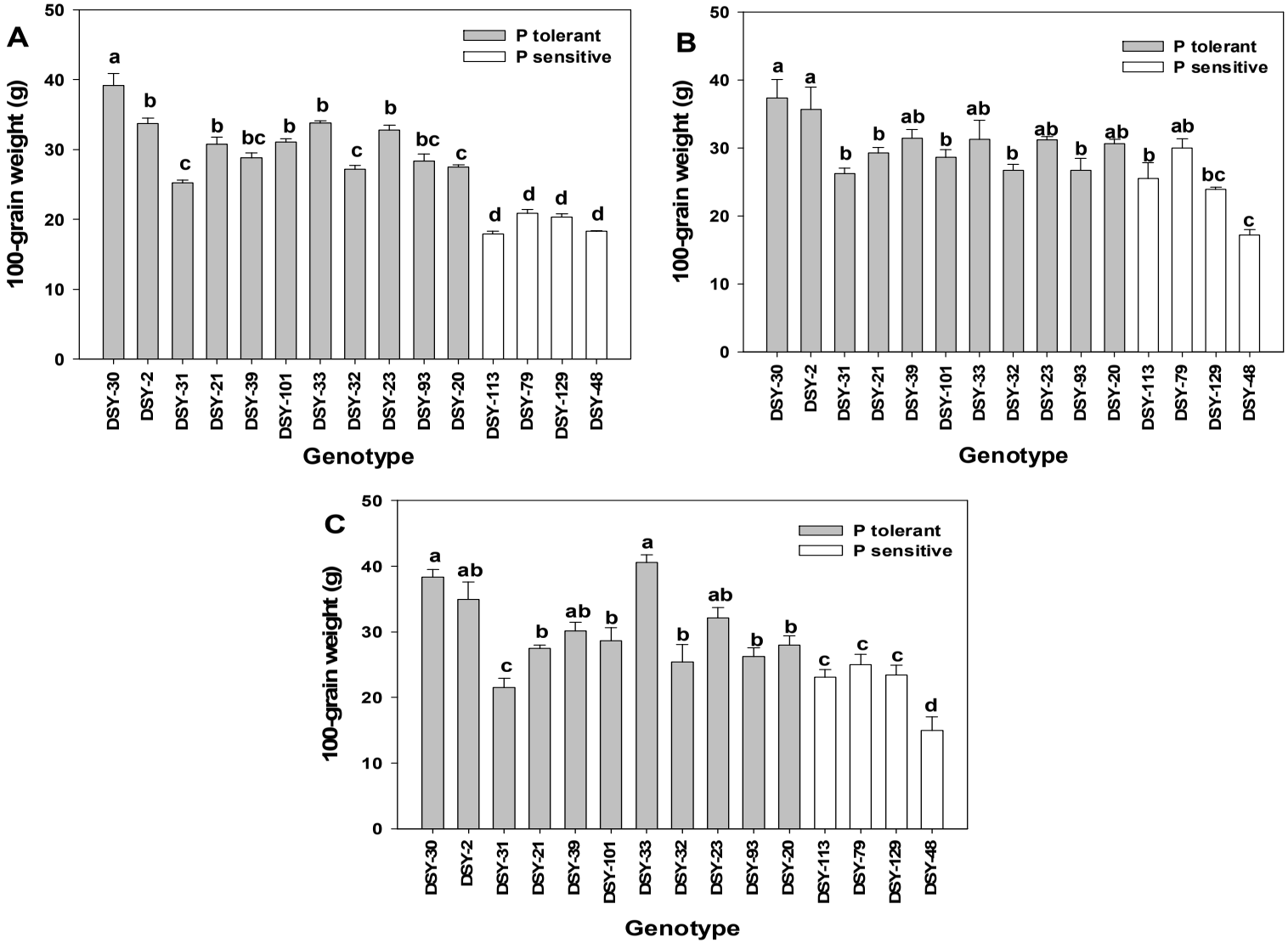

FIG. 3. Effects of low-P limitation on 100-grain weight of typical maize genotypes in different years. Each column represents the mean of three plants ± SD. A: the field experiment 1 (in 2007), B: the field experiment 2 (in 2008), C: the field experiment 3 (in 2009).

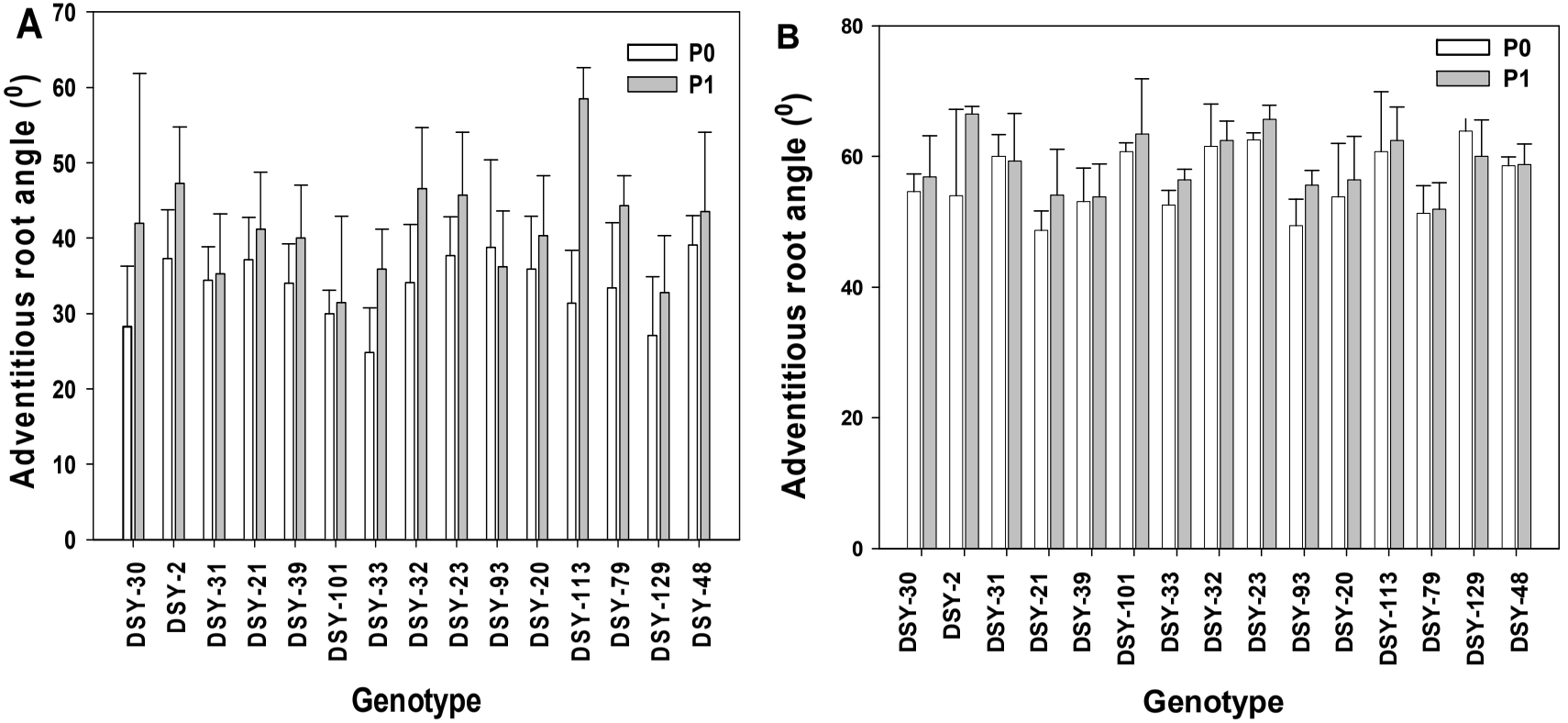

FIG. 4. Adventitious root angle of typical maize genotypes under low-P limitation and normal P conditions. Each column represents the mean of three plants ± SD. A: the field experiment 2 (in 2008), B: the field experiment 3 (in 2009).

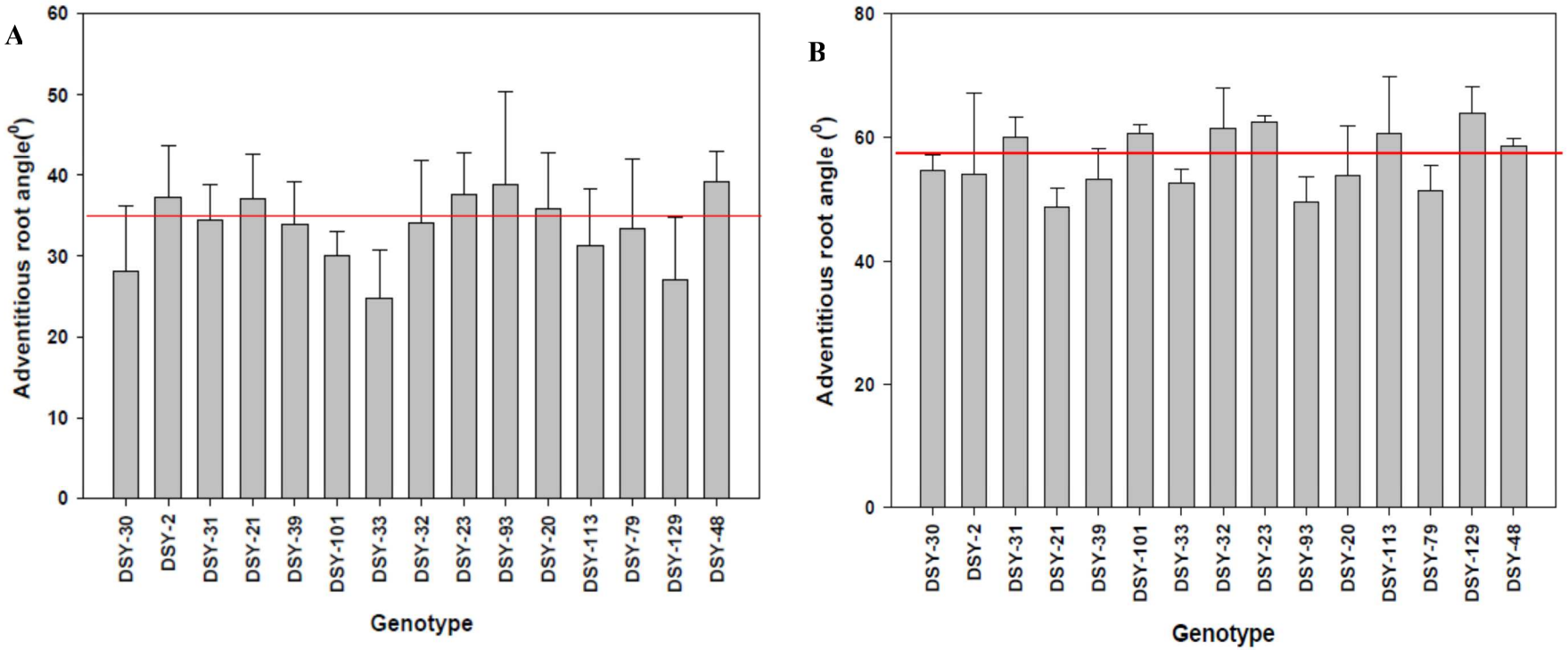

FIG. 5. Adventitious root angle of typical maize genotypes under low-P limitation. Red line: the mean value of adventitious root angle. Each column represents the mean of three plants ± SD. A: the field experiment 2 (in 2008), B: the field experiment 3 (in 2009).

3.1.3. P nutrient characteristics of maize genotypes at the seedling stage in the field experiments 2 and 3

3.1.3.1. Root: shoot ratio

As expected, the root/shoot ratio increased in response to P deficiency (Fig. 6). The reason for this phenomenon is that P deficiency during the early stage affected the plant growth, resulting in greater restriction of the shoots than the roots because carbon dioxide was converted to carbohydrates through photosynthesis and transported from the shoots to the roots. The root/shoot ratio of low-P tolerant genotypes was higher under low-P limitation condition than that of low-P sensitive genotypes, whatever in 2008 or 2009. These findings suggested that the Inhibition of the root/shoot ratio was more stable during the seedling stage.

There was a close relationship between the plant's P uptake and the soil P supply level. P uptake efficiency of different maize genotypes were lower under P deficiency as compared with that under P sufficiency in both field experiments (Fig. 7). Under P deficiency, P uptake efficiency in DSY-21 and DSY-23 (low-P tolerant genotypes) was significantly higher than that DSY-113 (low-P sensitive genotype) in the field experiment II (Fig. 7A). In comparison with low-P sensitive genotypes, the low-P tolerant genotypes were of higher P content under P deficiency, indicating that P uptake ability of low-P tolerant genotypes were relatively stronger in the field experiment III (Fig. 7B). P uptake efficiency in DSY-30, DSY-2, DSY-39, DSY-101, DSY-93 (low-P tolerant genotypes) was significantly higher than DSY-113, DSY-79, DSY-129, DSY-48 (low-P sensitive genotypes). The Inhibition of P uptake efficiency in low-P tolerant was much lower than most of low-P sensitive genotypes in the field experiment II and III, indicating that P uptake ability of low-P tolerant was relatively strong under low-P limitation.

3.1.3.2. Relationship between root length and biomass

Correlation analysis was performed to reveal relationship with root length and the total biomass (Fig. 8). Under P-deficiency condition, root length was significantly correlated with total biomass. The correlation coefficient was 0.61 ($P<0.05$). It was obvious that the biomass of maize genotype for tolerance to low-P limitation had the tendency to associate with this morphological characteristic. Otherwise, it may be used as a fast screening protocol to evaluate the maize lines at the seedling stage under low-P limitation in the field evaluation.

3.2. Experiment 2: ^{32}P isotope tracer

3.2.1. Selecting plant materials

To study the nutrient acquisition from sparingly soluble P forms and determine L-values, the ^{32}P tracer technique was used [17]. Based on 100-grain weight of three field experiments (2007, 2008 and 2009), DSY-30, DSY-2, DSY-32 were selected as low-P tolerant genotypes, and DSY-48 and DSY-79 were selected as low-P sensitive genotypes for the tracer experiment (Fig. 9, red circle: maize inbred lines).

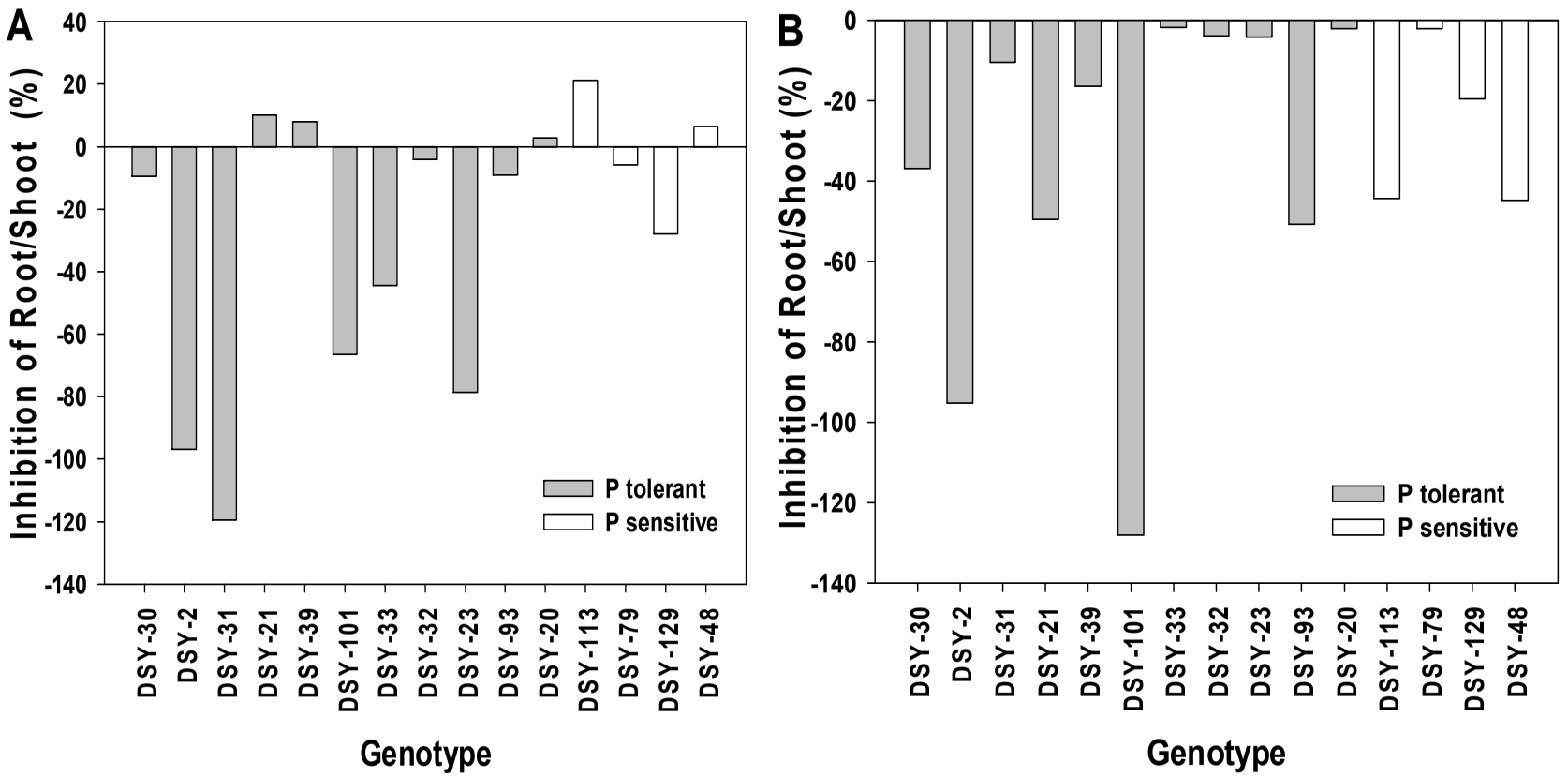

FIG. 6. Inhibition of root: shoot ratio. A: the field experiment 2 (in 2008), B: the field experiment 3 (in 2009).

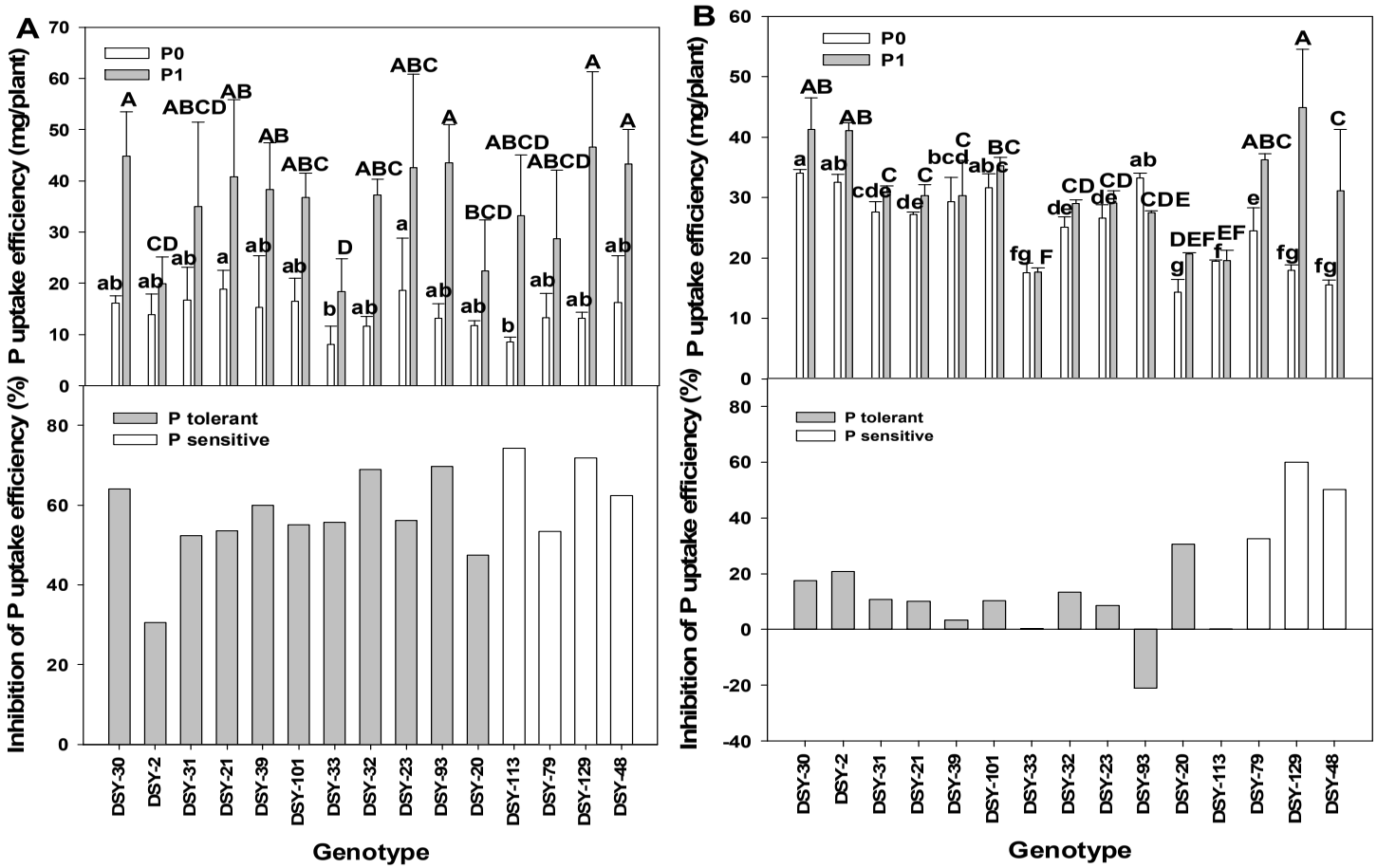

FIG. 7. P uptake efficiency and inhibition of typical maize inbred lines. A: the field experiment 2 (in 2008), B: the field experiment 3 (in 2009). Note: Different uppercase or lowercase letters indicate significant differences (P<0.05).

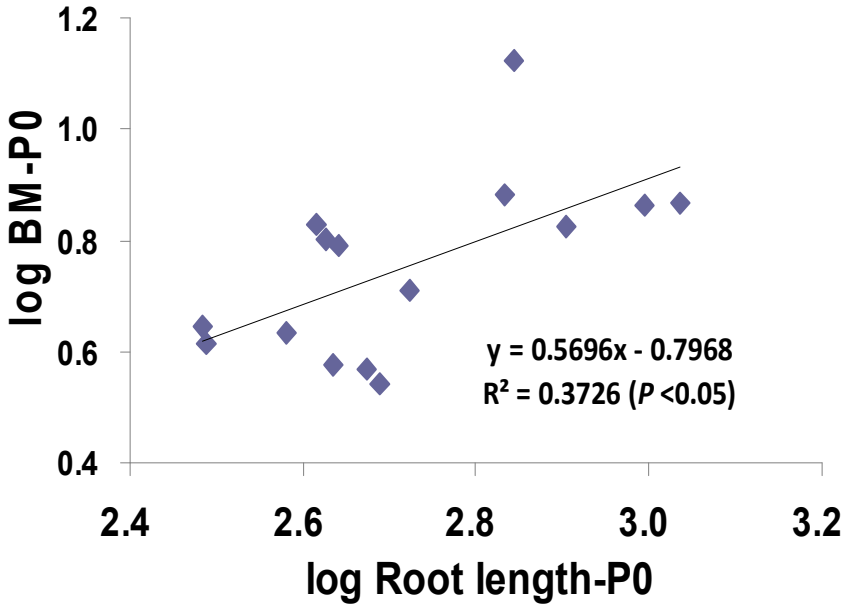

FIG. 8. Relationship between the root length and biomass at the seedling stage.

3.2.2. Dynamic variations of the P fractionations

To better understand the transformation dynamics of fertilizer P to different P fractions, the ^{32}P tracer technique was employed. Without P fertilizer, the ^{32}P activity in Ca_2-P decreased rapidly during the early period, but then decreased slowly. However, a reverse trend was observed in Ca_8-P, i.e., a rapid increase during the early period followed by a slow increase. The ^{32}P activity in Fe-P and Al-P increased steadily at a moderate rate and reached a steady state. However, the ^{32}P activity in O-P and Ca_{10}-P increased slowly and steadily during the entire test period of 25 d (Fig. 10A). When P fertilizer was added to the soil, the transformation dynamics of ^{32}P in all P fractions in the soil were consistent with the transformation dynamics without P fertilizer (Fig. 10B). However, the ^{32}P activity in different P fractions showed different characteristics. First, the ^{32}P activity in Ca_2-P was significantly higher than activity without P fertilizer. Second, the ^{32}P activity in slowly available P (Ca_8-P, Al-P and Fe-P) and unavailable P (O-P and Ca_{10}-P) was significantly lower than without P fertilizer. These results suggested that the rate of water-soluble P that transformed to slowly available and unavailable P in the soil with deficient P was higher than in the soil with sufficient P. The possibility of water-soluble P transformation to unavailable P (such as O-P) in the soil with sufficient P was lower than in the soil with deficient P.

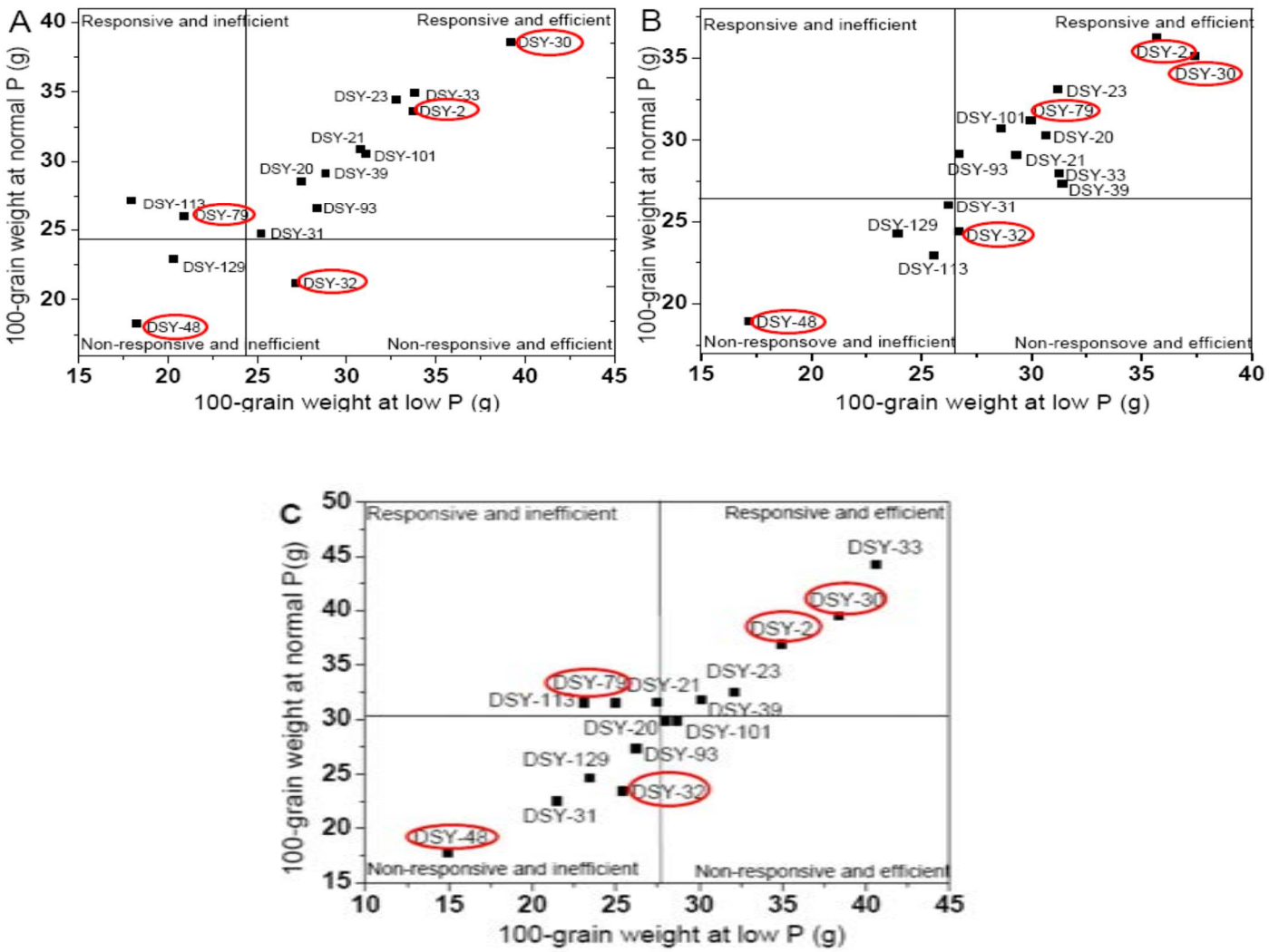

FIG. 9. 100-grain weight of typical inbred lines of maize under P0 and P1 treatments in field experiment 1, 2 and 3. A: the field experiment 1(in 2007), B: the field experiment 2 (in 2008), C: the field experiment 3 (in 2009).

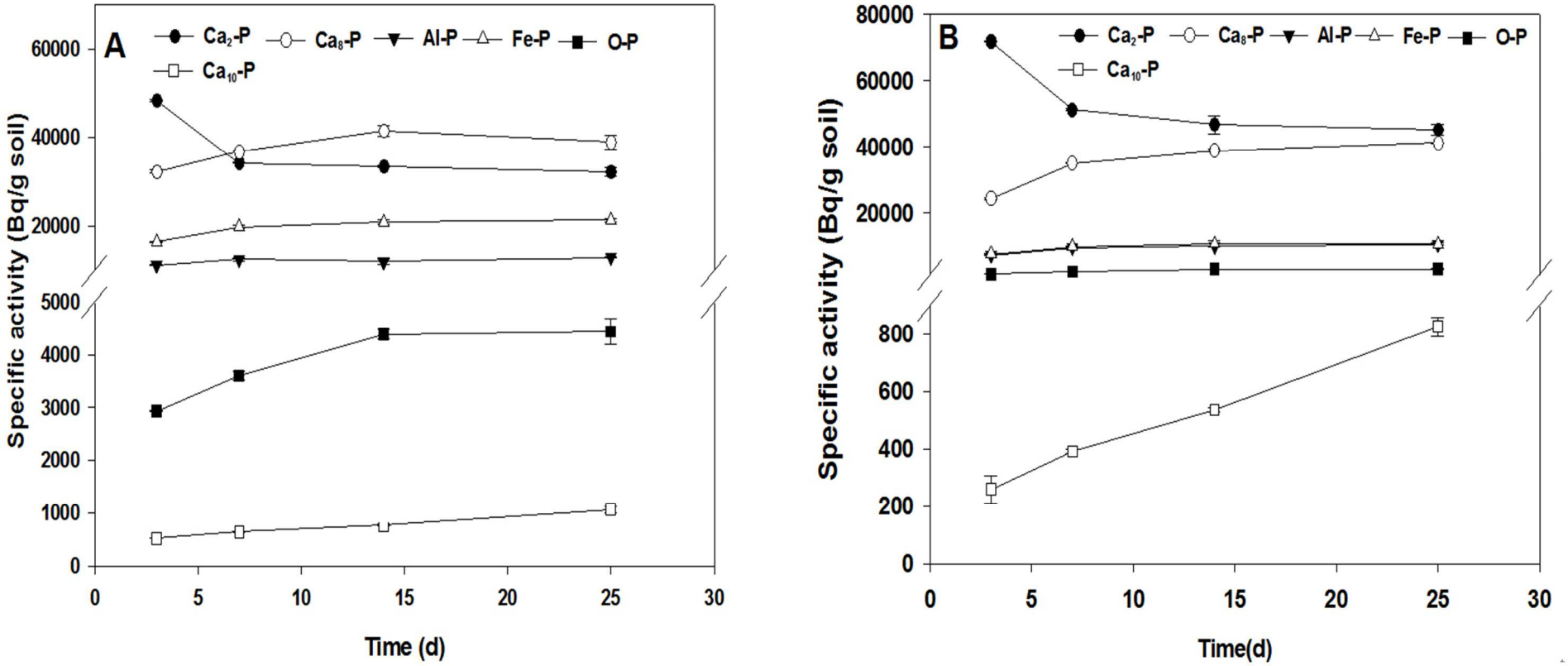

FIG. 10. Temporal changes in the ^{32}P specific activity of the inorganic P fractions. A: no P-fertilizer; B: with P-fertilizer.

3.2.3. Inorganic P and L-value

This experiment was performed using radioactive ^{32}P to estimate the plant available soil P. The L-value has considerable theoretical advantages as a measurement of plant available soil P. As shown in Tables 2 and 3, the DSY-30 and DSY-2 cultivars had higher L-values and intermediate P consumption, which indicated they activated soil P in different forms, and absorbed it under low-P limiting conditions. These cultivars showed a slight dependency on insoluble and soluble P; therefore, DSY-30 and DSY-2 were likely to be the low-P tolerant genotypes.

The L-value of DSY-32 was the highest under low-phosphorus limitation, which indicated that DSY-32 activated and utilized different Pi forms in soil, and reduced the dependence on soil soluble P (Table 2). After P fertilizer was applied, the L-value of DSY-32 decreased rapidly (Table 3), which suggested that more P from fertilizer was utilized by DSY-32. Therefore, DSY-32 was a typically low-P tolerant genotype.

TABLE 2. L-VALUES OF MAIZE GENOTYPES WITHOUT EXTERNAL P FERTILIZER

Variety	Specific activity of		L-value
	Plant (Bq μg^{-1})	Soil (Bq g^{-1} soil)	(μg P g^{-1} soil)
DSY-30	22.4 ± 2.5†	4216 ± 144	190 ± 28
DSY-2	20.0 ± 0.1	4484 ± 154	225 ± 7
DSY-32	7.8 ± 1.0	3965 ± 136	513 ± 45
DSY-79	11.4 ± 0.8	3572 ± 122	315 ± 32
DSY-48	64.9 ± 2.9	3623 ± 124	56 ± 1

†Values following means are ± standard errors

TABLE 3. L-VALUES OF MAIZE GENOTYPES WITH EXTERNAL P FERTILIZER

Variety	Specific activity of		L-value
	Plant (Bq μg^{-1})	Soil (Bq g^{-1} soil)	(μg P g^{-1} soil)
DSY-30	16.8 ± 0.9†	4015 ± 339	239 ± 32
DSY-2	14.0 ± 1.0	3517 ± 120	252 ± 26
DSY-32	20.4 ± 1.7	3793 ± 130	251 ± 5
DSY-79	8.3 ± 0.4	3821 ± 131	460 ± 17
DSY-48	28.8 ± 2.7	5089 ± 312	134 ± 36

†Values following means are ± standard errors

Although the L-value of DSY-79 was higher than DSY-30 and DSY-2, the radioactivity of the shoot was also higher, indicating that DSY-79 required much higher P content in the plant to maintain normal crop growth. Therefore, DSY-79 was a typical low-P sensitive genotype. The L-value of DSY-48 was significantly lower than the other maize genotypes with or without external P fertilizer. One probable explanation for this result is that DSY-48 had a high dependency on soil available P and was sensitive to the supply of soil available P.

4. DISCUSSION

The primary objective of most maize breeding programs is the evolution of high yielding and well adapted cultivars. Breeding for improved varieties is a continuous process and requires, primarily, a thorough knowledge of the genetic mechanism governing yield and yield components [18]. Tang et al. [19] reported that grain yield had positive significant correlation with 100-grain weight (P<0.05). Kanaka [20] also reported that over dominance

was present for 100-grain weight. The 100-grain weight was also likely to play a major role in the tolerance to low-P limitation, and could also be used as the best parameter to evaluate P efficiency in maize [21], which was supported by our data. 100-grain weight of low-P tolerant genotypes were higher than DSY-113, DSY-79, DSY-129 and DSY-48 (low-P sensitive genotypes) under low-P limitation in our study, which suggested that 100-grain weight could be an indicator for screening tolerant maize genotypes. Similar 'specific mechanism(s)' has been described [22]: a truly tolerant genotype requires less nutrient than a sensitive genotype for normal metabolic processes. Therefore, a genotype with a small decrease in yield with a decrease in P supply is more tolerant to P limitation than one with a large decrease, provided the compared germplasm could achieve similar yield when sufficient P is available.

Root architectural plasticity may be an important factor in the acquisition of immobile nutrients such as P by plants. It was well known that plant tolerance to P limitation is closely related with root characteristics [23]. A study conducted by [24] found that adventitious rooting enhanced P acquisition with shallow growth angles. These early reports were supported by our results: that an adventitious root angle may be correlated with low-P adaptation. Adventitious root angles of maize genotypes were observed more frequently in the P0 groups than the P1 groups. This is likely because P availability is greatest near the surface in most natural and agricultural soils [25]. The results also showed that low-P tolerant genotypes had shallower adventitious root angles than low-P sensitive under low-P limitation, which indicated that maize genotypes with shallower roots had greater growth in low-P soil than deep-rooted genotypes [26].

With less mobile ions like P, uptake was often closely related to root length [27]. There have been a number of reports on root length under P-deficient conditions. Root elongation by P deficiency was observed in Arabidopsis [28], barley [29], horsegram [30], and rice [31]. The screening of maize varieties in the present study revealed root elongation induced by P deficiency. Varieties in the low-P tolerant groups were found to elongate their roots specifically under P deficient conditions, which could result in roots exploring more soil volume (Table 1). The result was consistent with previous reports of maize root system growth and development as influenced by P deficiency [32]. P uptake is also dependent on root surface area and volume in contact with soils [33]. Our results suggested that low-P tolerant genotypes could accelerate the growth of root surface and volume under low-P limitation.

Effects of P deficiency on root biomass are more controversial. In a P starvation experiment, increased root dry weight was found in 12 day-old maize plants [33], and it was also reported that tolerant varieties of broad bean usually have higher root biomass [34, 35]. In contrast, no effect of P deprivation was observed on maize root biomass [36]. However, authors generally agree that P deficiency in maize leads to a higher root: shoot ratio [37]. The previous reports were supported by our results: that the root: shoot ratio of low-P tolerant genotypes was higher under low-P limitation than that of low-P sensitive genotypes, in both experiments in 2008 and 2009. This response indicated that dry matter accumulated more in the roots of low-P tolerant genotypes than that of low-P sensitive genotypes. We suggest that the relationship between root length and the total biomass for those tolerant and sensitive varieties may be used as a fast screening protocol at the seedling stage in the field evaluation.

An understanding of the relationship with morphological and physiological characteristics is fundamental for identification and utilization of P efficient germplasm [3]. In this study, changes of root length induced by P deficiency were reflected in the biomass correspondingly. Close relationships of the total biomass with root length were revealed. The

finding is expected since longer roots could be more efficient in absorbing P from soils low in available P, which affected biomass accumulation and distribution. This suggested that root length was a reliable trait in screening the germplasm for P efficiency.

Zhang et al. [1] reported that in the seedling stage, P uptake efficiency was the main contributor to P tolerance, because a higher P uptake ability under P limitation may also contribute to the tolerance to P limitation for a germplasm, regardless of its nutrient requirement. In our study, there existed significant genotypic differences in P uptake efficiency among different maize inbreds (Fig. 7). Low-P tolerant genotypes could keep the higher uptake efficiency and absorb more P from soil to satisfy the demand of their growth under low-P conditions, while low-P sensitive genotypes could not.

The elucidation of the transformation dynamics of P fractions is important for a better understanding of the availability of different P fractions. In different soil types, the availability of a certain P fraction to a plant differs significantly. Jiang and Gu [38] reported that the Ca_2-P fraction was the main one for rapid availability of P to plants, whereas Ca_8-P, Fe-P and Al-P were slowly available, and Ca_{10}-P and O-P were unavailable. Previous studies have shown that the primary P form was Ca_2-P after P fertilizer was applied to calcareous soil [39, 40]. Our results are consistent with this conclusion. Additionally, our data using the ^{32}P tracer technique indicated that the ^{32}P activity reached a maximum before 3 d and then decreased rapidly during 3 to 7 d after ^{32}P-labeled fertilizer P was applied to Langfang low-P soil. Simultaneously, the ^{32}P in other P fractions, including Ca_8-P, Fe-P, Al-P, O-P and Ca_{10}-P increased at different rates, i.e., Ca_2-P transformed to other P fractions over time. Therefore, we proposed that Ca_2-P in this soil was a rapidly transforming phase and the main P fraction available to the plant. A continuously increasing trend of ^{32}P activity was observed in Ca_8-P, Fe-P and Al-P during the entire 25 d test period, and this rate was moderate. Therefore, Ca_8-P, Fe-P and Al-P should be described as moderate transforming phases. Among these three P fractions, the ^{32}P activity and its increasing rate in Ca_2-P and Ca_8-P exhibited maxima, which might be partly due to the higher concentration of Ca than Fe and Al in calcareous soil. Therefore, Ca_8-P might be another important available P fraction in Langfang low-P soil. During the test period of 25 d, the ^{32}P activity in Ca_{10}-P and O-P increased very slowly, which suggested that Ca_{10}-P and O-P were slow transforming phases (Fig. 9). Additionally, after fertilizer P application, the ^{32}P activity and its increasing rate in O-P decreased rapidly, which indicated that a larger proportion of water-soluble P in P deficient soil without external P fertilizer would transform to the plant-unavailable O-P fraction, i.e., a plant would have more difficultly acquiring P from P deficient soil. The result presented further evidence that external P fertilizer was essential to vigorous crops growing in the Langfang low P soil.

In low-P soil, the P efficiency of plants differs among genotypes within a given plant species [11, 41, 42]. The screening criterion for low-P tolerant materials differs among plant species [43–45]. This experiment was performed using radioactive ^{32}P to estimate the plant available soil P. The L-value has considerable theoretical advantages as a measurement of plant available P from the soil. The L-values were determined after maize plants were grown in Langfang low-P soil for 25 d to evaluate the genotype P efficiency. Together with our data, the P efficiency for five maize genotypes was significantly different. DSY-32, which is a typically low-P tolerant genotype, actively regulated the P utilization ratio between the soil P and the exogenous fertilizer P. When exogenous P was supplied, DSY-32 preferentially absorbed exogenous P, otherwise it would try to exploit soil P when no exogenous P was supplied. However, another low-P tolerant genotype, DSY-2, exhibited a different low-P tolerant pathway that efficiently utilized soil P regardless of exogenous P application. This result suggested that different maize genotypes have very different low-P tolerant pathways

(Tables 2 and 3). Therefore, it was viable to fully exploit the limited P resources in low-P soil by the screening and planting of low-P tolerant crop species. Additionally, the results showed that the L-value was a very useful parameter for evaluation of plant P efficiency.

5. CONCLUSIONS

This field study clearly demonstrated that maize genotypes differ in their ability to take up P from the low-P soil, and that these differences were attributed to the morphology and physiology of the plants relative to their germplasm base. Based on these results, an effective method of increasing P efficiency was to develop P tolerant cultivars that could achieve a high yield under P deficiency. The results indicated that soil P availability during maize seedling development was critical for early growth and grain yield of maize. The inhibition of the root: shoot ratio, root length, root surface area, root volume and P uptake efficiency were tentatively defined as screening indices for low-P tolerant genotypes during the seedling stage. The relationship between root length and total biomass for those typical genotypes could be used as a very fast screening protocol at the seedling stage in the field evaluation under low-P limitation. In addition, the 100-grain weight was defined as the screening indexes of low-P tolerant genotypes during the mature stage.

The ^{32}P tracer technique provides a powerful alternative to better understand soil P availability and sources of P pools in a low P soil-plant system. The results indicated that the rate of water-soluble P that transformed to slowly available and unavailable P in the soil with deficient P was higher than in the soil with sufficient P. Ca_2-P was a quick transforming phase; Ca_8-P might be another important available P fraction while Ca_{10}-P and O-P were slow transforming phases. The L-value determination showed low-P tolerant cultivars regulated soil P use efficiency and plant P content.

The study exploited the physiological-biochemical mechanism on P uptake and P transport of selected maize genotypes in low-P soil, through a field experiment associated with the use of ^{32}P tracer. Since P deficiency had slight effects on low-P tolerant genotypes as compared with low-P sensitive genotypes, it was demonstrated that differences of tolerance to P deficiency existed among different maize genotypes.

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PHOSPHORUS USE EFFICIENCY BY BRAZILIAN UPLAND RICE GENOTYPES EVALUATED BY THE ^{32}P DILUTION TECHNIQUE

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Abstract

The objectives of this work were to identify the most efficient upland rice genotypes in phosphorus (P) utilization, and to verify if P from the seed affects the classification of upland rice genotypes on P uptake efficiency. The experiment was conducted in a greenhouse of the Center for Nuclear Energy in Agriculture (CENA/USP), Piracicaba, São Paulo, Brazil, using the ^{32}P isotope technique, and plants were grown in pots with samples of dystrophic Typic Haplustox (Oxisol). The experimental design was completely randomized with four replications. The treatments consisted of 47 upland rice genotypes and two standard plant species, efficient or inefficient in P uptake. The results were assessed through correlation and cluster analysis (multivariate). The Carisma upland rice genotype was the most efficient in P uptake, and Caripuna was the most efficient on P utilization. The P derived from seed does not influence the identification of upland rice genotypes in P uptake efficiency.

1. INTRODUCTION

In low input farming systems, phosphorus (P) is one of the most important factors worldwide limiting crop yields. In Brazil, upland rice is typically grown in P deficient soils with high P-fixing capacity [1] and without fertilization in agricultural frontier areas, mainly in the Cerrado region. Low P input as fertilizer is one of the main factors that explain low upland rice grain yields in Brazil, on average 2 t ha^{-1} [2], compared to lowland rice of 4.5 t ha^{-1} [3]. Furthermore, upland rice is an important crop in Brazil, because it is grown by resource poor farmers as a subsistence crop, representing a minimum input production system.

There is a large natural inter- and intra-specific genetic variation for plant traits that are associated with P uptake efficiency, and development of transgenic plants can be used as a strategy for improving P uptake efficiency of crops that may represent a sustainable solution to increase yields [4]. Although success in developing nutrient efficient crop genotypes has been limited, this strategy should continue to receive top priority during the 21st century [5].

Upland rice genotypes differ in P use efficiency and efficient rice genotypes can be used in breeding programs [1, 6]. Identifying rice genotypes more efficient in P uptake is the first step to a successful breeding program, and is a strategy to reach high economic yields in low input systems. High P content in rice grains (the majority as phytate) contributes little to human nutrition because micronutrients such as iron and zinc are binding to phytate [7]. In addition, continued removal of P from the fields in rice grain at harvest results in depletion of soil P reserves in low input agricultural systems [8].

There are several definitions and calculation methods for P use efficiency (divided into P uptake efficiency and P utilization efficiency). Here we define P uptake efficiency as the ability of upland rice genotypes to take up P from soil assessed by the ^{32}P dilution technique [9], and P utilization efficiency as the ability to produce grain yield under low available P supply [10]. The advantage of the ^{32}P isotopic dilution method compared to others is the possibility of eliminating the influence of seed derived P when comparing P uptake efficiency by crop species or genotypes, by the L-value [9, 11, 12].

The ability of different plant species (canola, white lupin, pigeon pea, soybean, sunflower and wheat) for absorbing less available forms of soil P was compared using the ^{32}P isotope dilution technique, and it was observed that the white lupin was more efficient [9]. Using this same technique in a study of 22 plant species [13], it was observed that white lupin, upland rice, eucalyptus, cotton and pigeon pea were the most efficient in P uptake, while sunhemp, cowpea and soybean were classified as less efficient species. An important factor usually not considered in studies assessing genotypic variation for P uptake efficiency is the P content present in seeds. Genotypes that have seeds with higher P content can be classified, by mistake, as more efficient in P uptake than others with less P content of seeds. Comparing wheat genotypes for their efficiency in P uptake, it was found that the tolerance to P deficiency was higher in genotypes of durum wheat (*Triticum durum* L.) in relation to wheat (*Triticum aestivum* L.) [14, 15]. In this study, the greater tolerance of durum wheat genotypes was attributed to higher P content in seeds in relation to wheat.

The objectives of this study were to identify upland rice genotypes more efficient in P uptake using the ^{32}P isotope dilution technique and upland rice genotypes more efficient in P utilization. Furthermore, we aim to verify if P from the seed affects the classification of upland rice genotypes on P uptake efficiency.

2. MATERIALS AND METHODS

2.1. Experimental

The experiment was conducted in the greenhouse at Center for Nuclear Energy in Agriculture (CENA/USP), located at latitude 22°42'30" S, longitude 47°38'01" W and 554 m altitude, in Piracicaba, São Paulo, Brazil. The study were performed in 3.0 l plastic pots, lined with polyethylene bags, containing 2.5 kg of air-dried soil, collected from the 0 to 0.20 m of a dystrophic Typic Haplustox [16]. The soil samples were dried, sieved in a 2 mm mesh sieve and homogenized. The soil had 280, 70 and 650 g kg^{-1} content of clay, silt and sand, respectively, and the following chemical characteristics: pH (0.01 mol l^{-1} $CaCl_2$, 4.5; organic matter, 18.0 g dm^{-3}; resin extracted P, 5 mg dm^{-3}; K, 0.6 $mmol_c$ dm^{-3}; Ca, 11.5 $mmol_c$ dm^{-3}; Mg, 5.2 $mmol_c$ dm^{-3}; H + Al, 35.4 $mmol_c$ dm^{-3}; CEC, 52.7 $mmol_c$ dm^{-3}; sum of bases, 17.3 $mmol_c$ dm^{-3}; base saturation, 32.8%, according to methodology described by [17]; and P by Mehlich-1, 3 mg dm^{-3} [18].

After application of lime (Calcium Carbonate Equivalent = 110%) to raise the base saturation to 50% for the upland rice, according to the official recommendation of Bulletin 100 [19], the soil was incubated for 30 days and the moisture content was maintained at approximately 70% of water holding capacity.

To evaluate the efficiency of genotypes of upland rice for P uptake, a mixture of triple superphosphate (20 mg P kg^{-1} soil) as a source of readily available P to plants, and Patos phosphate rock (150 mg P kg^{-1} soil) were applied to raise the total P content of soil to 170 mg P kg^{-1} in each pot. N and K were applied at rates of 200 mg N kg^{-1} as urea and 200 mg K kg^{-1} as potassium sulphate. Fertilization with micronutrients, in the three experiments was done applying nutrient solution in all treatments at rates of 0.5 mg B kg^{-1}, 1.5 mg Cu kg^{-1}, 3.0 mg Fe kg^{-1}, 2.0 mg Mn kg^{-1}, 3.0 mg Zn kg^{-1} and 0.1 mg Mo kg^{-1}.

The experimental design was completely randomized with four replications. The treatments consisted of 47 upland rice genotypes and two standard species described in the literature as efficient or inefficient in P uptake: Sunhemp (*Crotalaria juncea* L.) as inefficient in absorbing P [12] and white lupin (*Lupinus albus* L.) as efficient [19, 12].

The upland rice genotypes evaluated were: Araguaia, Arroz Preto, Beira Campo, Bico Ganga, BRS Aimoré, BRS Apinajé, BRS Aroma, BRS Bonança, BRS Caripuma, BRS Colosso, BRS Curinga, BRS Monarca, BRS Pepita, BRS Primavera, BRS Sertaneja, BRS Soberana, BRS Talento, BRSMG Conai, BRSMG Relâmpago, Cabaçu, Cambará, Canastra, Carajás, Carisma, Cateto Seda, Centro América, Cuiabana, Douradão, Guanai, Guape, Guaporé, IAC 25, IAC 47, IAC 60 dias, IAC 202, IAC 1246, Ipê, Jaguary, Maravilha, Maranhão, Montaninha 90 dias, Progresso, Rio Paranaíba, Rio Verde, Tangará, Xingú and Zebu.

The soil was labeled with ^{32}P by applying a solution with 9.25 MBq of ^{32}P and 0.2 mg P kg^{-1} carrier. Eight seeds of the upland rice varieties or five seeds of the two standards were sown in each pot, and the final population was thinned to three plants pot^{-1}. Soil moisture was maintained at approximately 70% of water retention capacity during the experiment.

The above-ground part of the plant of each genotype was taken at two samplings: (i) first, two plants, from the total of three cultivated in each plot, were harvested at 40 days after emergence, and (ii) the one remaining plant at the stage of panicle full maturity. The plant samples were separated into shoots (leaves, stems, rachis and rice husks) and grain.

The seed-contained P was discounted for calculating the L-value, which was used to compare the efficiency of P uptake among the genotypes, considering that from the total P stored in the seeds of rice genotypes, 60% is used for plant growth [11], i.e., 40% of seed P is not used by the plant (remains in the cotyledon).

2.2. Calculations of phosphorus and ^{32}P

With shoot dry matter (Sdm), grain weight (Gw), P concentration in Sdm and Gw, the P contents in the shoot and in grain were calculated:

$P\,uptake = \text{Pconcentration}\, x\, \text{Sdm}$, where Sdm is shoot dry matter.

$P\,content = \text{Pconcentration}\, x\, Gw$, where Gw is grain weight.

With the data of plant P content and the ^{32}P activity of the plant, the specific activity (SA), the L-value, and the L-value subtracting the amount of seed-derived P from the total P content of the shoot were calculated [9, 10].

$$SA = \frac{^{32}P}{^{31}P}$$

where SA is specific activity (dpm μg^{-1} P); ^{32}P is radioisotope activity in the plant (dpm); ^{31}P is plant P content (μg P $plant^{-1}$);

$$Lvalue = X\left(\frac{SA_0}{SA_p} - 1\right)$$

where L-value (mg P kg^{-1} soil); SA_0 is specific activity of the applied solution (dpm μg^{-1} P); SA_p is specific activity of plant (dpm μg^{-1} P); X is amount of applied P;

$$L-s\,value = \left(Y\frac{\left(X_T - Z\right)}{Y_T} - X\right)$$

where: L-s value is L-value subtracting P in the plant derived from the seed (mg of P kg^{-1} soil); Y is the ^{32}P activity in the applied solution (dpm); X_T is plant P uptake (mg); Y_T is ^{32}P activity in the plant shoot dry matter (dpm); X is the rate of ^{31}P carrier applied pot^{-1} (mg); Z is the total P content derived from seed (mg).

2.3. Statistical analysis

The results of Sdm, Gw, P concentration and P content in the shoot or in the grain, specific activity (SA), L-value and L-value subtracting the P derived from the seed (L-s value) were submitted to analysis of Pearson linear correlation and hierarchical cluster analysis with the objective for grouping the similar genotypes. Cluster analysis of upland rice genotypes was carried out with the SAS 9.1 - "Statistical Analysis System" [20] and SYSTAT version 10.2 software programs, using the UPGMA (un-weighed pair group arithmetic average clustering) The cluster analysis was preceded by the standardization of data before the Euclidian distances calculation, as the variables presented different scales. After standardization, all the variables were equally important in the determination of these distances. Final results of the groups were presented as dendrograms. The P uptake efficiency by plants is inversely proportional to SA and directly proportional to L-value and L-s value.

The upland rice genotypes were grouped into four or five groups, aiming at achieving greater homogeneity within each group and greater heterogeneity among the different groups. The results are presented and discussed in three parts: (1) first sampling - shoot; (2) second sampling - shoot, and (3) second sampling - grain. The term shoot dry matter (Sdm) refers to all above ground plant organs (leaves, stalks, husks and rachis) except the grain.

3. RESULTS AND DISCUSSION

3.1. First sampling - shoot

Plant data for the first sampling are given in Table 1.

TABLE 1. MEAN SHOOT DM YIELD (SDM) OF 47 UPLAND RICE GENOTYPES, P CONCENTRATION (P CONC), P UPTAKE, SPECIFIC ACTIVITY (SA), L-VALUE AND L-VALUE DISCOUNTING THE P FROM THE SEED (L-S VALUE) IN THE FIRST SAMPLING

Genotype	Sdm ($g\ pot^{-1}$)	P conc ($g\ kg^{-1}$)	P uptake ($mg\ pot^{-1}$)	SA (dpm μg^{-1} P)	L-value ($mg\ kg^{-1}$ soil)	L-s value ($mg\ kg^{-1}$ soil)
Cuiabana	2.07	1.53	3.16	137.29	12.34	12.18
Caripuna	2.50	1.45	3.63	213.81	7.86	7.87
Relâmpago	2.63	1.44	3.77	200.52	8.39	8.38
Maravilha	2.66	1.58	4.20	103.04	16.81	16.75
Xingú	2.69	1.44	3.88	119.29	14.25	14.12
Ipê	2.75	1.60	4.39	152.76	11.08	10.98
Aroma	2.78	1.46	4.06	173.75	9.71	9.72
Canastra	2.80	1.34	3.76	136.66	12.40	12.32
Carisma	2.81	1.72	4.81	80.37	21.24	21.16
Carajás	2.91	1.49	4.32	158.15	10.69	10.66
IAC 202	2.95	1.48	4.38	140.00	12.10	12.09
Colosso	2.95	1.64	4.83	135.93	12.47	12.46
Progresso	2.95	1.52	4.49	105.28	16.17	16.09
Araguaia	2.99	1.55	4.63	129.26	13.14	13.12
Rio Verde	3.00	1.47	4.41	160.72	10.61	10.57
Arroz Preto	3.02	1.25	3.77	95.90	17.67	17.50
Bonança	3.05	1.46	4.44	152.64	11.11	11.10
Zebu	3.05	1.55	4.73	106.84	15.95	15.81
Guaporé	3.10	1.58	4.88	152.11	11.13	11.11
Talento	3.10	1.47	4.55	105.13	16.20	16.09
Pepita	3.10	1.72	5.33	130.04	13.05	12.95
Sertaneja	3.11	1.43	4.41	97.20	17.53	17.50
Primavera	3.14	1.53	4.78	126.48	13.43	13.36
Tangará	3.15	1.26	3.95	214.96	7.81	7.77
Apinajé	3.20	1.56	4.99	122.64	13.85	13.80
Montanhinha 90 dias	3.21	1.43	4.61	171.16	9.86	9.85
Guanai	3.23	1.34	4.32	153.87	10.99	10.99
Conai	3.24	1.60	5.18	138.10	12.27	12.27
Cambará	3.27	1.54	5.03	127.68	13.29	13.23
Monarca	3.28	1.25	4.11	164.62	10.27	10.25
Curinga	3.29	1.48	4.88	162.81	10.38	10.45
Douradão	3.39	1.49	5.02	154.87	10.96	10.92
IAC 47	3.42	1.37	4.68	132.41	12.81	12.73
Beira Campo	3.45	1.34	4.59	218.79	7.67	7.65
Aimoré	3.50	1.59	5.57	173.49	9.73	9.70
Soberana	3.51	1.51	5.28	169.53	9.96	9.95
Rio Paranaíba	3.52	1.27	4.48	102.31	16.71	16.58
Cateto Seda	3.54	1.30	4.60	186.54	9.03	9.01
Centro América	3.66	1.44	5.28	97.96	17.38	17.29
IAC 1246	3.73	1.28	4.78	158.50	10.67	10.57
Cabaçu	3.80	1.35	5.14	157.02	10.77	10.76
Maranhão	3.82	1.31	5.00	134.41	12.62	12.62
Bico Ganga	3.87	1.45	5.61	109.53	15.52	15.48
IAC 25	3.92	1.47	5.77	142.28	11.91	11.88
IAC 60 dias	3.94	1.26	4.94	145.46	11.76	11.75
Jaguary	4.04	1.33	5.39	195.96	8.61	8.60
Guape	4.36	1.29	5.63	187.21	8.96	8.91
Average	3.22	1.45	4.65	145.43	12.32	12.27
CV (%)	10.90	10.22	10.38	12.21	12.78	12.65

The results obtained with the two standard species were: (i) White lupin - Sdm = 1.15 g pot^{-1}, P in Sdm = 2.03 mg pot^{-1}, SA = 39.06 dpm mg^{-1} P, L-value = 43.99 mg P kg^{-1} soil and L-s value = 2.6 mg P kg^{-1} soil, (ii) Sunhemp - Sdm = 5.16 g pot^{-1}, P content in Sdm = 5.77 mg pot^{-1}, SA = 193.32 dpm mg^{-1} P, L-value = 6.88 mg P kg^{-1} soil and L-s value = 6.67 mg P kg^{-1} soil. The white lupin plant was, as expected, more efficient in absorbing P (the lowest SA, and highest L-value L and L-s value) than all upland rice genotypes evaluated in this study. The Sdm of rice is one of the main parameters related to grain yield of this crop, and P increases due to an increase in the number of tillers and leaf area [21]. The values of Sdm of 47 upland rice genotypes correlated significantly and negatively with Sdm P concentrations (–0.466***) and positively with Sdm P contents (0.785***). Therefore, the dilution effect was observed in Sdm P, i.e., increasing Sdm decreased the Sdm P concentrations, although the total P uptake was higher. From these three variables, the cluster analysis identified the following five groups of upland rice genotypes (Fig. 1):

1st: Aimoré, Soberana, Centro América, Bico Ganga, IAC 25, Jaguary and Guape;
2nd: Cabaçu, Maranhão, IAC 1246, IAC 60 dias, Rio Paranaíba, Cateto Seda, Beira Campo, IAC 47 and Guanai;
3rd: Monarca, Tangará, Arroz Preto, Canastra, Aroma, Xingú, Relâmpago and Caripuna;
4th: Maravilha, Ipê, Progresso, Carajás, IAC 202, Rio Verde, Bonança, Talento, Sertaneja, Montaninha 90 dias, Douradão, Curinga, Araguaia, Zebu, Primavera, Guaporé, Apinajé, Cambará, Conai, Colosso, Carisma and Pepita;
5th: Cuiabana.

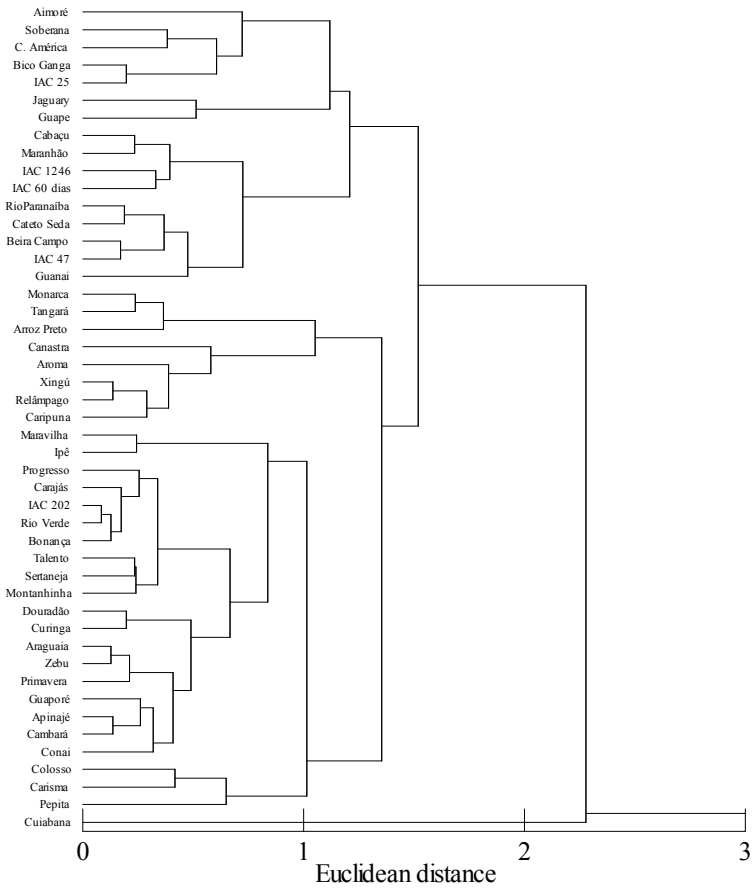

FIG. 1. Dendrogram resulting from the hierarchical cluster analysis of 47 genotypes of upland rice, based on the variables of shoot dry matter (Sdm), concentration and accumulation of P in Sdm. First plant sampling.

Among all the correlations between variables of upland rice genotypes, taken in the first sampling, the SA and L-value (–0.962 ***) x the L-s value (–0.960***), and L-value x the L-s value (0.999***) were the variables that showed higher Pearson correlation coefficients. By hierarchical cluster analysis with both variables SA and L-value (Fig. 2) as with the SA and L-s value (Fig. 3), upland rice genotypes were classified for the P uptake efficiency in the following four groups:

1st: very efficient, Carisma;
2nd: efficient, Arroz Preto, Sertaneja, Centro América, Maravilha, Rio Paranaíba, Talento, Progresso, Zebu and Bico Ganga;
3rd: medium efficiency, Xingú, Apinajé, Primavera, Cambará, Araguaia, Pepita, IAC 47, Maranhão, Colosso, Canastra, Cuiabana, Conai, IAC 202, IAC 25, IAC 60 dias, Guaporé, Bonança, Ipê, Guanai, Douradão, Cabaçu, Carajás, IAC 1246, Rio Verde, Curinga, Monarca, Soberana, Montaninha 90 dias, Aimoré and Aroma;
4th: less efficient, Cateto Seda, Guape, Jaguary, Relâmpago, Caripuna, Tangará and Beira Campo.

The Carisma genotype was the best for P uptake efficiency, and did not form a group with any other genotype (Figs. 2 and 3). Furthermore, we observed that the two dendrograms (Figs. 2 and 3) are similar, meaning that there was no difference in P uptake efficiency among groups of upland rice genotypes based on L-values or P in the plant derived from seed, because the genotypes grouped by SA, L-value and L-s value were similar.

3.2. Second sampling - shoot

The Sdm values correlated significantly and positively with Sdm P concentrations (0.486***) and P content in Sdm P (0.884***). Therefore, there was a response in shoot production to an increase of P concentration in plant tissue. The dendrogram obtained by grouping these three variables, in the second sampling, is shown in Fig. 4. The 47 upland rice genotypes were classified into four groups:

1st: Cuiabana, Ipê, Cabaçu and Zebu (genotypes with higher values of Sdm, Sdm P concentration and P uptake);
2nd: Cateto Seda, Beira Campo, Guaporé, Xingú, Sertaneja, Araguaia, Caripuna, Rio Parnaíba, IAC 47, Maranhão, IAC 1246, Arroz Preto, Guape, Monarca, Canastra, Maravilha, Rio Verde, Progresso, Curinga, Bonança, Pepita, Montaninha 90 dias, Carisma, Jaguary, Carajás, Aroma, IAC 202, Talento, Cambará, IAC 25 and Soberana;
3rd: Tangará, Relâmpago, Aimoré, Conai, Douradão, Centro América, Colosso, Primavera, Apinajé, Guanai and IAC 60 dias (genotypes with lower values of Sdm, P concentration and content in Sdm);
4th: Bico Ganga. It did not group with any other genotypes, because although it had high Sdm production, P concentration and P uptake were low (Fig. 4).

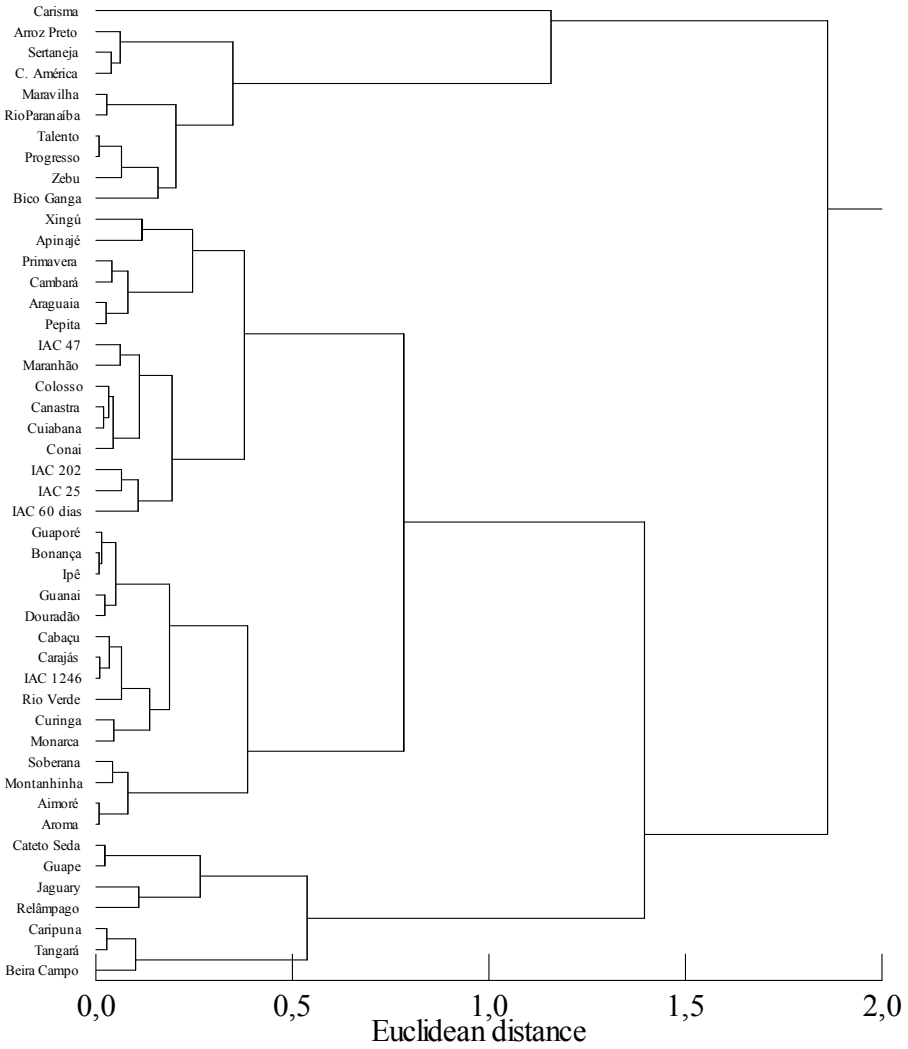

FIG. 2. Dendrogram resulting from hierarchical cluster analysis of 47 genotypes of upland rice, based on specific activity (SA) and L-value. First plant sampling.

3.3. Second sampling – grain

The grain dry matter, P concentration and P content of grain are given in Table 2. The Gw values correlated significantly and negatively with its P concentrations (–0.512***) and positively with its P contents (0.711***). The grain P concentration decreased with increasing Gw due to the dilution effect of P in vegetal tissue. The positive correlation between grain yield and its P content indicates that it is possible to increase grain production of upland rice with increasing plant P content, as observed by [23] for common bean, suggesting the use of bean genotypes more efficient in P utilization to increase grain yield.

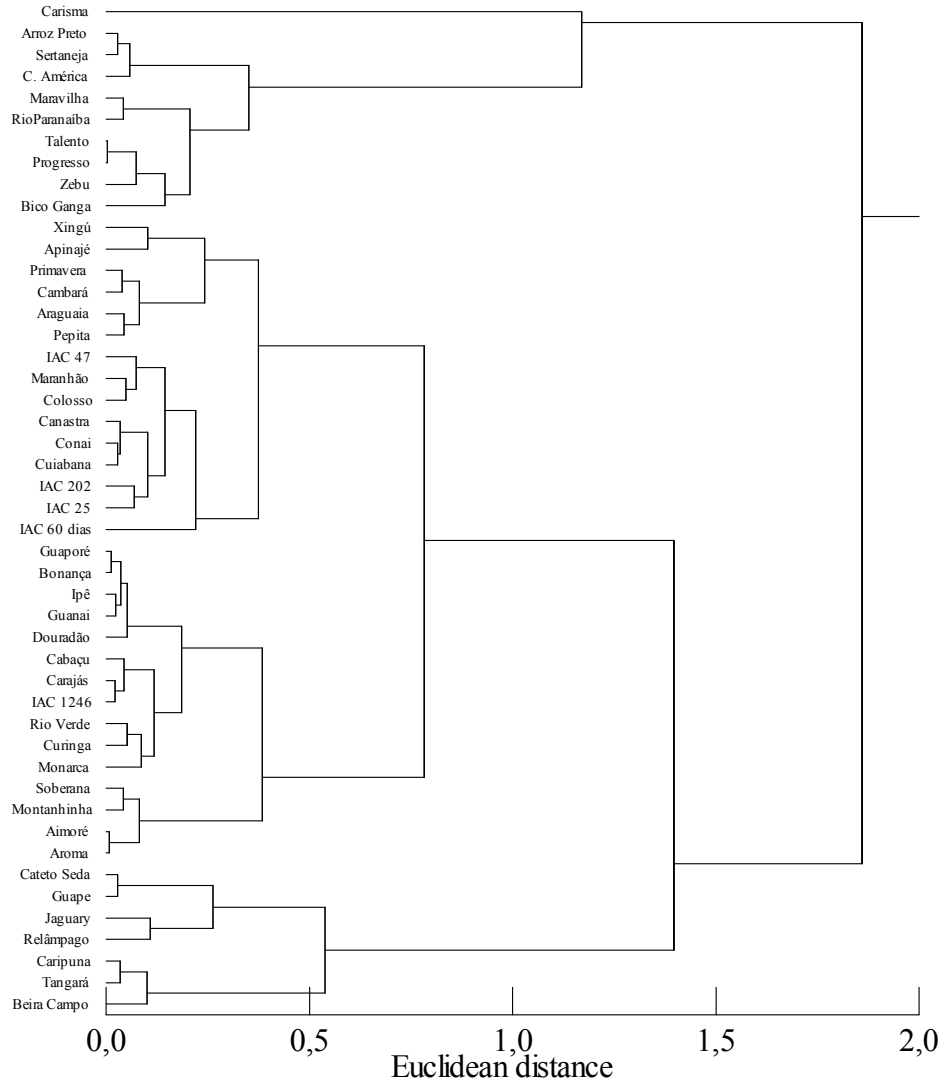

FIG. 3. Dendrogram resulting from hierarchical cluster analysis of 47 genotypes of upland rice, based on specific activity (SA) and L-value discounting the P in plant derived from seed (L-s value). First plant sampling.

From the analysis of hierarchical clustering of variables Gw concentration and content of P, the following five groups were identified, homogeneous and distinct from varieties of upland rice genotypes (Fig. 5) and were classified as:

1st: highly productive and highly rich in grain P content (genotype Caripuna);
2nd: very productive and very rich in grain P content (genotypes Bico Ganga, Sertaneja, IAC 202, Colosso and Rio Parnaíba);
3rd: productive and rich in grain P (genotypes Cambará, Relâmpago, Tangará, Aroma, Monarca, Guanai, Progresso, Cuiabana, Aimoré, Arroz Preto, Rio Verde, Xingú, Zebu, Cabaçu, Araguaia, Douradão, Centro América, Maravilha, IAC 25, IAC 60 dias, Primavera, Bonança, IAC 1246, Ipê, Montaninha 90 dias, IAC 47, Talento, Carisma, Conai, Pepita, Beira Campo, Apinajé, Guaporé, Maranhão, Jaguary, Curinga, Canastra and Cateto Seda);
4th: moderately productive and moderately rich in P in the grains (genotype Guape);
5th: less productive and low grain P (genotypes Soberana and Carajás).

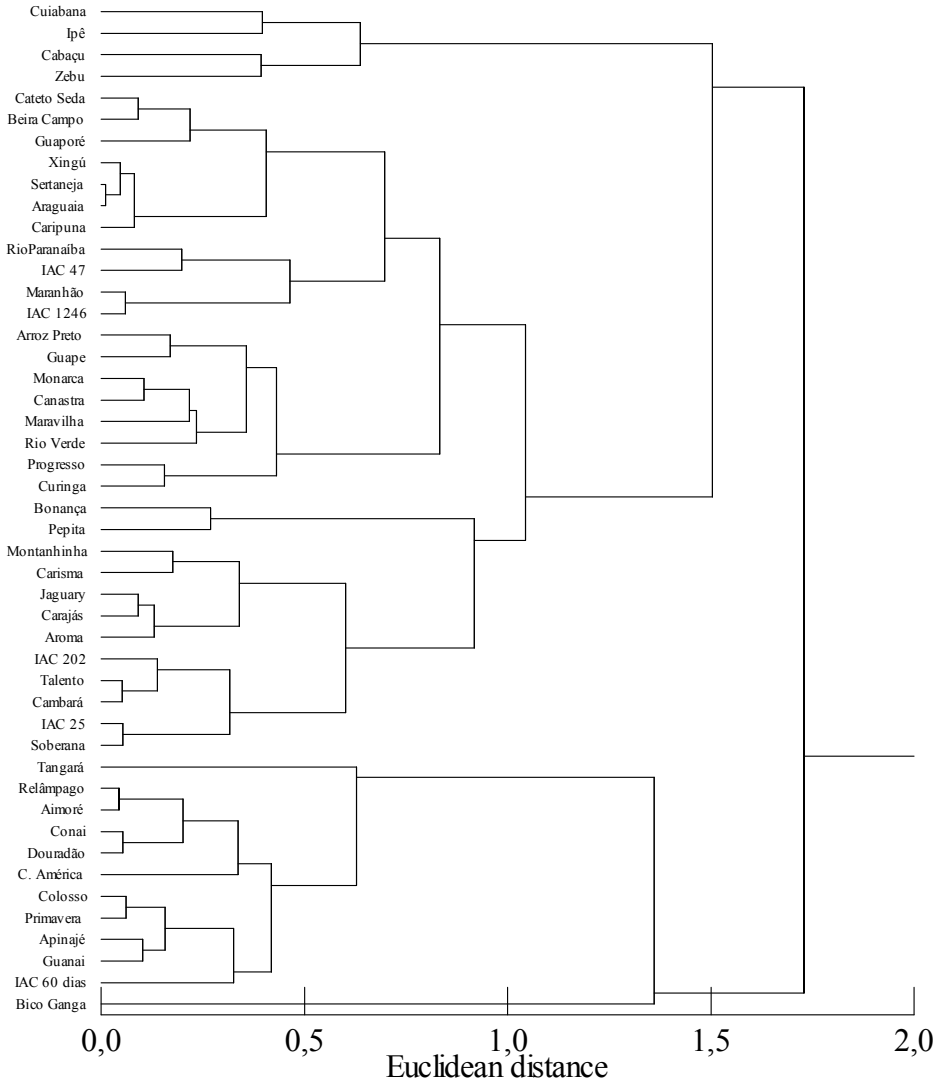

FIG. 4. Dendrogram resulting from hierarchical cluster analysis of 47 genotypes of upland rice, based on shoot dry matter (Sdm), concentration and accumulation of P in Sdm. Second plant sampling.

The Guape genotype was not grouped with any other genotype due to its low yield, but high accumulation of P in the grain, indicating that this genotype was not efficient in converting the plant accumulated P. Caripuna showed Gw similar to other genotypes, but was not grouped with any other, as the accumulation of P in the plant was higher than of other upland rice genotypes. In this experiment, we observed higher Gw and its P content in Rio Parnaíba compared to Araguaia, and these genotypes were classified as very productive and productive, respectively. Differences in P uptake and grain yield among upland rice genotypes grown in soil with low available P (P Mehlich-1 = 2.2 mg kg^{-1}) were also observed in the field [24].

TABLE 2. MEAN GRAIN DRY MATTER YIELD 47 UPLAND RICE GENOTYPES, P CONCENTRATION AND P CONTENT IN GRAIN IN THE SECOND SAMPLING

Genotype	Grain yield (g plant^{-1})	P concentration (g kg^{-1})	P content (mg plant^{-1})
Soberana	10.66	2.01	21.35
Guape	11.21	2.45	27.47
Carajás	11.47	1.88	21.45
Zebu	12.43	2.24	27.77
Cabaçu	12.46	2.10	26.21
Xingú	12.64	2.22	28.06
Araguaia	12.83	2.04	26.12
Centro América	12.88	1.92	24.73
Douradão	12.88	2.03	26.12
Arroz Preto	13.25	2.29	30.34
IAC 60 dias	13.51	1.98	26.75
Rio Verde	13.61	2.22	30.18
Primavera	13.64	2.02	27.56
IAC 25	14.04	1.97	27.69
Montanhinha 90 dias	14.10	2.12	29.89
Progresso	14.15	1.82	25.68
Bonança	14.17	2.02	28.65
IAC 1246	14.25	2.05	29.21
Ipê	14.34	2.08	29.85
Maravilha	14.48	1.94	28.11
Cuiabana	14.74	1.84	27.02
Aimoré	14.81	1.87	27.59
Jaguary	14.81	2.15	31.83
Guanai	14.96	1.71	25.52
Monarca	15.18	1.72	26.00
Carisma	15.25	1.95	29.80
Conai	15.35	1.94	29.71
Relâmpago	15.41	1.76	27.00
Tangará	15.47	1.73	26.78
Aroma	15.49	1.70	26.38
Pepita	15.60	1.93	30.03
Curinga	15.72	2.27	35.60
Beira Campo	15.78	1.91	30.09
Maranhão	15.88	2.09	33.15
Apinajé	16.15	1.94	31.38
Guaporé	16.36	2.00	32.70
Cambará	16.61	1.67	27.68
Canastra	16.79	2.11	35.41
Cateto Seda	17.37	2.20	38.31
Talento	17.60	1.95	34.26
Rio Paranaíba	17.69	1.79	31.63
IAC 47	17.79	1.99	35.44
Caripuna	18.18	2.39	43.34
Colosso	18.52	1.65	30.50
Sertaneja	20.20	1.88	37.91
IAC 202	20.39	1.69	34.43
Bico Ganga	20.59	2.04	41.87
Average	15.14	1.98	29.88
CV (%)	12.32	12.19	12.15

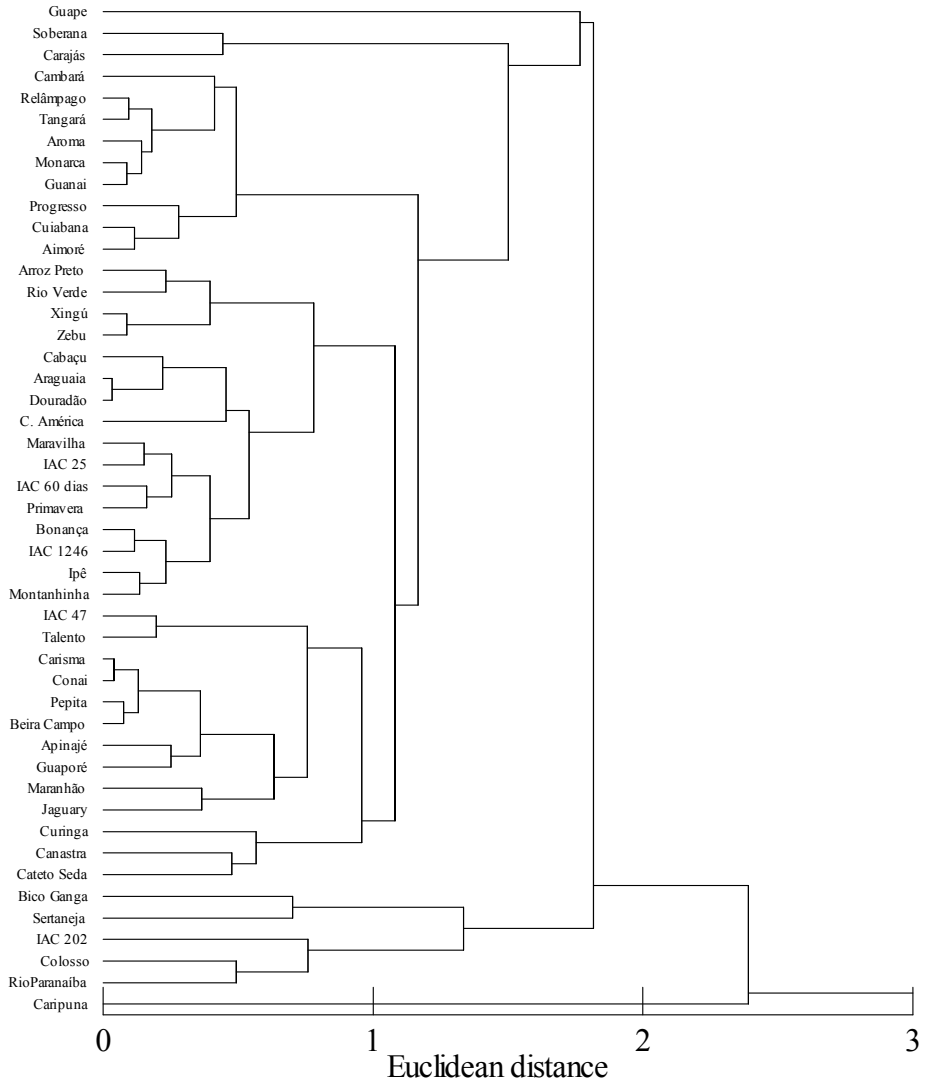

FIG. 5. Dendrogram resulting from hierarchical cluster analysis of 47 genotypes of upland rice, based on grain dry matter yield (Gw), and accumulated P concentration in Gw. Second plant sampling.

Although Carisma was the most efficient upland rice genotype in P uptake (Figs. 2 and 3), it was not classified in the group of the genotypes most productive in grain. Therefore, considering the definition of efficiency on P utilization by crops [10], Carisma was not the most efficient in P utilization.

In the second group of upland genotypes Arroz Preto, Sertaneja, Centro América, Maravilha, Rio Paranaíba, Talento, Progresso, Zebu and Bico Ganga were more efficient in P uptake (Figs. 2 and 3). Bico Ganga, Sertaneja and Rio Parnaíba were the highlighted genotypes, because these genotypes were classified in the second group that produced more grain (Fig. 5).

There was no significant correlation between SA, L-value and L-s value of 47 upland rice genotypes (measured in the first sampling) with Gw, P concentration and P content in the grain (measured in the second sampling). This indicates that upland rice genotypes more efficient in P uptake are not necessarily the most efficient in converting P taken up into grain.

The P amount required by plants can be reduced by using efficient upland rice genotypes in P use [25]. The identification of upland rice genotypes more efficient in P uptake and P utilization is a strategy to reduce P fertilizer rates besides allowing its cultivation in soils poor in P, and yet obtain high economic grain yields.

4. CONCLUSIONS

- The upland rice genotype Carisma was the most efficient in P uptake;
- The Caripuna upland rice genotype was the most productive in grain yield under conditions of low available soil P (genotype more efficient in P utilization);
- The P derived from seed in the plant, when the ^{32}P L-value technique is used, did not affect the identification and classification of upland rice genotypes.

ACKNOWLEDGMENTS

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EVALUATION AND SELECTION OF COMMON BEAN (*PHASEOLUS VULGARIS L.*) GENOTYPES FOR ROOT TRAITS ASSOCIATED WITH PHOSPHORUS (P) ACQUISITION EFFICIENCY AND THE USE OF ^{32}P ISOTOPE IN STUDIES ON P UPTAKE BY ROOT HAIRS

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Abstract

Low phosphorus (P) availability is one of the main edaphic constraints limiting crop production and productivity in most of the tropical agro-ecosystems. Several root traits are known to be associated with P acquisition efficiency in low P soils. These root traits include root hairs. Computer modeling, laboratory and field studies show the depletion of ^{32}P-phosphate around roots and that the depletion zone is influenced by the length and density of root hairs. We conducted a study involving a series of experiments with the objective of evaluating the variability of root traits associated with P uptake efficiency among common bean (*Phaseolus vulgaris* L.) genotypes, and to understand the mechanisms of long root hairs leading to the increase in P uptake in common bean. The study included (a) the screening of common bean genotypes in the laboratory and in the field for root traits, and (b) the use of radioactive phosphorus (^{32}P) in the experiments conducted in the greenhouse. For laboratory screening, seedlings were germinated in paper rolls in a growth media for 3 days before evaluation for basal root whorl number (BRWN), basal root number (BRN), basal root growth angle (BRGA) and root hair length (RHL). Common bean genotypes were planted in the field with low P for 45 days after planting (DAP) before evaluation. For the ^{32}P study four contrasting genotypes for root hairs were grown for 28 DAP in the greenhouse using 15-20 liter pots filled with a mixture of sand and vermiculate as the growth media. The radioactive P was incorporated in the growth medium in the form of alumina-P fertilizer. Normal phosphorus (non-radioactive ^{31}P) was included in the nutrient solution in the form of calcium phosphate, $Ca_3(PO_4)_2$, and supplied through irrigation. Screened genotypes exhibited different root traits associated with P uptake efficiency, and that a given genotype can have one or more root traits responsible for it P uptake efficiency. Data analysis of radioactivity present in the plant tissue among contrasting genotypes showed that long root hair genotypes had greater ^{32}P uptake compared with short root hair genotypes. In addition, a strong positive correlation ($R^2 = 0.8703$) was observed between specific activity of ^{32}P plant tissue and shoot dry weight of the four genotypes contrasting for root hairs. These results suggests that (a) a genotype can exhibit one or more root traits responsible for P uptake efficiency; (b) long root hairs increase total P uptake by releasing organic compounds (root exudates) that could help to solubilize P otherwise not readily available to inefficient genotypes with short root hairs.

1. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important sources of protein for over a billion people worldwide, providing in addition to protein, vitamins and minerals for people in the developing countries situated in the tropics [1, 2]. Meanwhile, bean production is often limited by low soil fertility, especially low P availability, which is often found in weathered tropical soils, because of the intense leaching and weathering conditions and high P fixation by Fe and Al oxides [3]. Low P availability is especially problematic for

legumes like common bean, since root nodules responsible for N_2 fixation have a high P requirement. Application of P fertilizer is only a partial solution since they are costly, nonrenewable, potentially harmful to the environment, and also marginally effective because of immobilization by the soil. Therefore, several authors have suggested that genetic improvement for P efficiency in crops would be more economical and practical than reliance on chemical P fertilizers alone. As it has been widely demonstrated that genotypes vary in response to high nutrient availability (thus the success of several efforts in that regard, e.g. the 20th century green revolution), we should accept that genotypes also vary in response to low nutrient availability, which has been demonstrated by several authors. Lynch et al. [4] have demonstrated genotypic variations in response to P availability in common bean and other crops, and have referred to nutrient efficiency as the ability of a genotype to grow and yield at sub-optimal nutrient supply. The root system is an important factor for plant productivity. Plants evolved a wide range of adaptations to enhance P and water acquisition from the soil. Important root traits for P acquisition efficiency are root hair length and density.

The study of root hairs is of a great importance in many disciplines of plant science. In cell biology root hairs are important as single cell models [5] and in plant nutrition as nutrient uptake organs [6]. The capacity of plants to absorb both water and mineral nutrients from the soil is related to the plant's ability to develop an extensive and well-located root system. Of the total root surface area, root hairs can contribute up to 67%. This is a very valuable contribution provided by root hairs. Cost / benefit analysis of C respired per unit of P acquired suggests that the extension of the root surface area through root hairs is an efficient plant strategy for improving P uptake at low costs in P stressed ecosystems [7]. In addition, it has been demonstrated that the role of root hairs in P acquisition through studies in the laboratory and computer modeling showing the depletion of ^{32}P-phosphate around roots, and that the depletion zone was influenced by the length and density of root hairs [8], involving low C cost for maintenance.

The present study had two main objectives, namely, (a) to evaluate common bean materials for root traits conferring P uptake efficiency both in laboratory and in the field; and (b) to determine and quantify the contribution of root hairs for P acquisition in low P soils. We hypothesize that (a) different genotypes employ different mechanisms (root traits) P acquisition efficiency, and (b) the increased P content in plant tissue of efficient genotypes grown in soils with low P is due to the ability of acquiring P made available through dissolution of insoluble P by organic compounds from root exudates released in increased amounts induced by long and dense root hairs.

2. MATERIALS AND METHODS

2.1. Genotype evaluation for root traits associated with P uptake efficiency

One hundred ninety six (196) genotypes selected from CIAT were evaluated in the field experiments for root traits associated with P acquisition efficiency. G 19833, an Andean genotype considered to be P efficient, with shallow basal roots, three basal root whorls, 12 basal roots and many adventitious roots, and DOR 364, a P inefficient genotype from the Mesoamerican gene pool, with deep basal roots, two whorls and 8 basal roots, were included as checks. The field experiment was conducted from June to August of 2010 in Rock Springs, Pennsylvania, USA. The experimental design was a randomized complete block design (RCBD) with 4 replications, and each experimental unit was composed of one row of 1.6 m with eight plants. The spacing between rows was 0.7 m and between plants in a row was 0.2 m. The experiment was planted under low P availability. Weed and pest control, and irrigation were applied as needed.

Root crowns of 3 representative plants per replication were harvested 45 DAP. The following traits were measured: (i) adventitious root length, branching and diameter; (ii) basal root length, branching and diameter, (iii) primary root length, branching and diameter; (iv) basal root angle; and (v) number of nodules. Actual counts were taken for total number of adventitious and basal roots, and basal root whorls. Root length, angle from horizontal and diameter were measured with a ruler, protractor and caliper, respectively. The root diameter was measured in the main. Root branching (density) was obtained by counting the number of lateral roots in a representative segment of 2 cm in each root class. Shoots were dried at 60^{o} for 2-3 days for determination of the shoot dry weight. Minitab statistical software (2010 Minitab Inc., USA) was used to analyze the data.

2.2. Root hair imagining

Basal roots were briefly stained with diluted Typan blue (0.05%) for better visualization of root hairs. Root hair images were visualized with a light microscope and images were captured at 40x magnification. Images were taken 2 cm above emerging new root hairs. Image analysis software (http://rsbweb.nih.gov/ij/download.html) called Image J, was used to measure root hair length and density. Root hair length of each genotype was measured in 5 different representative segments per replication. The root hair density was measured by counting the number of root hairs in a representative area. Root hair density was then converted to number of root hairs per mm^2. Genotypes were then grouped in three categories based on root hair length: short (<0.4 mm), intermediate (0.4-0.6 mm) and long root hairs (>0.6 mm).

2.3. Use of ^{32}P isotope for determination of root hair contribution to P uptake

To test the above mentioned hypothesis, we employed a technique involving ^{32}P in the form of alumina-P in which common bean genotypes contrasting for root hair characteristics were used in the study conducted in the greenhouse. The study consisted in using radioactive phosphorus (^{32}P) using 15-20 liter pots filled with a mixture of sand and vermiculate as the growth medium. The radioactive P was incorporated in the growth medium in the form of Alumina-P fertilizer, and normal ^{31}P in the form of calcium phosphate, $Ca_3(PO_4)_2$, was included in the nutrient solution and supplied through irrigation. The solid phase buffered alumina P fertilizer was prepared according to Lynch et al. [9], and regulated the availability of ^{32}P in the treatments. All other nutrients (including micronutrients) were applied in the growth mediim as nutrient solution dissolved in the drip irrigation water.

Plants with contrasting root traits were grown for 35 DAP, and plant sub-samples were collected for laboratory analysis, which consisted of the determination of radioactivity (CPM g^{-1} plant tissue), using a liquid scintillation analyzer, and total P content in the plant tissue, using a colorimetric absorption spectrophotometer (Lambda 25). Specific activity was calculated using the following formula: SA = C (cps. g^{-1} DW) / ($^{32}P + ^{31}P$).

Four Recombinant Inbred Lines (RILs) contrasting in root hair length and density were used in this study. Two lines were selected from DOR364 x G19833 RIL population and the other two lines were selected from G2333 x G19839 RIL population of common bean. The selection of DO364 x G19833 RILs was based on [10] and the selection of the G2333 x G19839 was based on root hair evaluation at 10 DAP in the field. Long root hairs genotypes used in this study were RILs DxG53 and GxG41, and short root hair genotypes were DxG11 and GxG23.

The experiment was conducted under controlled greenhouse conditions, using a Completely Randomized Design Block (CRBD), with a total of 4 genotypes contrasting in root hair length, making a total of 2 treatments (2 genotypes per treatment), with 3 replications. Pots with the following dimensions: 30 cm deep and 25 cm in diameter were used. Pots were filled with a mixture of 50% Vermiculite, 45% sand and 5% alumina P, making a total volume of 20 liters per pot. At 35 DAP, plant shoots were collected from all treatments, and shoot dry weight determined.

3. RESULTS

3.1. Genotype evaluation for root traits associated to P acquisition efficiency

Field measurements showed significant differences among genotypes for adventitious root number, branching, and diameter, basal root whorl number, basal root number, branching, diameter and growth angle, primary root branching and diameter, and number of nodules ($P \leq 0.01$) (Fig. 1). Considerable variation in root traits was found among common bean genotypes evaluated in 8-day old seedlings. Significant differences in 165 genotypes were detected in basal root whorls number (BRWN), (F value = 8.2***), number of basal roots (F = 7.7***) and root hair length (F = 6.3***) evaluated in 8-day old seedlings. BRWN varied from 1 to 4. Most genotypes had 2 whorls. The average root hair length measured on basal roots varied from 0.19 to 0.78 mm (Table 1), and there was a positive correlation between root hair length and density.

High correlation between basal root number and basal root whorl number evaluated in 8-day old seedlings was found ($R^2 = 0.9$, $P \leq 0.01$). Basal root number evaluated in 45-day old plants (Table 2) was moderately correlated with BRWN measured in 8-day old seedlings ($R^2 = 0.522$, $P \leq 0.01$). Similarly to data from 8-day old seedlings, a strong and significant correlation was found between basal root number and BRWN ($R^2 = 0.88^{***}$) measured in the field. In this study substantial variation occurred in root traits among common bean genotypes. Useful root traits conferring tolerance to low P such as high number of basal root whorls, basal root number, basal root growth angle (BRGA) can be found in most of common bean accessions. Information on genotypic diversity of root traits and sources of useful root traits is important in breeding programs for development of genotypes adapted to a specific stress.

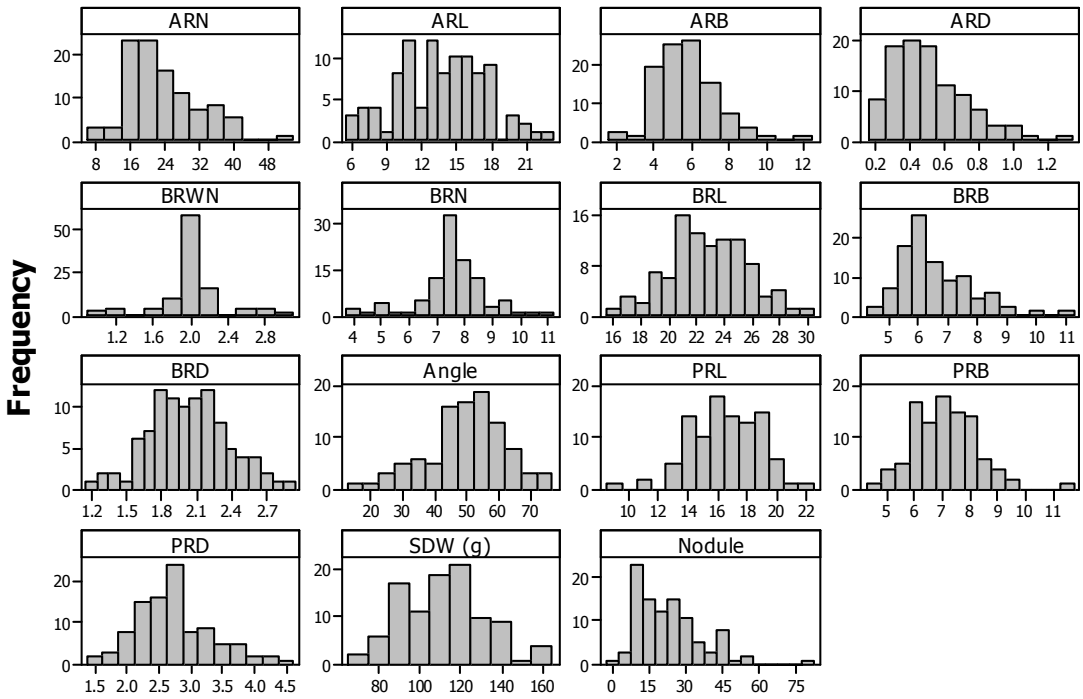

FIG. 1. Phenotypic variation of root traits of the genotypes evaluated in the field. Adventitious root number (ARN); Adventitious root length (ARL); Adventitious root branching (ARB); Adventitious root diameter (ARD); Basal root whorl number (BRWN); Basal root number (BRN); Basal root length (BRL); Basal root branching (BRB); Basal root diameter (BRD); Basal root growth angle (Angle); Primary root length (PRL); Primary root branching (PRB); Primary root diameter (PRD); number of nodules per plant, and shoot dry weight (SDW). Branching correspond to number of lateral roots in 2 cm root segment. ARN, BRWN and BRN are counts per plant.

3.2. Root hair contribution to P uptake

Genotypes GxG 23 had less shoot dry weight (1.28 g) followed by DxG 11 and GxG41, both with 1.37 g each. Genotype DxG 53 had the greatest shoot dry weight (1.65 g). Genotype DxG53, which is a long-haired line, had significantly higher shoot dry weight compared to the rest of the genotypes (Fig. 2).

TABLE 1. ROOT HAIR LENGTH IN SELECTED GENOTYPES THAT CAN BE USED IN THE BREEDING PROGRAMS. ROOT HAIR LENGTH (RHL) CLASSIFICATION: SHORT: < 0.4 MM; LONG: > 0.6 MM

Genotype	Gene pool	BRWN	BRN	Basal RHL (mm)	Category	PR RHL (mm)	Category
AFR 298	Andean	2	6.5	0.705	Long / high	0.73	Long / high
G 14665	Andean	3.5	13.25	0.65	Long / high	0.795	Long / high
Sel. 63 crema	Andean	2.5	8	0.71	Long / high	0.695	Long / high
PVA 773	Andean	3	10	0.295	Short/ low	0.4	Short/ low
SUG 47	Andean	2.75	11.75	0.38	Short/ low	0.4	Short/ low
SEA 5	Mesoam.	2	7.25	0.69	Long / high	0.745	Long / high
VAX 1	Mesoam.	2	8	0.67	Long / high	0.735	Long / high
SXB 418	Mesoam.	2	7.75	0.42	Short/ low	0.33	Short/ low

TABLE 2. ROOT TRAITS OF SIX COMMON BEAN GENOTYPES EVALUATED IN THE FIELD. THE DATA ARE AVERAGE OF 4 REPLICATIONS MEASURED 45 DAYS AFTER PLANTING

Genotype	Basal root growth angle	Basal root number	Basal root whorl number	Adventitious root number
BAT 477	67.5a	7.2c	4a	5.5b
Tio Canela	62a	7c	3b	6.25b
SEQ 1003	57a	8.25bc	3b	4.25b
Bonus	37b	10.5b	2.5c	8ab
LIC-04-3-1	27b	14a	2c	12.5a
LIC-04-2-1	26b	11b	2c	7.5ab

Means within a column followed by the same letter are not significantly different (P<0.05)

Total radioactivity (RA) of ^{32}P was assessed both in the stems and in the leaves of the 4 genotypes. Efficient (long root haired) genotypes had significantly higher accumulation of ^{32}P in plant tissue (Fig. 3). ^{32}P activity in the leaves ranged from 7681 to 10775 CPM g^{-1} DW, while for the stems ^{32}P activity ranged from 4123 to 6792 CPM g^{-1} DW.

Efficient genotypes had greater total P compared to inefficient genotypes (Fig. 4). In addition, long root hair genotypes DxG53 and GxG 41 had greater accumulation of ^{32}P in plant tissue compared to short root hair genotypes GxG23 and DxG11. In fact efficient genotypes (GxG 41 and DxG 53) accumulated 25.3 and 33.1% respectively, while inefficient genotypes (DxG11 and GxG 23) had percentages of ^{32}P accumulation of only 10.0% and 11.2%, respectively (Fig. 5). Efficient genotypes had lower specific activity values compared to inefficient genotypes, since these were able to uptake P from two different pools with a greater total P accumulation (Fig. 6).

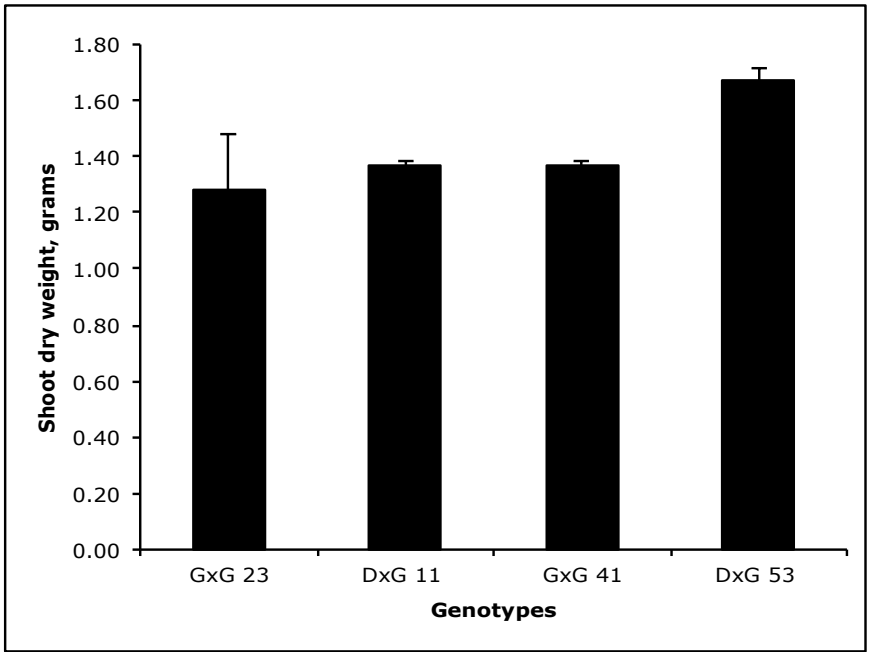

FIG. 2. Shoot dry weight of 4 common bean genotypes contrasting for root hairs, grown for 30 days in the greenhouse under low P availability and exposed to ^{32}P. Genotypes GxG 41 and DxG 53 have long root hairs, while genotypes GxG 23 and DxG 11 have short root hairs. Genotype DxG 53 (a RIL from DOR364xG19833), showed significantly high shoot dry weight compared to the rest of the genotypes, which showed no significant differences among them (Y axis represents shoot dry weight in grams).

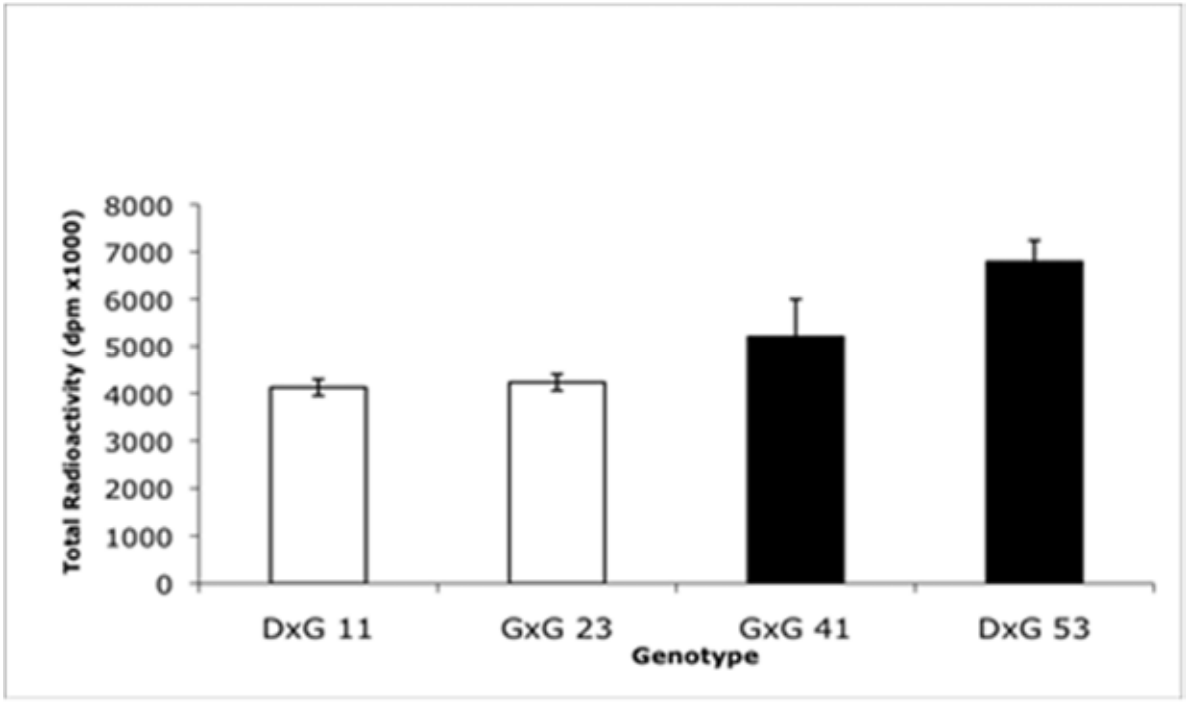

FIG. 3. Total radioactivity among four contrasting common bean genotypes for root hairs: short-root hair genotypes (open bars) and long root hair genotypes (solid bars).

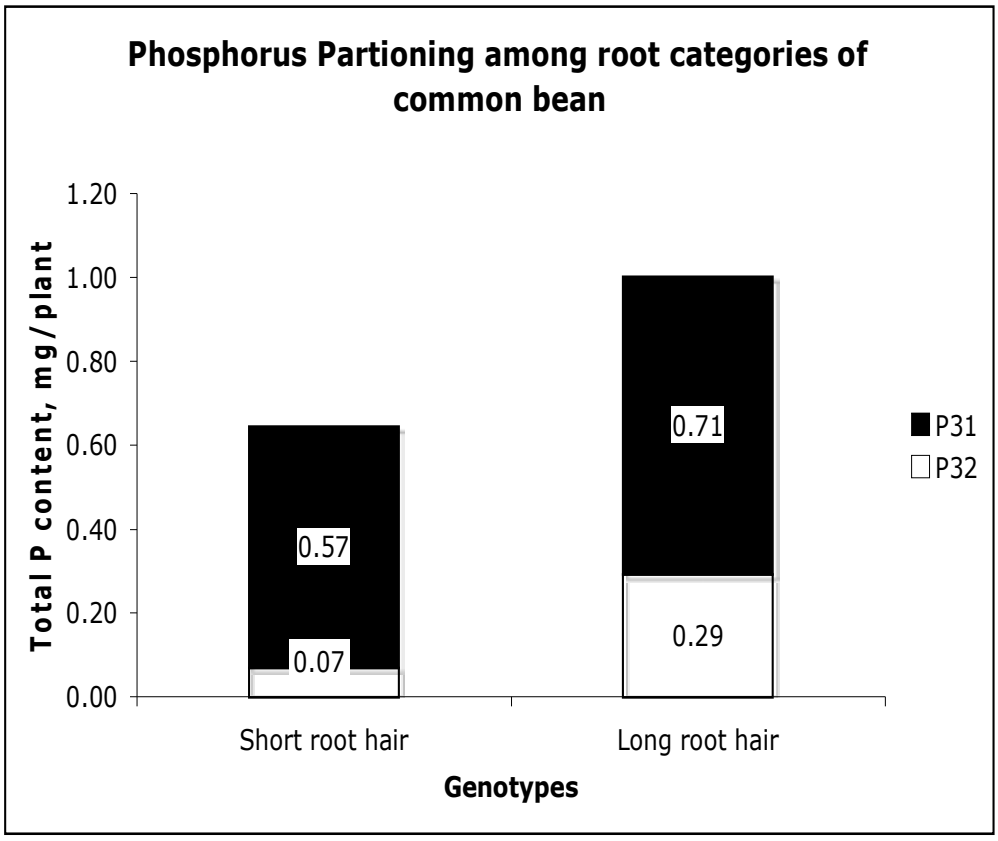

FIG. 4. Total P content per plant tissue between two common bean categories: Long root hair and short root hair genotypes. Long root hair category has greater portion of ^{32}P absorbed in plant tissue compared to short root hair genotypes.

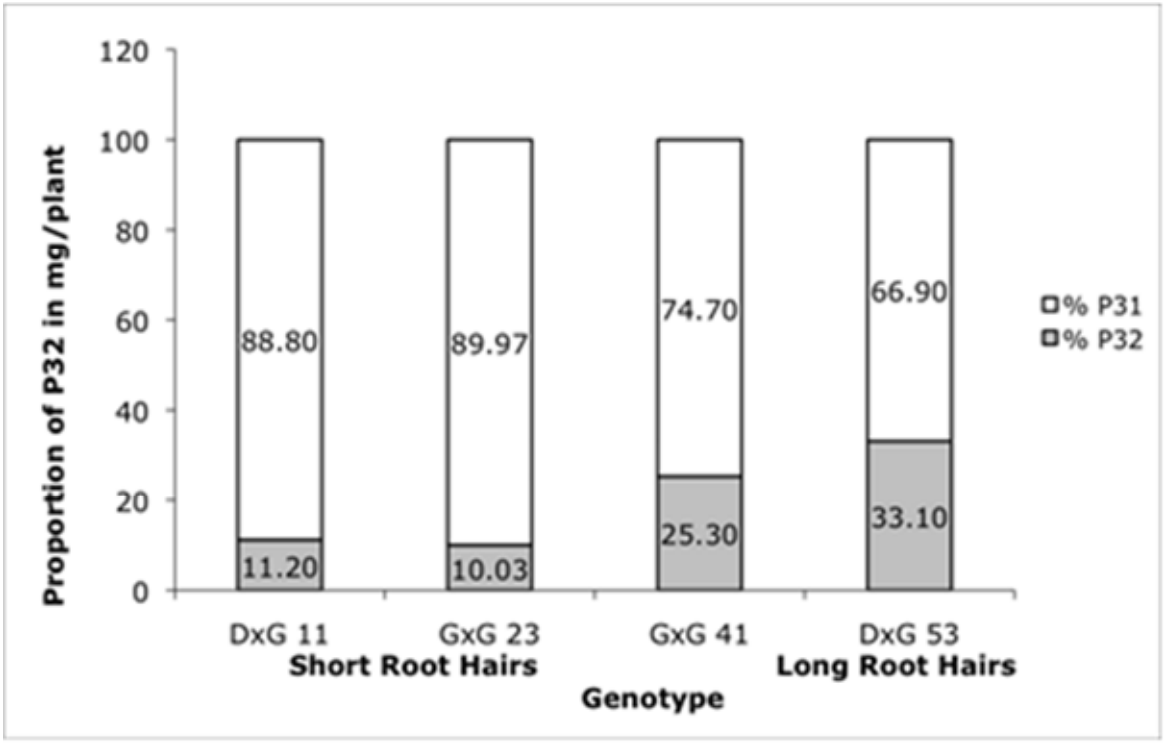

FIG. 5. Perceptual proportion of ^{32}P (blue bars) and ^{31}P (plum bars) in plant tissue of 4 genotypes contrasting for root hair length.

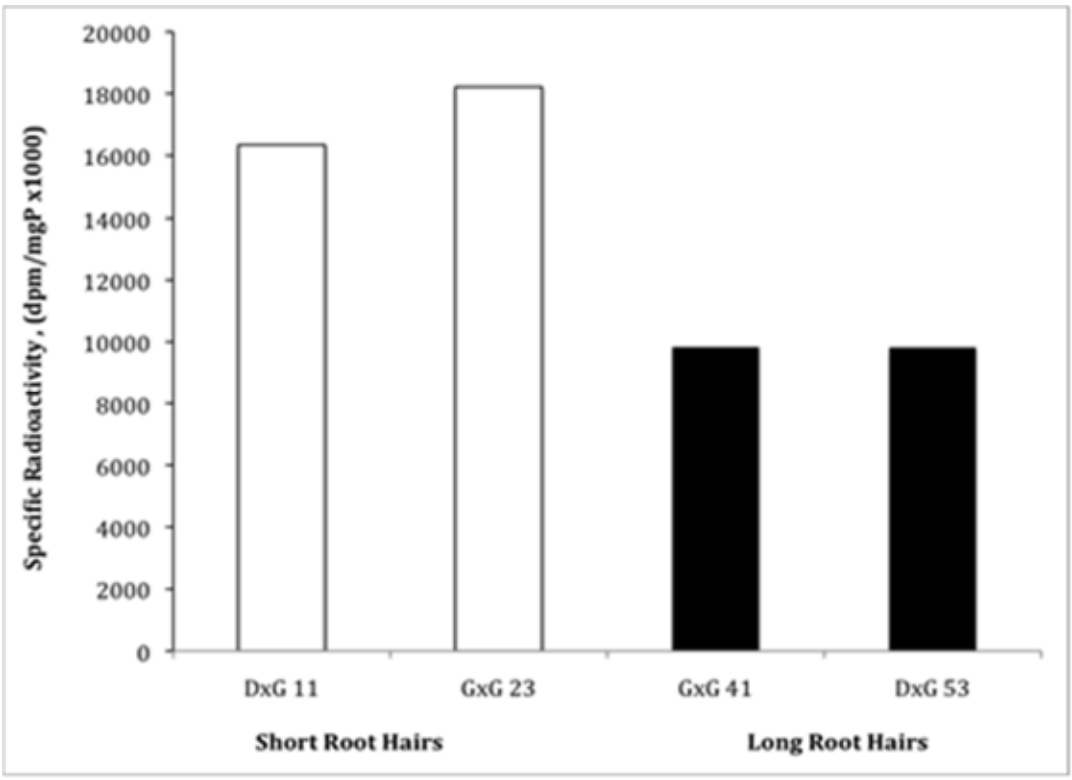

FIG. 6. Specific radioactivity in plant tissue of 4 genotypes contrasting for root hairs: Genotypes with short root hairs (open bars) and genotypes with long root hairs (full bars).

4. DISCUSSION

The results of genotype screening show that genotypes exhibit a number of root traits associated with P acquisition efficiency. A given genotype can have one or more root traits responsible for P acquisition efficiency. These traits include root hair length and density, basal root growth angle, adventitious rooting and basal root number.

The importance of root hairs for P acquisition in low P environments is very well documented [2]. Plant roots not only are responsible for absorbing nutrients, but they are also responsible for secretion of a variety of compounds (exudates) into the rhizosphere, leading to an increase of the amounts and forms of dissolved ions in the soil solution. Root hairs enable these processes to occur more effectively. A study involving nearly 20 genotypes contrasting for root hairs grown in the field with low P availability showed a strong correlation of root hair length to shoot dry weight and to shoot P content in plant tissue [10]. In fact, it has been estimated that of the total root surface area, root hairs can contribute up to 67%, a very significant contribution.

The results of this study attempt to give an explanation about one of the physiological mechanisms associated with root hairs' contribution to enhanced P uptake in plants grown in soils with low P. Less soluble Al-P applied in all of the four treatments could only be readily available to the plants after being dissolved from its source. We observed that genotypes with long root hairs were able to dissolve and take up more ^{32}P than genotypes with short root hairs. This supports our hypothesis that long root hair genotypes are able take up more P, in part by being able to dissolve P initially not readily available. The plant might achieve this by releasing some forms of organic compounds (root exudates) capable of dissolving P otherwise not readily available to the plant, and root hairs might be associated with the increased level of root exudates occurring in genotypes with long root hairs. The P availability in seeds from genotypes with long root hairs and genotypes with short hairs requires assessment.

We also observed that ^{32}P was found in grater amounts in the leaves than in the stems in all the treatments. This was probably due to the fact that P, which is relatively immobile in the soil, is readily mobile in plant tissue, and therefore, it could have been rapidly translocated to the growing younger leaves.

The availability of an additional source of P for long root hair genotypes (observed by an increase in the amount of ^{32}P in plant tissue, which can also be seen by looking at the proportion of ^{32}P in relation to total P (^{32}P + ^{31}P) in plant tissue (Fig. 5), was expressed by additional growth and vigor, and ultimately a relative increase in shoot dry matter accumulation in long root hair genotypes. In addition, a strong correlation between absorbed ^{32}P and shoot dry weight observed in this study seems to support the idea that an additional source of P available to hairy genotypes led to an increased accumulation of shoot dry matter, which can in turn lead to a better plant performance.

5. CONCLUSIONS

According to the results of our studies we can conclude that (a) genotypes have various root traits associated with P uptake efficiency, and that a given genotype can have one or more root traits that will help it to acquire P, when grown in low P soils (b) root hairs confer a significant contribution to P acquisition, confirming that genotypes with long root hairs have significantly better performance (greater total shoot dry weight and P content and concentration) compared to genotypes with short root hairs (c) the method that was used in

this study involving ^{32}P can also be used to quantify the contribution (degree of importance) of a particular root class (e.g. adventitious, basal, tap root, etc.) for overall P uptake by plants.

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PHOSPHORUS USE EFFICIENCY BY BRAZILIAN COMMON BEAN GENOTYPES ASSESSED BY THE ^{32}P DILUTION TECHNIQUE

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Abstract

The objectives of this work were to identify the most efficient common bean (*Phaseolus vulgaris* L.) genotypes on phosphorus (P) utilization, and verify if P from the seed affects the classification of common bean genotypes on P uptake efficiency when the ^{32}P isotopic dilution technique is used. The experiment was conducted in a greenhouse, and plants were grown in pots with surface samples of a dystrophic Typic Haplustox. The treatments consisted of 50 common bean genotypes and two standard plant species, efficient or inefficient in P uptake. The results were assessed through correlation and cluster analysis (multivariate). Sangue de Boi, Rosinha, Thayú, Grafite, Horizonte, Pioneiro and Jalo Precoce common bean genotypes were the most efficient on P uptake, and Carioca 80, CNF 10, Pérola, IAPAR 31, Roxão EEP, Aporé, Pioneiro, Pontal, Timbó and Rudá were the most efficient in P utilization. The P derived from seed influences the identification of common bean genotypes for P uptake efficiency.

1. INTRODUCTION

Common bean is one of the main crops grown in the off-season under irrigation in the Cerrado (Savannah) areas of Brazil [1]. The low P content and the high P fixation capacity in the Cerrado soils is one of the main limitations to agricultural productivity. To reach satisfactory production of beans it is necessary to apply high rates of P fertilizer [2]. Besides liming and fertilizer application to minimize such problems, another strategy would be to identify and to explore the use of the genotypic differences in common bean for efficiency in P use (uptake and utilization), which can reduce the expenses with P fertilizers [3, 4]. The P recovery efficiency by the bean plant is less than 10% in the Cerrado soils, depending on the application rate [5]. This low value of P efficiency by beans can be increased with the use of genotypes more efficient in P uptake. There are common bean genotypes which can increase P uptake significantly through the capacity to modify the rhizosphere by organic acid

exudation, surface root architecture and longer basal root hairs under stressed conditions [6]. In other words some common bean cultivars can increase soil P use efficiency by different adaptations [7, 8].

The objectives of this study were to identify common bean genotypes more efficient in P uptake using the ^{32}P isotope dilution technique, and also for P utilization. Furthermore, we investigated whether P from the seed affects the classification of common bean genotypes in P uptake efficiency when the ^{32}P isotopic dilution technique is used.

2. MATERIALS AND METHODS

2.1. Experimental

The experiment was conducted in the greenhouse at the Center for Nuclear Energy in Agriculture (CENA / USP), located at latitude 22°42'30" S, longitude 47°38'01" W and 554 m altitude, in Piracicaba, Sao Paulo, Brazil.

The study were performed in 3.0 l plastic pots, containing 2.5 kg of air-dried soil, collected from the 0 to 0.20 m layer of a dystrophic Typic Haplustox [9]. The soil had 280, 70 and 650 g kg^{-1}content of clay, silt and sand, respectively, and the following chemical characteristics: pH (0.01 mol l^{-1} $CaCl_2$), 4.5; organic matter, 18.0 g dm^{-3}; P extracted by resin, 5 mg dm^{-3}; K, 0.6 $mmol_c$ dm^{-3}; Ca, 11.5 $mmol_c$ dm^{-3}; Mg, 5.2 $mmol_c$ dm^{-3}; H + Al, 35.4 $mmol_c$ dm^{-3}; CEC, 52.7 $mmol_c$ dm^{-3}; sum of bases, 17.3 $mmol_c$ dm^{-3}; base saturation, 32.8%, according to methodology described by [10] and P by Mehlich-1, 3 mg dm^{-3} [11].

After application of lime (Calcium carbonate equivalent = 110%) to raise the base saturation to 70%, according to the official recommendation of Bulletin 100 [12], the soil was incubated for 30 days, maintaining the moisture content at approximately 70% of water holding capacity. To evaluate the efficiency of bean genotypes for P uptake, a mixture of triple superphosphate (20 mg P kg^{-1}) as a source of readily available P, and Patos rock phosphate (150 mg P kg^{-1}) were applied to raise the total soil P content to 170 mg P kg^{-1} soil in each pot. N and K were applied at rates of 200 mg N kg^{-1} as urea and 200 mg K kg^{-1} as potassium sulfate. Micronutrients were also applied as nutrient solution in all treatments at rates of 0.5 mg B kg^{-1}, 1.5 mg Cu kg^{-1}, 3.0 mg Fe kg^{-1}, 2.0 mg Mn kg^{-1}, 3.0 mg Zn kg^{-1} and 0.1 mg Mo kg^{-1}.

The experimental design was completely randomized with four replications. The treatments consisted of 50 common bean genotypes and two standard species described in the literature as efficient or inefficient in P uptake: Sunhemp (*Crotalaria juncea* L.) as inefficient in absorbing P [13] and white lupin (*Lupinus albus* L.) as efficient [13, 14]. The common bean genotypes evaluated in this study were: Carioca 80, Rudá, Aporé, Princesa, Pérola, Requinte, Pontal, BRS Horizonte, BRSMG Talismã, BRSMG Pioneiro, IPR Colibri, IAC Tybatã, IAC Alvorada, LP01-38, CV-48, CNF 10, Roxão EEP, Sangue de Boi, Roxão, Roxinho, Safira, BRS Timbó, Roxo 90, BRS Pitanga, Gen 99TG50-47, Ouro Negro, Ônix, Macanudo, Chapecó, Xodó, Diamante Negro, Xamego, IAC UNA, BRS Valente, BRS Grafite, FT Nobre, BRS Triunfo, Thayú, Rosado, Rosinha de Cipó, Rosinha G2, Rosinha, Rosinha Brilhosa, Rubi, BRS Vereda, IAC Boreal, Gen99TGR1-10, Jalo Precoce, IAPAR 31 and Gen99TG34-50.

The soil was labeled with ^{32}P solution (9.25 MBq of ^{32}P) and 0.2 mg P kg^{-1} carrier. Five bean seeds were sown in each pot and the plants were thinned to three plants pot^{-1}. Soil

moisture was maintained at approximately 70% of water retention capacity during plant development.

The above-ground parts of the plants were sampled on two occasions: (i) two plants from the total of three grown in each plot were harvested at 30 days after emergence, and (ii) the one remaining plant, at the stage of grain physiological maturity. The shoot samples were separated into stem, branches, leaves, bark and grain. The P contained in seed was discounted for calculating the L-value, which was used to compare the efficiency of P uptake among the genotypes, considering that from the total P stored in the seeds of bean genotypes, 60% is used for plant growth [15], i.e. 40% of seed P is not used by the plant and remains in the cotyledon.

2.2. Calculations of P and ^{32}P

With shoot dry matter (Sdm), grain weight (Gw), P concentration in Sdm and Gw the P content in the shoot and in grain were calculated.

P uptake = P concentration × Sdm , where Sdm is shoot dry matter.

P content = P concentration × Gw, where Gw is grain weight.

With the data of plant P concentration and 32P activity of the plant, the specific activity, L-value, and the L-value subtracting the amount of seed-derived P from the total P content of shoots were calculated [14,16].

$$SA = \frac{^{32}P}{^{31}P}$$

where SA is specific activity (dpm μg^{-1}P); ^{32}P is radioisotope activity in the plant (dpm); ^{31}P is plant P content (µg of P $plant^{-1}$);

$$Lvalue = X\left(\frac{SA_0}{SA_p} - 1\right)$$

where L-value (mg P kg^{-1} soil); SA_0 is specific activity of applied solution (dpm μg^{-1} P); SA_p is specific activity of the plant (dpm μg^{-1}P); X is amount of applied P;

$$L-svalue = \left(Y\frac{\left(X_T - Z\right)}{Y_T} - X\right)$$

where L-s value is L-value subtracting P in the plant derived from the seed (mg of P kg^{-1} soil); Y is ^{32}P activity in the applied solution (dpm); X_T is plant P uptake (mg); Y_T is ^{32}P activity in the plant shoot dry matter (dpm); X is ^{31}P carrier applied rate pot^{-1} (mg); Z = total P content derived from the seed (mg).

2.3. Statistical analysis

Sdm, Gw, P concentration and P content in the shoot or in the grain, specific activity (SA), L-value and L-value subtracting the P derived from the seed (L-s value) were submitted to analysis of Pearson linear correlation, and hierarchical cluster analysis with the objective for grouping similar genotypes. Cluster analysis of bean genotypes was carried out with the SAS 9.1 - "Statistical Analysis System" [17] and SYSTAT version 10.2 software programs, using the UPGMA (un-weighed pair group arithmetic average clustering) The cluster analysis

was preceded by the standardization of data before the Euclidian distances calculation, as the variables presented different scales. After standardization, all the variables were equally important in the determination of these distances. Final results of the groups were presented as dendrograms. The P uptake efficiency by plants is inversely proportional to SA and directly proportional to L- and L-s values.

The common bean genotypes were grouped into four or five groups, aiming to achieve greater homogeneity within each group and greater heterogeneity among the different groups. The results are presented and discussed in three parts: (1) first sampling - shoot; (2) second sampling - shoot, and (3) second sampling - grain. The term shoot dry matter (Sdm) refers to all above ground tissues (stem, branches, leaves and bark of legumes) except the grain.

3. RESULTS AND DISCUSSION

3.1. First sampling - shoot

Plant data for the first sampling are given in Table 1. The results obtained with the two standard species were: (i) White lupin - Sdm = 0.56 g pot^{-1}, P in Sdm = 1.34 mg pot^{-1}, SA = 17.71 dpm g^{-1} P, L-value = 47.44 mg P kg^{-1} soil and L-s value = 29.5 mg P kg^{-1} soil, (ii) Sunhemp - Sdm = 3.49 g pot^{-1}, P content in Sdm = 5.40 mg pot^{-1}, SA = 75.49 dpm mg ^{1} P^{-}, L-value = 10.26 mg P kg^{-1} soil and L-s value = 9.95 mg P kg^{-1} soil. The white lupin plant was, as expected, more efficient in absorbing P (the lowest SA, and highest L-value and L-s values) than all common bean genotypes evaluated in this study.

Mean values of shoot dry matter (Sdm), P concentration (P conc) and P uptake (P uptake) in Sdm, specific activity (SA), L-value and L-value discounting the P from the seed (L-s value) of 50 common bean genotypes at the first sampling are given in Table 1.. The values of Sdm correlated significantly and negatively with P concentration (–0.625***) and positively with P uptake (0.675***). P uptake in Sdm increased with increasing Sdm, but the P concentration in plant tissue decreased by the dilution effect of P in the Sdm. By cluster analysis with these three variables (Fig. 1), the following five groups of common bean genotypes were identified:

1st: Rubi;
2nd: Grafite, CV-48, Pioneiro, Xamego, IAC UNA, Carioca 80, Roxão, Horizonte, Thayú, Triunfo, Sangue de Boi, Gen99TG3450, Timbó, Talismã, Pontal, Pérola, Requinte, Gen99TG50-47, Vereda, IAC Alvorada and Rosinha Brilhosa;
3rd: Pitanga, Roxo 90, FT Nobre, Ônix, LP01-38, Rudá, Colibri, Aporé, Tybatã, Macanudo, Rosinha Cipó, Princesa, Xodó, Valente, Diamante Negro, CNF 10, Roxinho, IAPAR 31, Rosado and Chapecó;
4th: Gen99TGR1-10, Ouro Negro, Rosinha, Safira, Rosinha G2, Jalo Precoce and Roxão EEP; and
5th: IAC Boreal.

TABLE 1. MEAN VALUES OF SHOOT DRY MATTER (SDM), P CONCENTRATION (P CONC) AND P UPTAKE IN SDM, SPECIFIC ACTIVITY (SA), L-VALUE AND L-VALUE DISCOUNTING THE P FROM THE SEED (L-S VALUE) OF 50 COMMON BEAN GENOTYPES AT THE FIRST SAMPLING

Genotype	Sdm ($g\ pot^{-1}$)	P conc ($g\ kg^{-1}$)	P uptake ($mg\ pot^{-1}$)	SA (dpm μg^{-1} P)	L-value ($mg\ kg^{-1}$ soil)	L-s value ($mg\ kg^{-1}$ soil)
Chapecó	5.33	1.42	7.57	56.06	11.81	11.00
IAPAR 31	5.56	1.51	8.38	65.40	10.10	9.38
Rosado	5.62	1.45	8.11	63.48	10.42	9.28
Roxinho	5.77	1.51	8.69	51.04	13.00	12.23
CNF 10	5.78	1.51	8.71	58.81	11.25	10.25
Rubi	5.80	1.71	9.92	49.47	13.43	12.43
Diamante Negro	5.82	1.52	8.82	65.87	10.03	9.29
Pitanga	6.00	1.33	7.97	51.16	12.98	11.83
Grafite	6.06	1.59	9.60	44.86	14.82	13.44
Valente	6.07	1.46	8.86	60.08	11.02	10.05
CV-48	6.20	1.56	9.65	53.00	12.51	11.05
Xodó	6.32	1.43	9.04	58.42	11.33	10.69
Princesa	6.43	1.43	9.20	57.95	11.43	10.61
Colibri	6.47	1.32	8.50	66.46	9.94	9.18
Rosinha de Cipó	6.47	1.43	9.26	53.42	12.41	11.58
FT Nobre	6.47	1.27	8.24	47.68	13.95	12.50
Aporé	6.49	1.34	8.65	57.00	11.62	10.28
Roxo 90	6.53	1.23	8.03	76.04	8.66	7.81
Xamego	6.53	1.52	9.93	56.38	11.75	11.08
IAC UNA	6.56	1.51	9.93	52.85	12.54	11.34
Horizonte	6.58	1.55	10.18	44.28	15.02	13.80
Macanudo	6.59	1.36	8.95	55.39	11.96	11.15
Pioneiro	6.60	1.49	9.82	43.33	15.37	14.33
Tybatã	6.60	1.34	8.82	50.49	13.15	11.77
Carioca 80	6.63	1.51	10.01	53.68	12.34	11.36
Vereda	6.66	1.43	9.51	51.21	12.99	11.96
Thayú	6.67	1.57	10.44	46.21	14.40	13.54
Roxão	6.68	1.51	10.10	52.91	12.53	10.85
Ônix	6.68	1.27	8.47	49.53	13.41	12.20
Rudá	6.79	1.30	8.83	52.53	12.62	11.75
Pérola	6.80	1.44	9.81	53.40	12.41	11.37
LP01-38	6.85	1.23	8.46	54.93	12.08	10.81
Gen99TG50-47	6.88	1.41	9.70	48.87	13.58	11.08
Requinte	6.91	1.42	9.78	59.88	11.05	10.31
Talismã	6.97	1.45	10.12	57.35	11.54	10.71
Rosinha Brilhosa	7.01	1.33	9.33	54.86	12.07	11.23
Pontal	7.02	1.42	9.96	55.58	11.92	10.99
IAC Alvorada	7.05	1.35	9.49	60.09	11.01	9.86
Timbó	7.20	1.39	10.03	49.39	13.44	12.63
Gen99TG34-50	7.22	1.38	9.97	46.67	14.23	12.51
Sangue de Boi	7.40	1.37	10.13	47.30	14.05	13.06
Triunfo	7.42	1.38	10.25	51.75	12.82	11.61
Safira	7.86	1.33	10.45	52.61	12.60	11.86
Ouro Negro	7.98	1.19	9.45	58.96	11.23	10.31
Rosinha G2	7.99	1.31	10.48	57.90	11.44	10.72
Jalo Precoce	8.01	1.31	10.51	39.72	16.77	15.14
Gen99TGR1-10	8.14	1.15	9.34	51.52	12.88	11.39
Mean	6.78	1.40	9.44	53.80	12.51	11.43
CV (%)	12.46	9.10	9.43	12.58	12.42	12.42

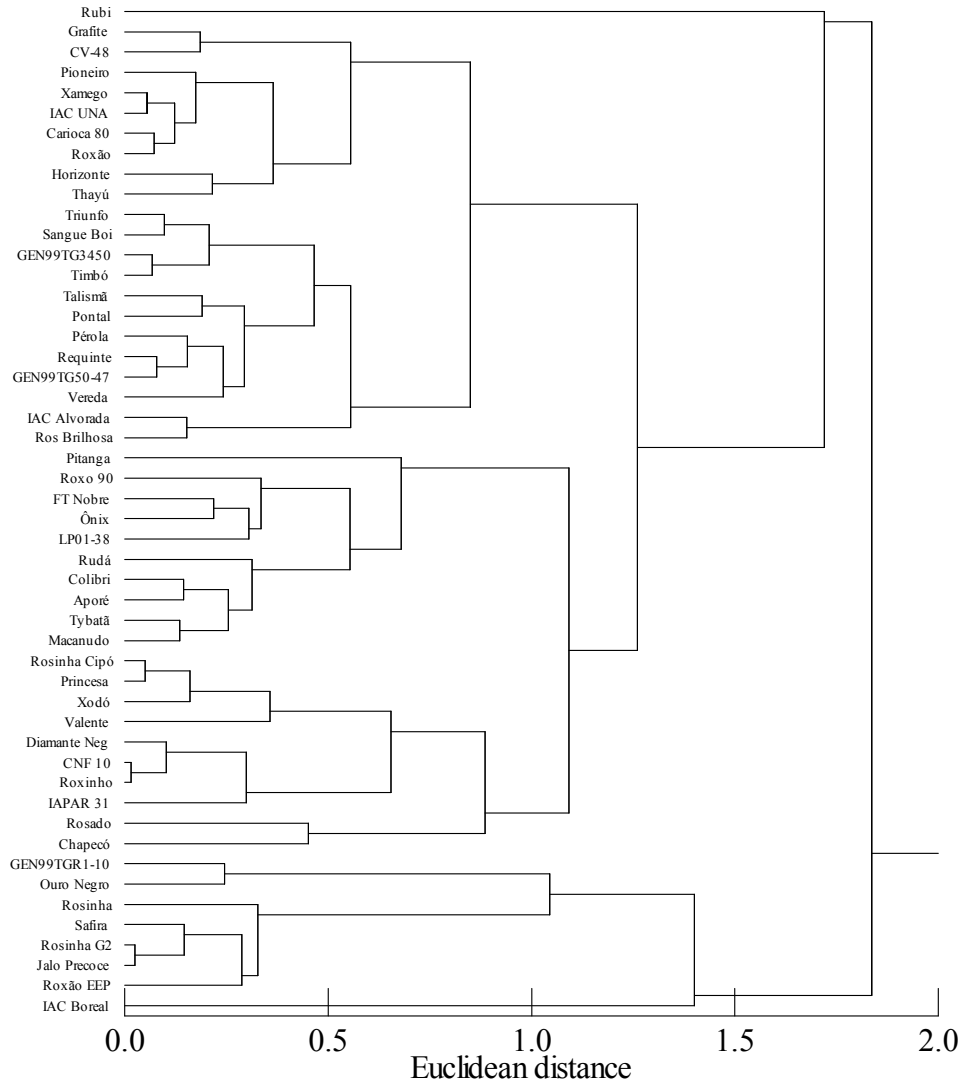

FIG. 1. Dendrogram resulting from hierarchical cluster analysis of 50 common bean genotypes, based on shoot dry matter (Sdm), P concentration and P uptake at the first sampling.

Of the five groups of common bean genotypes formed by hierarchical cluster analysis (Fig. 1) and considering the values of Sdm and P uptake shown in Table 1, it was found that the genotype IAC Boreal presented higher values of Sdm and P uptake, while the genotypes in contrast, Pitanga, Roxo 90, FT Nobre, Ônix, LP01-38, Rudá, Colibri, Aporé, Tybatã, Macanudo, Rosinha Cipó, Princesa, Xodó, Valente, Diamante Negro, CNF 10, Roxinho, IAPAR 31, Rosado and Chapecó presented lower values of Sdm and P uptake. Jalo Precoce was classified in the second group that presented higher values of Sdm and P uptake. Among eight common bean genotypes grown under a low P rate (24 mg dm^{-3} P_2O_5) in the substrate (pots with 16 kg of sand) and harvested at 45 days after germination, genotypes BAT 477, Jalo Precoce and Roxo produced the most Sdm [4].

Rubi did not group with the other genotypes, because it had high P concentration and P uptake, but low production of Sdm. This probably indicates that this genotype was not efficient in utilizing P taken up to produce the Sdm, at the beginning of plant development. We emphasize that genotypes more productive in terms of Sdm, are not necessarily more efficient in P uptake under conditions of low P availability in the substrate; the P in the plant

derived from the seed, from which the plant originates, is an important source of P at the early developmental stage, in evaluating the efficiency of P uptake by plants. Moreover, the production of Sdm also involves the concept of efficiency of use, and genotypes more efficient in P use are those that best convert the nutrient uptake into Sdm. It was observed that the variables SA, L-value and L-s value of 50 common bean genotypes, harvested in the first sampling, were the ones with the highest Pearson correlation coefficients between them. The SA correlated significantly and negatively with the L-value (–0.982***) and the L-s value (–0.960***), and the L-value correlated positively with the L-s value (0.974***).

By cluster analysis with the variables SA and L-value (Fig. 2) four groups of common bean genotypes were identified:

1st: low efficiency in P uptake (Roxo 90);
2nd: moderately efficient in P uptake (Colibri, Diamante Negro, IAPAR 31, Rosado, IAC Alvorada, Valente, Requinte, Ouro Negro, CNF 10, Xodó, Princesa, Rosinha G2, Talismã, Aporé, Xamego, Chapecó, Pontal, Macanudo, LP01-38 and Rosinha Brilhosa);
3rd: efficient in P uptake (Carioca 80, Rosinha Cipó, Pérola, CV-48, Roxão, IAC UNA, Safira, Rudá, Triunfo, Gen99TGR1-10, Vereda, Pitanga, Roxinho, Tybatã, Ônix, Rubi, Timbó, Roxão EEP, GenTG50-47, IAC Boreal, FT Nobre, Sangue de Boi, Rosinha, Gen99TG3450 and Thayú);
4th: very efficient in P uptake (Grafite, Horizonte, Pioneiro and Jalo Precoce).

Grafite, Horizonte, Pioneiro and Jalo Precoce genotypes were those with lower values for SA and higher L-values. Thus, these common bean genotypes were classified as more efficient in P uptake. It is noteworthy that in this classification of genotypes for the P uptake efficiency, P was not discounted in the plant from the seeds. In another study evaluating the efficiency of P uptake by eight genotypes of common bean, without the use of the technique with ^{32}P, the Jalo Precoce was also classified as efficient in P uptake [4].

By cluster analysis with the variables SA and L-s value (Fig. 3), four groups of common bean genotypes were identified:

1st: low efficiency in P uptake (Roxo 90);
2nd: moderately efficient (Colibri, Diamante Negro, IAPAR 31, Rosado, IAC Alvorada, Valente, Requinte, Ouro Negro, CNF 10, Aporé, Talismã, Princesa, Rosinha G2 and Xodó);
3rd: efficient in P uptake (Rosinha Brilhosa, Macanudo, Pontal, Chapecó, Xamego, LP01-38, Roxão, CV-48, IAC UNA, Pérola, Carioca 80, Rosinha Cipó, Gen99TGR1-10, Triunfo, Rudá, Safira, Vereda, Pitanga, Tybatã, Gen99TG50-47, IAC Boreal, Roxinho, Ônix, Rubi, Roxão EEP, Timbó, FT Nobre and Gen99TG3450);
4th: very efficient in P uptake (Sangue de Boi, Rosinha, Thayú, Grafite, Horizonte, Pioneiro and Jalo Precoce).

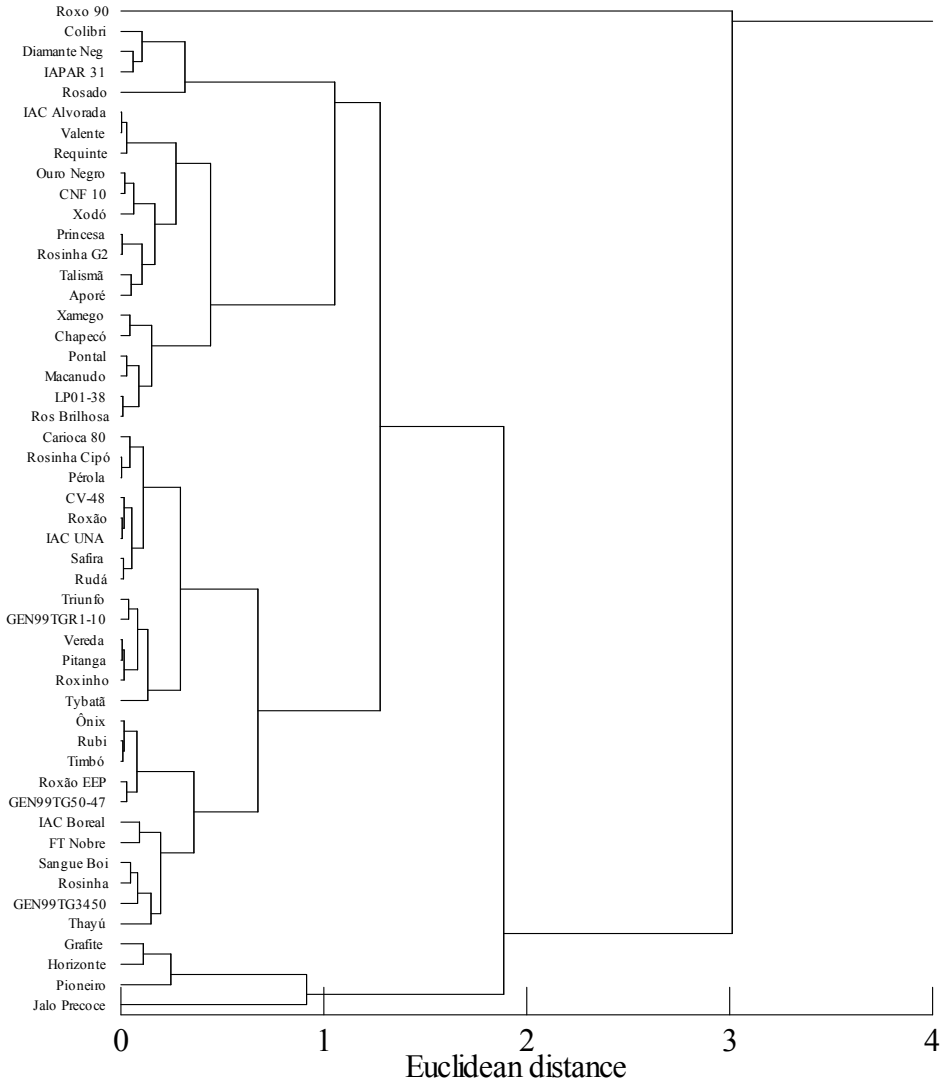

FIG. 2. Dendrogram resulting from hierarchical cluster analysis of 50 common bean genotypes based on specific activity (SA) and L-value at the first sampling.

Xamego, Chapecó, Pontal, Macanudo, LP01-38, Rosinha Brilhosa, Sangue de Boi, Rosinha and Thayú genotypes were classified into different groups, when the hierarchical cluster analysis was performed based on L-value discounting (Fig. 3) or not (Fig. 2), the P in the plant derived from the seed. Therefore, the P present in the seed affected the assessment and classification of common bean genotypes on P uptake efficiency.

Xamego, Chapecó, Pontal, Macanudo, LP01-38 and Rosinha Brilhosa genotypes were placed in the third group, when the P in the plant derived from the seed was discounted in the calculation of the L-value, and were classified as efficient in P uptake. Sangue de Boi, Rosinha and Thayú genotypes were grouped in the fourth group, when discounting the seed P, and were classified as very efficient in P uptake.

When plants are grown under P limiting condition, the roots become a strong drain of carbohydrates and this causes major limitation to the growth of the shoot than the root [18]. Roots of bean plants grown under conditions of P deficiency had much higher concentrations of sugars in the roots compared with plants with an adequate P supply, due to the increased shoot translocation of photo-assimilates [19]. In another study it was observed that the

difference in the selection of bean genotypes and the P-use efficiency and dry matter production was related to the translocation of P from roots to shoots [4]. The root architecture is another factor that differentiates the P uptake among genotypes, and relates to the spatial configuration of the root system, i.e. the geometry of the development of the root axes [20].

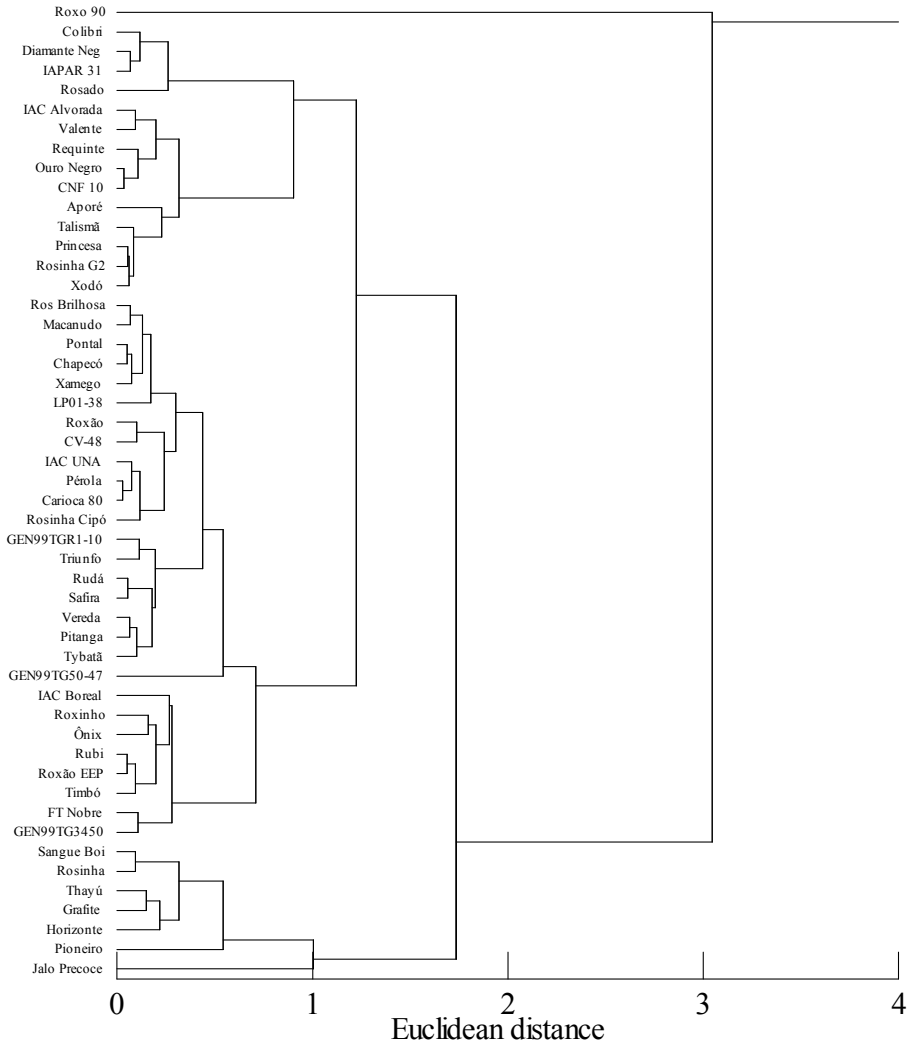

FIG. 3. Dendrogram resulting from hierarchical cluster analysis of 50 common bean genotypes, based on specific activity (SA) and L-value, discounting the P in the plant derived from the seed at the first sampling.

3.2. Second sampling - shoot

Mean values of shoot dry matter (Sdm), P concentration (P conc) and P uptake (P uptake) in Sdm of 50 common bean genotypes AT the second sampling are given in Table 2. The Sdm of common bean genotypes in the second sampling, correlated significantly and positively with P uptake (0.587***), but not with P concentration.

TABLE 2. MEAN VALUES OF SHOOT DRY MATTER (SDM), P CONCENTRATION (P CONC) AND P UPTAKE (P UPTAKE) IN SDM OF 50 COMMON BEAN GENOTYPES AT THE SECOND SAMPLING

Genotype	Sdm (g plant^{-1})	P conc (g kg^{-1})	P uptake (mg plant^{-1})
CNF 10	6,12	0,68	4,19
Macanudo	6,25	0,56	3,48
Ouro Negro	6,35	0,76	4,80
Rosinha G2	6,38	0,84	5,34
Gen99TG50-47	6,41	1,00	6,38
Aporé	6,47	0,62	3,98
Carioca 80	6,71	0,62	4,14
Sangue de Boi	6,90	0,94	6,52
Safira	6,96	0,72	4,99
CV-48	6,99	0,76	5,30
Colibri	7,03	0,98	6,86
Gen99TG34-50	7,32	0,79	5,77
LP01-38	7,37	0,76	5,57
Xamego	7,37	0,80	5,87
Princesa	7,43	0,72	5,36
IAPAR 31	7,45	0,78	5,78
Pontal	7,52	0,83	6,23
Jalo Precoce	7,53	0,87	6,58
Horizonte	7,55	0,89	6,71
IAC Alvorada	7,59	0,64	4,86
Rubi	7,67	0,71	5,43
Xodó	7,69	0,74	5,70
Pioneiro	7,82	0,60	4,71
Valente	7,85	0,74	5,77
Triunfo	7,88	0,71	5,57
Roxão EEP	7,96	0,59	4,67
Talismã	7,99	0,79	6,32
Pérola	8,03	0,63	5,03
Pitanga	8,11	0,58	4,65
Rosinha	8,13	0,86	6,95
IAC UNA	8,20	0,65	5,31
Rosinha de Cipó	8,22	0,94	7,69
Chapecó	8,25	0,91	7,52
Diamante Negro	8,36	0,78	6,48
Rosinha Brilhosa	8,37	0,87	7,30
FT Nobre	8,39	0,67	5,62
Roxinho	8,43	0,73	6,18
Vereda	8,48	0,64	5,39
Rudá	8,63	0,82	7,08
Timbó	8,63	0,54	4,68
Rosado	8,64	0,57	4,94
Gen99TGR1-10	8,79	0,82	7,18
Roxo 90	8,89	0,75	6,64
Thayú	8,89	0,58	5,14
Requinte	8,90	0,85	7,55
Ônix	9,05	0,80	7,28
Roxão	9,26	1,15	10,68
Mean	7,91	0,76	5,99
CV (%)	13,10	14,26	15,12

The dendogram obtained by grouping these two correlated variables is presented in Fig. 4.

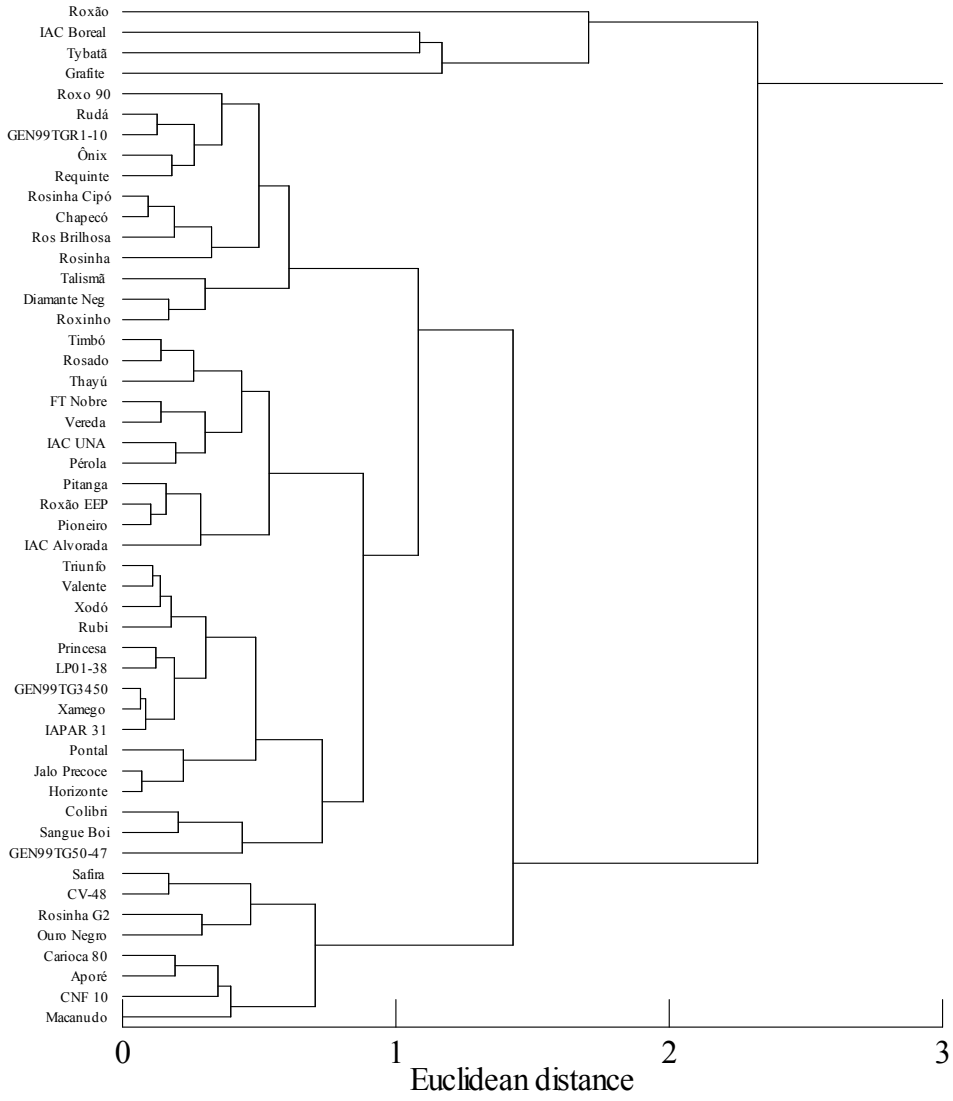

FIG. 4. Dendrogram resulting from hierarchical cluster analysis of 50 common bean genotypes, based on shoot dry matter (Sdm) and P uptake in Sdm at the second sampling.

The four genotype groups formed were:

1st: Roxão (genotype with highest values of dry matter yield and P accumulation in MSPA);

2nd: IAC Boreal, Tybatã and Grafite;

3rd: Roxo 90, Rudã, Gen99TGR1-10, Ônix, Requinte, Rosinha Cipó, Chapecó, Rosinha Brilhosa, Rosinha, Talismã, Diamante Negro, Roxinho, Timbó, Rosado, Thayú, FT Nobre, Vereda, IAC UNA, Pérola, Pitanga, Roxão EEP, Pioneiro, IAC Alvorada, Triunfo, Valente, Xodó, Rubi, Princesa, LP01-38, Gen99TG3450, Xamego, IAPAR 31, Pontal, Jalo Precoce, Horizonte, Colibri, Sangue de Boi and Gen99TG50-47;

4th: Safira, CV-48, Rosinha G2, Ouro Negro, Carioca 80, Aporé, CNF 10 and Macanudo (genotypes with lower Sdm and P uptake).

3.3. Second sampling - grain

Mean values of grain yield (Gw), P concentration (P conc) and P uptake (P uptake) in Gw of 50 common bean genotypes at the second sampling are presented in Table 3. The grain yield was significantly correlated with bean Sdm ($Y = –2610.23 + 5.58\ X – 0.0013\ X^2$, $R^2 = 0.60$ **), where Y = grain yield (kg ha^{-1}) and X = Sdm (kg ha^{-1}), and the maximum productivity of approximately 3200 kg ha^{-1} of grain was obtained with the production of 2098 kg ha^{-1} of Sdm [5] . It was observed in this study that a quadratic model was used to explain the relationship between grain yield and Sdm, which are two quantitative variables and dependent. It is recommended to apply Pearson linear correlation analysis to these types of variables.

Considering the 50 common bean genotypes evaluated in the second sampling, a linear and positive correlation was observed between Sdm and Gdm, but with a low correlation coefficient (0.292***). This indicates that the relationship between dry matter yield and grain yield depends on the bean genotype, i.e. this effect does not seem to be general with all common bean genotypes. For example, Roxão showed higher values of dry matter yield and P uptake, but was ranked as moderately productive and moderately rich in grain P (Fig. 5). Thus, in comparative studies of production between genotypes, even if conducted in a greenhouse, the plants should be developed to the production of grain. There was a significant and positive correlation between the P content in grain and bean grain productivity [5]. Therefore, according to these authors, it is possible to increase the productivity of common bean by increasing the absorption and accumulation of P in grains, with the use of efficient genotypes.

The Gdm values correlated significantly and negatively with P concentrations in the Gdm (–0.654***) and positively with P content in the Sdm (0.604***). The P content in the Gdm was higher with increasing dry matter yield, but decreased P concentrations, so probably there was a dilution on P content in Gdm. By cluster analysis with these three variables the following five groups of common bean genotypes were identified (Fig. 5):

1st: little productive and high P in grain (Talismã);
2nd: little productive and low P in grain (Ouro Negro and Horizonte);
3rd: moderately productive and moderately rich in grain P (IAC Boreal, Roxão, Jalo Precoce, Gen99TG50-47, Gen99TGR1-10, Rubi, Gen99TG3450, Rosinha G2, Princesa, Safira, Rosinha Brilhosa, Macanudo, IAC Alvorada, Pitanga, CV-48, Diamante Negro, Rosinha Cipó, LP01-38, Grafite, Colibri, Chapecó and Xamego);
4th: productive and rich in P in grain (Valente, Rosado, Ônix, Roxo 90, Tybatã, Roxinho, IAC UNA, Thayú, Sangue de Boi, Rosinha, Vereda, FT Nobre, Xodó, Requinte and Triunfo);
5th: very productive and very rich in grain P (Carioca 80, CNF 10, Pérola, IAPAR 31, Roxão EEP, Aporé, Pioneiro, Pontal, Timbó and Rudá).

TABLE 3. MEAN VALUES OF GRAIN YIELD (GW), P CONCENTRATION (P CONC) AND P UPTAKE (P UPTAKE) IN GW OF 50 COMMON BEAN GENOTYPES AT THE SECOND SAMPLING

Genotype	Gw (g plant^{-1})	P conc (g kg^{-1})	P uptake (mg plant^{-1})
IAC Boreal	6.48	3.62	23.45
Talismã	6.74	4.51	30.30
Ouro Negro	6.86	3.95	26.99
Roxão	6.87	3.29	22.57
Horizonte	7.15	3.78	27.02
Jalo Precoce	7.26	3.24	23.54
Rosinha G2	7.30	3.44	25.09
Princesa	7.34	3.49	25.57
Gen99TGR1-10	7.46	3.30	24.65
Gen99TG50-47	7.49	3.20	23.93
Rubi	7.64	3.34	25.48
Gen99TG34-50	7.81	3.22	25.04
Safira	7.85	3.41	26.77
Xamego	8.04	3.06	24.56
IAPAR 31	8.11	3.83	31.09
Rosinha Brilhosa	8.18	3.28	26.84
CV-48	8.19	3.44	28.21
Chapecó	8.26	3.16	26.06
IAC Alvorada	8.39	3.31	27.75
CNF 10	8.46	3.62	30.66
Macanudo	8.46	3.27	27.60
Pitanga	8.47	3.32	28.09
Carioca 80	8.58	3.49	29.94
Pérola	8.64	3.62	31.21
Roxão EEP	8.69	3.82	33.10
Triunfo	8.72	3.33	29.05
Rosinha de Cipó	8.73	3.15	27.44
Grafite	8.74	2.92	25.55
Diamante Negro	8.76	3.18	27.82
LP01-38	8.84	3.11	27.44
Colibri	8.91	2.98	26.49
Requinte	9.14	3.23	29.50
Aporé	9.16	3.58	32.78
Xodó	9.30	3.19	29.62
Thayú	9.59	3.04	29.12
Roxinho	9.63	2.87	27.63
FT Nobre	9.64	3.17	30.50
Rudá	9.65	3.32	32.01
Pioneiro	9.67	3.41	32.94
Pontal	9.68	3.39	32.80
Rosinha	9.69	3.09	29.97
IAC UNA	9.70	2.85	27.57
Timbó	9.71	3.31	32.14
Sangue de Boi	9.76	3.03	29.56
Tybatã	9.77	2.92	28.47
Vereda	9.85	3.10	30.54
Roxo 90	9.95	2.95	29.17
Mean	8.64	3.29	28.20
CV (%)	12.68	10.56	9.70

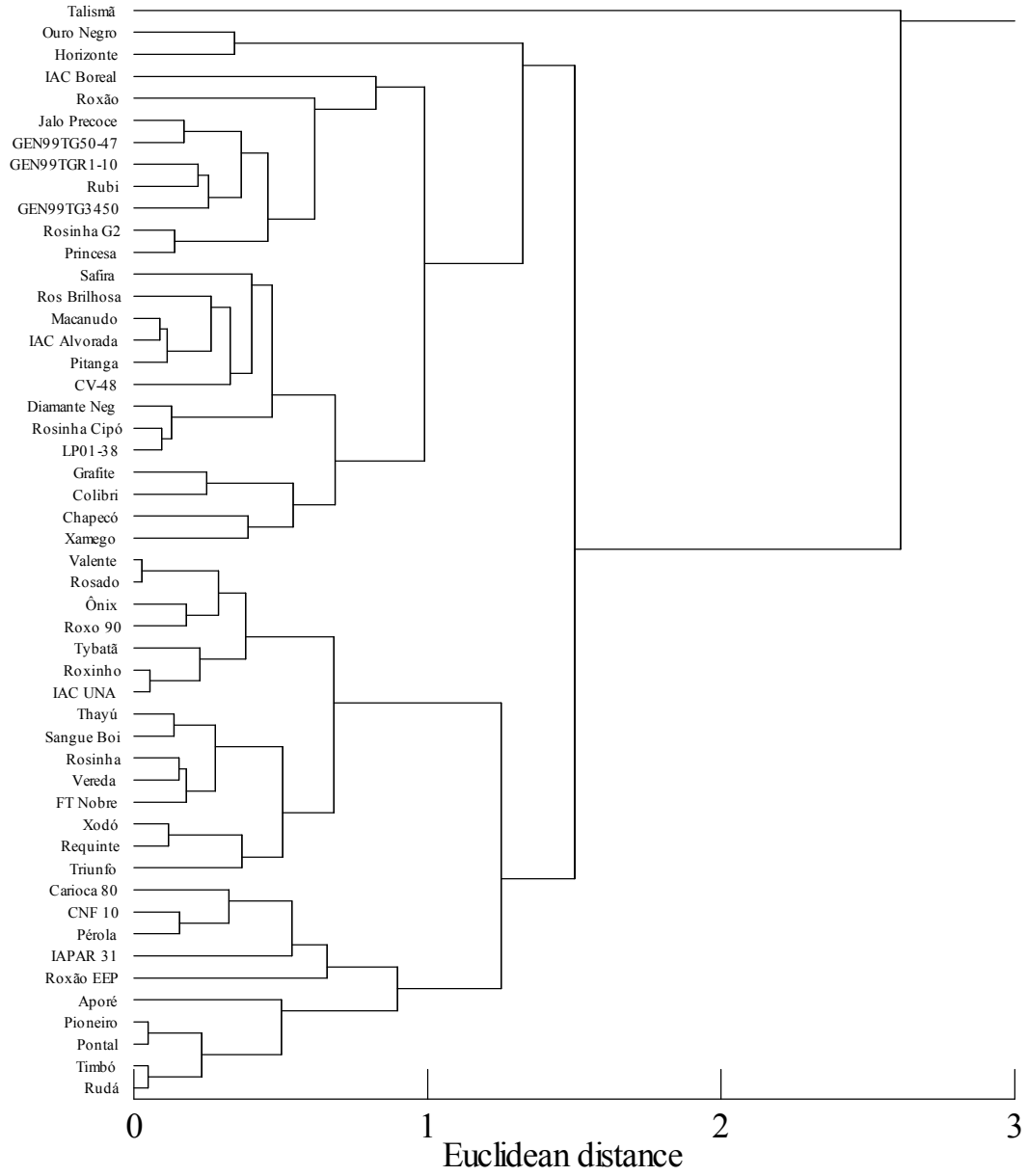

FIG. 5. Dendrogram resulting from hierarchical cluster analysis of 50 common bean genotypes, based on grain dry matter yield (Gdm), P concentration and P content in Gdm at the second sampling.

Talismã did not form a group with other genotypes because it had low grain yield, but high P concentration and P content of Gdm. Therefore, other groups were classified in four groups in terms of grain yield and P in Gdm.

Carioca 80, CNF 10, Pérola, IAPAR 31, Roxão EEP, Aporé, Pioneiro, Pontal, Timbó and Rudá were the most productive genotypes when grown under low P availability conditions. The 10 bean genotypes most productive in terms of grain had higher P utilization efficiency, defined as the ability to convert the element taken up by the plants into agricultural product of commercial value (leaf, fruit, root and stem) [21]. The efficiency of utilization (EU) is generally associated with productivity, that is, the greater the EU, the higher is the grain yield [22]. Among these genotypes, only Pioneer was rated as efficient in P uptake (Fig. 3).

4. CONCLUSIONS

- The common bean genotypes Sangue de Boi, Rosinha, Thayú, Grafite, Horizonte, Pioneiro and Jalo Precoce were the most efficient in P uptake;

- The common bean genotypes Carioca 80, CNF 10, Pérola, IAPAR 31, Roxão EEP, Aporé, Pioneiro, Pontal, Timbó e Rudá were the most productive in grain under conditions of low available soil P (genotype more efficient in P utilization);

- The seed derived P in the plant, when the ^{32}P L-value technique is used, affects the identification and classification of common bean genotypes.

ACKNOWLEDGMENTS

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SELECTION OF COMMON BEAN LINES, RECOMBINANT INBRED LINES AND COMMERCIAL GENOTYPES TOLERANT TO LOW PHOSPHORUS AVAILABILITY IN AN ACRISOL SOIL ON THE BASIS OF ROOT TRAITS AND GRAIN YIELD

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Abstract

Common bean (*Phaseolus vulgaris* L.) is the most important food legume for human consumption worldwide and especially in Latin America and Africa, but low soil phosphorus (P) availability limits grain production in these areas. For these reason eighty five recombinant inbred lines (RILs) of BAT 477 x DOR 364 and twenty commercial bean genotypes were sown in plots in an Acrisol soil with low P availability to evaluate nine root traits and grain yield. The study was carried out in Pinar del Rio province in Cuba between November 2006 and February 2009. The plots received basal fertilization (N and K) and P fertilization between 15 and 90 kg P_2O_5 ha^{-1}. Ten plants were sampled from each plot at R_6 pod fill to evaluate root traits and shoot biomass, and at R_9 physiological maturity to estimate grain yield. The 85 RILs showed great variability for root traits, grain yield and P stress tolerance calculated as relative grain yield. The commercial bean lines also showed large diversity in yield parameters. Principal Component Analysis showed that there were high and significant correlations between root traits (basal root number, primary root depth, adventitious root length and adventitious root number) and grain yield parameters (grain yield at 15 P level and relative grain yields). Adventitious root traits showed the greatest correlation with yield under low P. Promising RILs included 75.1.1, 60.1.1, 38.1.1, 14.1.1 and 38.1.1 and promising commercial bean lines included ICA Pijao, BAT 482, ICA 23, BAT 24 and BAT 832.

1. INTRODUCTION

Common bean (*Phaseolus vulgaris,* L.) is the most important food legume for human consumption worldwide, and especially in Latin America and Africa [1]. Low phosphorus (P) availability is a primary constraint to common bean production, and P fertilization is frequently not economically feasible for resource-limited farmers in the developing world, and is often inefficient in tropical soils due to fixation of P in insoluble forms by iron and aluminium oxides. Therefore fertilization is not an adequate solution in itself; rather, bean

genotypes that are capable of producing economic yields at low levels of native or added P are desirable. Such genotypes may be superior due to a greater ability to recover P from fertilizer and / or native fractions of soil P and / or due to greater productivity per unit of P absorbed [2].

Substantial genotypic variation in adaptation of bean to low P availability has been linked with root traits that enhance the efficiency of soil foraging [3]. For instance, it was affirmed that root gravitropism determines the relative distribution of plant roots in different soil layers [4], and therefore may influence the acquisition of shallow soil resources. Adventitious roots play an important role in P acquisition, as they are localized near the soil surface where P is relatively abundant [5]. The effect of low soil P availability on the increase of adventitious rooting in common bean could be used as a strategy to increase P acquisition for this legume [6].

Soil P deficiency is widespread as a limiting factor for food production in the tropics and subtropics because most of the forms in which it occurs are poorly available to crops [7]. P fertilization with water soluble sources (e.g. single and triple super phosphate, DAP, MAP in Cuba) is a solution to overcome this constraint, but third-world small farmers, including small farmers in Cuba, generally lack the resources to invest in this input. In addition, the efficiency of P fertilization is low in many tropical and subtropical soils because of fixation into plant-unavailable forms.

At present Cuban agriculture faces a fertilizer deficit with negative consequences for the quantity and quality of agricultural production. Soil nutrient deficiencies, mainly nitrogen (N) and P, coupled with soil acidity occur in many of the small agricultural areas. The use of plants with low adaptability to these constraining factors, as well as inadequate management practices among others, limit agricultural production. At the same time there is no improvement of soil fertility and consequently agriculture production. In global terms, 45% of agricultural lands in Cuba have low fertility and 40% are affected by acidity.

Therefore, as a strategy to improve common bean production in tropical and subtropical infertile soils, it is essential to evaluate plant root traits linked to high P acquisition from low P bioavailability through the selection of more adapted germplasm to soil constraints. This work was carried out to identify common bean lines (RILs and commercial cultivars) for use in Cuba that were more tolerant of P deficiency in an Acrisol soil on the basis of root traits and grain yield.

2. MATERIALS AND METHODS

Three experiments were carried out to characterize the root traits of 106 common bean lines (85 recombinant inbred lines and 19 commercial lines) used in Cuba.

2.1. Laboratory screening

2.1.1. Experiment 1

Four plants of 15 recombinant inbred lines (RILs) i.e. (1.1.1, 2.1.1, 5.1.1, 6.1.1, 14.1.1, 16.1.1, 20.1.1, 22.1.1, 27.1.1, 30.1.1, 31.1.1, 33.1.1, 34.1.1, 36.1.1) and two commercial lines (UPR 56 and UPR 70) were evaluated using Jonathan Lynch's methodology "cigar roll method" (personal communication). A rating scale of 1 to 9 was used to rank crown root architecture (Table 1).

TABLE 1. ROOTS TRAITS AND RATING SCALE USED TO EVALUATE BEAN LINES

Root traits	Rating scale
1. Adventitious root length (ARL)	1 = 1 cm to 9 = 15–20 cm
2. Adventitious root Number (ARN)	Actual number plant^{-1}
3. Adventitious root branching (ARB)	1 = no lateral branching to 9 = multiple lateral branches with up to 4 orders of branching
4. Basal root length (BRL)	1 = absent to 9 = 20–30 cm (width of excavation)
5. Basal root number (BRN)	Actual number of basal roots plant^{-1}
6. Basal root branching (BRB)	1 = no lateral branching to 9 = multiple lateral branches with up to 4 orders to branching
7. Basal root depth (BRD)	1 = horizontal to 9 = vertical
8. Primary root depth (PRD)	1 = no taproot left to 9 = 20 – 30 cm (depth of excavation)
9. Primary root branching (PRB)	1 = no lateral branching to 9 = multiple lateral branches with up to 4 orders of branching
10. Combined adventitious roots	Sum of results of rating scale analysis of the 1, 2 and 3 root traits
11. Combined basal roots	Sum of results of rating scale analysis of the 4, 5 and 6 root traits
12. Combined primary roots	Sum of results of rating scale analysis of the 7, 8 and 9 root traits

2.1.2. Experiment 2

Seventeen commercial common bean lines in Cuba (BAT 24, BAT 58, BAT 93, BAT 304, BAT 482, BAT 832, Guama 23, Rosas M112, Bolita 42, Güira 89, Jamapa, CC – 259R, ICA Pijao, Bonita 11, Red Kloud and Velazco Largo) were also evaluated using the cigar roll method (Table 1).

2.2. Field evaluation – Experiment 3

Eighty five recombinant inbred lines (Table 2) as well as BAT 477 and DOR 364 were evaluated between November 2006 and February 2007 for root traits.

TABLE 2. RECOMBINANT INBRED LINES EVALUATED IN EXPERIMENT 3

Recombinant inbred lines				
1.1.1	22.1.1	45.1.1	65.1.1	83.1.1
2.1.1	25.1.1	46.1.1	66.1.1	84.1.1
3.1.1	26.1.1	47.1.1	67.1.1	85.1.1
4.1.1	27.1.1	48.1.1	68.1.1	88.1.1
6.1.1	28.1.1	50.1.1	69.1.1	89.1.1
7.1.1.	30.1.1	53.1.1	70.1.1	90.1.1
8.1.1	31.1.1	54.1.1	71.1.1	91.1.1
9.1.1	33.1.1	55.1.1	72.1.1	92.1.1
10.1.1	34.1.1	56.1.1	73.1.1	93.1.1
12.1.1	35.1.1	57.1.1	74.1.1	94.1.1
13.1.1	35.1.4	58.1.1	75.1.1	95.1.1
14.1.1	36.1.1	59.1.1	76.1.1	96.1.1
15.1.1	38.1.1	60.1.1	77.1.1	97.1.1
17.1.1	39.1.1	61.1.1	79.1.1	98.1.1
19.1.1	41.1.1	62.1.1	80.1.1	99.1.1
20.1.1	42.1.1	63.1.1	81.1.1	128.1.1
21.1.1	43.1.1	64.1.1	82.1.1	

The soil was an Acrisol from Viñales, Pinar del Rio province in the west of Cuba. The physic-chemical characteristics were: pH (H_2O), 4.8; pH KCl, 3.8; Organic matter (Walkley-Black method), 3.02%; available pP (Bray-Kurtz I method, 4.4 mg P kg^{-1}; exchangeable bases (cmol kg^{-1}); Ca, 0.75; Mg, 0.43; Na, 0.10; K, 0.05; N total (Kjeldhal method), 0.115%; exchangeable acidity 0.380; exchangeable H, 0.013; exchangeable Al, 0.367; Al saturation, 23.1 %.

The plots size was 2.8 m^2 (two rows at 0.70 m × 2 m long) and plots were surrounded by 0.5 m of untreated soil. All plots received basal fertilization of 15 kg–N ha^{-1} as urea and 60 kg K_2O ha^{-1} as KCl. The common bean seeds were inoculated with *Rhizobium* sp. strain 6bIII from the Soil Institute Collection in Cuba.

Each RIL was evaluated at two P levels (15 and 90 kg P_2O_5 ha^{-1}) using triple super phosphate (TSP) as the P source. The experiment was arranged in a two factor randomized block design with 87 genotypes × 2 P fertilization levels, where every row was considered to be one replicate. Three plants of each plot were sampled at the R_6 – R_7 growth stage on January 3, 2007 for root characteristics evaluation (Table 1). Ten plants of each treatment were harvested at the R_9 growth stage (85 to 95 days after sowing) to determine grain yield.

2.3. Statistical analysis

The statistical software MSTAT-C version 2.10 [8] was used to perform the analyses of variance at $P<0.05$ for differences of means traits among genotypes, P treatment and interactions, and to calculate the correlation between parameters. The root traits evaluated and grain yield parameters were used to perform a Principal Component Analysis (PCA) using software XLSTAT [9].

3. RESULTS

3.1. Root traits in bean lines evaluated under laboratory conditions

Great differences in eight roots traits were found in 17 bean lines evaluated in the first experiment. The rating scale used during root evaluation showed that ARL ranged from 2 to 3, ARN from 1 to 5, ARB from 1 to2, BRL from 2 to 4, BRN from 1 to 5, BRB from 1 to 3, PRD from 4 to 7 and PRB from 2 to 5 (Table 3).

TABLE 3. MINIMUM, MAXIMUM, AVERAGE AND STANDARD DEVIATION OF 8 ROOT TRAITS IN 15 RILS AND 2 COMMERCIAL LINES OF COMMON BEAN IN THE FIRST LABORATORY EXPERIMENT

Root traits	Minimum	Maximum	Average	Standard deviation
1. Adventitious root length (ARL)	2	3	2.84	0.43
2. Adventitious root number (ARN)	1	5	3.38	0.88
3. Adventitious root branching (ARB)	1	2	1.10	0.20
4. Basal root length (BRL)	2	4	3.66	0.62
5. Basal root number (BRN)	1	5	3.56	1.06
6. Basal root branching (BRB)	1	3	1.44	0.54
7. Primary root depth (PRD)	4	7	5.75	0.71
8. Primary root branching (PRB)	2	5	3.54	0.76

Principal component analysis (PCA) showed that the first fourth components explained more than 80% the variance (Table 4). Correlated positively to F1 were adventitious root length, and adventitious root number (Table 5). On the contrary, negatively

related were basal root number, primary root depth and primary root branching. Positively related to F2 were adventitious root numbers, basal root length, primary root depth and primary root branching but negatively correlated with basal root number. The F3 component was only positively linked to two variables, adventitious root branching and basal root branching (Table 5). Adventitious root branching, basal root length and basal root number were correlated with F4.

TABLE 4. PRINCIPAL COMPONENT ANALYSIS OF 8 ROOT TRAITS IN 15 RILS AND 2 COMMERCIAL LINES OF COMMON BEAN IN THE FIRST LABORATORY EXPERIMENT

Parameter	F1	F2	F3	F4
Characteristic value	2.20	1.94	1.43	0.92
Variability (%)	27.47	24.25	17.88	11.47
% accumulated	27.47	51.72	69.61	81.09

TABLE 5. CORRELATION COEFFICIENTS BETWEEN 8 ROOT TRAITS AND FACTORS FROM THE PRINCIPAL COMPONENT ANALYSIS OF 15 RILS AND 2 COMMERCIAL COMMON BEAN LINES IN THE FIRST LABORATORY EXPERIMENT

Root traits	F1	F2	F3	F4
1. Adventitious root length (ARL)	0.80	0.29	0.21	0.13
2. Adventitious root number (ARN)	0.44	0.76	– 0.17	0.08
3. Adventitious root branching (ARB)	0.17	0	0.81	0.33
4. Basal root length (BRL)	– 0.28	0.58	0.27	0.38
5. Basal root number (BRN)	– 0.76	– 0.38	– 0.03	0.48
6. Basal root branching (BRB)	– 0.17	– 0.18	0.79	–0.45
7. Primary root depth (PRD)	– 0.61	0.67	0.03	0.06
8. Primary root branching (PRB)	– 0.53	0.57	0.04	–0.45

The PCA analysis showed that the lines with the best adventitious root traits were 36.1.1 and 27.1.1 (Fig. 1). The lines with the highest primary root depth, primary root branching and basal root lengths were 20.1.1, 30.1.1 and 31.1.1 and the lines with the most basal root number and basal root branching were 16.1.1 and 20.1.1.

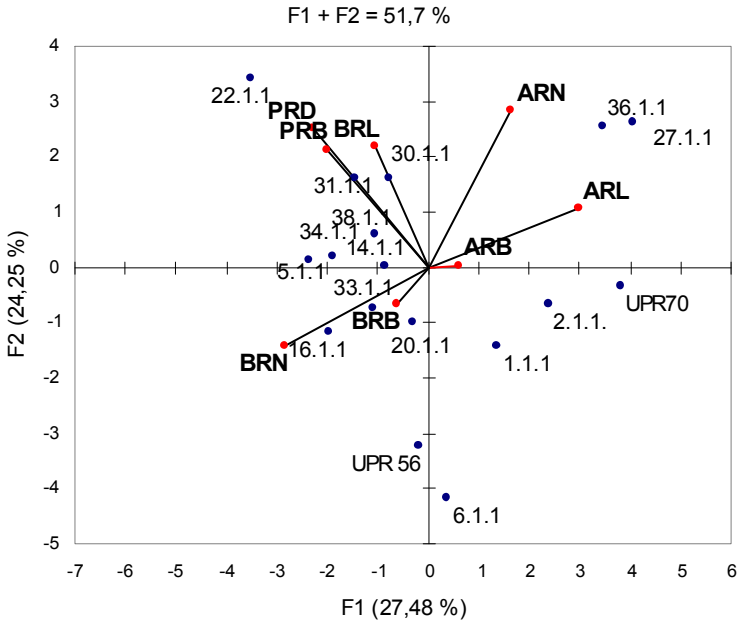


FIG. 1. Coordinates of variables and factors in principal component analysis of 15RILs and 2 commercial lines (numbered) of common bean in the first laboratory experiment. Root trait abbreviations are given in Table 3.

Dendrogram analysis permitted the identification of three clusters according to root traits for 17 bean lines evaluated in the first laboratory experiment (Fig. 2, Table 6). Class 1 (C1) included 6 lines (1.1.1, 2.1.1, 6.1.1, 20.1.1, UPR 56 and UPR 70) having low production of adventitious root number (ARN); Class 2 (C2) included nine lines (5.1.1, 14.1.1, 16.1.1, 22.1.1, 30.1.1, 31.1.1, 33.1.1, 34.1.1 and 38.1.1) having high primary root depth (PRD), basal root length and basal root number and Class 3 (C3) included 2 lines (27.1.1 and 36.1.1) having high adventitious root number (ARN) and adventitious root length (ARL).

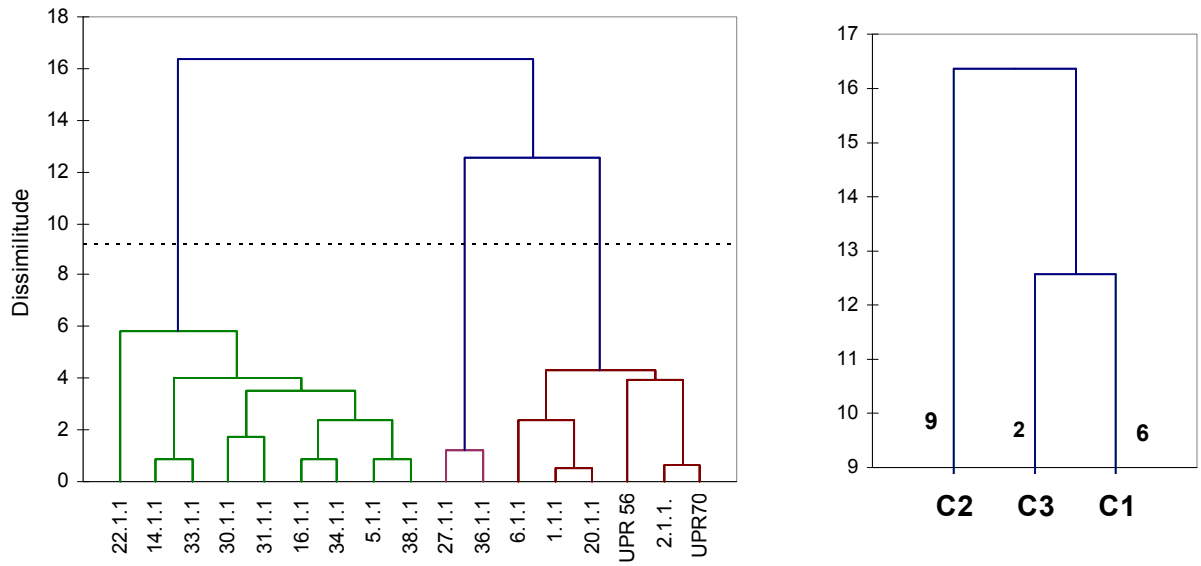


FIG. 2. Dendrogram of 8 root traits in 15 RILs and 2 commercial lines of common bean in the first laboratory experiment.

TABLE 6. CLASS "CENTROID" OF DENDROGRAM ANALYSIS OF 8 ROOT TRAITS OF 15 RILS AND 2 COMMERCIAL LINES OF COMMON BEAN IN THE FIRST LABORATORY EXPERIMENT

Class	ARL	ARN	ARB	BRL	BRN	BRB	PRD	PRB
1	2.88	3.00	1.13	3.13	3.33	1.50	5.08	3.04
2	2.67	3.28	1.11	4.00	4.14	1.50	6.22	3.92
3	3.50	5.00	1.00	3.75	1.63	1.00	5.63	3.38

PCA showed that commercial lines evaluated in the second experiment were more variable in root traits than RILs (Table 7). ARL ranged from 2 to 5, ARN from 2 to 6, ARB from 1 to 3, BRL from 1 to 6, BRN from 0 to 5, BRB from 0 to 4, BRD from 0 to 7, PRD from 4 to 7 and PRB from 1 to 6. The first four components of PCA explained 82 % of the variance (Table 8). Positive correlations with F1 were BRL, BRN, BRB and BRD, but negatively ARL (Table 9). Positively correlated to F2 were ARL, ARN, BRL and PRD, but negatively correlated with ARB and PRD. Correlated positively to F3 were ARL, BRB and PRB, but negatively with PRD. F4 was negatively correlated with ARN and PRB.

Dendrogram analysis identified fives class for the 17 commercial lines evaluated (Table 10, Fig. 3, Fig. 4). Class 1 included 3 lines (BAT 24, Guama 23 and Velazco Largo) with high ARL, ARN, BRL, BRD and PRD. Class 2 included 7 lines (BAT 58, BAT 832, Rosas, M 112, Bolita 42, Bonita 11 and Red Kloud) with medium to high BRD and PRD. Class 3 included 1 line (BAT 93) with low production of BRN, BRB, BRD and PRD. Class 4 included 3 lines (BAT 304, BAT 482 and ICA Pijao) with high BRD and PRD, and Class 5 included 3 lines (Guira 89, Jamapa and CC 25 – 9R) with high BRD and ARL.

TABLE 7. MINIMUM, MAXIMUM, AVERAGE AND STANDARD DEVIATION OF 9 ROOT TRAITS IN 17 COMMERCIAL COMMON BEAN LINES IN THE SECOND LABORATORY EXPERIMENT

Root traits	Minimum	Maximum	Average	Standard deviation
1. Adventitious root length (ARL)	2	5	3.2	0.67
2. Adventitious root number (ARN)	2	6	3.9	1.06
3. Adventitious root branching (ARB)	1	3	1.2	0.43
4. Basal root length (BRL)	1	6	3.7	1.07
5. Basal root number (BRN)	0	5	3.5	1.26
6. Basal root branching (BRB)	0	4	1.5	0.75
7. Basal root depth (BRD)	0	7	5.6	1.58
8. Primary root depth (PRD)	4	7	5.5	0.66
9. Primary root branching (PRB)	1	6	3.2	1.04

TABLE 8. PRINCIPAL COMPONENT ANALYSIS OF 9 ROOT TRAITS IN 17 COMMERCIAL COMMON BEAN LINES IN THE SECOND LABORATORY EXPERIMENT

Parameter	F1	F2	F3	F4
Characteristic value	3.22	1.87	1.35	0.94
Variability (%)	35.88	20.77	15.02	10.44
% accumulated	35.88	56.65	71.67	82.12

TABLE 9. CORRELATION COEFFICIENTS BETWEEN 9 ROOT TRAITS AND FACTORS FROM THE PRINCIPAL COMPONENT ANALYSIS OF 17 COMMERCIAL COMMON BEAN LINES IN THE SECOND LABORATORY EXPERIMENT

Root traits	F1	F2	F3	F4
1. Adventitious root length (ARL)	–0.476	0.553	0.574	0.247
2. Adventitious root number (ARN)	0.014	0.831	0.288	–0.100
3. Adventitious root branching (ARB)	0.162	–0.454	0.211	0.795
4. Basal root length (BRL)	0.835	0.458	–0.218	0.008
5. Basal root number (BRN)	0.881	0.014	–0.089	0.150
6. Basal root branching (BRB)	0.704	0.138	0.521	0.009
7. Basal root depth (BRD)	0.883	0.107	–0.239	0.015
8. Primary root depth (PRD)	–0.187	0.543	–0.324	0.412
9. Primary root branching (PRB)	0.438	–0.362	0.637	–0.210

TABLE 10. CLASS "CENTROID" OF DENDROGRAM ANALYSIS OF 8 ROOT TRAITS OF 17 COMMERCIAL BEAN LINES IN THE SECOND LABORATORY EXPERIMENT

Class	ARL	ARN	ARB	BRL	BRN	BRB	BRD	PRD
1	3.7	5.6	1.2	5.3	4.7	1.8	6.8	6.0
2	2.9	3.0	1.4	3.6	4.1	1.5	5.5	5.4
3	4.5	5.0	1.0	1.0	0.0	0.0	0.0	5.8
4	3.1	3.5	1.0	3.3	2.3	1.2	6.0	6.0
5	3.2	4.3	1.2	3.8	3.3	2.3	6.3	4.6

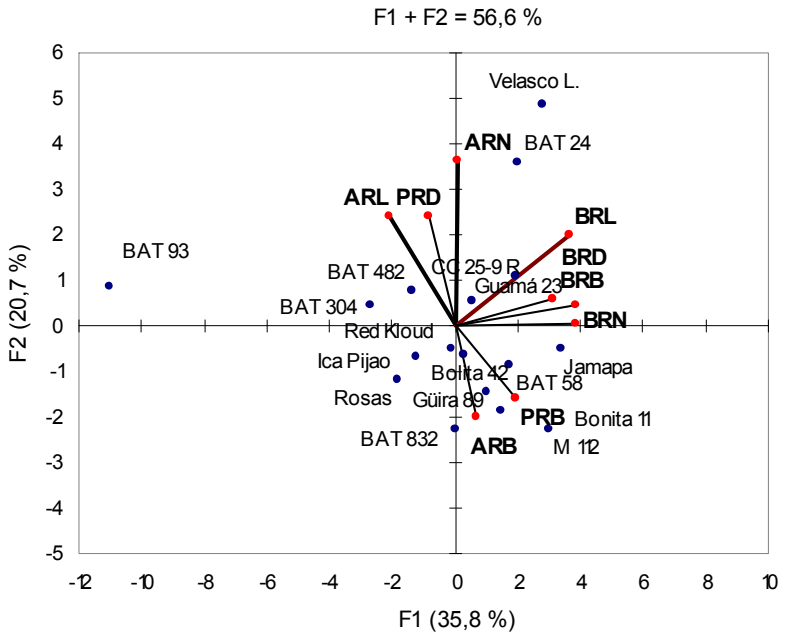


FIG. 3. Coordinates of variables and factors in principal component analysis of 17 commercial lines (numbered) of common bean in the second laboratory experiment. Root trait abbreviations are given in Table 7.

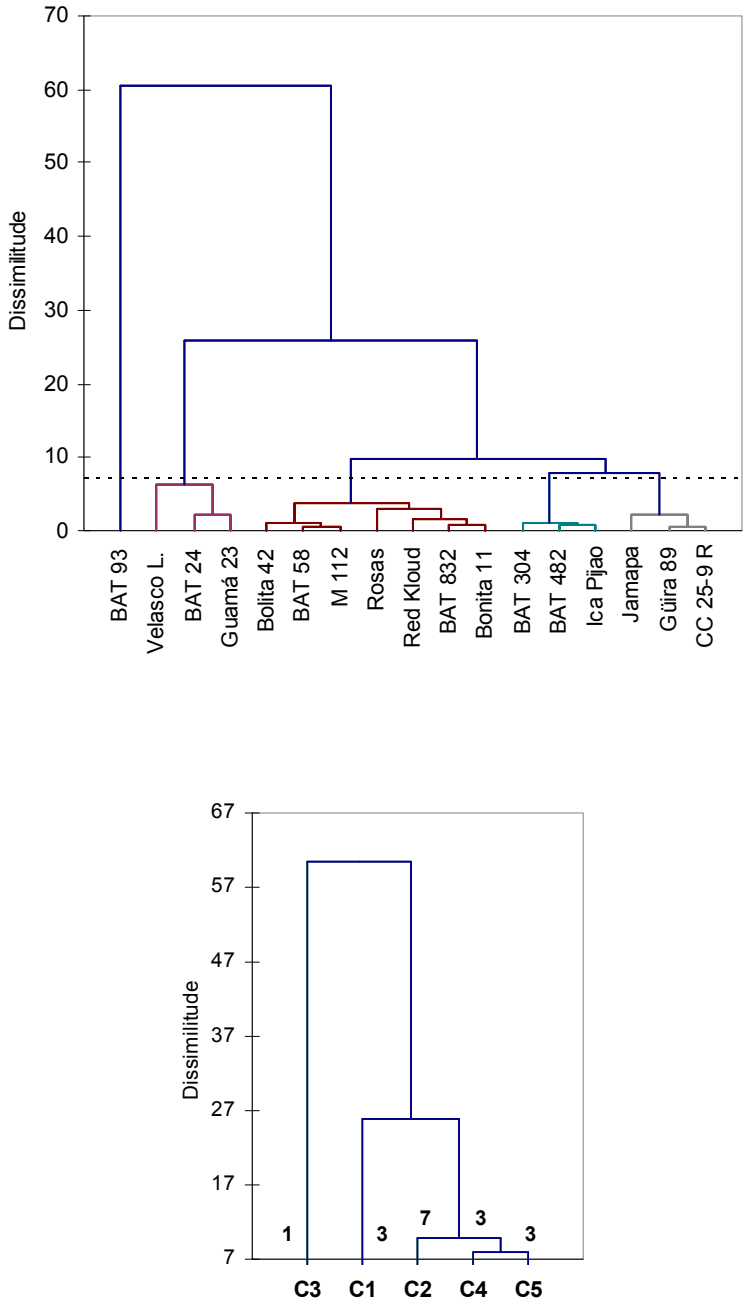


FIG. 4. Dendrogram of 9 root traits in 17 commercial lines of common bean in the second laboratory experiment

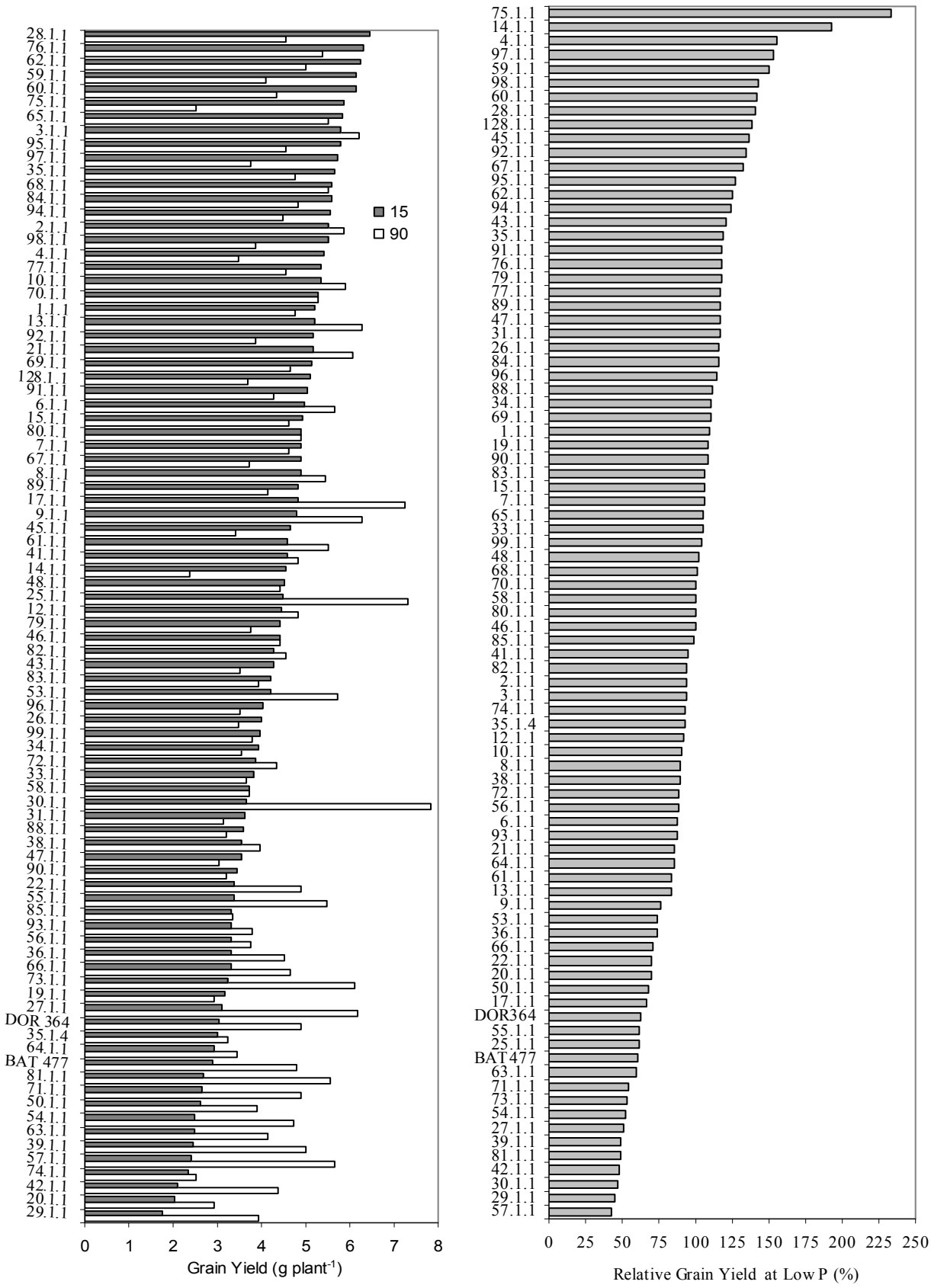


FIG. 5. Grain yield and relative grain yield of 85 RILs, BAT 477 and DOR 364 at two P levels in the field experiment.

3.2. Root traits and grain yield in bean lines evaluated under field conditions

Grain yield (GY) and relative grain yield (RGY) at 15 and 90 P_2O_5 fertilization rates varied among the RILs (Fig. 5). The GY at 15 P ranged from 1.77 to 6.43 g plant^{-1} while at 90 P from 2.36 to 7.84 g plant^{-1}. The relative grain yield (RGY) ranged from 43 to 234 %.

Large differences in root traits were observed in 85 bean RILs evaluated in the field experiment (Table 11). When the rating scaling was applied to ARL, ARN and ARB root traits the values ranged from 2 to 8, 2 to 13 and 1 to 8, respectively. The basal root branching, number and depth were fewer than adventitious traits, so BRB, BRN and BRD ranged from 2 to 7, 2 to 5 and 1 to 6, respectively (Table 11). Primary root traits depth and primary root branching ranged from 1 to 4 and 1 to 3, respectively.

TABLE 11. MINIMUM, MAXIMUM, AVERAGE AND STANDARD DEVIATION OF 9 ROOT TRAITS AND 2 YIELD PARAMETERS IN 85 RILS OF COMMON BEAN GROWN AT 2 P LEVELS IN THE FIELD EXPERIMENT

Root traits and yield parameters	Minimum	Maximum	Average	Standard deviation
1. Adventitious root length (ARL)	2	8	4.11	1.32
2. Adventitious roots numbers (ARN)	2	13	6.17	2.44
3. Adventitious root branching (ARB)	1	8	2.46	0.90
4. Basal root length (BRL)	2	7	3.37	0.89
5. Basal root number (BRN)	2	5	3.20	0.79
6. Basal root branching (BRB)	1	6	2.67	1.14
7. Basal root depth (BRD)	1	7	4.28	1.63
8. Primary root depth (PRD)	1	4	2.17	0.98
9. Primary root branching (PRB)	1	3	1.75	0.65
10. Grain yield at 15P (g plant^{-1})	1.77	6.43	4.28	1.17
11. Relative grain yield (%)	42.78	233.69	98.88	33.08

PCA performed with nine root traits and two parameters of grain yield showed that the first five components explained 77 % of the variance (Table 12).

TABLE 12. PRINCIPAL COMPONENT ANALYSIS OF 9 ROOT TRAITS AND 2 YIELD PARAMETERS IN 85 RILS OF COMMON BEAN GROWN AT TWO P LEVELS IN THE FIELD EXPERIMENT

Parameter	F1	F2	F3	F4	F5
Characteristic value	3.32	2.59	1.85	1.75	1.30
Variability (%)	23.74	18.49	13.20	12.50	9.27
% accumulated	23.74	42.23	55.43	67.93	77.20

Thus PCA showed that the F_1 component was correlated with all traits studied except basal root length, grain yield at 15P and relative grain yield, which were negatively correlated (Table 13). Correlated with F_2 were adventitious root length, adventitious root number, grain yield at 15P, and relative grain yield, but correlated negatively with primary root depth and primary root branching. Correlated to F_3 were basal root number, primary root depth, primary root branching, grain yield at 15P and relative grain yield. Correlated with F4 and F5 components were basal root depth, basal root number, adventitious root number, primary root depth and primary root branching (Table 13). Higher correlations were observed between the yield parameters (GY at 15P and RGY) and F2 and F3 components, and also between these components and adventitious root length, adventitious root number, basal root number and primary root depth.

Results of the PCA (Fig. 6, Table 14 and Fig. 7,) show the greatest ARL and ARN were found in RILs 22.1, 36.1.1, 47.1.1, 97.1.1 and 99.1.1; the greatest BRD, BRN and ARB were observed in RILs 77.1.1, 71.1.1 and 22.1.1; superior production of BRB was seen in RILs 39.1.1, 96.1.1 and 30.1.1; greater PRD and PRB were observed in RILs 56.1.1, 72.1.1, and 54.1.1 and lines BAT 477 and DOR 364; high BRL was observed in RILs 48.1.1 and 7.1.1; lines with high grain yield at 15P and relative grain yield were RILs 60.1.1, 75.1.1, 85.1.1, 95.1.1 and 99.1.1.

TABLE 13. CORRELATION COEFFICIENTS BETWEEN 9 ROOT TRAITS, 2 YIELD PARAMETERS AND FACTORS FROM THE PRINCIPAL COMPONENT ANALYSIS OF 85 RILS OF COMMON BEAN GROWN AT 2 P LEVELS IN THE FIELD EXPERIMENT

Root traits and yield parameters	F1	F2	F3	F4	F5
1. Adventitious root length (ARL)	0.559	0.635	0.236	–0.102	0.185
2. Adventitious roots numbers (ARN)	0.373	0.708	0.165	–0.073	0.402
3. Adventitious root branching (ARB)	0.695	0.068	0.037	–0.315	–0.323
4. Basal root length (BRL)	–0.432	–0.149	–0.128	–0.246	0.389
5. Basal root number (BRN)	0.252	0.095	0.520	0.534	–0.350
6. Basal root branching (BRB)	0.609	–0.183	0.187	–0.454	–0.343
7. Basal root depth (BRD)	0.304	0.106	0.118	0.791	0.189
8. Primary root depth (PRD)	0.279	–0.432	0.544	–0.144	0.406
9. Primary root branching (PRB)	0.489	–0.366	0.373	–0.042	0.514
10. Grain yield at 15P (g plant^{-1})	–0.456	0.396	0.638	–0.180	–0.039
11. Relative grain yield (%)	–0.350	0.556	0.605	–0.221	0.013

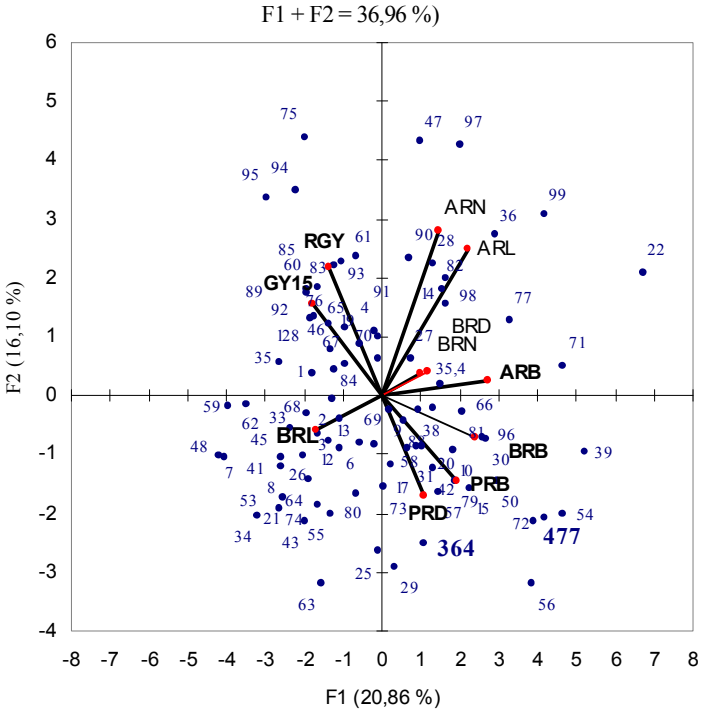
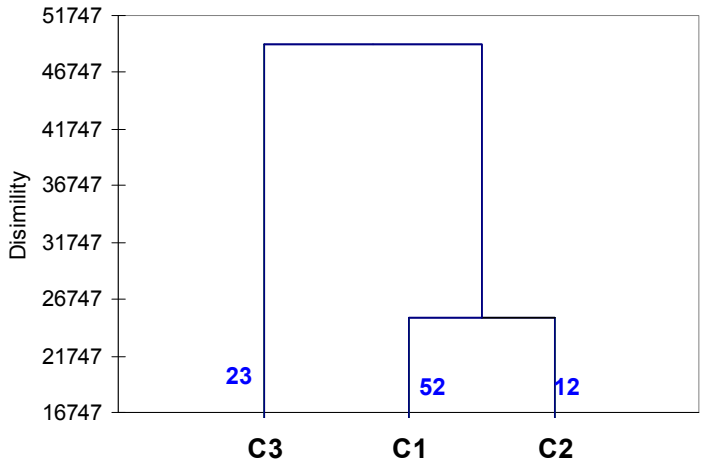


FIG. 6. Coordinates of variables and factors in principal component analysis of 85 RILs (numbered) of common bean at two P levels in the field experiment. Root trait abbreviations are given in Table 11.

TABLE 14. CLASS "CENTROID" OF DENDROGRAM ANALYSIS ON 85 RILS, BAT 477 AND DOR 364 FOR 9 ROOT TRAITS AND 2 GRAIN YIELD PARAMETERS AT 2 P LEVELS IN THE FIELD EXPERIMENT

Class	ARL	ARN	ARB	BRL	BRN	BRB	BRD	PRD	PRB	GY 15	RGY
1	3.9	6.2	2.3	3.6	3.2	2.5	4.2	2.2	1.7	4.5	103.6
2	4.4	6.7	2.5	2.8	3.3	2.8	3.8	2.3	1.6	5.5	154.3
3	4.4	5.9	2.8	3.2	3.1	3.0	4.6	2.1	1.9	3.1	59.3



C_3	C_1	C_1	C_2
9.1.1	1.1.1	61.1.1	4.1.1
17.1.1	2.1.1	62.1.1	14.1.1
20.1.1	6.1.1	64.1.1	28.1.1
22.1.1	7.1.1	65.1.1	45.1.1
25.1.1	8.1.1	68.1.1	59.1.1
27.1.1	10.1.1	69.1.1	60.1.1
29.1.1	12.1.1	70.1.1	67.1.1
30.1.1	13.1.1	72.1.1	75.1.1
36.1.1	15.1.1	74.1.1	92.1.1
39.1.1	19.1.1	76.1.1	97.1.1
42.1.1	21.1.1	77.1.1	98.1.1
50.1.1	26.1.1	79.1.1	128.1.1
53.1.1	31.1.1	80.1.1	
54.1.1	33.1.1	82.1.1	
55.1.1	34.1.1	83.1.1	
57.1.1	35.1.1	84.1.1	
63.1.1	35.1.1	85.1.1	
66.1.1	38.1.1	88.1.1	
71.1.1	41.1.1	89.1.1	
73.1.1	43.1.1	90.1.1	
81.1.1	46.1.1	91.1.1	
BAT 477	47.1.1	93.1.1	
DOR 364	48.1.1	94.1.1	
	56.1.1	95.1.1	
	58.1.1	96.1.1	

FIG. 7. Dendrogram of 85 RILs of common bean, and lines BAT 477 and DOR 364 for 9 root traits and 2 yield parameters at 2 P levels in the field experiment.

4. DISCUSSION

In summary, there was great variability for root traits and grain yield in the 85 RILs and 20 commercial lines of common bean evaluated. High and significant correlations were found for grain yield or relative grain yield at 15P and basal root number, primary root depth, adventitious root length and adventitious root numbers in the 85 RILs. Lines identified under laboratory conditions, such as 22.1.1, with favourable primary and basal root traits were also found to be promising in the field. This high diversity is desirable both to select contrasting RILs and commercial common bean cultivars as potentially elite material for breeding programmes to improve grain yield at low soil P, and also because of the interest in introducing material with high adaptability to low P input systems (soil and P fertilizer) in small farmer's plots.

In addition, these results are similar to earlier studies which recognized that bean adaptation to low P availability in soil is associated with root traits [3, 4], and which identified adventitious roots as playing the principal role in P acquisition [5, 6]. It is likely that this trait is probably associated with enhanced grain yield when common bean lines are grown in soil with low P availability as was observed in the present study.

In Cuba, new common bean lines are often introduced for 'on-farm' trials, but the identification of superior genotypes with greater productivity in low-nutrient environments similar to those utilized by smallholder farmers in Cuba is rarely considered. In this work, the grain yields at low P availability of RILs such as 2.1.1, 5.1.1, 14.1.1, 22.1.1, 34.1.1, 38.1.1, 60.1.1 and 75.1.1 were observed to be high and stable, and thus theses RILs are promising material for further 'on-farm' studies.

The possibility of identifying promising material in early growth stages with the "cigar roll" method will permit evaluation of a greater number of lines in a cost-effective way. It was concluded that there is high variability associated with root traits and grain yield among the 102 bean lines evaluated. Correlations of grain yield parameter and root traits, such as basal root number, primary root depth, adventitious root length and adventitious root numbers, were observed.

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PHOSPHORUS USE EFFICIENCY FOR SYMBIOTIC FIXATION NITROGEN IN VOANDZOU (*VIGNA SUBTERRANEA*) USING ISOTOPIC EXCHANGE METHOD IN RHIZOTRON

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Abstract

Low bioavailability of nitrogen and phosphorus is one of the main constraints in the acid soils with high P-fixing capacity. Plants adapt to low nutrient availability through various biological and physico-chemical mechanisms. Since genetic variation of N_2 fixation exists in numerous legume species, optimization of symbiotic nitrogen fixation (SNF) under P deficiency could be a way to the replenishment of soil fertility in tropical soils. As the genetic potential of crops like *Vigna subterranea* (Bambara groundnut or voandzou) is little studied, although its agronomic potential is interesting for the farmers of Africa, a physiological study through legume screening for N_2 fixation was performed with 54 cultivars from Madagascar, Niger and Mali, inoculated with the reference strain of *Bradyrhizobium* sp. Vigna CB756 in hydroponic culture under P deficiency and sufficiency (30 and 75 µmol KH_2PO_4 $plant^{-1}$ $week^{-1}$, respectively), corresponding respectively to 28 and 70 mg P kg^{-1} of soil. Large variability of nodulation and plant biomass was found among cultivars. These two parameters were generally correlated and the slope of the plant biomass regression as a function of nodulation was considered as an indicator of the efficiency in use of the rhizobial symbiosis. For the two cultivars most tolerant to P deficiency, V1 and V4 from Madagascar, the increase in use efficiency of the rhizobial symbiosis under P deficiency was linked with an increase in nodulated root O_2 consumption linked to N_2 fixation, and in phytase gene expression observed on the nodule sections by *in situ* RT-PCR. As the complexity of P compartments makes it difficult to assess the P bioavailability in the plant rhizosphere, an isotopic ^{32}P exchange method was carried out in a rhizotron in order to assess the direct effect of the roots on P mobilization in rhizosphere soil, comparing V1 and V4 with 28 or 70 mg P kg^{-1} of soil. Throughout this study, the various rhizospheric mechanisms involved in the P mobilization for the plant nutrition were assessed by diffusive phosphate ion (Pd), soil acidification by pH decrease, organic anion complexation inducing a low Al and Fe content, and mineralization of organic P through phosphatase. The gross amount of diffusive Pi (Pd) was determined as a function of Cp and time (t) by coupling sorption-desorption experiments with subsequent isotopic dilution kinetics

in soil suspensions at steady-state. The Pd vs. (Cp, t) relationships varied significantly between treatments, indicating that roots modify soil properties and consequently re-distribute diffusive Pi between the soil solution and constituents. The Pd values were greater for the rhizosphere soil obtained with V1 after applying 28 mg P kg^{-1}.This could be attributed to a strong re-supplying capacity of the soil solution in Pd along the exchange time leading to a large P nutrition of voandzou. It is concluded that genotypic variability exists among voandzou cultivars for internal adaptation to P deficiency.

1. INTRODUCTION

Phosphorus deficiency remains the main constraint of agricultural production in various soils particularly the soils rich in Al and Fe sesquioxides. The phosphorus nutrition of plants is due exclusively to phosphate ions in the soil solution [1]. Phosphate ions in the soil solution account for 5 to 10% of total P absorbed annually by crops [2, 3]. The remaining quantities come from P linked to soil components, particularly sesquioxides and clays which permanently supply the soil solution of phosphate ions [4]. The bioavailability concept integrates the existence of a zone strongly influenced by plant roots in the soil. This soil-plant interface called the rhizosphere is defined as the soil volume around the living roots which is subject to their actions [5]. Thus, plants adapt to P availability through various biological and physico-chemical mechanisms. The dominant mechanism in soils under long-term cultivation and fertilization of field crops in temperate regions is the molecular diffusion of phosphate ions from the solid phase towards the soil solution [6]. Other biochemical processes that also contribute to the P bioavailability in soil are the excretion of such compounds as proton efflux and organic acids, and exo-enzymes like phosphatases. These excreted products involve such reactions as desorption, dissolution of mineral phosphate, cation complexation in interaction with phosphate ion and mineralization of organic P [5, 7] leading to phosphate ion release.

Many authors developed some devices in order to study closely the rhizospheric mechanisms contributing to P bioavailability. Development of the rhizobox or rhizotron [8–10] made it possible to refine the studies of the rhizosphere by using a thin layer of soil in direct contact with the plant roots, which is considered as the plant rhizosphere. The rhizosphere is the volume of a thin layer of soil immediately surrounding plant roots in which strong root activities exist due to different physico-chemical and biological mechanisms related to nutrient transfer at the soil-solution interface [5, 11, 12].Thus, it distinguishes from the ‘bulk” soil which is not influenced directly by growing roots. Various methods are used to assess the P bioavailability of soil. The conventional methods using chemical extractants are the most used of the current standard analyses because of their rapidity and cost effectiveness. Nevertheless, these chemical extractants can dissolve the P forms, either available or unavailable, for plants [4, 13]. The P extracted with anion exchange membrane or resin, most closely corresponds to the P absorbed by plants until now. Yet it cannot simulate specifically the sorption-desorption reaction of phosphate ions in soils because of the limited capacity of the inorganic P sink, the ions on the membrane surface (Cl^-, F^-, OH^-, CO^-) and the effect on the soil-solution pH.

The isotopic tracers are the tools of choice when the system to be analyzed is made up of multiple compartments and when the transferred quantities are weak compared to the quantities in the receiver compartment [14]. The method of isotopic exchange kinetics consists in surveying the behavior of phosphate ions transfer between the solid and soil solution phases by the marked ions ^{32}P or ^{33}P in the two phase system. The isotopic tracing allows the flux of phosphate ions to be quantified, which is likely to be transferred from the solid phase towards the soil solution and which is difficult to quantify by other methods to

characterize P bioavailability [15]. The results from the rhizosphere and non-rhizosphere soil enable the effect of rhizosphere mechanisms in terms of the P bioavailability to be estimated.

Legumes have been considered for a long time as an alternative technology for soil fertility restoration. In order to study the contribution of rhizosphere mechanisms which intervene in P bioavailability, our study focused on the legume voandzou (*Vigna subterranea*) inoculated with *Bradyrhizobium* sp. Vigna CB756. Following a previous selection of lines contrasting in P use efficiency for their nitrogen symbiotic fixation, two contrasting lines were grown in a rhizotron to compare rhizosphere and non-rhizosphere soils, with the isotopic technique using ^{32}P. The aim of this study was to assess the contribution of rhizosphere mechanisms which are involved in the P bioavailability and to test the genotype variability of voandzou.

2. MATERIALS AND METHODS

2.1. Experimental design

In order to study the voandzou effect on biochemical parameters at the rhizosphere scale, an experimental design of rhizotron culture was set up under glasshouse conditions. This device consists of separating a thin layer of soil from roots through use of a porous membrane whose mesh allows solute exchange between soil and roots while keeping roots from penetrating into the soil compartment [8–10]. Approximately 200 g of soil was put in a polyamide bag (45 x 20 cm) with 30 µm of mesh (Nytrel 0.2SPN, Fyltis-U.G.B., Lyon, France) to obtain a 1 mm thick soil layer to serve as the rhizosphere for the whole plant. Indeed, previous studies have shown that rhizospheric modification appears within 1 mm around the root area [16]. The plant roots were spread over the polyamide bag, thereafter rolled up and inserted into a 50 cm long PVC column. A filter paper ensured the connection between soil and the soil solution supplied at the basis of the column. Three tubes ensured soil oxygenation with compressed air at 400 ml^{-1} min^{-1}. The rhizotron columns were vertically maintained during the duration of the experiment. This system allowed an easy access to the collection of voandzou rhizosphere samples by avoiding root deterioration during soil separation by agitation.

The studied soil was sampled at 0-20 cm depth in Casevieille, France. It is a fersiallitic, chromic Cambisol according to the FAO-UNESCO classification [17]. This soil was marked by its high total P content with a low available P content (Table 1).

2.2. Culture conditions

Two contrasting cultivars of voandzou, previously selected among six cultivars for their SNF ability for the PUE, were chosen for the rhizotron culture. Phosphorus supplies for each treatment were 30 and 75 µmol KH_2PO_4 $plant^{-1}$ $week^{-1}$ corresponding respectively to 28 and 70 mg P kg^{-1} of soil. The experiment was carried out under glasshouse conditions with 20 / 33°C temperature during 16 / 8 h day / night cycle, with additional illumination of 400 µmol photons m^{-2} when needed, and 70% daily relative humidity.

The seeds were sterilized with 3% calcium hypochlorite for 20 min and rinsed by 5 washings with sterile distilled water. Thereafter, the sterilized seeds were transferred for germination in an incubator at 28-30°C on humidified wrapped filter paper in a slightly tilted vat in which humidity was controlled regularly.

After germination, the seeds were inoculated by soaking the roots for 30 min in a suspension of 100 ml of *Bradyrhizobium* sp. Vigna CB756 containing 10^9 bacteria ml^{-1}. The inoculum was prepared from a rhizobial culture preserved in tubes at 4°C on 120°C sterilized agar YEM (Yeast Extract Mannitol) medium: 900 ml distilled water; 100 ml of Bergersen concentrated solution containing 1 g KCl, 0.1 g $FeCl_3$, 0.4 g $CaCl_2$, 4.5 g $Na_2HPO_4.12H_2O$, 1 g $MgSO_4.7H_2O$, 1 g Yeast extract, 10 g mannitol and 15 g agar [18]. Four days after sowing the plantlets were transferred to a 40 l vat with 20 inoculated plants vat^{-1}, the roots being carefully passed through the hole of a rubber stopper with cotton wool fixed at the hypocotyl level, and grown with the following nutrient solution: $CaCl_2$, 1650 µM; $MgSO_4.7H_2O$,1000 µM; K_2SO_4, 700 µM; Fe EDDHA, 8.5 µM as sequestrene Fe; H_3BO_3, 4 µM; $MnSO_4.H_2O$, 6 µM; $ZnSO_4.7H_2O$, 1 µM; $CuSO_4.7H_2O$, 1 µM; $Na_2MoO_4.7H_2O$, 0.1 µM. The solution was changed every 2 weeks. Urea (2 mM) was supplied during the first two weeks. P was supplied in the form of KH_2PO_4 with an exponential distribution during successive weeks.

Four weeks after transplanting, the plants were transferred in the columns for the rhizotron culture. The two P level, 28 and 70 mg P kg^{-1} of soil, were supplied according to the exponential distribution of hydroponic culture until the 6th week. Three soil thin layers without plants were prepared with the same nutritive solution in order to be used as a control or non-rhizosphere soil ("bulk soil"). The plants were harvested after 12 days of soil contact. Shoot and root biomass were oven-dried at 60°C. Soil samples were preserved in the refrigerator at 4°C in order to keep them fresh for the analyses.

TABLE 1. PHYSICO-CHEMICAL PROPERTIES OF THE SOIL

Parameter	Value
Clay (%)	48,60
Fine silt (%)	21,80
Coarse silt (%)	17.80
Fine sand (%)	11.60
Coarse sand (%)	3.00
pH H_2O	7.2
pH KCl	6.1
βs (µmol OH^{-1}(q soil)$^{-1}$ (pH unit)$^{-1}$)	53.73
$CaCO_3$ (g kg^{-1})	1.48
Organic matter (g kg^{-1})	53.70
Organic carbon (g kg^{-1})	31
CEC (cmol (+) kg^{-1}) (Cobaltihexamine)	25
Ca^{2+} (cmol (+) kg^{-1}) (Cobaltihexamine)	22.30
Na^+ (cmol (+) kg^{-1}) (Cobaltihexamine)	0.12
Mg^{2+} (cmol (+) kg^{-1}) (Cobaltihexamine)	0.98
K^+ (cmol (+) kg^{-1}) (Cobaltihexamine)	0.21
P total (mg kg^{-1})	960
P ass (mg kg^{-1}) (P Dyer)	7.90
P Olsen (mg kg^{-1})	5.31
P $CaCl_2$ (mg kg^{-1})	0.06
N total (g kg^{-1})	2.88
C: N	10.80
Fe (cmol (+) kg^{-1}) (Cobaltihexamine)	<0.005
Fe (g kg^{-1}) (Ammonium oxalate)	1,63
Mn (cmol (+) kg^{-1}) (Cobaltihexamine)	0.013
Al (cmol (+) kg^{-1}) (Cobaltihexamine)	0.042
Al (g kg^{-1}) (Ammonium oxalate)	4.58

2.3. Soil analysis

Acid phosphatase activity, in particular phosphomonoesterase release by roots and soil micro-organisms, was measured from para-nitrophenyl-phosphate (*p*-NPP) hydrolysis in the soil according to method initially developed by [19] as modified by [20, 21] with aliquots of fresh soil immersed in the buffer solution at pH 6.5 and incubated at 37°C during 1 h. After filtration, absorbance was determined spectrophotometrically at 400 nm. Enzyme activities were expressed as µmol *p*-NPP g^{-1} soil h^{-1}. Measurement pH was in 1:5 ratio of soil: water [22]. Soil solution concentration of PO_4 ions was determined using the malachite green colorimetric method at absorbance 610 nm [23]. Total P content of soil and plant, was measured by colorimetry following drying, ashing and acidification.

2.4. Diffusive PO_4 ions transfer from the soil solid phase to the solution phase

The dynamic of diffusive PO_4 ions (Pd) was determined using the isotopic exchange method by sorption-desorption and subsequently following the isotopic dilution [24–26]. The experiment was carried out on a fresh soil suspension preserved at 4 °C at equilibrium. Three series of five amounts of P between 0 and 150 µg P g^{-1} soil, depending on the treatment, were added as KH_2PO_4 to the soil suspension to obtain a final concentration between 0.01 to 5 mg P l^{-1} in the soil solution, and to create a range of PO_4 ion concentrations in the soil solution allowing the determination of the diffusive PO_4 ions flux. Soil suspensions were shaken on a shaking table for 40 h to reach a steady state for the subsequent hours during which isotopic dilution analysis was carried out. To the soil suspension was added 0.1 ml of biocide (toluene) to avoid microbial activity, and isotopically labeled by introducing 0.1 ml of ^{32}P-labeled PO_4 ions with radioactivity (R) between 0.1 – 10^6 Bq ml^{-1} at time zero, and then shaken at 4°C. This tracer was uniformly and instantaneously dispersed. The amount R of carrier-free ^{32}P ions was negligible compared to total amount of P ions in solution, which was 10^5 to10^6 times higher, and did not disturb the equilibrium state of the soil suspension. After 4, 40 and 400 min, 2.5 ml of soil suspension were sampled with a plastic syringe and immediately filtered through a 0.2 µm membrane. The remaining radioactivity (r) of the soil suspension was counted by a liquid scintillation cocktail (Insta-gel Plus Packard, PerkinElmer, Boston, MA) using a liquid scintillation counter (Packard TR 1100, PerkinElmer, Boston, MA).

The amount of isotopically exchangeable P (E) includes both Pw as the amount of PO_4 present in the soil solution, and Pd as the gross amount of diffusive PO_4 ions transferred between solid and liquid phases. Pw (mg kg^{-1}) was calculated as follows:

$$Pw = (V/M)*Cp = 10*Cp \quad (1)$$

with V, the volume of distillated water (ml); M, the weight of soil; Cp, solution PO_4 ions concentration. The gross amount of diffusive PO_4 ions from the solid phase to the soil solution (Pd) was determined through the isotopic dilution principle: the isotopic composition ratio (IC) of PO_4 in the soil solution (Pw) is equal to IC in the solid phase (Pd). The amount of unlabeled soil PO_4 newly transferred to the solution can be measured by determining the amount of unlabelled PO_4 ions in solution (Pw) and the isotopic composition (IC) ratio:

$$r_t / Pw = (R - r_t) / Pd = R / E \quad (2)$$

with r, the radioactivity remaining in solution; R, the radioactivity introduced initially, r / Pw and (R – r) / Pd, the IC ratio of Pw and Pd.

The Pd value can be deduced from Eq. 2, giving:

$$Pd_{(t)} = \{Pw\ (r_t / R)^{-1}\} - Pw \qquad Pd_{(t)} = Pw\ \{(1 / (r_t/R)^{-1}) - 1\} \qquad (3)$$

Pd variation as a function of time and Cp was fitted using the kinetic Freundlich equation (Eq. 4)

$$Pd = \nu Cp^{w} t^{p} \quad \text{with } Pd < P \text{ mineral} \qquad (4)$$

with ν, the Pd value for t = 1 min at 1 mg P l^{-1}; w, the nonlinear increase in Pd values with Cp; *p,* the nonlinear increase in Pr values with time.

2.5. Statistics

The cultivar and treatment effects on soil characteristics were analyzed by a two-way analysis of variance (ANOVA). Estimates of ν, w and p kinetic Freundlich parameters were obtained using the nonlinear procedure: Proc Mixed of Statistical Analysis Software SAS [27]. PCA analysis was performed on nutrient content of the soil aliquot, kinetic Freundlich, soil and plant parameters in order to highlight the rhizosphere effect at the voandzou cultivars under limited on no available P.

3. RESULTS

3.1. Plant biomass and P concentration

Biomass was slightly affected by P supply, in particular for cultivar 1. Shoot biomass values varied from 1.34 to 1.54 g $plant^{-1}$ and root biomass from 0.56 to 0.79 g $plant^{-1}$ (Fig. 1). Similar P concentrations were observed for root and shoot biomass, around 1600 mg P kg^{-1}, except for cultivar 4 with 28 mg P kg^{-1} soil (Fig.1). P supply significantly increased the plant nodulation ($P< 0.001$; $R^2 = 0.50$) in particular for cultivar 1.

3.2. Solution PO_4 ions concentration, pH, and enzymatic activity of soils

P supply led to a significant response of soil solution PO_4 ions concentration (Cp) at two cultivars ($P<0.001$; $R^2 = 0.79$). The Cp at 70 mg P kg^{-1} of soil was ten times higher compared to 28 mg P kg^{-1} of soil at two cultivars (Fig. 2). In addition, a significant difference was observed between rhizosphere and non rhizosphere soil at the two P supplies. A high phosphatase activity was observed in “bulk soil” in comparison to rhizosphere soil. More microbial activity was highlighted under minimal P supply as 28 mg P kg^{-1} soil, compared to 70 mg P kg^{-1} soil, particularly for cultivar 1 (Fig. 2). The pH decreases of rhizosphere and bulk soil were from 7.3 to 7.1 according to P supply (Fig. 2) with the lowest value at 28 mg P kg^{-1} soil.

3.3. Nutrient composition of the soil solution

The nutrient composition in the soil solution of dry soil revealed the superiority of low added P (28 mg P kg^{-1} soil) in terms of NO_3-N, total N, Ca, Mg, Na and Cl (Table 2). Otherwise, the high P supplied as 70 mg P kg^{-1} soil was higher in terms of Al, Fe, K, Si and SO_4^{2-}-S. Cultivar 1 was marked by the lowest values of some parameters, in particular pH, organic C, total C, NH_4-N, total N, Mg, K, Cl and SO_4^{2-}-S. Results from a Principal Component Analysis of nutrient content in soil solution with plant and soil parameters summarize these observed trends (Fig. 3).

3.4. Dynamic calibration of the diffusive PO_4 ions (Pd) by the kinetic Freundlich equation

The isotopically exchangeable P transfer between the solid and soil solution phases were closely related to Cp and the isotopic exchange time (Fig. 4). The increase of P supply in the rhizosphere and bulk soil suspensions increased Cp for the two cultivars. Increases in diffusive PO_4 ion transferred from solid towards soil solution (Pd) phases were observed when soluble P at different rates was applied with exchange times of 4, 40 and 400 min. Pd values increased from 4 to 400 min at the same soil solution PO_4 ion concentration, while increasing Cp produced a further increase in Pr after addition of soluble P.

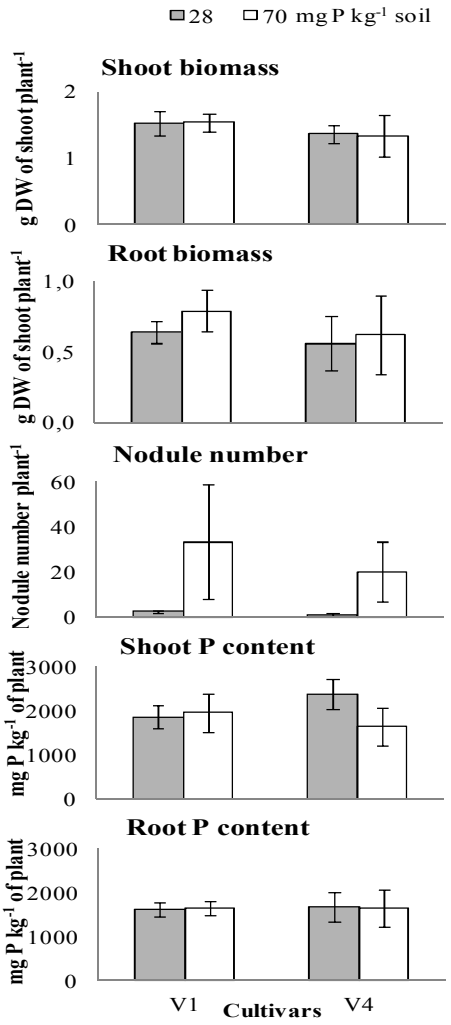

FIG. 1. Shoot and root biomass, nodule number and P concentrations of shoots and roots. Data represent the mean of 4 replicates at 45 days after transplanting. Errors bars represent standard errors.

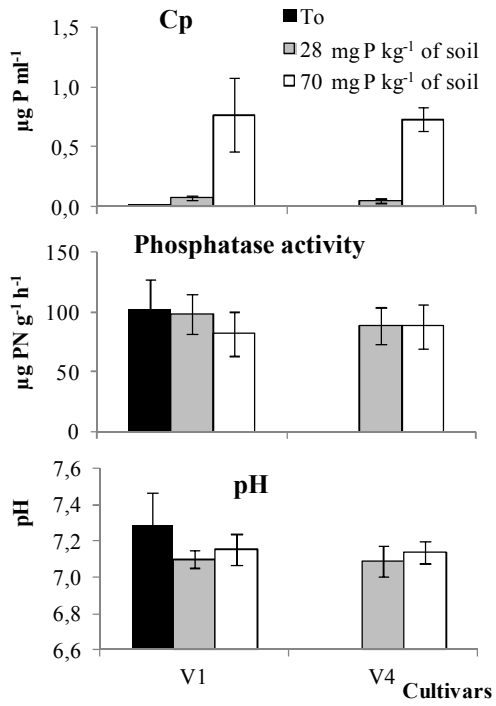

FIG. 2. Biochemical parameters of rhizosphere and bulk soil in terms of pH, phosphatase activity and Cp under two P supplies. The data represent the mean of 4 replications at 45 days after transplanting. Error bars represent standard errors.

TABLE 2. NUTRIENT CONCENTRATIONS IN THE SOIL SOLUTION FOR TWO CULTIVARS AT THREE LEVELS OF APPLIED P (0, 28 AND 70 MG P KG^{-1} SOIL)

Parameter†	Cultivar 1 at mg P kg^{-1} soil		Cultivar 4 at mg P kg^{-1} soil		O mg P kg^{-1}
	28	70	28	70	soil
pH	7.6	7.5	7.8	7.7	7.8
Organic C	50.2	49.5	70.8	83.6	60.0
Total C	58.9	59.6	79.7	92.8	70.2
NH_4^+-N	2.1	1.7	2.5	2.4	2.5
NO_3^--N (1)	1.9	1.1	1.6	1.3	2.2
Total N	6.2	5.3	6.5	6.2	5.7
Al	0.26	0.63	0.10	0.41	0.37
Ca	29.5	21.9	30.5	21.9	25.6
Fe	0.09	0.19	0.03	0.15	0.14
Mg	1.3	0.9	1.5	1.0	1.4
K	2.8	4.5	3.0	4.6	2.3
Si	0.1	0.4	0.1	0.4	0.1
Na	6.2	4.2	6.0	4.0	4.3
Cl	34.5	22.5	36.2	25.0	18.9
NO_3^--N (2)	3.3	3.3	3.3	0.05	3.2
SO_4^{2-}-S	4.9	4.4	5.2	6.7	4.4

†Units are mg l^{-1}; NO_3^--N (1), spectrocolorimetric method; NO_3^--N (2), ion exchange chromatography method

In order to assess the influence of rhizosphere activities on phosphate ion transfers between solid and soil solution phases, variations of Pd, Cp and time values according to P supply levels were fitted through a regression function called the kinetic Freundlich equation: $Pd = \nu Cp^w t^p$ (Table 3). The gross amount of Pi that diffuse in 1 min when the Cp value is 1 mg P l^{-1} , the ν parameter, significantly decreased at the lowest added P (28 mg P kg^{-1} soil),

being 77% lower than at the highest added P (70 mg P kg^{-1} soil) for all cultivars. A significant variation was observed for the ν parameter between rhizosphere and bulk soil, marked by a significant reduction for cultivar 1 with 28 mg P kg^{-1} soil as P supplied (Table 3). No significant difference was observed for the other parameters. However, w and p parameter values at low P supply were higher than the high P supply especially for cultivar 1.

4. DISCUSSION

A genotypic variation between the voandzou cultivars was observed, marked by a large P mobilization for cultivar 1 under the lowest rate of added P. This is in agreement with previous results for symbiotic nitrogen fixation optimization, P nutrition and genetic improvement among legume species through genotypes with effective rhizobial strains [28–31]. This genotypic variation was highlighted at the rhizosphere scale. It is known that growing roots absorb phosphate ions and diffusion is the main rhizospheric mechanism of Pi that contributes to replenish Pi in solution [1]. Thus, the plant available P was assessed by measuring both soil solution Pi concentration and the dynamics of diffusive Pi between solid and soil solution phases. Soil solution Pi concentrations were highest for the highest added P rate for all cultivars. By comparing the biomass and the uptake of P by plants, it seems that legumes used more soil and fertilizer P for biomass production under a low P availability compared to a high P availability.

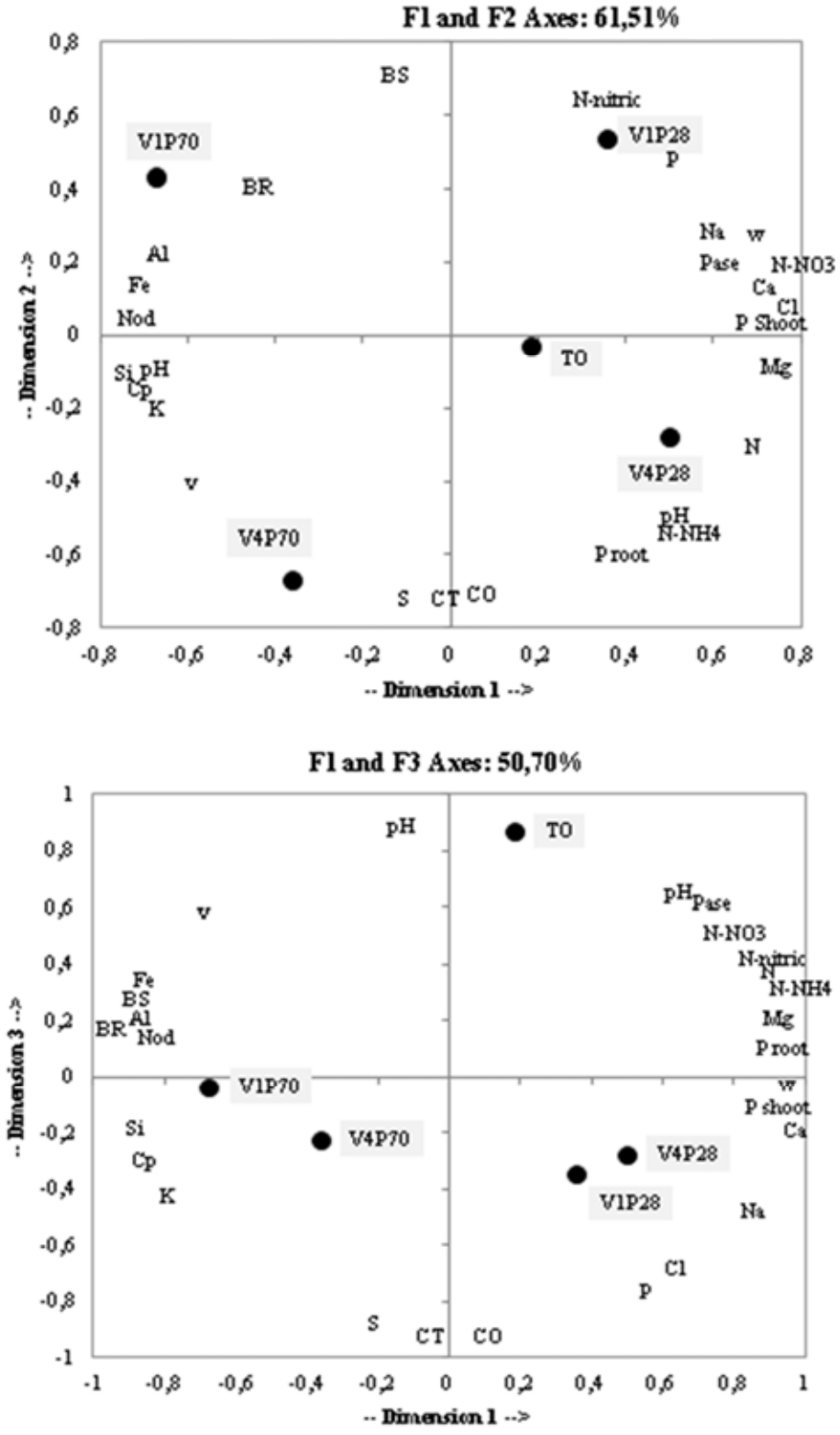

FIG. 3. Principal Component Analysis of nutrient content in soil the solution. V1P28, cultivar 1 with 28 mg P kg^{-1} soil; V4P28, cultivar 4 with 28 mg P kg^{-1} soil; V1P70, cultivar 1 with 70 mg P kg^{-1} soil; V4P70, cultivar 4 with 70 mg P kg^{-1} soil; BS, shoot biomass; BR, root biomass; P shoot, shoot P content; P root, root P content.

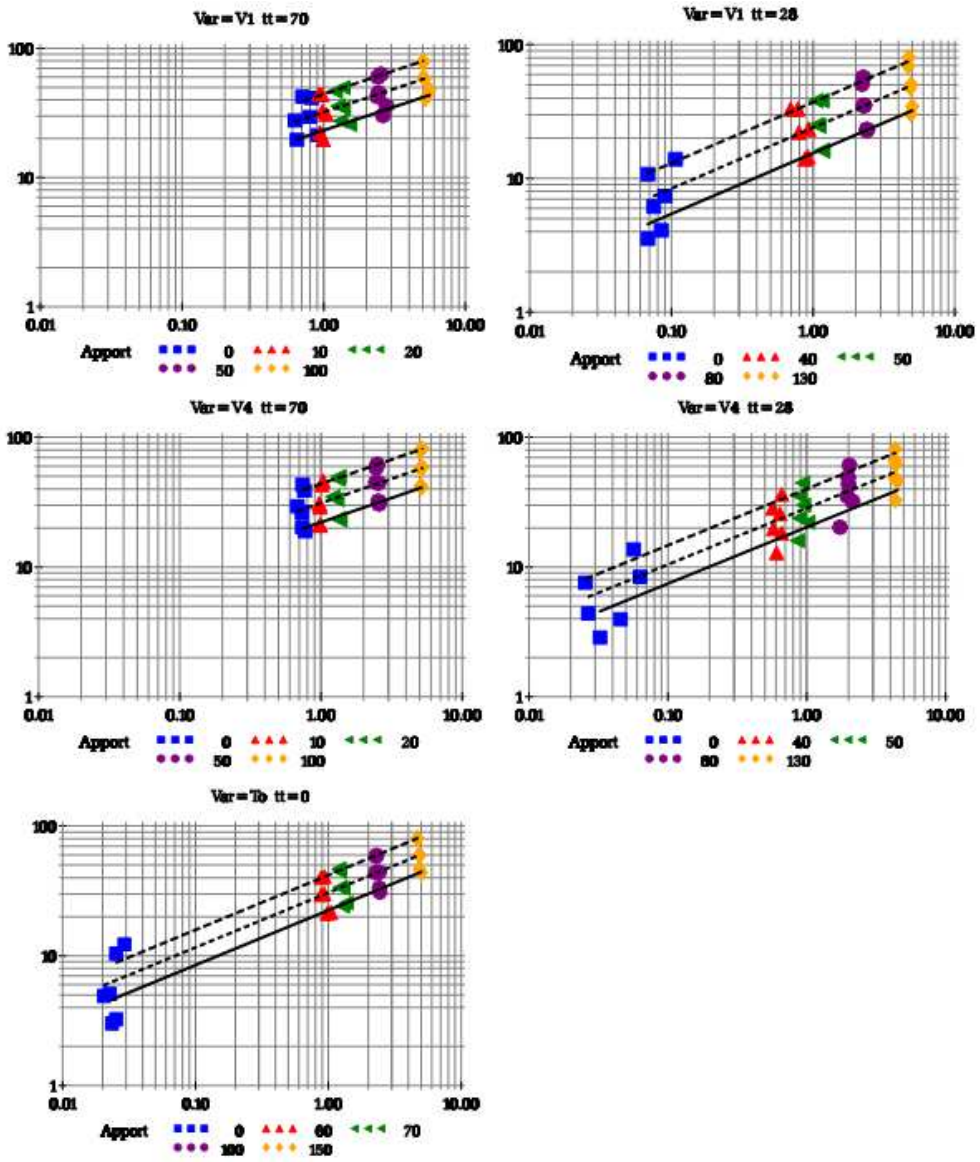

FIG. 4. The gross amount of diffusive phosphate ions (Pd) in relation with the soil solution Pi concentration (Cp) for the five rates of soluble P and the different periods of isotopic dilution (4, 40 and 400 min) for the different treatments in cultivar V1 and cultivar V4 under 28 and 70 mg P kg^{-1} of soil . Y-axis (Pd) and X-axis (Cp) are represented in log-log scales. Regression lines represent the three periods of isotopic dilution, 4, 40 and 400 min. P supply levels for the determination of diffusive PO_4 ions flux: 0, 10, 20, 50, 100 µg P g^{-1} soil for 70 mg P kg^{-1} soil; 0, 40, 50, 80, 130 µg P g^{-1} soil for 28 mg P kg^{-1} soil ; 0, 60, 70, 100, 150 µg P g^{-1} soil for 0 mg P kg^{-1} soil.

Modeling of diffusive PO_4 ion dynamics at the soil-solution interface showed that ν parameter had the lowest value at 28 mg P kg^{-1} soil, in particular for cultivar 1. The ν parameter is the PO_4 ions likely to supply P through diffusion to the soil solution after 1 min of isotopic dilution at PO_4 ions concentration of 1 mg P l^{-1}. The decrease of the ν parameter compared to "bulk" soil were 36% for cultivar 1 with 28 mg P kg^{-1} soil, 11% for cultivar 4 with 28 mg P kg^{-1} soil and 3% for cultivar 4 with 70 mg P kg^{-1} soil. These values were in contrast with those found for *Pisum sativum* under rhizotron culture, being around 7% lower than the bulk soil [32]. The comparison of this parameter allows an appreciation of the rhizosphere effect on soil physico-chemical properties. The decrease of diffusive PO_4 ions in the short-term, the ν parameter, may be explained by P plant nutrition and efficiency of crops

[33], which is marked by a large shoot and root biomass observed for cultivar 1 under low P supply (Fig. 1).

TABLE 3. MEAN KINETIC FREUNDLICH ($PD = NCP^{W}T^{P}$) PARAMETERS DESCRIBING THE TIME (T, MIN) AND CP DEPENDENCIES ON THE AMOUNT (PD, MG P KG^{-1}) OF PO_4 IONS TRANSFERRED BETWEEN SOIL AND LIQUID PHASES OF RHIZOSPHERE AND NON-RHIZOSPHERE SOIL SUSPENSIONS

Treatments†	v‡	w‡	p‡
V4 P28	16.5 ± 1.16	0.44 ± 0.03	0.15 ± 0.01
V4 P70	18.0 ± 0.37	0.38 ± 0.01	0.14 ± 0.01
V1 P28	11.9 ± 0.40	0.46 ± 0.01	0.19 ± 0.01
V1 P70	19.1 ± 0.43	0.37 ± 0.01	0.14 ± 0.00
TO	18.6 ± 0.32	0.42 ± 0.01	0.14 ± 0.00
Analysis of variance#			
P level (P)	0.038*	0.103ns	0.061ns
Cultivar (C)	0.282ns	0.303ns	0.239ns
(P) x (C)	0.049*	0.663ns	0.147ns

† TO, bulk soil; V1, cultivar 1; V4, cultivar 4; P28, 28 mg P kg^{-1} soil added; P70, 70 mg P kg^{-1} soil added
‡ v, w, p, kinetic Freundlich parameters; ±, standard error of the mean
# *, $P<0.05$; ns, not significant

The $Pd_{(Cp,\ t)}$ values were calculated for each 1-day interval for one week and the exchange rates (µg P g^{-1} day^{-1}) were deduced thereafter (Table 4).

TABLE 4. $PD_{(CP,\ T)}$ VALUES AND THE KINETICS OF THE RATE EXCHANGE IN $PD_{(CP,\ T)}$ DURING THE 1ST WEEK OF EXCHANGE IN RHIZOSPHERE AND "BULK" SOIL

Line	P level	Pd (µg P g^{-1})	Exchange time (day)						
	(mg P kg^{-1} soil)	Rate (µg P g^{-1} d^{-1})	1	2	3	4	5	6	7
V4	28	Pd (0.052. *t*)	13.37	14.82	15.73	16.42	16.97	17.43	17.84
V4	28	Rate	13.37	1.44	0.92	0.68	0.55	0.46	0.40
V4	70	Pd (0.73. *t*)	46.93	52.00	55.21	57.62	59.55	61.18	62.59
V4	70	Rate	46.93	5.07	3.22	2.40	1.93	1.63	1.41
V1	28	Pd (0.08. *t*)	14.68	16.75	18.09	19.11	19.94	20.64	21.25
V1	28	Rate	14.68	2.07	1.34	1.02	0.83	0.70	0.61
V1	70	Pd (0.77. *t*)	47.99	52.88	55.97	58.27	60.12	61.68	63.02
V1	70	Rate	47.99	4.89	3.09	2.30	1.85	1.55	1.35
T0	0	Pd (0.02. *t*)	9.44	10.37	10.96	11.39	11.74	12.04	12.29
T0	0	Rate	9.44	0.93	0.59	0.44	0.35	0.29	0.26

In order to analyze the potential release of Pi from the solid phase to the soil solution, the variation effect of Cp change and immediate exchange capacity of PO_4 ions were assessed from diffusive PO_4 ions values from the solid phase towards the soil solution (Pd) through a regression function with the kinetic Freundlich equation during one week. The comparison of the Pr daily increase rate (µg P g^{-1} day^{-1}) during one week deduced from Table 4 allowed close assessment of the effect of root activity on Pr while separating the effect of soluble P added and uptake P on Cp. For all treatments, Pd values increased with time during one week of exchange, while the Pr increase rate per day decreased with the exchange time. Compared to the first day of exchange, the Pr increase rate per day decreased by 3% for all treatments after one week of exchange except for cultivar 1 with 28 mg P kg^{-1} of soil (by 4%).

Similar results were reported during an experiment with maize and pea rhizosphere soil [32]. These results allowed the highest potential release of Pi in the soil solution to be

deduced for cultivar 1 with 28 mg P kg^{-1} soil, according to the exchange time. This may be explained by the rhizosphere mechanism suggesting that cultivar 1 was able to optimize the P assimilation in order to maintain a significant biomass level under the lowest rate of added P. Otherwise, the slight reduction of the ν parameter and the Pr variation could not only be explained by PO_4 ion diffusion but could be attributed to other mechanisms.

The Principal Component Analysis of microelements in rhizosphere and "bulk" soil solution (Fig. 3) with plant measurements showed the grouping of a low P supply (28 mg P kg^{-1} soil) and the bulk soil with biological indicators of rhizosphere effects, in particular NO_3^--N (2), acid phosphatase, shoot and root P concentration, Ca, Na, Cl and Mg, whereas the highest rate of added P (70 mg P kg^{-1} soil) was associated with soil indicators including those related to the soil solid phase such as soil solution Pi concentration Cp, Al, Fe, Si, K, pH, v parameter, nodulation and shoot and root biomass.

Indeed, plants adapt to their environments according to nutrient availability. With a low P level in the soil solution, some plant roots could develop a competitive capacity with the soil solid phase, in particular, by P assimilation at a very low PO_4 ion concentration for natural plant development [33]. Under 28 mg P kg^{-1} soil, plant P concentration and potential release of Pi into the soil solution and shoot biomass were greater for cultivar 1. Plants can mobilize a contrasting P level in biomass in particular for cultivar 1 (Fig. 1). This may be because rhizosphere acidification occurred and might increase the bioavailability of P sorbed onto metal oxides such as goethite. The release of H^+ in the rhizosphere was related to differential uptake of cations and anions by plant roots such as Na^+, Ca^{2+}, Mg^{2+} and to the root excretion of organic acid [33–35]. The legume plants adapt to environmental constraints, such as low PO_4 ions concentration, by the more important root excretions modifying the different biochemical properties of soil [36].

The increase of the organic anion exudation was shown by many authors with different species in response to P deficiency. These organic anions such as malate and citrate can be complexed with solid phase Al or Fe thereby reducing the availability of soluble Al while increasing the P bioavailability [33, 37–39]. This explains the low Al and Fe values observed at the lower P supply in comparison with the higher P supply.

As acid phosphatase was reported to indicate the organic P mineralization potential and biological activity of soils to enhance P availability, the excretion of acid phosphatase could also explain the plant adaptation. Under limited P availability, the increase of acid phosphatase excreted by roots was observed, in particular at the control and the low P supply (Fig. 2) [40]. Indeed, the level of soil orthophosphate ions could inhibit the phosphomonoesterase activities such as organic P and phytate hydrolysis. P supply of 10 µmol orthophosphate g^{-1} soil inhibited from 21 to 42% of acid phosphate activity and from 17 to 51% of alkaline phosphatase activity [41, 42], while a decreased P supply from 10 to 1 µmol of PO_4^{3-} g^{-1} of soil gave increased phosphatase activity resulting from kinetic competitiveness of orthophosphate substrate on enzymatic hydrolysis [41, 42]. A high enzymatic activity, important for organic P mineralization, in particular soil phytate, was a part of the adaptation mechanism of plants in P mobilization in rhizosphere soil, specifically for cultivar 1 with low P supply.

5. CONCLUSION

Plants differ from one cultivar to another with respect to their ability to mobilize, to use and to promote more nutrient availability than others. Our result indicated that a slight modification on the Pi exchangeability from solid to soil solution phases was observed at the lowest added P of 28 mg P kg^{-1} soil, particularly for cultivar 1, which probably occurred by local root-induced changes in pH and root secretion. High P efficiency was shown at the lowest added P level by changing root physiological and biochemical mechanisms and taking up greater amounts of P from the soil. Cultivar 1 was able to use efficiently more P under low PO_4 ions concentration in order to produce enough biomass compared with cultivar 4. The rhizosphere effect, mainly for cultivar, influenced the diffusive PO_4 ions dynamic between solid and soil solution phases under limited P availability. The different rhizospheric mechanisms contributing to the phosphate ions mobilization for the plant PO_4 nutrition were diffusion, acidification, complexation and mineralization. Otherwise, the potential release of Pi between solid and soil solution phases can be assessed by this quantitative approach coupling a sorption-desorption experiments with subsequent isotopic dilution kinetics.

This study showed that genotypic variation existed in the P use of voandzou, a difference which is particularly highlighted under limited P availability.

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EVALUATION OF SOYBEAN AND COWPEA GENOTYPES FOR PHOSPHORUS USE EFFICIENCY

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Abstract

Initial screening of one hundred and fifty-two (152) and fifty (50) genotypes of soybean and cowpea, respectively, were conducted at the early growth stage to evaluate root traits associated with phosphorus (P) efficiency. Fifty soybean genotypes were subsequently selected and evaluated on a tropical low P soil (Lixisol) for growth and yield under low and adequate P availability. Plants were sampled at twelve and thirty days after sowing and at maturity. Six cowpea genotypes were also selected and evaluated in pots filled with Alfisol under low, moderate and high P availability. Plants were sampled at forty days and assessed for shoot yield and nodulation under low P availability. Using Principal Component Analysis (PCA), Phosphorus Efficiency Index (PEI) was used to determine P efficiency of soybean and cowpea genotypes. A wide variation in root traits for soybean and cowpea at the early growth stage was found, and allometric analysis showed a significant correlation between the root and shoot parameters at this stage. The study provided an opportunity to compare root traits of newly developed cowpea genotypes (early maturing, medium maturing, dual purpose and Striga resistant lines) with older released cultivars. There were significant differences in root length among the groups. In general, dual purpose, Striga resistant and medium/early maturing genotypes showed the longest roots while the older varieties showed the least total root length. Field and pot results also showed differential growth of soybean and cowpea with low P availability. Further, PCA of the results indicated that soybean genotypes could be grouped into three distinct P efficiency categories. Retaining the PC and the relative weight for each genotype in combination with yield potential under high P, four categories of responsiveness to P were obtained. Cowpea genotypes were grouped into three P efficiency categories and two categories of responsiveness to P. The study also found genetic differences in nodulation under conditions of P stress. There were large genotypic variations for P uptake under high P levels but not under low P levels. The study showed that there was significant genotypic variation for root traits during early growth and genotypic differences for soybean and cowpea growth under low P.

1. INTRODUCTION

Tropical soils exposed to long periods of weathering have low organic matter, low cation exchange capacity and overall low inherent fertility. At present, the main nutrient limiting factors in sub-Saharan African soils are N and P. While N can be introduced to the soil through various organic inputs, there is no comparable process to N_2 fixation for the introduction of P into farming systems [1].

Soybean and cowpea are important food legumes because they are relatively cheap sources of protein in most developing countries. Soybean contains 30-50% protein while

cowpea contains between 21-23% protein [2]. Additionally, they fix atmospheric N_2, a fundamental process in soil nutrient management practices. In soybean it is estimated that 25–85% of its total shoot N content can come from fixation [3]. Despite their importance, yields of both legumes are extremely low. For example, cowpea grain yields on average farmers' fields are only half compared to yields obtained on researcher-managed on-farm fields [4]. Low soil availability of P accounts for low productivity in legumes [5].

Low P status in Ghanaian soils has been extensively documented [6–10]. Two main reasons may explain this occurrence, firstly P concentrations in the soil solution are generally low (<0.05 μg ml^{-1}) compared to nitrate-N concentrations (100 μg ml^{-1}) [11]. Anne and Lal [12] reported that soil available P in soils of West African is well below the critical level of 10.8 mg kg^{-1} required for grain legume production. Secondly, soils, especially those with low pH, have a high amount of soluble Fe and Al which react with P and render it insoluble and unavailable for plant use [13]. This is further compounded by the fact that, unlike N which comes to plants by mass flow (i.e., soil water moves towards the roots as the plant loses water through transpiration), P is absorbed mainly by diffusion through gradients created by root uptake, which means that plant roots have to grow to come into contact with new soil from which they can extract P. Root characteristics are therefore an important factor in P acquisition. A larger root system provides greater root-soil contact and thereby higher uptake of soluble P especially under low P availability [11].

The application of P amendments to these soils can greatly improve production levels, but non-availability and high costs limit their use practically by a majority of smallholder farmers. Reports indicate that a great percentage of Ghanaian farmers do not use inorganic fertilizers to increase the soil nutrient availability [14, 15].

In crops, roots are the vital structure for water and nutrient uptake, but data on root traits are rarely included in most screening and final breeding programs. Several researchers have shown that root traits are important in enhancing the root system and consequently nutrient uptake. For instance, Vieira et al. [16] reported that root hairs are important in P uptake, and lateral roots have been reported to enhance top soil foraging of P [17]. A better understanding of root physiological traits that improve P acquisition especially under low P stress would facilitate selection of more P efficient crop genotypes which would be important in P deficient soils [18]. It would therefore be desirable to discover crop genotypes which can access a greater proportion of the total soil nutrient that may otherwise be unavailable to them [19].

The objectives of this study were therefore to:

a) Characterize root and other traits of cowpea and soybean which contribute to efficient P uptake in low P soils using established analytical protocols at the seedling stage.
b) Evaluate the performance of genotypes with different root traits under limiting P conditions.
c) Evaluate whether genotypes with different root traits result in increased biomass production and grain yield under conditions of varying P availability

2. MATERIALS AND METHODS

In a study conducted at the Crop Science department, University of Ghana (UG), Legon, one hundred and fifty-two (152) soybean genotypes were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, and the Crop Research Institute (CRI), Fumesua, Ghana. Fifty cowpea genotypes were also obtained from IITA and

the Crop Science Department, University of Ghana (UG), Legon. The 50 cowpea genotypes included 18 early maturing, 6 medium maturing, 10 Striga resistant and 6 dual purpose genotypes obtained from IITA, and 10 from the Crop Science Department, UG. The latter from UG were improved varieties which have been released and grown extensively in most of the agro-ecological zones of Ghana. Seeds of each genotype were surface sterilized in 0.5% NaOCl for one minute rinsed and sown into PVC tubes of length 15.0 cm and 3.4 cm in diameter. The tubes contained a mixture of sawdust and rice husk in the ratio of 1:1 by volume. The tubes were placed in aluminium troughs containing 0.5mM $CaSO_4$ solution. Each genotype was replicated six times. The set-up was placed in a screen house and watered daily with tap water. Five days after planting (DAP) the seedlings were removed from the medium and shoots cut from roots. The roots were washed free of the substrate and growth parameters including primary root length, number of lateral roots on the primary root and total root length were evaluated. Root length was determined using a modified line-intersect method [20]. Counts were made on the intercept of roots with 2 cm vertical and horizontal grid lines with the aid of a tally counter. Counts were converted to length measurements using the formula:

$$\text{Root length(R)} = \text{number of intercepts} \times \text{length conversion factor}$$

where length conversion factor for the 2 cm grid square was 1.5714.

Data were also collected for shoot growth parameters and subjected to Analysis of Variance (ANOVA) with the differences between means compared using the least significant difference (LSD). Principal Component Analysis was performed to evaluate inter- and intra-specific differences among genotypes.

Fifty soybean genotypes were selected and used in a field experiment conducted at the UG farm, Legon. The soil at the experimental site was classified as Adenta series under the Ghanaian classification, and Vertic Lixisol under the FAO classification. For characterization, undisturbed soil samples were taken from 0-10 cm and 10-20 cm of the profile on April 30, 2008, and mixed to form a composite sample (about 5 kg). The soil was analysed for pH in water (electrometric), total N [21], organic carbon [22] and available P (Bray I), K, Ca and Mg were determined by extraction with NH_4OAC. Seeds of all the 50 genotypes were sown on May 8, 2008 in double rows one metre long. There were two levels of phosphorus, 0 and 30 kg P ha^{-1}. Triple super phosphate was applied in furrows along the individual rows of soybean, 5 DAP. At the same time, potassium was applied as muriate of potash at the rate of 30 kg ha^{-1} to all soybean rows. The combination of soybean genotypes and P treatments was replicated three times. Twelve and thirty DAP, four plants from each genotype were harvested for the determination of number of roots, root dry weight and shoot dry weight. Pod number per plant, 100 seed weight and seed yield were measured on 10 plants at maturity, when pods had dried and turned brown. Pods were removed from individual plants and counted. Pods were shelled by hand and the weight of seed determined at 12% moisture.

Six cowpea selected genotypes (IT03K-351-1, IT00K-901-5, IT93K-452-1, IT98K-1263, IT97K-819-118, Asontem) were evaluated in a pot experiment. The soil used for the study was Toje series (Ghanaian classification), or Alfisol (FAO classification). A sample of the soil was obtained from 0-15 cm depth from an uncultivated area at the UG, Legon, and characterized for its physical and chemical properties. Six litre capacity pots were filled with 5 kg P deficient soil, which was previously sieved with a 2 mm sieve. Three P treatments, as Triple Super Phosphate, were administered; P at 40 kg ha^{-1} (P_{40}), P at 20 kg ha^{-1}(P_{20}) and no P (P_0). The experimental design was a factorial randomized complete block with four

replications. Because of the laborious nature of root harvest and analysis, the experiment was divided into two plantings. A basal N application of ammonium sulphate at a rate of 50 kg–N ha^{-1} was applied before planting. Hoagland's plant nutrient solution, not containing P, was prepared by dissolving 0.4925 g $CaCl_2.2H_2O$, 1.875 g K_2SO_4, 3.075 g $MgSO_4.7H_2O$, 0.8043 g Fe (III) Na EDTA, 0.0155 g H_3BO_3, 0.009 g $MnCl_2.4H_2O$, 0.0008 g $CuSO_4.5H_2O$ and 0.0002 g $H_2MoO_4.H_2O$ in 1 litre of distilled water. The solution was adjusted to pH 5.5-6.0 with 0.1N NaOH and 20 ml applied to each pot 8 DAP. This was repeated weekly together with application of tap water three times a week.

Plants were harvested by cutting the shoot at the soil surface. Leaves were separated, counted and leaf area measured using an AM 100 leaf area meter (Delta-T Devices Burwell, Cambridge). Roots were retrieved by washing under running water. Nodules were counted dried and weighed. All plant parts were dried at a temperature of 65˚C for 48 h and milled for determination of N and P.

Phosphorus efficiency of soybean genotypes was determined by phosphorus efficiency index (PEI) [23], and assessed using principal component analysis (PCA) of standardized values of plant biomass and seed yield factors at low P, and relative values at low P to those obtained under high P supply. In cowpea, phosphorus efficiency was also assessed for nodulation under limiting P.

3. RESULTS

3.1. Soils used to evaluate soybean and cowpea genotypes for phosphorus efficiency

Characterization of the soil used for the soybean and cowpea genotypes is shown in Table 1. In common with most soils found in Ghana, the level of available P the soils used for the study were low. Similarly the total N was also low consistent with the relatively low OM status of the soil.

TABLE 1. PHYSICAL AND CHEMICAL CHARACTERISTICS OF SOILS

Parameter	Classification and properties	
FAO Classification	Lixisol	Alfisol
Ghanaian Classification	Adenta Series	Toje Series
Sand : Silt: Clay (%)	64: 6: 30	70: 4: 26
Bulk density (Mg m^{-3})	1.16	1.22
pH (KCl: H_2O)	5.3: 5.5	5.0
Organic C (g kg^{-1})	5.8	6.5
N (%)	0.08	0.08
Total P (mg kg^{-1})	95.5	201
Available P (mg kg^{-1})	5.8	7
Al Saturation (%)	18.5	16.0
Fe Saturation (%)	6.5	6
CEC (cmol kg^{-1})	2.9	2.8

3.2. Shoot and root characteristics of soybean and cowpea at early growth stage

There were significant differences among soybean and cowpea genotypes for the following parameters measured at the early growth stage; total root length, root number, root weight, shoot weight and root: shoot ratio within each group of soybean and cowpea genotypes (Table 2). The number of lateral roots in soybean genotypes varied from 4 to 50 with a mean of 30 while in cowpea it varied from 9 to 66. Tap root length varied between 5.1 and 18 cm. The average tap root length of cowpea genotypes was 13.1 cm. The lateral root density was obtained by dividing the tap root length by the number of lateral roots counted. There were no significant differences found among the genotypes in lateral root density.

There were significant differences in root biomass among soybean genotypes. However, cowpea showed no significant difference. Even though a high coefficient of variation was observed in root parameters of cowpea genotypes compared to soybean genotypes, cowpea genotypes obtained the highest number of lateral roots and total root length. This indicates that cowpea had more extensive root growth compared to soybean. Principal component analysis was conducted on soybean and cowpea genotypes using 5 plant parameters measured. The first two PCs accounted for 83% of the variability observed (Table 3). PC1 represented 60% whiles PC2 contributed 23% of the observed variability.

TABLE 2. ROOT AND SHOOT CHARACTERISTICS OF SOYBEAN AND COWPEA GENOTYPE 5 DAP

Trait	Soybean			Cowpea		
	Mean	Range	CV (%)	Mean	Range	CV (%)
Tap root length (cm)	-	-	-	13.1**	5.1-18.0	22.7
Number of lateral roots	30***	4–50	22.3	41**	9–66	36.0
Total root length (cm)	57.1***	24.9–103.1	24.9	103.6*	6.2–258.6	56.2
Lateral root length (cm)	–	–	–	100.3*	2.0–240.5	56.4
Lateral root density (number of roots/cm)	–	–	–	3.3ns	1.3–5.3	40.2
Root fresh weight (g)	0.3***	0.1–0.5	24.0	0.30ns	0.10– 0.50	56.1
Shoot fresh weight (g)	0.6***	0.3–1.2	15.0	0.67**	0.2– 1.2	18.6
Root: shoot ratio	0.46***	0.15–0.81	15.4	0.45ns	0.1–1.3	43.5
Total biomass (g)	0.92***	0.33–1.64	15.2	0.96***	0.52–1.36	23.9

*; $P<0.05$, **; $P< 0.01$, ***; $P< 0.001$

TABLE 3. EIGEN VALUES AND PROPORTIONS OF TOTAL VARIATION AMONG SOYBEAN GENOTYPES (N = 152) AND COWPEA GENOTYPES (N = 50) AS EXPLAINED BY THE FIRST TWO PRINCIPAL COMPONENTS

Parameter	F1	F2
Eigenvalue	3.62	1.37
Variability (%)	60.37	22.86
Cumulative %	60.37	83.23

There was wider variation observed in cowpea compared with soybean genotypes (Fig. 1) even though 50 genotypes in cowpea were studied compared with 152 in soybean. 86% of the cowpea genotypes had lateral root numbers higher than 30 which was the average for soybean genotypes, while only 2% of soybean genotypes had lateral root number higher than 41 which was the mean among cowpea genotypes.

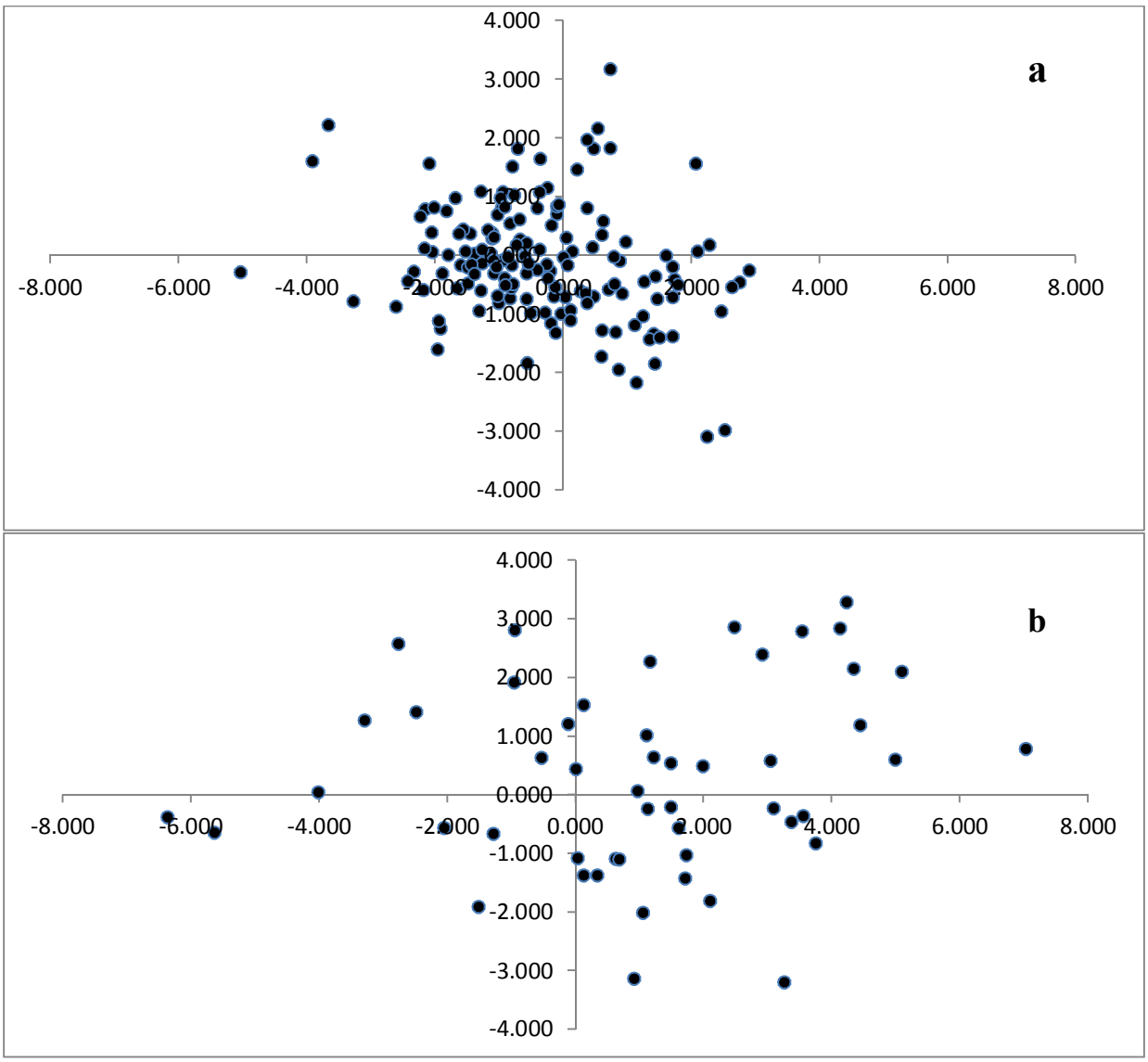

FIG. 1. Genotypic variation in roots and shoot parameters of soybean (a) and cowpea (b) as given by the first two principal components.

To eliminate the effect of variation in plant size the data were log transformed to show the allometric relationship between parameters more or less independent of the size of the plants (Fig. 2 and Fig. 3). There was strong allometric relationship between shoot weight and total root length and root weight in soybean and cowpea genotypes. Results show a similar allometric partitioning coefficient among soybean and cowpea genotypes.

The study also provided an opportunity to compare newly developed genotypes of cowpea [early maturing (EM), medium maturing (MM), Dual purpose (DP) and Striga resistance (SR) genotypes] with older genotypes released previously (ER). There were significant differences in root length among the genotypes. In general, EM genotypes had greatest tap root length followed by the SR and the DP genotypes (Table 4). The ER genotypes had the least taproot length.

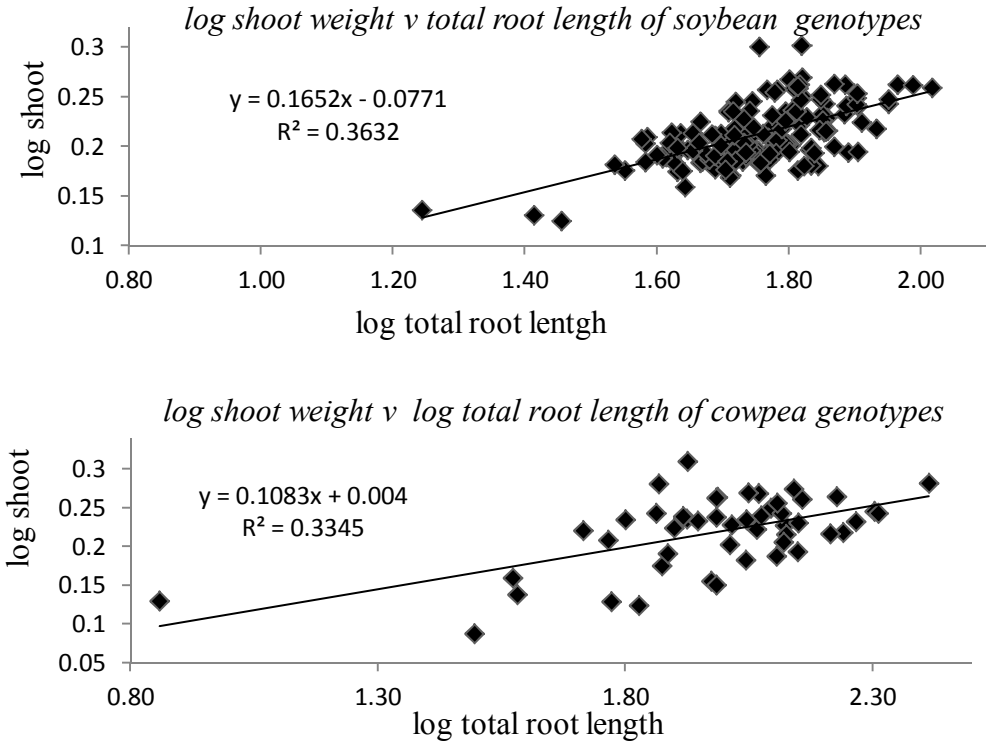

FIG. 2. Allometric relationship between total root length and shoot biomass (log scale base 10) of soybean and cowpea genotypes.

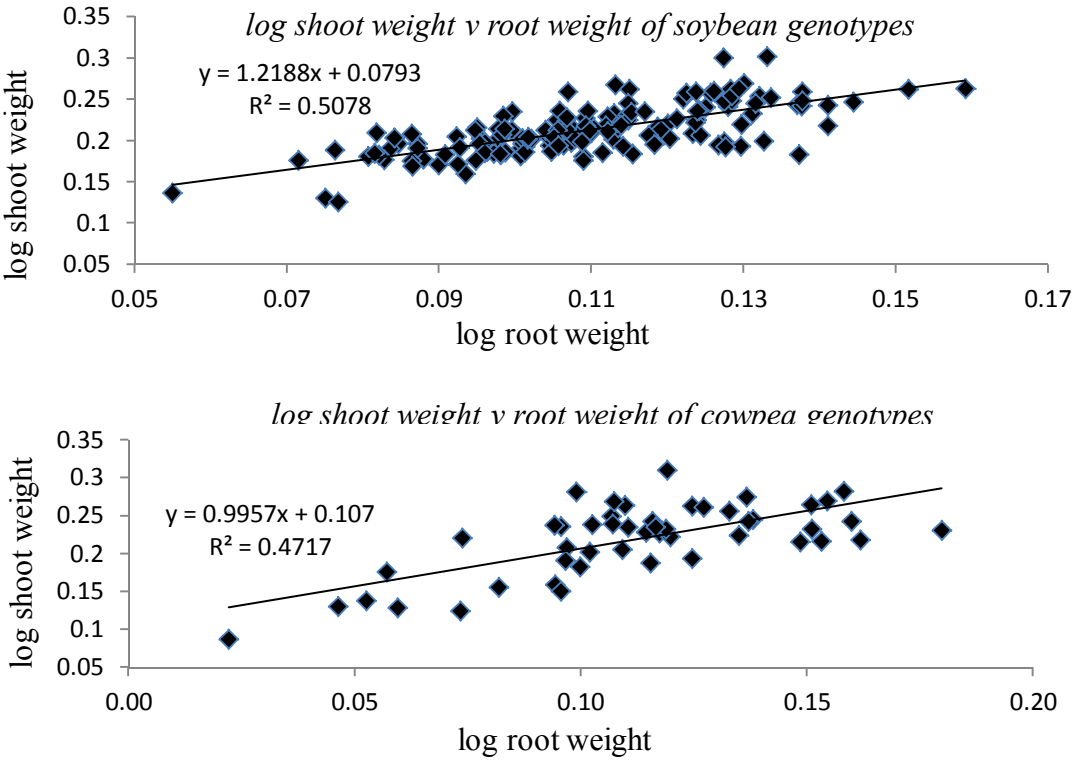

FIG. 3. Allometric relationship between root biomass and shoot biomass (log scale) of soybean and cowpea genotypes.

The EM genotypes, which had the greatest tap root length, also had the highest root dry weight while DP genotypes had the least root dry weight (Table 4). The root dry weight

was however not significantly different among the genotypes and within genotypes only EM genotypes showed significant differences. The DP genotypes had the greatest number of lateral roots, followed by the SR genotypes while the ER genotypes had the least number of lateral roots. Within genotypes there were no significant differences in lateral root number observed. The DP and SR genotypes of cowpea, which had the greatest number of lateral roots, also showed the highest lateral root densities of 3.8 and 3.5, respectively but differences were not significant. However, there were significant differences in lateral root density within ER genotypes.

TABLE 4. MEANS OF ROOT TRAITS OF DUAL PURPOSE, EARLY MATURING, LOCALLY RELEASED, MEDIUM MATURING AND STRIGA RESISTANT GENOTYPES OF COWPEA

Cowpea group†	Number of lateral roots	Root length (cm)			Lateral root density (roots cm^{-1} taproot)	Root weight	
		Tap	Total	Lateral		Fresh (g)	Dry (mg)
Dual purpose	50a	13.6a	141.5a	127.9a	3.8a	0.34a	17.6a
Early maturing	44b	13.8a	122.9a	109.1a	3.2a	0.33a	22.6a
Local released	30c	11.5a	66.3b	55.3b	3.0a	0.20c	19.2a
Medium maturing	34c	12.1a	85.0b	72.9b	3.4a	0.27b	22.4a
Striga resistant	47ab	13.6a	131.7a	118.2a	3.5a	0.34a	19.0a

† Number of genotypes within each classification; Dual purpose, n = 6; Early maturing, n = 18; Locally released, n = 10; Medium maturing, n = 6; Striga resistant, n = 10.
Means within a column followed by the same letter are not significantly different among the groups at $P<0.05$

The shoot dry weight among genotypes in ascending order was ER<EM<SR<MM<DP (Table 5). Plant height varied significantly (P<0.01) among and within cowpea genotypes. DP and ER genotypes showed significant differences within each genotype. There were however no significant differences within EM, MM and SR genotypes.

TABLE 5. MEANS OF SHOOT TRAITS OF DUAL PURPOSE, EARLY MATURING, LOCALLY RELEASED, MEDIUM MATURING AND STRIGA RESISTANT GENOTYPES OF COWPEA

Cowpea group†	Shoot fresh weight (g)	Shoot dry weight (mg)	Plant height (cm)	Root: shoot ratio	Total biomass (mg)
Dual purpose	0.77a	112.5a	6.2a	0.16b	130.1a
Early maturing	0.70b	98.8a	5.7b	0.25ab	121.4a
Locally released	0.43c	57.2b	5.0c	0.35a	74.1b
Medium maturing	0.73ab	112.0a	6.0ab	0.19ab	134.4a
Striga resistant	0.72b	103.1a	5.9ab	0.19ab	122.1a

† Footnote same as for Table 4

3.3. Phosphorus efficiency among soybean genotypes

3.3.1. Plant growth and yield

P application significantly increased the shoot and root dry weight of soybean genotypes (Table 6). On average both shoot and root biomass increased by more than 70% when P was applied. The significant increase in shoot biomass of more than 80% in soybean genotypes with P application was expected considering that the soil used was extremely low in available P. Under low P availability significant differences were observed among fifty soybean genotypes (Table 6). Shoot biomass ranged from 1.13g to 4.67g while root biomass ranged from 0.14g to 0.62 g. Relative shoot biomass, computed as shoot biomass of low P plants as % of that of high P plants ranged from 20.3 to 213.2%, while relative root dry weight ranged from 28 to 193.5%. A significant and positive correlation was observed

between the shoot biomass and root biomass under low and high P availability (Fig. 3) that was independent of differences in plant size. Seed yield also increased by as much as 99% among the 50 genotypes when P was applied. Seed yield varied significantly among soybean genotypes under P limiting conditions varying from 1.2 to 9.2 g per plant. Although 100 seed weight varied significantly among soybean genotypes, P application produced no significant difference in seed size.

TABLE 6. MEAN RESPONSES OF GROWTH PARAMETERS OF 50 SOYBEAN GENOTYPES GROWN IN A P-DEFICIENT SOIL UNDER LOW (LP) AND HIGH P (HP).

Growth parameters	Means				CV	Probability	
	LP	Range	HP	Range	(%)	P	G
Total root length 12 DAP (cm plant^{-1})	41.53	26.97- 63.64	28.49	13.95-49.56	155	***	***
Number of lateral roots 12 DAP	26	17- 41	23	13-37	115	***	***
Root DW 12 DAP (g plant^{-1})	0.04	0.02-0.07	0.03	0.02-0.05	123	***	***
Shoot DW 12DAP (g plant^{-1})	0.18	0.11-0.25	0.18	0.10-0.28	99	ns	**
Root: shoot ratio 12 DAP	0.22	0.13-0.48	0.17	0.09-0.31	128	***	***
Total root length 30 DAP (cm plant^{-1})	56.14	39.48-83.15	60.1	32.80-97.36	99	*	**
Number of lateral roots 30 DAP	21	9-40	20	6-30	109	*	***
Root DW 30 DAP (g plant^{-1})	0.35	0.14-0.62	0.47	0.23-0.84	86	***	*
Shoot DW 30 DAP (g plant^{-1})	2.14	1.13-4.67	3.01	1.03-6.25	82	***	*
Root: shoot ratio 30 DAP	0.17	0.11-0.24	0.16	0.10-0.25	6.4	*	*
Number of pods plant^{-1}	24	9-42	35	16-70	76	***	*
Seed yield (g plant^{-1})	3.26	1.23-9.22	5.34	1.31-13.29	83	***	**
100 seed weight	12.32	7.76-19.90	12.63	7.20-17.35	99	**	ns

†P, phosphorus; G, genotype; P x G interactions were not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns, not significant

3.3.2. Root traits

Total root length and number of roots was measured at 12 DAP and 30 DAP. At 12 DAP total length and number of roots varied significantly ($P<0.001$) among soybean genotypes, and when P was applied (Table 6). In general, P application reduced total root length of soybean genotypes with mean total root length being 20.7 and 18.3cm plant^{-1} for P0 and P30, respectively. Under P limiting conditions total root length varied from 9 to 32.3 cm plant^{-1} and the number of roots showing the same trend. On average, P application reduced the number of roots. At 30 DAP total root length and number of roots were significantly ($P<0.05$) increased under P limiting conditions.

3.3.3. Phosphorus use efficiency and phosphorus responsiveness

Phosphorus efficiency was assessed using principal component analysis of 6 growth parameters of the 50 genotypes. The 6 parameters at low P along with 6 indices at low P relative to high P differed significantly among accessions. Three principal components (PCs) of each genotype with Eigen values greater than one were retained whose cumulative contribution was 45%.

The relative weight of each principal component was weighted by the corresponding contribution rate accounting for variation of all growth traits. All genotypes were grouped into 3 clusters: cluster 1 (PEI <−0.01) as low efficient genotypes, cluster 2 (0.04 < PEI < 0.32) as moderately efficient genotypes and cluster 3 (PEI > 0.45) as highly efficient genotypes (Table 7). The first cluster consisted of 31genotypes that showed low efficiency. The second cluster was comprised of 13 moderately P efficient genotypes and third cluster consisted of 4 highly P efficient genotypes. Using Phosphorus Efficiency Index (PEI) and standardized values of

shoot biomass and seed yield at high P, the P efficiency of soybean genotypes was determined (Fig. 4).

TABLE 7. CATEGORISING 50 SOYBEAN GENOTYPES INTO LOW EFFICIENT (LE), MODERATE EFFICIENT (ME) AND HIGH EFFICIENT (HE), ACCORDING TO P EFFICIENCY INDEX (PEI)

ID	Cluster	PEI†	ID	Cluster	PEI
9	LE	0.03	23	LE	–0.24
5	LE	0.25	68	LE	–0.12
17	LE	0.04	107	LE	–0.27
59	LE	0.23	7	LE	–0.04
143	LE	0.27	92	LE	–0.11
48	LE	0.19	34	LE	–0.21
14	LE	0.06	84	LE	–0.20
65	LE	0.26	27	LE	–0.15
38	LE	0.19	3	ME	0.11
90	LE	0.20	77	ME	0.23
101	LE	0.28	57	ME	0.04
86	LE	0.10	55	ME	0.16
138	LE	0.46	137	ME	0.29
134	LE	0.20	4	ME	0.04
26	LE	0.10	46	ME	0.26
69	LE	0.05	67	ME	0.07
60	LE	0.05	106	ME	0.32
28	LE	0.01	145	ME	0.24
22	LE	0.12	43	ME	0.04
121	LE	0.03	94	ME	0.15
113	LE	0.35	45	ME	0.31
16	LE	0.04	129	HE	0.75
87	LE	0.02	141	HE	0.45
66	LE	0.20	104	HE	1.03
103	LE	0.04	10	HE	0.60

†All values in the column are negative

Standardized values of both shoot yield and seed yield were used because even though the main goal of breeding efficient genotypes is to increase yield, this trait however is influenced by several environmental conditions compared with shoot parameters in the field. Interestingly, the outcome was different for each trait among genotypes. Genotypes were grouped into four distinct categories - (i) efficient and responsive (ER); (ii) non-efficient and responsive (NER); (iii) non-efficient and non-responsive (NENR); and (iv) efficient and non-responsive (ENR).

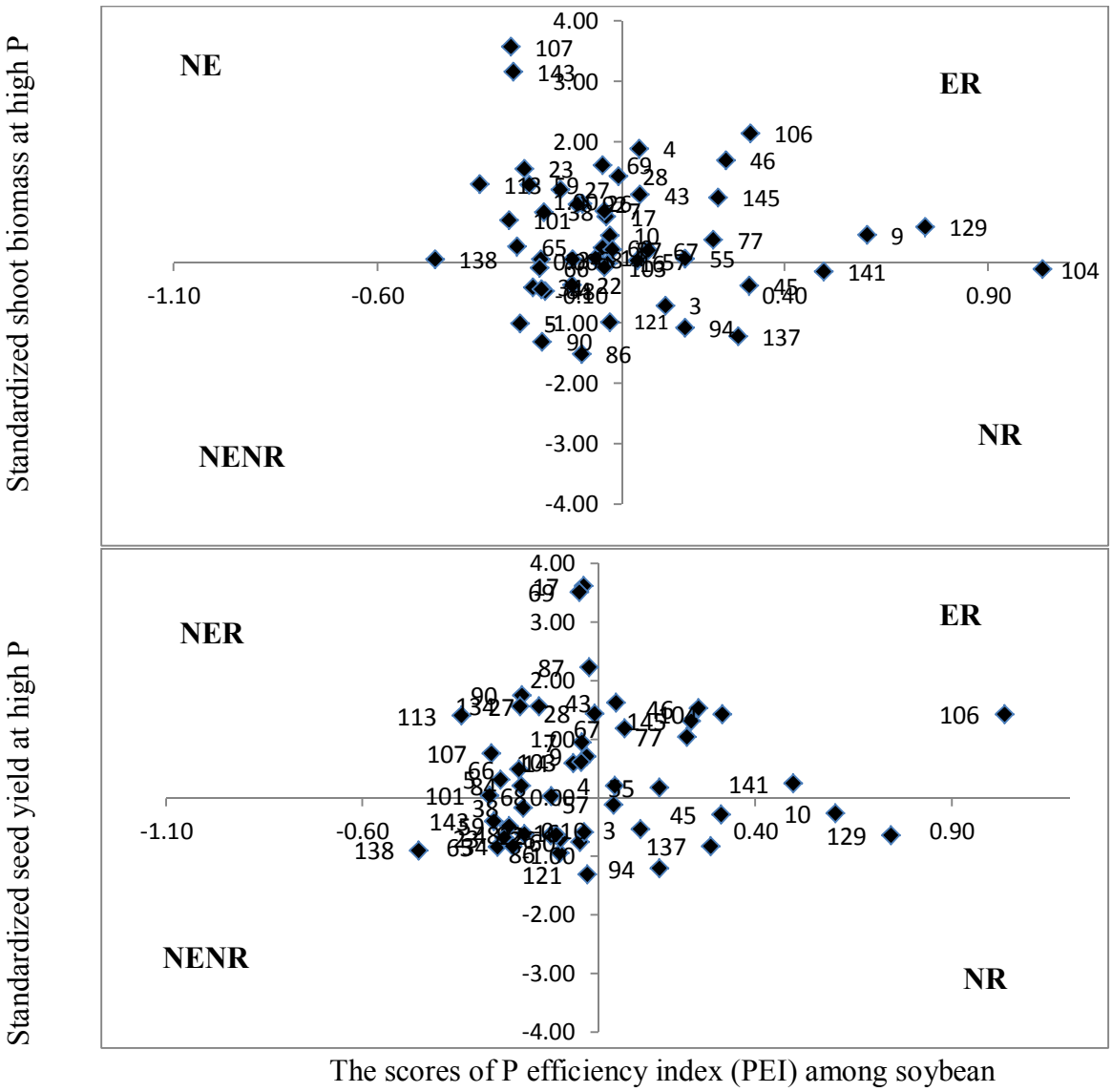

FIG. 4. Classification of soybean genotypes into four distinct responsive groups efficient and responsive (ER), non-efficient and responsive (NER), non-efficient and non-responsive (NENR) and efficient and non-responsive (ENR) according to P Efficiency Index (PEI) and standardized values of shoot biomass under high P. Standardized values of shoot dry weight (Xs) are estimated as the following function: $X_s = (X - \bar{X})/SD$

3.3.4. Correlation between root traits, plant biomass and yield

There was no significant correlation between root traits, biomass and yield in this study (Table 8). Shoot dry weight correlated positively with seed yield at 30 DAP under low P availability. Root dry weight correlated significantly and positively with shoot dry weight at 30 DAP under low P availability.

TABLE 8. CORRELATION MATRIX AMONG ROOT TRAITS AND SHOOT BIOMASS: ROOT NUMBER (RN), TOTAL ROOT LENGTH (TRL), SHOOT DRY WEIGHT (SDW), NUMBER OF PODS AND SEED YIELD

Parameter	12 DAP			30 DAP			SH	Number	Seed
	TRL	RN	RDW	TRL	RN	RDW	DW	of pods	yield
TRL 12 DAP	1	**0.59**	**0.15**	**0.19**	**0.03**	**0.18**	**0.17**	**0.04**	**0.26**
R N 12 DAP	0.49	1	**0.04**	**0.23**	**0.04**	**–0.01**	**–0.08**	**–0.08**	**–0.16**
RDW 12 DAP	–0.12	0.46	1	**0.08**	**0.21**	**0.06**	**–0.06**	**–0.10**	**0.03**
TRL 30 DAP	0.22	0.08	0.15	1	**0.43**	**0.42**	**0.33**	**–0.08**	**0.26**
RN 30 DAP	0.21	0.21	0.19	0.51	1	**0.08**	**–0.02**	**0.04**	**0.19**
RDW30 DAP	–0.25	–0.25	0.01	0.23	–0.01	1	**0.88**	**0.12**	**0.19**
SDW 30 DAP	–0.24	–0.03	–0.10	0.11	–0.14	0.87	1	**0.12**	**0.17**
Number of pods	–0.20	–0.10	0.01	–0.19	–0.19	0.22	0.27	1	**0.71**
Seed yield	–0.27	0.05	–0.12	–0.24	–0.33	0.27	0.32	0.64	1

3.3.5 Correlation between P efficiency and plant parameters

P efficiency was positively correlated to total root length at 30 DAP, shoot DW at 12 DAP, shoot and root DW at 30 DAP, number of pods plant^{-1}, seed yield and 100 seed weight at low P (Table 9).

TABLE 9. CORRELATIONS BETWEEN P EFFICIENCY AND PARAMETERS OF 50 SOYBEAN GENOTYPES GROWN IN A P-DEFICIENT SOIL WITHOUT (LOW P) OR WITH P ADDITION (HIGH P) AND LOW P AS A % OF HIGH P (LOW P/HIGH P)

Parameter	Correlation coefficients (r)		
	Low P	High P	Low P/high P
Total root length 12 DAP (cm plant^{-1})	0.17	0.20	–0.04
Number of lateral roots 12 DAP	–0.10	0.05	–0.19
Root DW 12 DAP (g plant^{-1})	–0.06	–0.02	0.06
Shoot DW 12DAP ((g plant^{-1})	0.30*	0.09	0.14
Root: shoot ratio 12 DAP	–0.17	0.05	–0.02
Total root length 30 DAP (cm plant^{-1})	0.29*	–0.01	0.23
Number of lateral roots 30 DAP	–0.06	–0.01	–0.13
Root DW 30 DAP (g plant^{-1})	0.80***	0.09	0.66***
Shoot DW 30 DAP (g plant^{-1})	0.97***	–0.11	0.64***
Root: shoot ratio 30 DAP	–0.37**	–0.10	–0.24
Number of pods plant^{-1}	0.24	0.14	0.10
Seed yield (g plant^{-1})	0.26	0.27	0.15
100 seed weight	0.29	0.23	0.04

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

However, P efficiency was negatively correlated to number of lateral roots at 12 DAP, root dry weight at 12 DAP, root: shoot ratio at 12 DAP and root: shoot ratio at 30 DAP (Table 9). Under low P, the highest correlation was observed between P efficiency and root and shoot DW at 30 DAP. At high P, similar trends were observed with the exception of shoot DW at 30 DAP, which was negatively correlated with P efficiency. Furthermore, P efficiency showed the highest positive correlation with the values of root and shoot DW at 30 DAP at low P as a percentage of high P (Table 9).

3.4. Phosphorus efficiency among cowpea genotypes

3.4.1. Shoot biomass

Shoot biomass increased significantly under moderate and high P limiting conditions. Among the six genotypes there was an average increase of 55% of shoot biomass under moderate P and 36 % increase of shoot biomass under high P conditions (Table 10).

Shoot biomass varied significantly ($P<0.01$) from 2.0 to 3.2 g. Genotype IT98K-1263 showed the highest shoot biomass while Soronko the least (Table 11). Genotypic variation in shoot dry weight was related to differences in area per leaf and number of leaves (Table 10), which accordingly affected total leaf area. In general, smaller leaves and lower leaf numbers were observed in plants grown in low P treatments (P0, P20) as compared to those with high P treatment (P40).

3.4.2. Nodulation, P uptake and P efficiency

Nodule number and nodule dry weight (NDW) increased significantly under moderate and high P conditions (Table 10). Generally NDW increased 24% under moderate P and a further rise of 11% under high P conditions. Significant variation was observed in nodulation of cowpea genotypes under P limiting conditions.

TABLE 10. MEANS AND LEVEL OF SIGNIFICANCE FOR GROWTH PARAMETERS OF 6 COWPEA GENOTYPES WITH LOW P (0 KG HA^{-1}) MODERATE P (20 KG HA^{-1}) AND HIGH P (40 KG HA^{-1}) AVAILABILITY

Parameter	P0	P20	P40	P0/P20 (%)	P0/P40 (%)	P	G	P × G
Leaf area(cm^2 $plant^{-1}$)	322	556	786	57.9	41.0	***	***	*
Leaf dry weight (g pot^{-1})	1.03	1.94	3.08	53.1	33.4	***	*	ns
Leaf number	28	36	52	77.8	53.9	***	*	ns
OLA	11.5	15.4	19.9	74.7	57.8	**	***	ns
Shoot dry weight (g pot^{-1})	2.41	4.33	6.39	55.7	37.7	***	ns	ns
Root dry weight (g pot^{-1})	0.79	1.38	1.77	57.3	44.6	***	*	ns
Root : shoot ratio	0.46	0.30	0.32	153.3	143.8	***	*	***
Nodule number	22	40	47	55.0	46.8	***	***	ns
Nodule dry weight (mg pot^{-1})	0.016	0.067	0.136	23.9	11.8	***	**	ns
P uptake (mg g^{-1})	10.9	23.7	42.8	46.9	25.5	***	**	ns
N uptake (mg g^{-1})	0.26	0.45	0.63	57.8	41.3	***	ns	ns
P utilization efficiency	0.21	0.61	1.08	34.4	19.4	***	*	ns
P uptake efficiency	14.30	9.70	9.28	147.4	154.1	***	*	ns

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns, not significant

Similarly, there were significant differences in root to shoot ratio, shoot P concentration, root P concentration, ratio of shoot to root P concentration, shoot P content, root P content, total P uptake, and P uptake efficiency amongst the genotypes and the P treatments. However, with the exception of leaf area and root to shoot ratio no significant interaction was observed (Table 10).

NDW varied from 3mg in IT93K-452-1 to 42 mg produced by IT98K-1263 under low P conditions (Table 11). It is worth noting that genotype IT98K-1263 was consistently the highest nodulating under all three P treatments.

TABLE 11. SHOOT, ROOT, NODULATION, P UPTAKE AND P UTILIZATION EFFICIENCY CHARACTERISTICS OF 6 COWPEA GENOTYPES GROWN UNDER 0, 20 AND 40 KG P HA^{-1}

Parameter†	IT03K-351-1			IT00K-901-5			IT93K-452-1			IT98K-1263			IT97K-819-118			Soronko		
	0	20	40	0	20	40	0	20	40	0	20	40	0	20	40	0	20	40
Leaf area (cm^2 pot^{-1})	201	446	649	319	492	624	371	526	822	433	565	864	295	362	467	189	859	905
Leaf number	27	37	59	32	41	53	34	31	70	27	32	37	36	43	63	17	34	29
Single leaf area (cm^2)	7.51	11.85	12.92	11.31	13.17	14.76	11.12	17.22	13.07	15.88	18.45	24.82	9.56	9.27	8.51	15.19	25.72	42.58
Leaf DW (g pot^{-1})	0.83	1.95	3.24	0.88	1.91	2.18	1.05	1.74	3.57	1.55	2.11	4.19	1.08	1.53	2.47	0.78	2.39	2.83
Stem DW (g pot^{-1})	1.56	2.66	5.06	1.40	2.58	3.26	1.50	2.53	4.61	1.61	2.60	3.00	1.02	1.95	3.03	1.24	2.12	2.99
Shoot DW	2.39	4.61	8.30	2.28	4.49	5.44	2.55	4.27	8.18	3.16	4.71	7.19	2.10	3.48	5.50	2.02	4.51	5.82
Root DW	0.86	1.39	1.77	0.78	1.56	2.07	0.95	1.29	1.88	0.97	1.68	1.87	0.54	1.11	1.28	0.65	1.24	1.76
Root to shoot ratio	0.62	0.29	0.22	0.48	0.40	0.41	0.42	0.32	0.29	0.31	0.37	0.27	0.27	0.24	0.26	0.62	0.24	0.32
Nodule number	24	40	47	27	42	50	8	23	31	41	59	67	25	29	45	8	50	42
Nodule DW (mg pot^{-1})	25.82	79.7	156.8	19.53	73.1	159.7	3.07	43.3	89.7	42.41	100	183.7	19.24	43.8	110.5	9.65	64.4	115.3
Shoot N uptake (mg g^{-1})	8.58	28.69	74.38	7.85	36.24	61.58	13.95	30.43	59.90	15.69	38.91	63.95	4.95	22.53	43.26	5.60	26.53	58.52
Shoot P uptake (mg g^{-1})	0.84	1.90	4.46	0.80	2.12	3.48	0.97	2.03	5.18	1.11	1.84	3.45	0.63	1.25	2.41	0.57	1.65	2.57
P uptake efficiency	0.97	1.36	2.52	1.03	1.36	1.68	1.02	1.58	2.76	1.14	1.09	1.84	1.17	1.12	1.88	0.87	1.33	1.46
P utilization efficiency	0.21	0.71	1.42	0.18	0.62	0.76	0.23	0.51	1.26	0.37	0.80	1.31	0.18	0.42	0.72	0.17	0.59	0.98

†DW, dry weight

The PEI value generated for the genotypes was positive for IT03K-351-1, IT00K-901-5, IT93K-452-1and IT98K-1263 (Table 12). On the other hand PEI values generated were negative for genotypes IT97K-819-118 and Soronko. Based on the PEI cowpea genotypes were classified under three levels of P efficiency of low, moderate and high (Table 12). Genotype Soronko was low, IT97K-819-118 moderate and IT03K-351-1, IT00K-901-5, IT93K-452-1 and IT98K-1263 were highly efficient.

TABLE 12. CATEGORISING 6 COWPEA GENOTYPES INTO LOW EFFICIENCY (LE), MODERATE EFFICIENCY (ME) AND HIGH EFFICIENCY (HE), ACCORDING TO P EFFICIENCY INDEX (PEI) GENERATED FROM PCA USING PARAMETERS UNDER LOW P (0 KG HA^{-1}) AND HIGH P AVAILABILITY (40 KG HA^{-1})

ID	Genotype	Cluster	PEI
8	IT03K-351-1	HE	0.059
11	IT00K-901-5	HE	0.064
14	IT93K-452-1	HE	0.121
22	IT98K-1263	HE	0.225
27	IT97K-819-118	ME	–0.345
48	Soronko	LE	–0.094

Based on PEI generated from PCA and growth potential of shoot dry weight, nodule dry weight and P uptake efficiency at high P, two categories were identified (Fig. 5). Genotypes 11 (IT 00K-901-5, early maturing), 14 (IT93K-452-1, early maturing), 8 (IT03K-351-1, early maturing) and 22 (IT98K-1263, medium maturing) were identified as efficient and responders (ER). On the other hand genotypes 27 (IT97K-819-118, Striga resistant) and 48 (Soronko, released variety) were classified as inefficient and responders. Genotypes were consistent in their classification by shoot dry weight, NDW and P uptake efficiency. However genotype 22 (IT98K-1263, medium maturing) was highly efficient in NDW and P uptake. Genotype 8 (IT03K-351-1, early maturing) was consistently highly efficient in shoot biomass, NDW and P uptake.

3.4.3. Correlation analysis between P efficiency and plant growth parameters

P efficiency was positively correlated with leaf area, single leaf area, dry weights of leaf stem, shoots and roots, root to shoot ratio, nodule number, NDW, shoot N and P uptake and P utilization efficiency, and negatively correlated to leaf number and P utilization efficiency at P0 (Table 13). At P40, P efficiency was positively correlated with all parameters measured but negatively correlated with leaf number. Likewise, P efficiency was positively correlated the relative indices at P0 as a % of those at P40, except leaf area, single leaf area, leaf dry weight and P uptake efficiency.

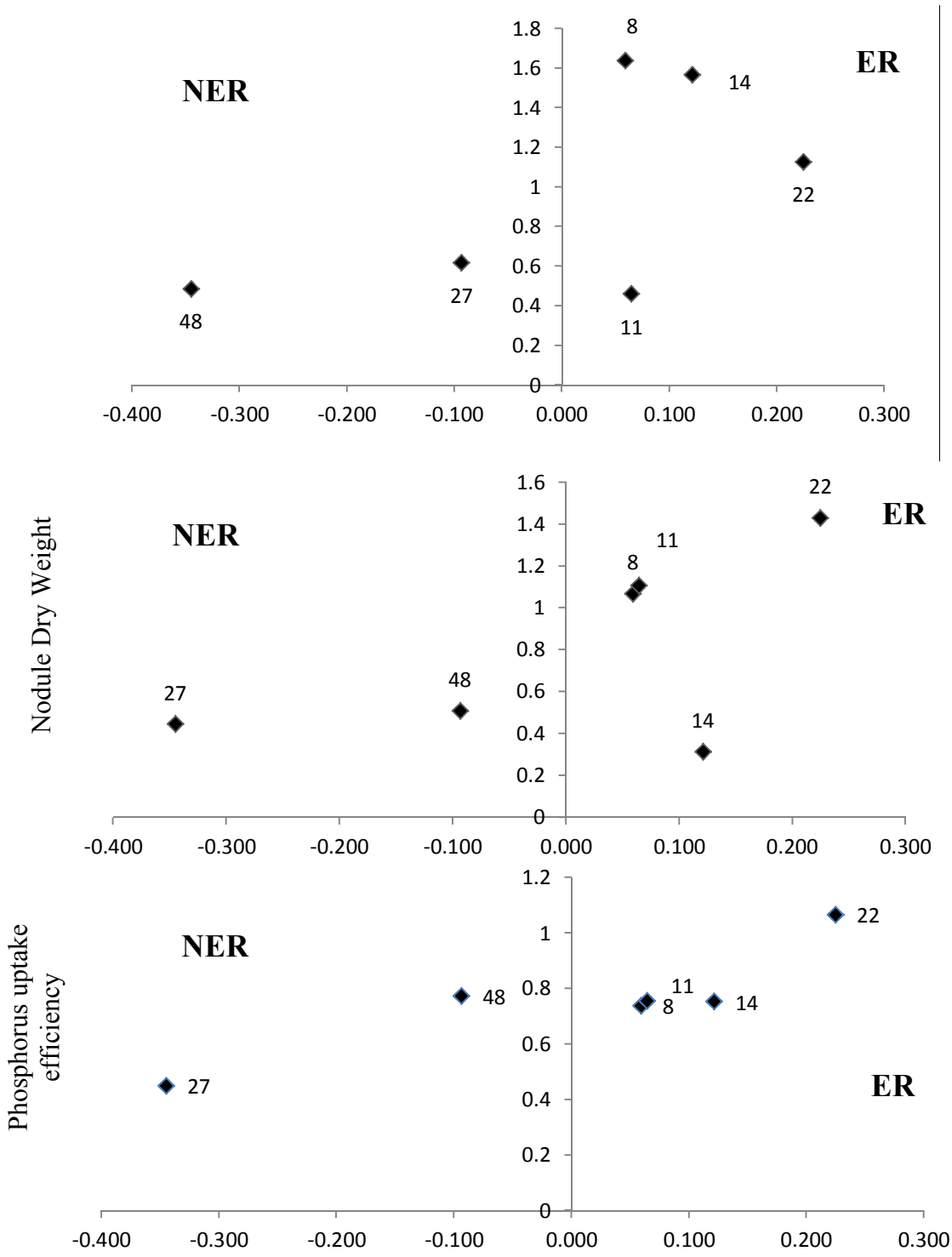

FIG. 5. Classification of cowpea genotypes into 2 distinct responsive groups according to P efficiency index and standardized values of shoot biomass, nodule dry weight and P uptake efficiency under high P availability.

TABLE 13. CORRELATIONS BETWEEN P EFFICIENCY AND PARAMETERS OF 6 COWPEA GENOTYPES GROWN IN A P-DEFICIENT SOIL WITHOUT (P0) OR WITH P ADDITION (P40) AND PARAMETERS AT P0 AS % OF THOSE AT P40 (P0/P40)

Parameter	Correlation coefficients (*r*)		
	P0	P40	P0/P40
Leaf area (cm^2 pot^{-1})	0.48	0.61	–0.21
Leaf number	–0.16	–0.15	0.16
Single leaf area (cm^2)	0.31	0.11	–0.43
Leaf dry weight (g pot^{-1})	0.37	0.67	–0.40
Stem dry weight (g pot^{-1})	0.96**	0.33	0.46
Shoot dry weight	0.77	0.60	0.09
Root dry weight	0.95**	0.85*	0.58
Root to shoot ratio	0.15	0.10	0.13
Nodule number	0.26	0.33	0.05
Nodule dry weight (mg pot^{-1})	0.33	0.52	0.03
Shoot N uptake (mg g^{-1})	0.84*	0.77	0.72
Shoot P uptake (mg g^{-1})	0.85*	0.66	0.07
P uptake efficiency	–0.12	0.31	–0.33
P utilization efficiency	0.67	0.69	0.01

*, $P<0.05$; **, $P<0.01$

4. DISCUSSION

4.1. Shoot and root growth at early growth stage

The significant differences and correlation found among genotypes within soybean and cowpea for total root length, root number, root weight, shoot weight and root: shoot ratio were similar to the findings of Mia et al. [24] who found significant differences in several root traits among legumes they studied during early growth. Interestingly, they reported that cowpea showed the widest genotypic variability for root traits, and formed an extensive root system by producing a large number of lateral roots, comparable to the findings in this study. Extensive lateral rooting systems have been shown to be important adaptive traits that enhance P uptake from low P soils [25].

Genotypic differences were observed in soybean and cowpea genotypes for lateral rooting at 5 DAP. This has been reported in early growth stage in both chickpea and cowpea [24]. The deployment of root architectural traits in plant breeding programs has great potential to alleviate P deficiency, a primary constraint to crop production in world agriculture [18]. The significant differences found in soybean and cowpea at the seedling stage provides an opportunity for screening a large number of genotypes for important root traits such as an extensive lateral root system.

4.2. Phosphorus efficiency in soybean and cowpea

Soybean and cowpea, like most legumes grown in Ghana, are grown under little or no nutrient application. In common with most soils found in Ghana, the levels of available P in the soils used for the study were low. Similarly, the total N was also low, consistent with the relatively low OM status of the soil. The most common stress that affects grain and forage production in Ghana is nutrient stress, particularly low P. The soil would therefore need some fertilization from an external source to sustain crop growth and permit continuous cultivation [26]. However this is beyond the economical reach of most Ghanaian farmers and has

prompted genotype screening and selection for tolerance to low soil P conditions as an important strategy to increase productivity [27].

We observed substantial variation among 50 soybean genotypes for growth in low P soil and under high P conditions for most of the parameters studied. Genotypic evaluation of crops should be under both low and high availability P since P efficiency is a complex quantity trait involving growth parameters [23]. As in soybean, genotypic variation was observed in 6 cowpea genotypes studied under P limiting conditions and high P conditions. Genotypic variation in P efficiency has been identified in cowpea [19, 28, 29].

The method used for P efficiency analysis was the PCA which is relatively new multiple-parameters screening method used in evaluating P efficiency. Due to the sensitive nature of parameters such as shoot biomass and P uptake to P availability, multiple- parameter screening methods are ideal because unlike single-parameter screening methods, they take into account the relative contributions of all parameters measured to P efficiency [23]. Utilizing the same method, 50 soybean genotypes could be grouped into 3 categories of P efficiency and 4 categories according to PEI, and in combination with P responsiveness, shoot dry weight and seed yield at high P. Shoot biomass and yield are important parameters in breeding, and shoot biomass is also an important determinant of seed yield [23]. Similar results were obtained by Pan et al. [23] using the same method of analysis for soybean genotypes.

The cowpea genotypes were also grouped under three categories of P efficiency. Consistent with the findings of others [23, 30], as well as the findings from this study, PEI generally places genotypes into three main categories of P efficiency of low, moderate and high. The 6 cowpea genotypes were further classified into two P responsive groups using the standardized values of shoot dry weight at high P and P efficiency. In contrast, the 50 soybean genotypes were classified under four P responsive categories. The categories obtained for cowpea were (i) efficient and responsive (ER); (ii) inefficient and responsive (NER). This grouping indicates that even though all 6 genotypes were good responders to P application, genotype was either efficient or inefficient.

IT03K-351-1, IT 00K-901-5, IT93K-452-1 and IT98K-1263 were more P efficient than genotypes IT97K-819-118 and Soronko. According to Sanginga et al. [19], genotypes with increased shoot dry weight with increasing levels of P, as observed in this study, distinguishes all genotypes as P-responders. Genotype 48 (Soronko) was identified as low efficiency under PEI, but was very responsive to P application. For instance, it had the highest leaf area when P was applied at 40 kg ha^{-1} and produced the highest percentage increase when P was applied. It is a typical cowpea variety which was produced during earlier breeding programmes by IITA to respond well to P application. In earlier IITA breeding programmes, yield parameters were predominantly targeted while factors such as P use and uptake efficiency and root traits may not have been deliberately included.

When the combination of PEI with P responsiveness at high P is considered, soybean genotypes 4, 43, 46, 55, 67, 77, 106 and 145 were consistently classified as efficient responders for both shoot and seed yield, and therefore were the best genotypes, particularly for the P-deficient soil used for the study. Genotypes categorized as the most P efficient are most responsive to increased P availability [30]. Remarkably, the genotypes listed above were classified as only moderately efficient using the PEI generated from the PC analysis. The following genotypes could be classified as highly efficient: 10, 104, 129 and 141 showed marked differences when classified according to shoot and seed yield potential at high P. Genotype 129 was an efficient responder in terms of potential shoot biomass at high P, while

genotype 10 was an inefficient responder, and genotypes 104 and 141 were efficient non-responders. In terms of potential yield at high P, genotypes 141 and 104 were efficient responders, and genotypes 10 and 129 were efficient non-responders.

P application significantly increased leaf dry weight, stem dry weight, root dry weight, leaf area, nodulation, P uptake and P uptake efficiency of all 6 cowpea genotypes. Increased shoot growth of cowpea genotypes in response to P application in low P soils has also been reported by several researchers [28, 29, 31–33]. The enhancing effects of P application on nodulation have been associated with increased nodule mass and number in cowpea as shown by this study and detailed by the other researches [19, 34, 35]. In contrast, Kolawole et al. [29] found a decrease in nodule number when P was applied in cowpea production.

4.3. Correlation among parameters

With the exception of shoot biomass and root biomass no significant correlation was found between PEI and most of the parameters studied in soybean genotypes. The study also indicated little correlation between the plants harvested at 5 DAP and shoots and yield parameters in the field. One possible explanation for the differences between the field and greenhouse results is that the field environment may have presented other environmental variables and stresses that could have difference observed in the plants. Lack of correlation between root traits and yield per plant under low P in the field could have been caused by the uncertainty associated with yield estimates from small plots in a single location and season.

5. CONCLUSIONS

The study concluded that there were significant genotypic variation for root traits at an early growth stage and genotypic differences for soybean and cowpea growth under low P. The PEI in combination with P responsiveness at high P showed that soybean genotypes 4, 43, 46, 55, 67, 77, 106 and 145 were efficient responders for both shoot and seed yield at high P, and therefore were the best genotypes particularly for the P-deficient soil used for the study. All 6 cowpea genotypes used in the P efficiency study, were good responders to P application, but IT03K-351-1, IT 00K-901-5, IT93K-452-1 and IT98K-1263 were more P efficient than IT97K-819-118 and Soronko.

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GENOTYPIC VARIATION IN PHOSPHORUS USE EFFICIENCY FOR SYMBIOTIC NITROGEN FIXATION IN VOANDZOU (*VIGNA SUBTERRANEA)*

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Abstract

Vigna subterranea, known as voandzou or Bambara groundnut as an African indigenous crop which is often neglected or under-used in African subsistence agriculture. Preliminary research and country perceptions have shown its agronomic and nutritional properties, in particular under atypical climates of arid and tropical areas, and in saline soils. There is a high potential to increase the production by optimizing symbiotic nitrogen fixation (SNF) through effective inoculation even in nitrate-rich environments. In this study, *Vigna subterranea* inoculated with the reference strain of *Bradyrhizobium* sp. Vigna CB756 was studied in order to assess the symbiotic fixation potential of different cultivars and landraces of Madagascar, Niger and Mali under low-P and sufficient-P conditions. Six voandzou cultivars inoculated with *Bradyrhizobium* sp. Vigna CB756, were grown under hydroaeroponic culture for 6 weeks supplied with four phosphorus levels of 15, 30, 75 and 250 µmol $plant^{-1}$ $week^{-1}$ in order to establish the response curve of voandzou to P supply, and to induce P deficient and sufficient levels. In another experiment five tolerant cultivars with high SNF and five sensitive cultivars with low SNF were chosen after a preliminary screening of 54 voandzou genotypes, including 50 landraces from Madagascar, Niger and Mali supplied with 2 P levels as P deficient and P sufficient (30 and 75 µmol $plant^{-1}$ $week^{-1}$) under hydroaeroponic conditions. Genotypic variation in SFN for the high phosphorus use efficiency (PUE) was observed among the 54 cultivars and landraces. Variability was especially related to the nodule and shoot biomass, nodule permeability, nodule respiration and gene phytase expression. Contrasting cultivars and landraces in terms of PUE for SNF were selected for further evaluation under field conditions.

1. INTRODUCTION

The improvement of symbiotic nitrogen fixation (SNF) in legumes makes it possible to mitigate N deficiency of many soils, to promote the availability of some soil nutrients such as P, and consequently to improve the crop's production, especially in countries with low use of inputs [1]. In addition, variability between landraces exists for certain traits related to N_2 fixation [2]. In this way, researches for SNF optimization among legume species were undertaken by numerous researchers [3–9]. A Thompson Boyce Institute report in 1978 mentioned that the genetic factors implied in nodulation should be used in the legume selection in particular for the specific nodulation traits (weight, number), including the inoculation of adequate rhizobia [10]. This concept was confirmed by the orientation of the research carried out during recent years, which involves the integration of plant-soil-microorganisms system. Indeed, the knowledge of the legume's capacity to use nutrients under sufficiency or deficiency of P would allow better adaptation of legumes in farming systems for productivity improvement. Thus, one way is the study of legume genotype-rhizobia combinations in N_2 fixation improvement [11]. Indeed, genetic variability in SNF under P sufficiency or deficiency was the subject of many studies for numerous species of legumes including *Phaseolus vulgaris* [1, 12, 13], *Vigna unguiculata* [14], *Glycine max* [2] and *Vigna subterranea* [3]. The increase of nodular respiration under P deficiency was observed for *Glycine max* [15] and *Phaseolus vulgaris* [16].

Vigna subterranea, known as the vernacular name of voandzou or Bambara groundnut as an African indigenous crop, often neglected or under-used in African subsistence agriculture [17]. Preliminary research and country perceptions show the potentiality of its agronomic and nutritional properties, in particular under atypical climates of arid and tropical areas, and in saline soils. The genetic potential is little known, and some previous work is available only in old publications or little-known languages [17, 18]. A genotype selection of *Vigna subterranea* was initiated in multi-locational field trials in Africa, on the basis of their vigor, resistances against fungal diseases and yield, in particular in Nigeria, Burkina Faso, Camerounn and Botswana, showing a large special-temporal effect from one year to another [19–21]. Great variation of yield from 200 to 3000 kg ha^{-1} among farming systems [18, 22] and spatial variability for the same landraces makes it possible to formulate the hypothesis of a differentiation in the symbiotic effectiveness of native *Bradyrhizobia* nodulating *Vigna subterranea*. Moreover, the inoculation of *Vigna subterranea* with a *Bradyrhizobium* sp. strain showed a significant increase of production in Togo and Senegal. These observations indicate the potential to increase the production of *Vigna subterranea* via SNF optimization [18]. The nodulation of *Vigna subterranea* was mainly depending on symbiotic nitrogen fixation even in nitrate-rich environments [3].

In the present study, *Vigna subterranea* was inoculated with the reference strain of *Bradyrhizobium* sp. Vigna CB756 in order to assess the symbiotic fixation potential of different cultivars and landraces of Madagascar, Niger and Mali under P deficiency versus P sufficiency. The results of this experimentation allow us to highlight and to select contrasting lines of voandzou in P use efficiency (PUE) for SNF.

2. MATERIALS AND METHODS

2.1. Biological material and symbiosis culture

The experiment was carried out in hydroaeroponic culture under glasshouse conditions during 2010 with 20/33°C temperature during 16 / 8 h day / night cycle coupled with an intense ventilation of nodulated-roots, a complementary illumination of 400 µM photons m^{-2}

when needed, and 70% daily relative humidity. These conditions allowed an optimal expression of genetic potential of N_2 fixation while controlling closely the mineral nutrition mainly for N, P and for other essential elements.

The seeds were sterilized with 3% calcium hypochlorite for 20 min and rinsed by 5 washings with sterile distilled water. They were then transferred for germination on humidified wrapped filter paper in a slightly tilted vat and placed in an incubator at 28-30°C. After germination, inoculation was performed by soaking the roots of seedlings for 20 min in a suspension of *Bradyrhizobium* sp. Vigna CB756 containing 10^9 bacteria ml^{-1}. The inoculum was prepared from a rhizobial culture preserved in tubes at 4°C on 120°C sterilized agar YEM (Yeast Extract Mannitol) medium: 900 ml distilled water, 100 ml of Bergersen concentrated solution (which is prepared with a mixture of 1 g KCl; 0.1 g $FeCl_3$; 0.4 g $CaCl_2$, 4.5 g $Na_2HPO_4.12H_2O$ and 1 g $MgSO_4.7H_2O$, firstly in 100 ml of distilled water, then adjusted to 1 l), 1 g Yeast Extract, 10 g mannitol and 15 g agar [23]. From one of the preserved tubes, some strains were taken and put on 100 ml of liquid YEM (without agar), and maintained at 28°C for 24 h. The germinated seeds were carefully transferred for hydroaeroponic culture into each 40 l vat with 20 inoculated plants per vat. The roots of each seedling were carefully passed through the hole of a rubber stopper with cotton wool fixed at the hypocotyl level. They received the following nutrient solution that was changed every 2 weeks: $CaCl_2$, 1650 µM; $MgSO_4.7H_2O$, 1000 µM; K_2SO_4, 700 µM; Fe EDDHA, 8.5 µM as sequestrene Fe; H_3BO_3, 4 µM; $MnSO_4.H_2O$, 6 µM; $ZnSO_4.7H_2O$, 1 µM; $CuSO_4.7H_2O$, 1 µM; $Na_2MoO_4.7H_2O$, 0.1 µM. 2000 µM of urea was supplied for all treatments during the first two weeks' culture. P was supplied in the form of KH_2PO_4 with an exponential distribution for the two levels: 30 µmol $plant^{-1}$ $week^{-1}$ and 75 µmol $plant^{-1}$ $week^{-1}$.

In the first experiment, six voandzou (*Vigna subterranea*) cultivars (kindly supplied by FOFIFA, Madagascar), inoculated with *Bradyrhizobium* sp. Vigna CB756, were grown under hydroaeroponic culture during six weeks in order to test the effectiveness of the rhizobia-legume symbiosis. Four P levels, 15, 30, 75 and 250 µmol $plant^{-1}$ $week^{-1}$, were supplied under KH_2PO_4 form in order to establish the response curve of voandzou to P supply and to deduce P deficient and sufficient levels. Four replications were applied for each treatment.

In the second experiment, a preliminary screening of 54 voandzou genotypes including 14 landraces and 2 cultivars from FOFIFA in Madagascar, 36 landraces from Niger and 2 cultivars from Mali, was performed with one replicate during six weeks in containers under hydroaeroponic conditions. Two P supplies of P deficient as 30 µmol $plant^{-1}$ $week^{-1}$ and P sufficient as 75 µmol $plant^{-1}$ $week^{-1}$ deduced from the previous experiment were applied in order to screen the most tolerant and sensitive cultivars to P sufficiency *versus* P deficiency. Five tolerant cultivars with high SNF and 5 sensitive cultivars with low SNF were chosen from this prescreening on the basis of their nodule and shoot biomass under P deficiency. These 10 most contrasting cultivars were grown under P deficiency and P sufficiency in a randomized block design with 8 replicates. Three weeks after transplanting, the four most contrasting cultivars, 2 tolerant and 2 sensitive, were transferred for oxymetric measurement with three replications into 1 l bottles wrapped with aluminum foil to maintain darkness in the rooting environment. These bottles contained the same nutrient solution as the containers previously described.

2.2. Measurements of nodulated root O_2 uptake

In order to assess the tolerance mechanisms in SNF under P deficiency, the nodulated root gas exchange was performed with the four most contrasting cultivars and landraces. The consumption of O_2 by the nodulated roots (Conr) was measured *in situ* between 09.00 and 16.00 h with an oxymeter (Abiss, Verpillère, France) at the 6th week after transplanting. The level of the nutrient solution in each bottle was reduced to one-third of the volume one day before the Conr measurement for putting the whole nodule population in direct contact with the gas phase. The measurement was performed with the oxymeter connected to a recorder and a peristaltic pump to ensure the circulation and continuous homogenization of the gas phase between the nodulated roots and the oxymeter, with 400 ml^{-1} of flow [24].

The O_2 consumption was quantified according to a known volume of the bottle at different O_2 levels: 21, 25, 30, and 40 kPa O_2. Conr was calculated as $Conr = \Delta pO_2 (V / 24.2) (60 / t)$, with $\Delta pO_2 = pO_{2\ initial} - pO_{2\ final}$ in % of atmospheric pressure; V in l, volume of the gas phase under the experimental conditions; t in min, time between the initial and final measurement of O_2; 24.2 in l, volume of 1 pure gas mol under the experimental conditions [25]. Conr is expressed in µmol O_2 consumed $plant^{-1}$ h^{-1}.

2.3. In situ RT-PCR

Nodules of 3 mm diameter were randomly harvested at early flowering from replicated plants of each P treatment, thoroughly washed with DEPC- (diethyl pyrocarbonate) treated water, then fixed in freshly prepared PFA (v/v) (2% paraformaldehyde, 45% ethanol and 5% acetic acid) and stored overnight at 4°C. Fixed nodules were extensively rinsed with four washings of DEPC-treated water over 30 min (2 x 5 min and 2 x 10 min) with agitation to remove PFA. Thereafter, the nodules were included in low melting 9% (m/v) agarose dissolved in filtered phosphate-buffered saline (PBS; 5 mM Na_2HPO_4, 300 mM NaCl, pH 7.5) and cut into 50 µm thick sections using a microtome. The resulting sections were collected into tubes containing 0.5 ml of DEPC-treated water and freed from residual agarose by three washes with DEPC-treated water at 60°C. For reverse transcription, the fixed sections were transferred to PCR tubes and incubated in 40 µl RT mix [RT 1X Reaction Buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT) (Promega, Madison, WI, USA); 0.31 mM dNTP and 0.75 µM gene specific reverse primer (5'-TTC ACC TCT AGA ATC CCA T-3'). The samples were then heated at 65°C for 5 min, transferred on ice for 2 min and added with Moloney murine leukemia virus (M-MLV) reverse transcriptase H(−) (Promega) to a final concentration of 5 U μl^{-1} followed by incubation at 42°C during 1 h. After reverse transcription, the RT mix was removed, and the samples were washed three times with 100 µl DEPC-treated water. After removing the last washing, they received 40 µl of of PCR mix (1× PCR buffer, Invitrogen, Carlsbad, CA, USA), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.25 µM each of the gene-specific primer pair (Forward: 3'-GGA CAT GTT CAT GCC TAT GAG-5'; Reverse: 5'-TTC ACC TCT AGA ATC CCA T-3'), 0.25 nM digoxigenin-11-2′-deoxyuridine 5′-triphosphate (Dig-11-dUTP; Roche Diagnostics, Mannheim, Germany) and 1 U Taq DNA Polymerase (Invitrogen). Thermocycling was performed at 95°C for 3 min and 30 cycles (95°C for 30 s; 55°C for 30 s**;** 72°C for 45 s**,** 72°C for 2 min). Negative controls (no-RT) were prepared by omitting the reverse transcription with the samples in 40 µl of DEPC-treated water during the RT step and treating alike the other samples during the following steps.

For the detection of the amplified cDNA, the PCR mix was removed after amplification, and the samples were washed three times each for 10 min in 200 µl PBS under gentle agitation, and then incubated in 100 µl blocking solution (2% BSA, in PBS, with 0.3%

triton) for 30 min under gentle agitation in darkness at 37°C. Then the blocking solution was removed and replaced by 100 µl of alkaline phosphatase-conjugated anti-dioxygenin-Fab fragment (Roche Diagnostics) diluted 1: 1000 in 2% BSA. The samples were incubated at room temperature for 90 min and then washed three times for 10 min in PBS to remove excess antibody. Detection of alkaline phosphatase was carried out using the ELF-97 (enzyme-labeled fluorescent) endogenous phosphatase detection kit (Molecular Probes, Leiden, The Netherlands). The ELF substrate was diluted 1: 40 in the alkaline detection buffer (Molecular Probes, Leiden, The Netherlands), vigorously shaken, and then filtered through a 0.22 µm filter (Millex®-GV, Millipore, Bedford, USA) to remove any aggregates of the substrate that may have formed during storage. Samples were incubated in 20 µl ELF substrate-buffer solution in the dark for 20 min, then washed 3 x 1 min with wash buffer (PBS with 25 mM EDTA and 5 mM levamisole, pH 8.0) before the samples were mounted. Observations were made using an Olympus BX61® microscope equipped with an epifluorescence condenser, a Hoechst / DAPI filter set and color view camera.

2.4. Biomass, P content and statistical analysis

The plants were harvested at the end of the 6th week at the flowering stage. The shoot was separated from the root at the cotyledonary node, and then weighted after drying for 48 h at 80°C. Nodules were excised from roots, counted and weighted. The effect of P supply was estimated from the response curves of the biomass and the critical P supply was deduced at the maximum growth. In order to determine the contribution of P supply to plant growth, the response curves were established from biomass values with the software Excel Microsoft Office XP. The genotypes and P treatment effects were analyzed by two-way analysis of variance (ANOVA). Correlation and regression analyses were performed to complete the analysis.

3. RESULTS

3.1. Response curves of biomass to P supplies

A significant difference in the response to P supply was observed for the cultivars 1 and 3 in terms of nodulation and plant biomass compared to cultivars 4 and 6 (Fig. 1). The maximum nodulation was 27 and 32 mg nodule DW plant^{-1} for cultivars 1 and 3, respectively, *versus* around 7 mg nodule DW plant^{-1} for cultivars 4 and 6 (Fig. 1A). The critical P supply, i.e. for which plants could express their genetic potentials in terms of nodulation, were around 125 µmol Pi plant^{-1} week^{-1} for cultivars 1, 4 and 6 *versus* between 150 and 175 µmol Pi plant^{-1} week^{-1} for cultivar 3. The shape of the curves showed a decrease in the nodular biomass after reaching the critical P supply. From these response curves, 30 *versus* 75 µmol Pi plant^{-1} week^{-1} were selected for P sufficiency and deficiency in subsequent experiments. Cultivar 1 was the most sensitive to P supply in terms of nodule, shoot and root biomass while cultivars 4 and 6 presented a low response slope for all biomass parameters. Cultivars 1 and 4, which were respectively sensitive and tolerant to P supply, were selected as controls in the screening experiments.

3.2. Screening of voandzou for nodulation under P sufficiency *versus* P deficiency

The first screening of 58 cultivars and landraces of voandzou without replication enabled us to preselect contrasting landraces as tolerant and sensitive. A large variability among cultivars and landraces of voandzou was evident (Fig. 2). The nodulation was higher for Madagascar landraces than for Niger and Mali cultivars and landraces. The results observed under P deficiency exceeded those under P sufficiency for Niger and Mali landraces.

The cultivars and landraces with high nodulation ability under P deficiency harboured more than 0.2 g nodule DW plant^{-1} (Fig. 2). Under P deficiency, a significant nodulation, corresponding to a high SNF, was recorded in landrace 7 followed by V1, V4, 8 and 1, 11 compared to low nodulation in cultivars 2, 10, N'jibawolo and 4.

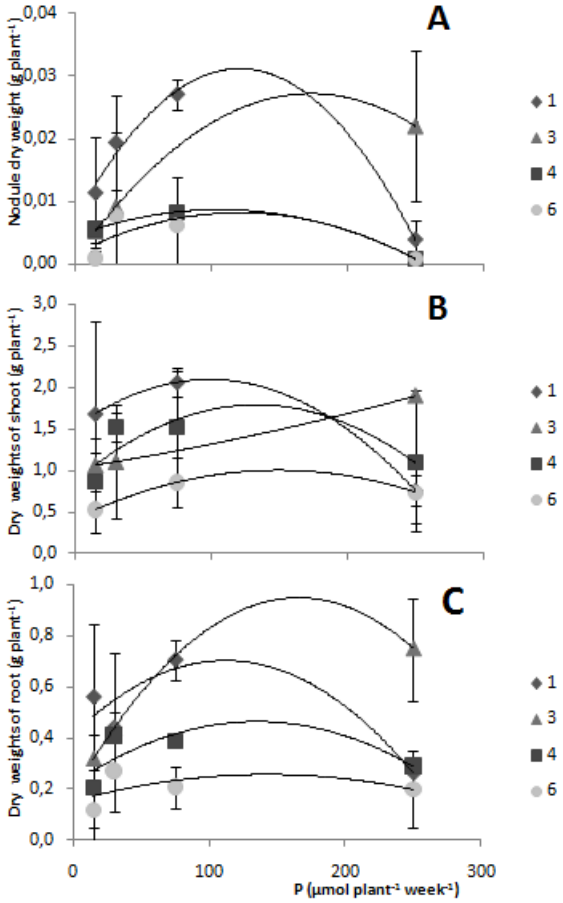

FIG. 1. Response curves of growth of nodules (A), shoots (B) and roots (C) as a function of weekly P supplies. Data are means of 4 replicates for 4 voandzou genotypes harvested at 45 DAS. Two P levels were identified as P sufficient and P deficient: 30 versus 75 µmol Pi plant^{-1} week^{-1}.

Thus, the first screening of Madagascar, Niger and Mali cultivars and landraces highlighted 10 contrasting cultivars and landraces whose tolerant lines for shoot and nodule biomass were the cultivar VMDV4 and the 4 landraces VMD4, VMD7, VMD8 and VMD10, while the sensitive lines were the cultivar VMDV1 and the 4 landraces VMD1, VMD2, VMD11 and N'jibawolo.

3.3. Efficiency in use of rhizobial symbiosis (EURS)

The SNF was closely related to biomass produced during the culture marked by a significant relationship between nodule and shoot biomass (Fig. 3). Genotypic variability was observed in some cultivars and landraces where high nodulation was associated with high shoot biomass. The shoot biomass of landrace 7 and cultivar 1 were significantly the highest, with more than 4 g DW plant^{-1}, and nodulation between 0.2 g and 0.4 g nodule DW plant^{-1} (Fig. 3) compared with landraces 8, 11 and 1, and cultivar 4.

The plants response to SNF was assessed by the regression line of shoot biomass as a function of the nodule biomass. Thus, EURS between different cultivars and landraces was calculated from the regression slope. The contrasting lines for EURS were the landraces 2, 7 and cultivar 1, with circa 14 g shoot DW g^{-1} DW nodule under P deficiency compared to the others (Fig. 3). Interestingly, low nodulation with weak nodule biomass in landrace 2 was compensated by a great EURS. The other landraces presented a lower EURS under P deficiency than under P sufficiency. Such tolerant cultivars as V4 and 1 were in this group.

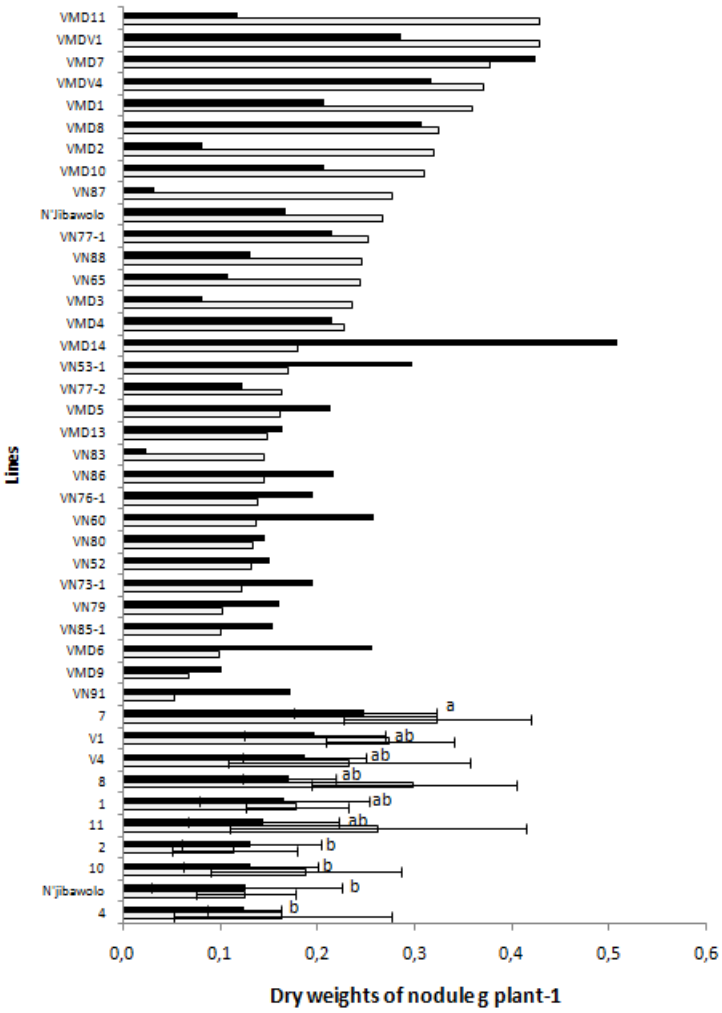

FIG. 2. Nodule growth of voandzou cultivars under P sufficiency (white bar) versus P deficiency (black bar) under hydroponic conditions at 45 DAS. 58 cultivars were prescreened without replication. The 10 most contrasting cultivars from prescreening based on nodule biomass were replicated. Error bars represent standard deviations from 8 replicates. Difference in means at P<0.05 is indicated by different letters under P deficiency.

3.4. Nodulated-root respiration

The rhizospheric concentration of O_2 (pO_2) in contact with the nodulated-root was significantly related to nodulated root O_2 consumption (Conr) for landrace 11 ($P = 0.038$), for cultivar 1 ($P = 0.040$); for landrace 7 ($P = 0.012$); and for cultivar 4 ($P = 0.002$) (Fig. 4). Indeed, the increase of pO_2 induced an increase of Conr to a maximum value corresponding to the critical oxygen pressure ($pO_2 = 40\%$). The Conr at ambient rhizospheric pO_2 (21% O_2) showed that roots and nodules respiration for the plants growth and maintenance under P

deficiency was 76% for cultivar 1, 30% for landrace 11 and 11% for landrace 7 of that under P sufficiency. However, Conr values for the cultivar 4 under P deficiency was 182% of that under P sufficiency (Fig. 4). The same observation was made on Conr at 40% O_2 which revealed the maximum respiration of the plants under exposure to a critical pO_2.

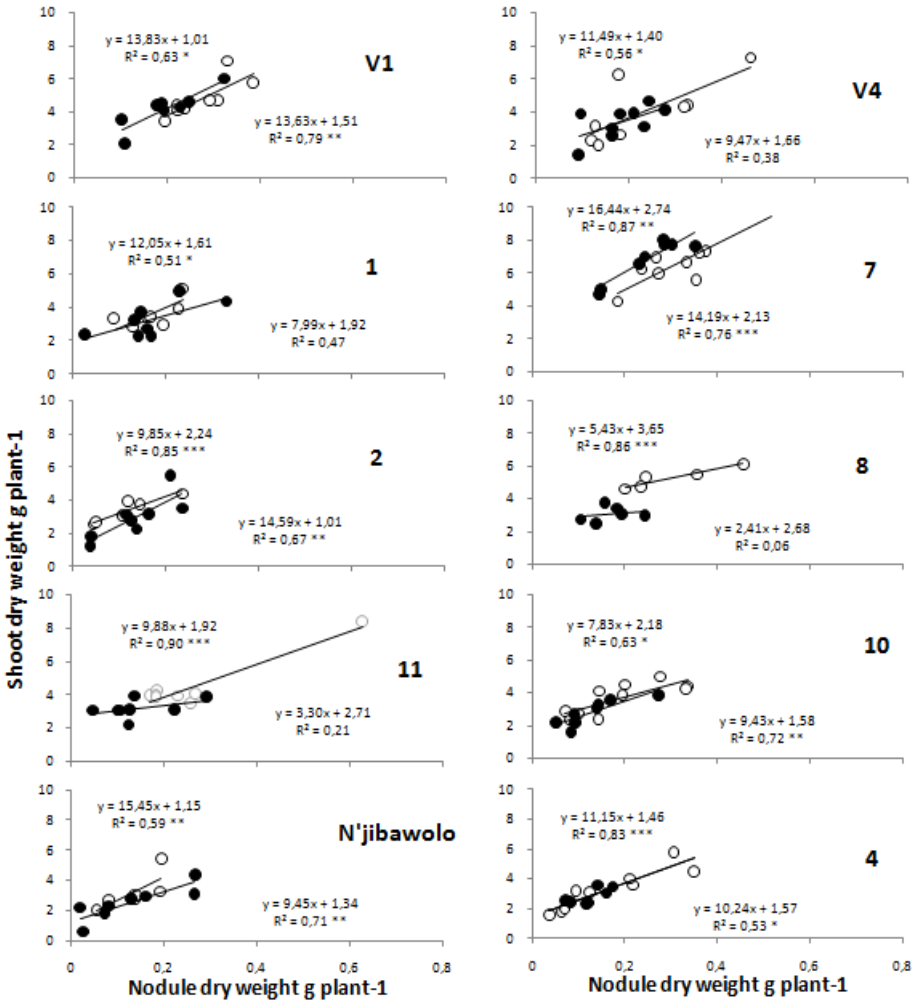

*FIG. 3. Efficiency in use of rhizobial symbiosis of 10 cultivars under P sufficiency (white circle) versus P deficiency (black circle). Data are individual values for 10 voandzou cultivars and landraces harvested at 6 weeks after transplanting. Symbols *, **, *** indicate P<0.05, P<0.01 and P<0.001, respectively.*

The general tendency of the regression curves reported the superiority of tolerant landrace 7 and cultivar 4 under P deficiency compared to P sufficiency in contrast with the sensitive landrace 11 and cultivar 1. Under P deficiency, the slope reflecting the nodule permeability was higher for landrace 7 than for cultivar 4 (Fig. 4). Nodule permeability to O_2 was calculated by dividing the slope of regression by nodule areas as previously described [14, 16, 24, 26]. Nodule permeability under P deficiency was similar for cultivar 4 and landrace 7, with 0.60 and 0.46 µm s^{-1}, respectively, which were 4 times higher than under P sufficiency.

3.5. Microscopic analysis of nodules

Under P deficiency, the cortical cells of "inner cortex" were larger in landrace 7 and cultivar 4 with marked increase in cell size and intercellular space. The superiority of Conr

under P sufficiency for cultivar 1 could be explained by a greater elongation of intercellular spaces in the "inner cortex" compared with P deficiency (Fig. 4).

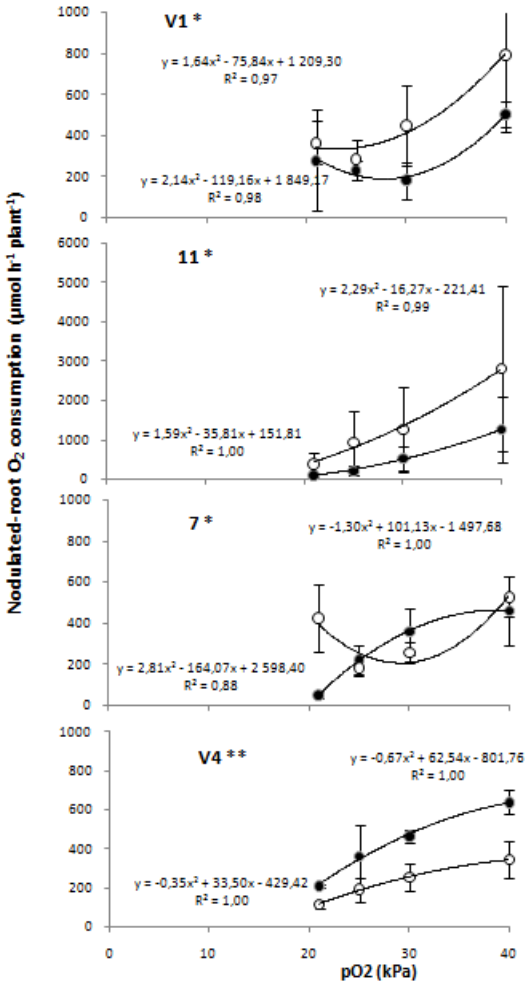

*FIG. 4. Nodulated root O_2 consumption to increasing rhizospheric pO_2 for 4 cultivars under P sufficiency (white circle) versus P deficiency (black circle). Data are means of 3 replicates between the 40th and 44th day after transplanting in serum bottles. Errors bars represents the standard error of the mean. Significant correlation at P<0.05 and P<0.01 are indicated by symbols * and **, respectively.*

More expression of the phytase gene, as bright green points, especially around the vascular trace, the "inner cortex" and the infected zone was observed in particular for landrace 7 under P deficiency (Fig. 5). An increase in phytase gene expression was also observed on intercellular spaces for cultivar 4 under P deficiency. The expression of the phytase gene in the nodule cross-section could explain the cultivar tolerance to P deficiency through the biomass results.

4. DISCUSSION

This study highlights contrasting lines of voandzou for SNF and N_2-dependent growth under P deficiency, in agreement with Greder et al. who concluded that genotypic selection on the basis of nodule weight is justified [27]. Thus, the selection of 58 cultivars and landraces of Madagascar, Niger and Mali allowed the prescreening of cultivars and landraces with nodulation above 0.2 g nodule DW plant^{-1} and shoot biomass above 2 g DW shoot plant^{-1}

under P deficiency. The lower nodules and shoot biomass of cultivars and landraces of Niger and Mali compared with those of Madagascar (Fig. 2) suggest that the genetic potentiality of the different voandzou selected in terms of biomass differ according to the origin of seeds in different agro-ecological regions, whose development is influenced by environmental factors, in particular temperature and photoperiod [28]. It confirms indeed that a great inter-landrace variation exists in the genetic resources of voandzou [29]. Numerous authors emphasized the variability of legume SNF experimental conditions, which reflect the genetic ability of the different legume species for N_2 fixation and yield under environmental constraints [10]. Thus, the critical P requirement of voandzou (Fig. 1) is lower than that of other legume species like *Glycine max* [15] and *Phaseolus vulgaris* [26, 30], but higher than that of *Vigna unguiculata* [14] and *Acacia mangium* [31].

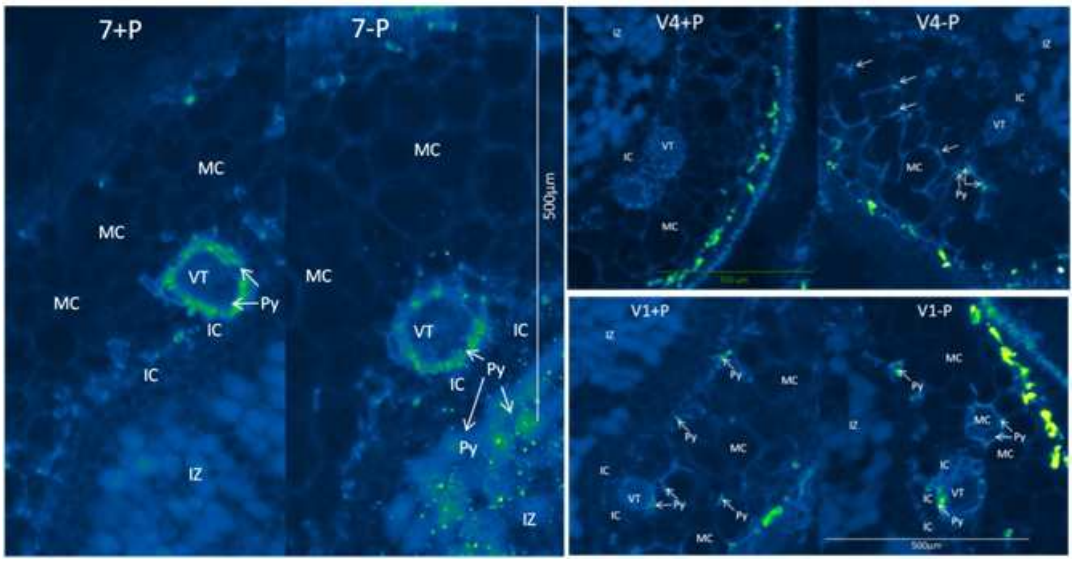

FIG. 5. Cross-section of a voandzou nodule. MC: Middle cortex, IC: Inner cortex, VT: Vascular trace, Py: Gene phytase expression, IZ: Infected zone. Scale bar: 500µm. Under P deficiency, the tolerant cultivars (7 and V4) were marked by an increase of cell size and intercellular space coupled with more gene phytase expression. 7+P: Landrace 7 under P sufficiency; 7-P: Landrace 7 under P deficiency; V4+P: Cultivar 4 under P sufficiency; V4-P: Cultivar 4 under P deficiency.

The inoculation of voandzou with *Bradyrhizobium* sp. Vigna CB756, initially developed for *Vigna unguiculata* cultivars, showed a great expression of genetic potential in the P use for nodulation and biomass, in particular cultivar 1 with a great response of P compared with cultivar 6. This genotypic variation could be attributed to the effectiveness of the rhizobia-legume symbiosis where some genotypes have selectivity for a highly effective strain selected for high nodulation and consequently for high productivity [10]. Kishinevsky et al. reported from their experiments with 20 landraces of voandzou, that the plant genotype and the *Bradyrhizobium* strain are important factors to be considered in a breeding program for a high N_2 fixation rate [5].

As a result of this pre-screening, ten contrasting lines were identified, including five tolerant lines in terms of shoot, root, and nodule biomass under P deficiency, and five sensitive in terms of the productivity decrease by P deficiency (Fig. 2). The most contrasting lines confirmed their high SNF potential by high SNF in terms of nodules and shoot biomass, in particular landrace 7, 11, cultivars V4 and V1. This great potential was highlighted by a better EURS under P deficiency for such lines as landrace 7, 2 and cultivar V1 (Fig. 3).

Furthermore, EURS under P deficiency was much higher than under P sufficiency for landraces 7 and 2, illustrating the tolerance of these lines to P deficiency. These results are in agreement with those obtained on contrasting lines of bean [14] and *Vigna unguiculata* [26].

The increase of O_2 consumption of nodulated root (Conr) in response to the variation of rhizospheric O_2 concentration for all the lines reveals an important energy respiratory requirement for SNF. Under ambient rhizospheric condition, in particular under pO_2 of 21%, the root and nodule respiration for plant growth and maintenance is limited by P deficiency in comparison with P sufficient, specifically for the sensitive cultivars V1 and 11. In addition, a 4 fold increase in nodule respiration, with a value 4 times higher in nodule permeability under P deficiency compared with P sufficiency, is in agreement with previous results for soybean, common-bean and [14–16]. Nodule permeability controls nodule respiration which provides ATP for the N_2 reduction in the infected zones of the nodule [13, 16, 26]. An alternative respiratory coupled with a large O_2 consumption by ATP produced under P deficiency can explain this increase in nodule permeability [15, 16, 24]. Thus, limitation of nodulated root respiration under ambient pO_2 was higher for the sensitive cultivars V1 and 11 than for the tolerant ones (Fig. 4).

The image analysis of histological observations on nodule cross-sections under P deficiency (Fig. 4) confirms that the cortical cells of the "inner cortex" increase in size when nodule permeability is increased, in agreement with previous observations of increases in cell size and intercellular space of the inner cortex in soybean, cowpea, and alfafa, and the nodule permeability to O_2 diffusion in soybean [15, 32, 33]. The orthophosphate ions could be implicated in the osmotic pressure regulation in the "inner cortex" cells [15]. *In situ* RT-PCR image analysis of the nodule cross-section showed a larger increase in the expression of the phytase gene in nodules under P deficiency, and specifically around the vascular trace, in the "inner cortex" and the infected zone for landrace 7. The nodulated root phytase activity in terms of the expression of acid phosphatase is in accordance with previous findings [4] for the lines of bean contrasting in their PUE for SNF, where the expression of acid phosphatase varied among the tissues, and was higher in the cells of the "inner cortex". The phytase gene expression is linked with the change of nodule permeability associated with the adaptation to P deficiency, specifically for Landrace 7 and cultivar 4, where the nodule permeability to O_2 was increased 4 times under P deficiency, suggesting a link, either direct or indirect, between phytase activity and the regulation of the respiration linked to SNF by nodule permeability.

5. CONCLUSION

Genotypic variation of the SNF for the PUE was observed and highlighted in the 54 cultivars and landraces from Madagascar, Niger and Mali. Variability was especially related to the nodule and shoot biomass, nodule permeability, nodule respiration and gene phytase expression. These experiments made it possible to select the more contrasting cultivars and landraces in terms of PUE for SNF, in particular the most tolerant ones such as landrace 7, cultivar 4, and the most sensitive ones such as cultivar 1, to be further compared under field conditions.

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GENOTYPIC VARIATION IN PHOSPHORUS USE EFFICIENCY FOR SYMBIOTIC NITROGEN FIXATION IN COWPEA *(VIGNA UNGUICULATA)*

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Abstract

Cowpea (*Vigna unguiculata* L. Walp) is an important food legume. In Africa, it is mostly cultivated under such environmental constraints as drought and pest, and nutrient deficiency. In particular low soil phosphorus strongly limits crop production for the poor farmers with limited access to P fertilizers. Therefore breeding cowpea for the tolerance to P deficiency is considered as an alternative to increase the productivity of traditional cowpea-cereal cropping systems in soils with low P availability. This paper reports cowpea genotypic-variation in P use efficiency for symbiotic nitrogen fixation as a contribution to select tolerant cowpea lines under P deficiency. Eighty cowpea cultivars inoculated with the reference strain of *Bradyrhizobium* sp. *Vigna* CB756 were pre-screened as a single replicate under hydroaeroponic culture for 6 weeks under P deficiency *versus* P sufficiency, namely 15 *vs* 30 µmol plant^{-1} week^{-1}. Large variability in nodule number per plant, and in shoot growth as a function of nodule mass, was observed among the diversity of cowpea lines. From this pre-screening experiment, the 40 cowpea lines showing the highest SNF-potential, i.e. high nodulation linked with high N_2-dependent growth under P sufficiency, and the most contrasting tolerance to P deficiency, i.e. highest *vs* lowest N_2-dependent growth under P deficiency, were grown again in glasshouse hydroaeroponics with 6 replicates. As an illustration of the most contrasting lines, the nodulation was decreased under P deficiency by less than 20% for IT82E-18 whereas by more than 80% for IT95K-1105-5 or SUVITA 2. The variations in nodulation were correlated with variations in growth with mean value of additional growth per unit increase in nodule biomass of 23 g shoot DW g^{-1} nodule DW under P sufficiency, showing 3 lines showing exceptionally high potential for symbiotic nitrogen fixation, *versus* 28 g shoot DW g^{-1} nodule DW showing large variation among lines. Most of the tolerance to P deficiency was due to increase in the nodule function rather than conservation of nodule mass. In conclusion, the screening in glasshouse hydroaeroponics made it possible to sort cowpea lines in SNF potential to fix N_2, and in tolerance to P deficiency.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is an important food legume and mostly cultivated in tropical savannas of African semi-arid and arid areas [1]. It is an essential component of cropping systems covering over 9.3 million ha making this legume an important source of quality nourishment to the urban and rural populations in various

developing country [2]. However, most African soils have low inherent levels of available P. Soil-P availability during plant development is an important determinant of growth, symbiotic nitrogen fixation (SNF) and grain yield of legumes [3]. Plants adapt to this environmental constraint through various morphological and physiological mechanisms as root hair development, higher root surface to increase P acquisition efficiency, and exudation of phosphatase and organic acids to increase P availability [4, 5].

Current research on cowpea breeding has concentrated on development of improved cowpea breeding lines, combining disease and insect resistance with quantitative traits such as higher grain yield, and with tolerance to extreme temperature and adaptation to drought [6, 7]. Nevertheless, the contribution of cowpea to soil fertility through SNF under such an abiotic constraint as P deficiency prompted variety screening under P deficiency for SNF [7, 8]. Contrasting cowpea genotypes for tolerance to P deficiency was considered as an alternative for the soil with low P availability, which strongly limits crop production for the poor farmers with limited accessibility to P fertilizers [8, 9]. Indeed, growth, nodulation and N_2-fixation of cowpea could be inhibited by P deficiency, whereas tolerant genotypes can access P which is not usually available to the average genotypes under low P conditions. However, few publications describe the physiological mechanisms by which cowpea adapts to P deficiency. The optimization of SNF for P use efficiency (PUE) in legume species were already undertaken for genotypic variation by numerous researchers [1, 2, 8]. Genotypic variability of cowpea in PUE for symbiotic nitrogen fixation has been found to be associated with such rhizospheric functions as nodulated-root proton efflux and respiration under low-P soils [1]. The contrasting genotypes increased the soil P availability by about 50% after a cultural cycle. Thus, cowpea breeding could significantly affect the productivity of traditional cowpea-cereal cropping systems. The present study aimed at screening a diversity of cowpea genotypes in PUE for SNF under hydroaeroponic culture.

2. MATERIALS AND METHODS

2.1. Biological materials

The seeds of cowpea 24-125B-1, 524B, 58-53, 58-57, APAGBAALA, BAMBEY 21, CB27, CB46, CC-27, UCR CC-36, DANILA, IAR7/8-5-4-1, IFE BROWN, IRON CLAY, IT82E-18, IT84S-2049, IT84S-2246, IT85F-3139, IT85F-867-5, IT90K-284-2, IT93K-2046-1, IT93K-503-1, IT93K-693-2, IT97K-437-1, IT95K-1105-5, IT95K-1479, IT95K-1491, IT95M-190, IT95M-303, IT96D-602, IT96D-610, IT97K-207-15, IT97K-461-4-1, IT97K-499-35-1, IT97K-499-39, IT97K-556-6, IT97K-569-9, IT97K-819-132, IT98D-1399, IT98K-128-2, IT98K-205-8, IT98K-205-8, IT98K-498-1, IT98K-555-1, IT98K-558-1, IT98K-589-2, IT99K-407-8, IT89KD-288, KVx-396-4-5-1, KVx403, KVx421-25, KVx525, KVx544-6-151-3, KVx61-1, MELAKH, Mounge, MOURIDE, NDIAMBOUR, PRIMA, Sasaque, SUVITA 2, TVu-7778, UCR Sh50-17-9-1, UCRP-24, UCR 779, YACINE, were kindly supplied by Jeff Ehlers from the University of California (USA) where the lines were selected for adaptation to water deficiency for the Sahelo-Sudanian pedo-climatic conditions. The additional lines DD 07, DMI 07, IT90K-372-1-2, KA85-19, KB85-23, KVx30-309-6G, TN121-80, TN256-87, TN259-87, TN27-80, TN3-78, TN5-78, TN88-63, and BAMBEY, Mouride, were supplied by INRAN (Niger) and ISRA (Senegal).

2.2. Symbiosis culture

The seeds were sterilized with 3% calcium hypochlorite for 20 min and rinsed by 5 washings with sterile distilled water. They were then transferred for germination on humidified wrapped filter paper in a slightly tilted vat and placed in an incubator at 28-30°C.

After germination, the inoculation was performed by soaking the roots of seedlings for 20 min in a suspension of *Bradyrhizobium* sp. *Vigna* CB756 containing 10^9 bacteria ml^{-1}. The inoculum was prepared from a rhizobia culture preserved in tubes at 4°C on 120°C-sterilized agar YEM (Yeast Extract Mannitol) medium containing 900 ml distilled water, 100 ml of Bergersen concentrated solution consisting of a mixture of 1 g KCl, 0.1 g $FeCl_3$, 0.4 g $CaCl_2$, 4.5 g $Na_2HPO_4.12H_2O$ and 1 g $MgSO_4.7H_2O$, 1 g Yeast extract, 10 g mannitol and 15 g agar [10]. From one of the preserved tubes, some strains were taken and transferred into 100 ml of liquid YEM, and maintained under agitation at 28°C for 24 h.

The germinated seeds were carefully transferred for hydroaeroponic culture into each 40 l vat with 20 inoculated plants per vat. The roots of each seed were carefully passed through the hole of a rubber stopper with cotton wool fixed at the hypocotyl level. They received the following nutrient solution that was changed every 2 weeks: $CaCl_2$, 1650 µM; $MgSO_4.7H_2O$, 1000 µM; K_2SO_4, 700 µM; Fe EDDHA, 8.5 µM as sequestrene Fe; H_3BO_3, 4 µM; $MnSO_4.H_2O$, 6 µM; $ZnSO_4.7H_2O$, 1 µM; $CuSO_4.7H_2O$, 1 µM; $Na_2MoO_4.7H_2O$, 0.1 µM. 2000 µM of urea was supplied for all treatments during the first two weeks' culture. Each P was supplied in the form of KH_2PO_4 with an exponential distribution for the two levels: 15 and 30 µmol $plant^{-1}$ $week^{-1}$ as P deficiency and P sufficiency, respectively.

The experiment was carried out in a glasshouse at 20/33°C temperature during 16/8 h day/night cycle coupled with an intense ventilation of nodulated-roots, a complementary illumination of 400 µmol photons m^{-2} s^{-1} when needed, and 70% daily relative humidity. These conditions allowed an optimal expression of genetic potential of nitrogen fixation while controlling closely the mineral nutrition mainly for N, P and for the other elements.

2.3. Biomass measurements and statistical analysis

The plants were harvested at the end of the 6^{th} week at the flowering stage. The shoot was separated from the root at the cotyledonary node, and then weighted after 48 h at 80°C. Nodules were excised from roots, counted and weighted. The effect of P supply was estimated from the response curves of the biomass, and the critical P supply was deduced at the maximum growth. In order to determine the contribution of P supply to plant growth, the response curves were established from biomass values with the software Excel Microsoft Office XP. The genotype and P treatment effects were analyzed by a two-way analysis of variance (ANOVA). Correlation and regression analysis were performed to complete the analysis.

3. RESULTS

3.1. Pre-screening for nodulation under P sufficiency *versus* P deficiency

Large variability in nodule number per plant was observed among the diversity of cowpea line used as a single replicate in the pre-screening experiment (Fig. 1). Under P sufficiency the highest number of nodules was more than 700 $plant^{-1}$, e.g. IT90K-284-2, whereas the lowest nodulation was 10 $plant^{-1}$, e.g. Mouride, 58-53, IT97K569-9, IT97K-461-4-1 and KA85-19. The mean nodulation was 300 $plant^{-1}$ for one third of the lines, and below

100 plant^{-1} for another third of the lines. Under P deficiency, the highest number of nodules was between 300 and 500 plant^{-1} for YACINE, NDIAMBOU, IRON CLAY, IFE BROWN, IAR7/8-5-4-1, IT99K-407-8, IT98K-555-1 and IT97K-819-132, whereas more than two thirds of the lines had less than 200 nod plant^{-1}.

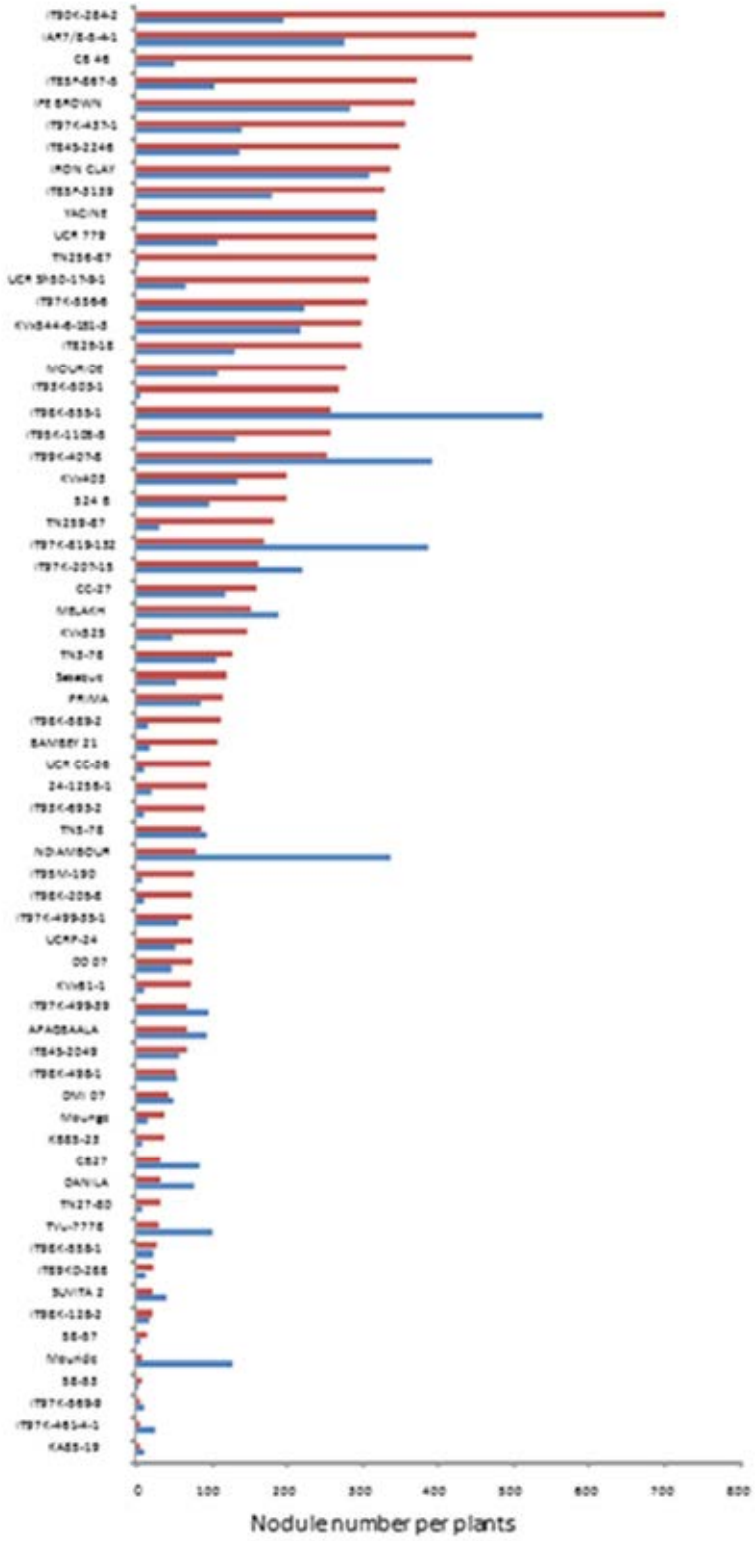

FIG. 1. Variation in nodule number per plant among cowpea lines under P sufficiency (■) versus P deficiency (■). Data are single values per line.

The nodule mass of the lines as a function of nodule number is shown in Fig. 2. The significant relation between both parameters established that the mean individual-nodule mass was 0.3 mg nod^{-1} under P sufficiency and 0.6 mg nod^{-1} under P deficiency. However the biggest nodule reached almost 2 mg nod^{-1} whatever the P supply, although bigger nodules were more frequent among lines with lower nodule number.

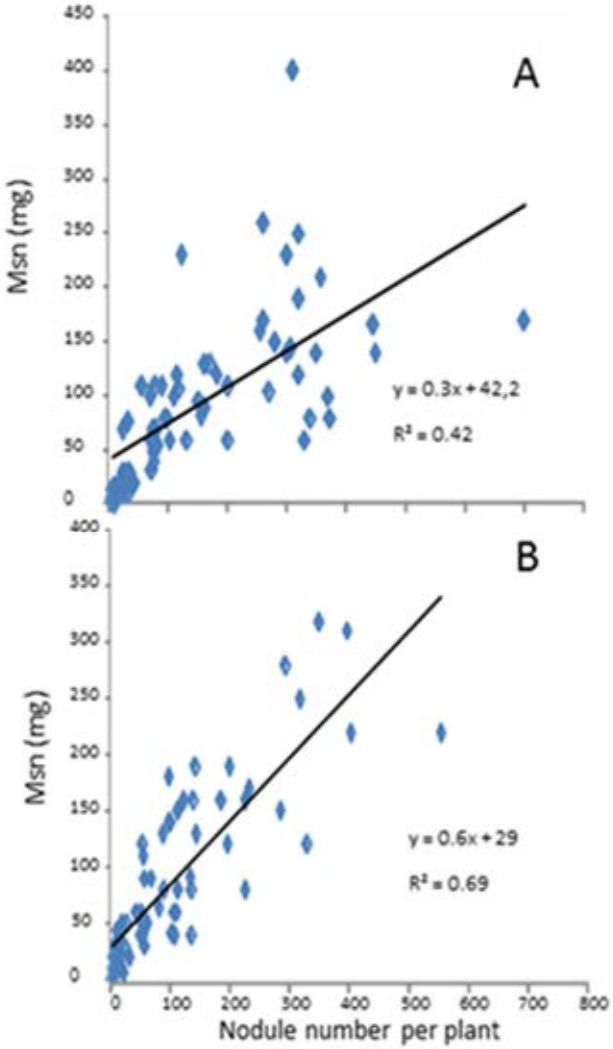

FIG. 2. Relation of nodule growth as a function of nodule number under P sufficiency (A) versus P deficiency (B) for cowpea lines shown in Fig. 1. Data are single values per line.

The shoot growth of the lines as a function of nodule mass is shown in Fig. 3. The significant relation between both parameters established that the mean growth per nodule mass was around 5 g shoot DW g^{-1} nod DW without any significant difference between P supply, although the growth without nodulation, i.e. dependent on seed N and starter N, was twice as high under P sufficiency (0.6 g DW shoot $plant^{-1}$) than under P deficiency (0.3 g DW shoot $plant^{-1}$). However, the largest N_2-dependent growth was close to 10 and 15 g shoot DW g^{-1} nod DW, under P sufficiency and P deficiency, respectively, among lines harboring more than 0.2 g DW nod $plant^{-1}$. The shoot growth was also significantly correlated with root growth with a mean shoot: root ratio of 2.2, whatever the P supply. By contrast, there was no significant correlation between root growth and nodule mass or number (data not shown). The distribution of biomass among the three organs for the diversity of the lines tested in the pre-screening is shown in Fig. 4.

3.2. Relation between root nodulation and plant growth among most contrasting lines

From the data in Figs. 2 and 3 lines were chosen having the maximal SNF-potential, i.e. high nodulation linked with high N_2-dependent growth under P sufficiency, and the most contrasting tolerance to P deficiency, i.e. highest or lowest N_2-dependent growth under P deficiency. They were grown again in glasshouse hydroaeroponics with 6 replicates.

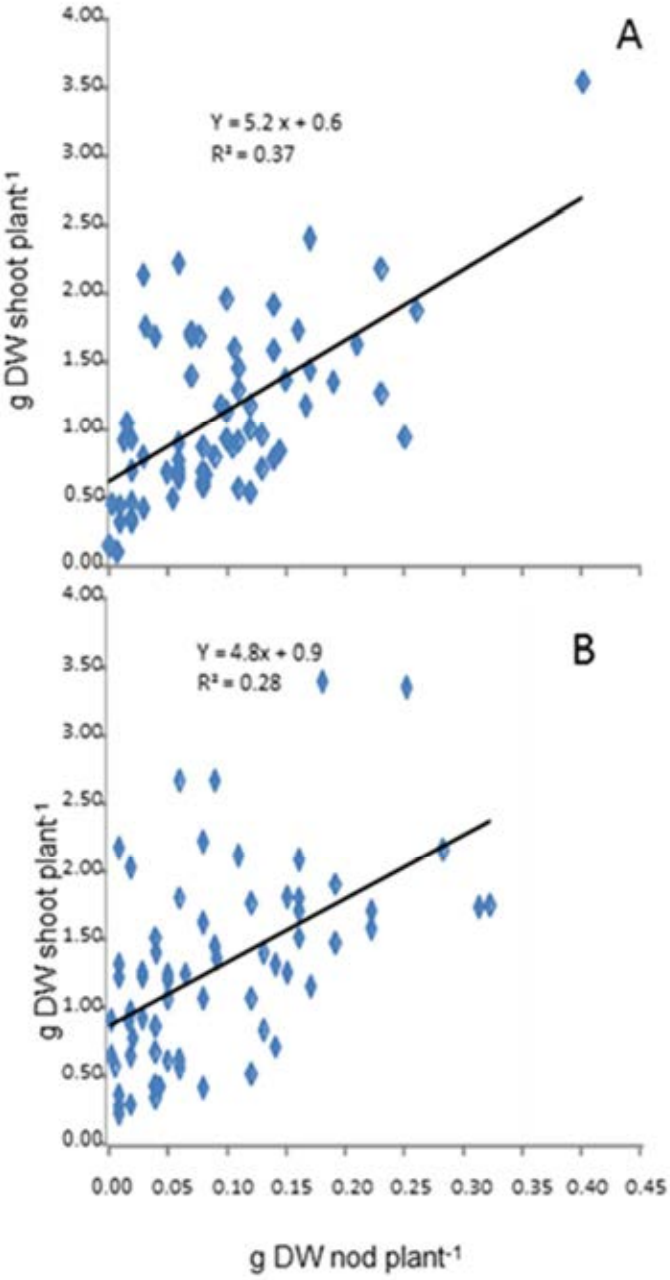

FIG. 3. Relation of shoot growth as a function of nodule number for cowpea lines under P sufficiency (A) versus P deficiency (B). Data are single values per line.

Lines having more than 400 mg DW nod plant^{-1} under P sufficiency are shown in Fig. 5. Among these lines, IT82E-18 show the highest nodulation under P deficiency, by contrast with such lines as IT95K-1105-5 or SUVITA 2 where nodulation decreased by more than 80% under P deficiency. The variation in nodulation, as shown in Fig. 5, were correlated with variation in growth, as illustrated in Fig. 6, showing that total biomass in shoots varied from more than 12 g DW plant^{-1} to almost 2 g DW plant^{-1} under P sufficiency, and from more than

8 g DW plant^{-1} to less than 2 g DW plant^{-1} under P deficiency. Three lines were found to have high nodulation with an exceptionally high potential for symbiotic nitrogen fixation, supporting a growth of more than 10 g DW shoot plant^{-1}.

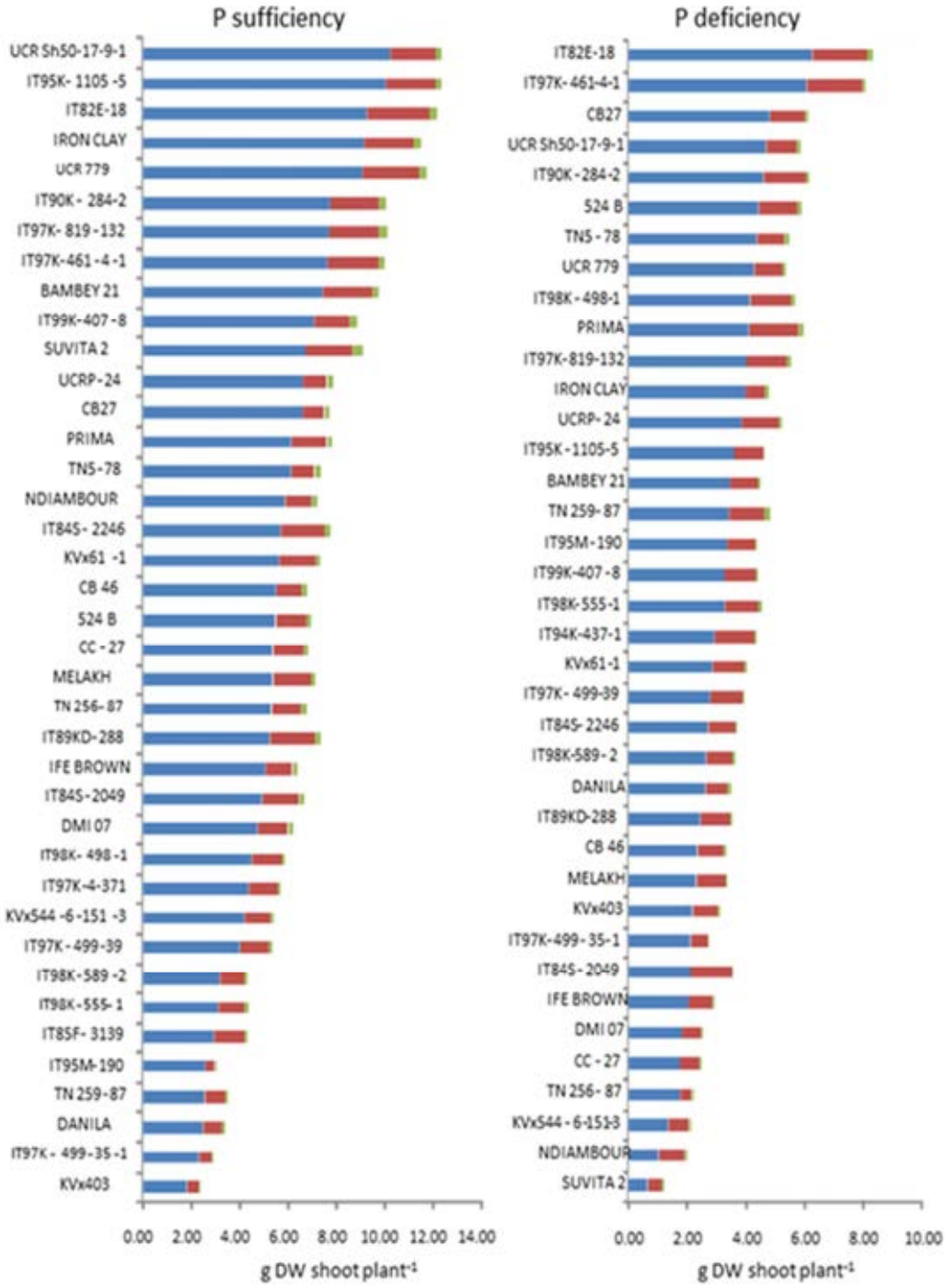

FIG. 4. Variation in total growth and distribution in shoot (■), root (■) and nodule (■) per plant among cowpea lines under P sufficiency (left) versus P deficiency (right). Data are single values per line.

Under P sufficiency, the relation between both parameters was linear between 100 and 330 mg DW nod plant^{-1}. The mean value of additional growth per unit increase in nodule, namely the efficiency in use of the rhizobial symbiosis (EURS) was 23 g DW shoot g^{-1} DW

nod. Under P deficiency, the mean EURS was 28 g DW shoot g^{-1} DW nod, i.e. slightly and significantly higher than under P sufficiency. In addition, there was more variation in EURS for the lines under P deficiency than under P sufficiency. The two lines showing the highest growth under P deficiency were TN259-87, IT82E-18 having higher EURS than the other lines. Three other lines tolerant to P deficiency, TN5-78, UCR Sh50-17-9-1 and IRON CLAY, also showed higher EURS than the rest of the lines. Thus most of the tolerance was due to increase in the nodule function rather than conservation of nodule mass under P deficiency. The two most sensitive lines to P deficiency were IT84S-2049 and IT95K-1105-5, combining low nodulation with low EUSR, whereas the sensitivity of three other lines, IT97K-499-35-1, NDIAMBOUR and SUVITA 2, was due to low EURS.

4. DISCUSSION

Eighty-one cowpea lines were screened under hydroaeroponic culture for adaptation to P deficiency and phosphorus use efficiency (PUE) for symbiotic nitrogen efficiency (SNF). The pre-screening measurement of shoot, root and nodule biomass under P sufficiency *versus* P deficiency (Fig. 4) indicated the existence of useful genetic variation among cultivars for PUE: SNF as confirmed by the subsequent experiment with 6 replicates for the most contrasting genotypes (Fig. 5). Nodulation measurements revealed 21 cowpea lines with high nodulation under P sufficiency, namely ITKOK-284-2, IAR7/8-5-4-1, CB46, IT85F-867-5, IFE BROWN, IT97K-437-1, IT84S-2246, IRON CLAY, IT85F-3-139, YACINE, UCR 779, TN256-87, UCR Sh50-17-9-1, IT97K-556-6, KVx544-6151-3, IT82E-18, MOURIDE, IT93K-503-1, IT98K-555-1, IT95K-1-105-5, IT99K-407-8, while 14 lines were highlighted under P deficiency with more than 150 mg dry weight nodule per plant, namely ITKOK-284-2, IAR7/8-5-4-1, IFE BROWN, IRON CLAY, IT85F-3-139, YACINE, IT97K-556-6, KVx544-6151-3, IT98K-555-1, IT99K-407-8, IT97K-819-132, IT97K-207-15, MELAKH and NDIAMBOUR (Fig. 2). These nodulation results were higher than those found bunder P deficiency [1], but lower than those found under P sufficiency [1, 11]. These discrepancies may be explained by the temperature and the natural illumination of the glasshouse that might have been lower in this work than during the previous experiments.

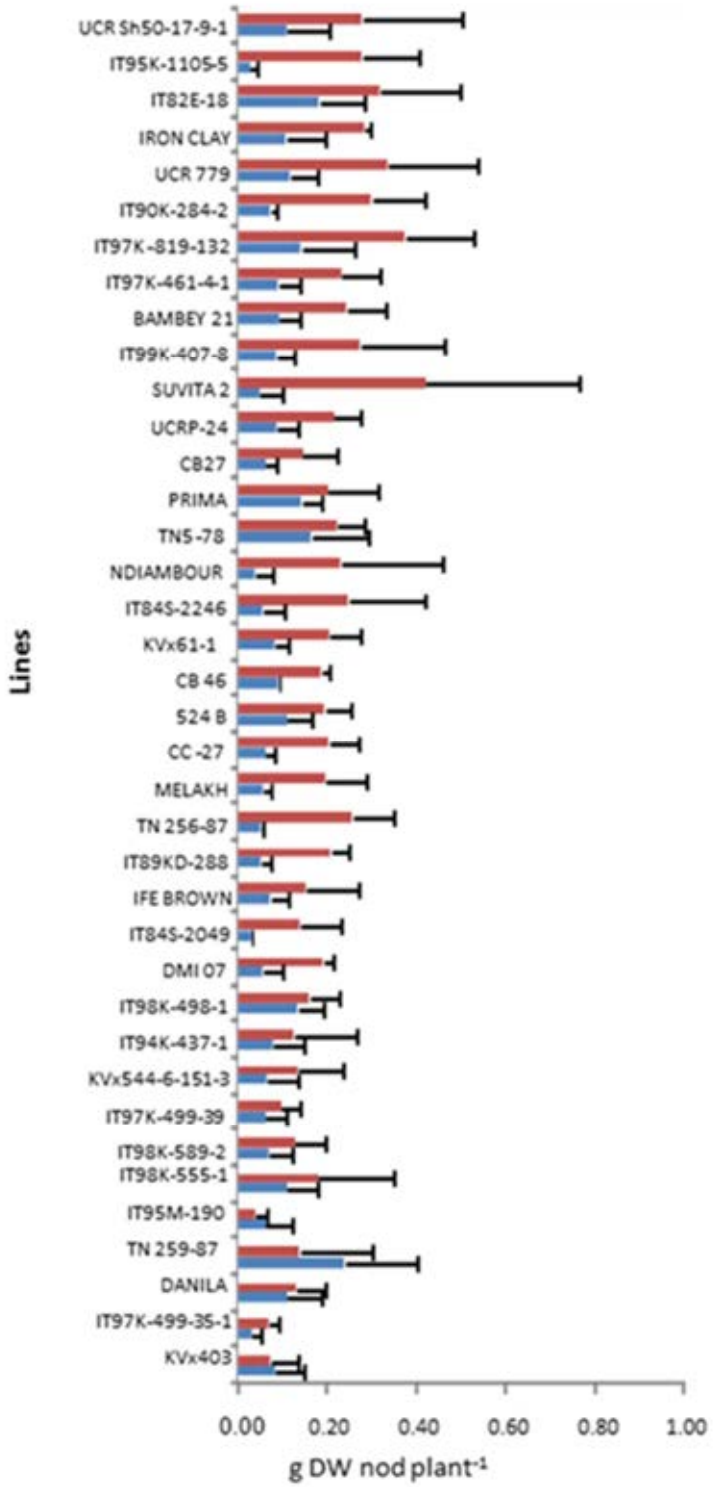

FIG. 5. Variation in nodule DW per plant under P sufficiency (■) versus P deficiency (■) among the most contrasting cowpea lines for tolerance to P deficiency (Fig. 1). Data are means and bars represent standard deviations of 6 replicates for each line.

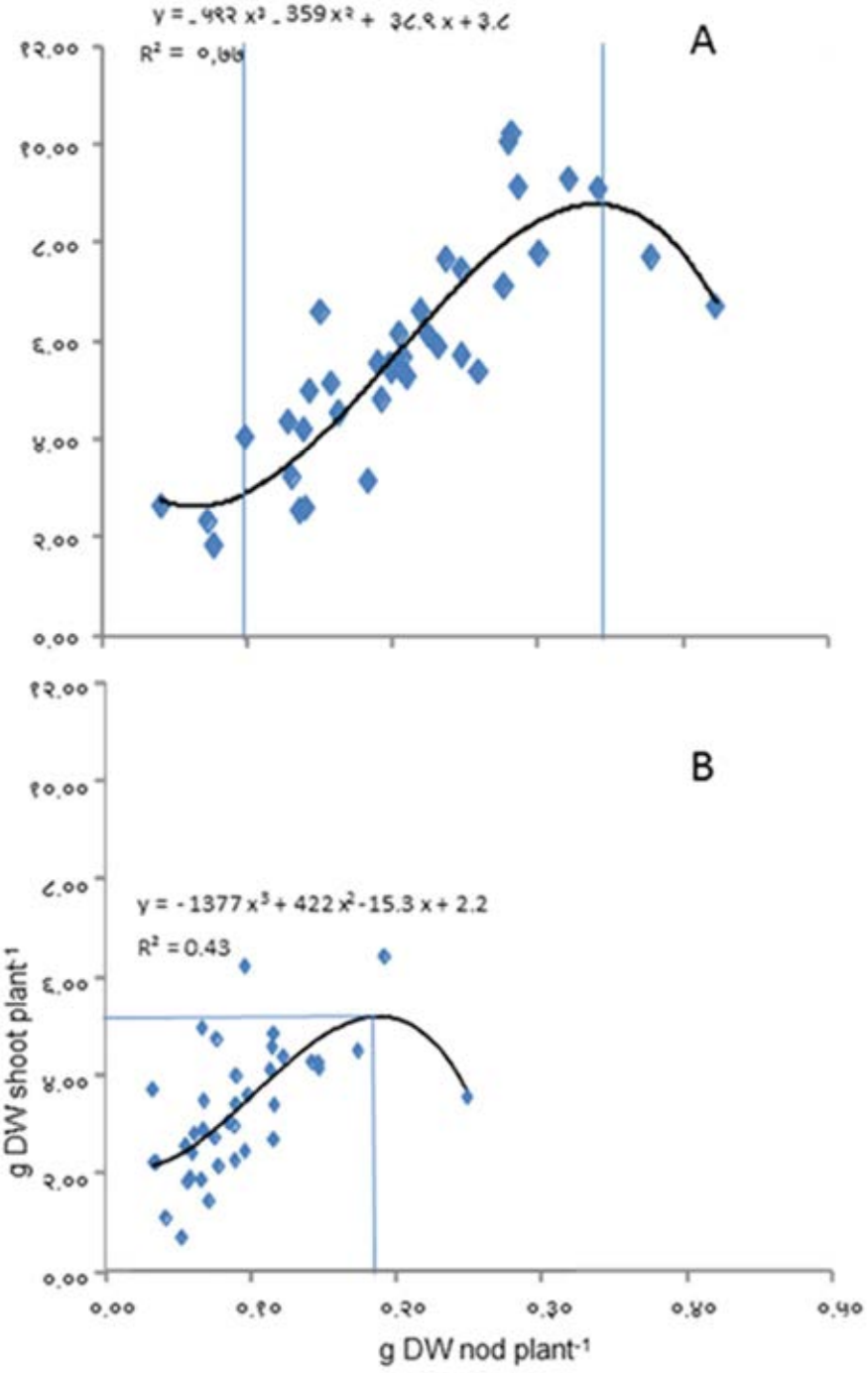

FIG. 6. Relation of shoot growth as a function of nodule mass for cowpea lines under P sufficiency (A) versus P deficiency (B) shown in Fig. 5.

Furthermore, plant biomass is an important indicator used in many allometric relationships. Thus variations in biomass production are used as a selection criterion for genotype assessment of nutrient efficiency at the seedling stage [12, 13]. Shoot biomass data under P deficiency (Figs. 4 and 6) are in agreement with that observed previously [1], but the values under P sufficiency were mostly higher than those found previously on cowpea lines [1, 14]. Previous studies reported that the low efficiency, shown by such lines as Melakh or Bambey-21, could be explained by early maturity with more effects of environmental constraints during vegetative- than reproductive- stages [15]. Gerloff reported that the intolerance to P deficiency is often due to inefficient P-acquisition mechanism [16]. Studies

on pigeonpea reported that genotypic difference in response to P deficiency may be related to the magnitude of resistance of the cultivar to a decrease in shoot growth, as the P deficiency affects leaf initiation and photosynthate per unit leaf area [17]. Tolerant hybrid-cultivars with low restriction of leaf number and leaf area by P deficiency, could partition more assimilates for shoot growth than sensitive cultivars where partitioning to roots was important for survival [17]. Efficient lines such as TN259-87, IT82E-18, TN5-78, UCR Sh50-17-9-1, IRON CLAY, UCR 779, IT97K-819-132, PRIMA, 524B, IT98K-498-1, IT98K-555-1 and DANILA with more than 150 mg nodule dry weight per plant, and with more than 4 g dry weight of shoot per plant (Figs. 5 and 6), were less inhibited by P deficiency. Furthermore, responsive lines which gave a higher plant biomass than other lines under high P sufficiency were also highlighted as UCR Sh50-17-9-1, IT95K-1105-5, IT82E-18, IRON CLAY, UCR 779, IT90K-284-2, IT97K-819-132, BAMBEY-21, SUVITA 2, IT84S-2246 and TN 256-87, with more than 225 mg nodule DW plant^{-1}, and with more than 5 g shoot DW plant^{-1}. These physiological features were considered as the most appropriate for an effective screening procedure to detect intra-specific differences [16].

The shoot biomass correlation with the nodule mass (Figs. 3 and 6) made it possible to define the efficiency in use of the rhizobial symbiosis (EURS), as estimated by the slope of regression, as in previous work with other legume species [1]. The high EURS under P deficiency (Fig. 6) confirms the results from pre-screening (Fig. 3). It suggests that internal remobilization of acquired P through SNF ability may help the tolerant cultivars to establish higher shoot biomass under P-deficiency. It highlights tolerant lines by high individual nodule mass that combines with high EUSR under P deficiency compared to that under P sufficiency. This agrees with the tolerance of nodulation to P deficiency that was attributed to a lower immobilization of P in nodule structure under P deficiency than under P sufficiency [18, 19], although greater P concentration was found in nodules than other tissues such as shoot and root.

Under P-limiting conditions, N_2-fixing legumes adopt some strategies to enhance N and P acquisition, involving: (i) root morphology by decreased growth rate and increased growth per unit of P uptake, and also by root development; (ii) remobilization of internal inorganic P at the cellular level, (iii) alternative respiratory pathways, (iv) secretion of acid phosphatase and organic acids [20–23]. Efficient N_2-fixing legumes are capable of maintaining a high metabolic activity in their root nodules under P deficiency as a mechanism of tolerance [24]. Increase in the acid phosphatase and phytase activities in nodules of *Phaseolus vulgaris* under P deficiency suggested the role of these enzymes in adaptive mechanisms for N_2-dependent legumes [25]. The up-regulation of acid phosphatases is mainly the means for releasing and recycling inorganic P from both internal and external resources as phytate [26]. Bio-physiological mechanisms to adjust nodule metabolism as a response to P deficiency are developed by legumes such as increased nodule O_2 permeability [19, 27] and plasma membrane intrinsic proteins and tonoplast intrisinc protein aquaporins in the inner cortex cells [28], involving changes in shape and volume of inner-cortex cells in response to stimuli affecting nodule permeability [29].

Also, such nodule enzyme activities as phosphoenolpyruvate carboxylase and malate dehydrogenase, involved in C cycling and energy substrates for N_2 fixation, and phytase, presumably involved in remobilizing P, were suggested as indicators of genotypic variation in legume species such as *P. vulgaris* [30], *Medicago sativa* [19] and *Vigna subterranea* [31]. Many studies reported that species and cultivars differ in their ability to take up specific elements, including P, due to several morphological, physiological, and biochemical mechanisms [32, 33]. Advanced studies of Arabidopsis, common bean (*P. vulgaris*), and

white lupin revealed genetic and biochemical factors as a result of gene expression changes that mediate plant adaptations to P deficiency [22]. Lastly, P nutrition can also affect legume SNF by specific effects on rhizobial growth and on nodule formation and function [34].

5. CONCLUSION

The screening in glasshouse hydroaeroponic culture made it possible to sort cowpea lines with high SNF potential *versus* significantly much lower potential to fix N_2, and with tolerance *vs* sensitivity to P deficiency. Thus contrasting lines of cowpea in PUE for SNF such as UCR Sh50-17-9-1, IT82E-18, IRON CLAY, UCR 779 and IT97K-819-132, are now available to test in farmers' fields whether they can be proposed as an alternative technology in low P soils.

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SELECTION OF GREEN MANURE SPECIES FOR EFFICIENT ABSORBTION OF POORLY-AVAILABLE FORMS OF SOIL PHOSPHORUS

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Abstract

Green manuring is an agronomic practice in which plants or their residues are added to the soil, improving of the soil physical, chemical and biological attributes, and increasing organic matter and fertility levels through nutrient cycling. It is estimated that green manures can increase P bioavailability. The integration of plant species in crop rotations to immobilize P is one of the most promising agronomic measures to improve the availability of P for the main crop. This study aimed to assess 21 species of green manure and a standard plant species (*Lupinus albus*) on their ability to absorb the available forms of P by the ^{32}P isotopic dilution technique. It also aimed to determine if the isotopically exchangeable P, the L-values, differed when calculated with or without taking seed N into account. The results were statistically correlated and analyzed by hierarchical clustering (HCA) in order to group similar plant species. Jack bean was the most efficient species in P utilization while the *Stylosanthes* spp. were the most efficient in P uptake. The seed-derived P affected the P uptake efficiency evaluated by L-value technique.

1. INTRODUCTION

Green manuring is an agronomic practice, in which plant material or crop residues are added to soil, promoting the improvement of the soil physical, chemical and biological attributes, and increase the soil organic matter and its fertility level [1] due to the retention of nutrients [2]. Conditioner plants that have a greater ability to recycle phosphorus can recover the organic and inorganic fractions of low availability and reduce the high amounts of phosphate applied to soil [3], especially when grown in soils with low fertility.

Legumes, when used as green manures, have the ability to utilize insoluble phosphates [4–6] and to release P in available form after mineralization. Thus, in cultivated soil with green manure, the reservoir of available P is significantly enriched [7, 8]. Grasses have also been used, mainly in the Cerrado region of Brazil, due to higher resistance to drought, higher biomass production and lower seed cost. Moreover, the high temperature associated with high humidity in summer, promotes rapid decomposition of plant residues with low C: N ratios [9].

Isotopic labeling of plant tissue is widely used in studies of green manure nutrient dynamics, especially for N, S [10] and P [6, 7, 11], using ^{15}N, ^{35}S, ^{32}P and ^{33}P, respectively. This technique has the great advantage of being able to separate the nutrient derived from the soil from that derived from the applied green manure, making it possible to determine the exact green manure nutrient utilization by the crop. It also allows direct measurement of the differences between plants in their absorption capability of less available forms of soil P [12].

The aim of this study was to use the ^{32}P isotopic technique (L-value), to compare the P uptake efficiency of a range of green manure species, and to verify if the P content in the plant derived from the seeds of the green manure affects the L-value.

2. MATERIALS AND METHODS

2.1. Experimental

The experiment was conducted in the greenhouse at the Center for Nuclear Energy in Agriculture (CENA/USP), Piracicaba (22º42'30" S, 47º38'01" W), São Paulo State, Southeast Brazil. The dystrophic Typic Haplustox soil [13] used in this study was collected from the 0.0–0.2 m depth, dried, sieved in a 2 mm mesh sieve and homogenized. The soil had clay, silt and sand contents, respectively, of 280, 70 and 650 g kg^{-1}, and the following chemical characteristics: pH (0.01 mol l^{-1} $CaCl_2$), 4.5; organic matter, 22.5 g kg^{-1}; P extracted by resin, 6.25 kg^{-1}; K, 0.75 $mmol_c$ kg^{-1}; Ca, 14.4 $mmol_c$ kg^{-1}; Mg, 6.5 $mmol_c$ kg^{-1}; H + Al, 44.2 $mmol_c$ kg^{-1}; CEC, 65.9 $mmol_c$ kg^{-1}; sum of bases, 21.6 $mmol_c$ kg^{-1}; base saturation, 32.8%, according to methodology described by [14]; and P by Mehlich-1, 3.7 mg kg^{-1} [15].The soil was limed according to [14], to reach 70% base saturation, and incubated for 30 days prior to the beginning of the experiment, maintaining the moisture content at approximately 70% of water holding capacity.

The study was conducted in 3.0 l plastic pots, lined with polyethylene bags, containing 2.5 kg of air-dried soil. A ^{32}P radioisotope solution (74 MBq pot^{-1}, carrier free) diluted in 200 ml l^{-1} of distilled water was used to label the soil. After applying this solution, the soil was incubated for 20 days to reach isotopic equilibrium ($^{32}P/^{31}P$).

The experimental design was complete randomized with three replications. The treatment consisted of 21 green manure species: *Brachiaria brizantha* cv. Marandú, *B. brizantha* cv. Xaraés, *B. ruziziensis, Calopogonium muconoides,* sunhemp (*Crotalaria juncea*), *and four other species of Crotalaria (C. breviflora, C. mucronata, C. ochroleuca and C. spectabilis), Stylosanthes guianensis* cv. Campo Grande, *S. guianensis* cv. Mineirão, jack bean *(Canavalia ensiformis),* pigeon pea cv. Fava larga *(Cajanus cajan)*, dwarf pigeon pea *(Cajanus cajan)*, sunflower cv. IAC-Iarama *(Helianthus annuus),* sunflower cv. IAC-Uruguai *(Helianthus annuus)*, lab lab (*Dolichos lablab),* millet *(Pennisetum glaucum),* dwarf velvet bean *(Mucuna deeringiana),* grey velvet bean *(Mucuna cinereum)* and black velvet bean *(Mucuna aterrima)*. White lupin *(Lupinus albus)* was used as the standard species, described in the literature as efficient in P uptake [5, 12, 16, 17].

Six seeds of the bigger sized species (jack bean, pigeon pea, sunflowers, lab lab, velvet bean and white lupin) and twelve seeds of the others were sown in each pot and thinned to two plants pot^{-1} seven days after emergence (DAE). N and K were applied at rates of 100 mg N kg^{-1} as urea and 100 mg K kg^{-1} as KCl. The pots were watered daily with deionized water and were maintained at 70% of water retention capacity.

The plant shoots were harvested 50 DAE, oven-dried at 60°C for 72 hours, weighed and ground to pass a 20 mesh sieve. The plant samples were digested with a mixture of nitric and perchloric acids and analyzed for ^{32}P activity in a Liquid Scintillation Counter by the Cerenkov effect [18], and the total P concentration was determined by the method described by [19].

2.2. L-values

With plant accumulated P values and ^{32}P / ^{31}P specific activities (SA), the L-value and L-value discounting the P in the plant derived from seed [12, 20] were calculated from the following equations [21]:

SA = ^{32}P / ^{31}P; where SA is the specific activity (disintegration per minute, dpm µg P^{-1}); ^{32}P is radioisotope activity in the plant (dpm); ^{31}P is P content in the plant (µg P pot^{-1});

L-value = (A_0 / SA_f – 1); where L-value (mg P kg^{-1} soil); A_0 is ^{32}P activity of the applied carrier-free solution (dpm); SA_f is the specific activity of the plant (dpm µgP^{-1}).

L-s value = A_0 / SA_f; where L-s value is the L-value calculated considering the P in the plant subtracting the P derived from the seed (mg of P kg^{-1} soil); A_o is carrier free ^{32}P activity of the applied solution (dpm); SA_{fs} = $^{32}P/^{31}P$, where ^{32}P is the plant ^{32}P activity and ^{31}P is the P content of the plant. The plant P content subtracting the seed derived P was calculated, assuming that 60% of P contained in the seed is used in plant development [22].

The L-value is by definition the soil P availability obtained through the isotopic dilution technique. Under similar soil P conditions, the higher the L-value the more efficient is the plant species in obtaining P.

2.3. Statistical analysis

Data of shoot dry matter (SDM), P concentration and P uptake in SDM, L-value and L-s value of the green manure species were arranged in a matrix, which was statistically analyzed using Pearson linear correlation and hierarchical cluster analysis (HCA). The statistical method of HCA was used in order to verify the similarities among the species of green manure, by calculating the Euclidean distance among the samples.

The SAS 9.1 - "Statistical Analysis System" [23] and the SYSTAT version 10.2 software programs, using the UPGMA (un-weighted pair group arithmetic average clustering), were used to perform binary grouping to define groups according to the degree of similarity between the species [24]. The cluster analysis was preceded to the standardization of data before the Euclidian distances calculation, as the variables presented different scales. After standardization, all variables will be equally important in the determination of these distances. Final results of the groups were presented as dendrograms. The P uptake efficiency by plants is inversely proportional to SA and directly proportional to L-value and L-value discounting the P in the plant derived from the seed.

3. RESULTS AND DISCUSSION

The results obtained with the white lupin as the standard species were: SDM = 1.77 g pot^{-1}, P in SDM = 1.66 mg pot^{-1}, SA = 9.05 dpm g^{-1}, L-value = 43.20 mg P kg^{-1} soil and L-s value = 5.93 mg P kg^{-1} soil. White lupin was more efficient in absorbing P, resulting in the

lowest SA, and the highest L and L-s values among the green manure species (Tables 1 and 2).

TABLE 1. MEANS AND STANDARD DEVIATIONS OF SHOOT DRY MATTER (SDM), P CONCENTRATION IN SDM (P CONC), P UPTAKE AND SEED P UPTAKE (P SEED) OF 21 SPECIES OF GREEN MANURE

Species	SDMM (g)	P conc (g kg^{-1})	P uptake (mg)	P seed (mg)
Brachiaria ruziziensis	3.37 ± 0.05	1.06 ± 0.05	3.57 ± 0.12	0.042
Brachiaria marandú	3.08 ± 0.23	1.05 ± 0.11	3.19 ± 0.22	0.042
Brachiaria xaraés	2.43 ± 0.33	1.18 ± 0.09	2.88 ± 0.52	0.042
Calopogonium	3.50 ± 0.17	1.27 ± 0.06	4.41 ± 0.03	0.102
Crotalaria breviflora	2.20 ± 0.13	1.61 ± 0.12	3.51 ± 0.07	0.132
Sunhemp	6.26 ± 0.11	0.80 ± 0.04	4.99 ± 0.31	0.372
Smooth rattlepod	2.10 ± 0.05	1.22 ± 0.04	2.57 ± 0.10	0.048
Crotalaria ochroleuca	3.43 ± 0.18	1.15 ± 0.07	3.95 ± 0.17	0.054
Showy rattlepod	3.55 ± 0.11	1.17 ± 0.04	4.16 ± 0.28	0.150
Stylosanthes cv. Campo Grande	0.99 ± 0.16	1.85 ± 0.04	1.82 ± 0.28	0.042
Stylosanthes cv. Mineirão	0.60 ± 0.13	1.74 ± 0.29	1.09 ± 0.33	0.042
Jack bean	16.10 ± 0.47	0.71 ± 0.02	11.46 ± 0.66	7.872
Pigeon pea	4.73 ± 0.13	1.03 ± 0.04	4.87 ± 0.11	0.804
Dwarf pigeon pea	4.73 ± 0.28	1.08 ± 0.04	5.07 ± 0.11	0.456
Sunflower cv. Iarama	5.17 ± 0.16	1.10 ± 0.03	5.70 ± 0.35	0.786
Sunflower cv. Uruguai	5.30 ± 0.12	1.06 ± 0.05	5.61 ± 0.32	0.576
Lab lab	5.24 ± 0.28	1.14 ± 0.05	5.94 ± 0.04	1.494
Millet	3.79 ± 0.24	0.87 ± 0.02	3.32 ± 0.28	0.042
Dwarf velvet bean	7.37 ± 0.61	0.86 ± 0.02	6.30 ± 0.37	3.726
Grey velvet bean	8.30 ± 0.59	0.86 ± 0.02	7.12 ± 0.41	5.634
Black velvet bean	10.08 ± 0.56	0.84 ± 0.01	8.45 ± 0.45	6.204

The values of plant SDM of 21 green manure species correlated significantly and negatively with P concentrations in SDM (–0.733***) and positively with P uptake in SDM (0.975***). The increase of SDM reduced the P concentration in SDM, while the total P uptake was higher.

The cluster analysis identified the following six groups of green manure species (Fig. 1):

1st: jack bean;
2nd: black velvet bean,
3rd: grey velvet bean and dwarf velvet bean;
4th: *Stylosanthes* cv. Minerão and *Stylosanthes* cv. Campo Grande;
5th: lab lab, sunflower cv. Uruguai, sunflower cv. Iarama, dwarf pigeon pea, pigeon pea and sunhemp;
6th: *Crotalaria breviflora*, *Crotalaria macronata* , *Brachiaria brizantha* cv. Xaraés, *Crotalaria spectabilis*, *Crotalaria ochroleuca*, *Brachiaria brizantha* cv. Marandú and *Brachiaria ruziziensi*s.

Among the five groups of green manure species (Fig. 1) and the values presented in Table 1, jack bean presented superior values of SDM and higher values of P uptake. The variables that showed higher Pearson correlations coefficients were: SA and L-value (–0.949***), SA and L-s value (–0.095^{ns}) and L-value and L-s value (–0.0012 ^{ns}).

TABLE 2. MEANS AND STANDARD DEVIATIONS OF SPECIFIC ACTIVITY (SA), L-VALUE AND L-VALUE DISCOUNTING THE P IN PLANT DERIVED FROM SEED (L-S) AND VARIATION (Δ) BETWEEN L-VALUE AND L-S VALUE OF 21 SPECIES OF GREEN MANURE

Species	SA (dpm μg^{-1} P)	L-value (mg P kg^{-1} soil)	L-s value (mg P kg^{-1} soil)	Δ (%)
Brachiaria ruziziensis	138.33 ± 14.83	2.79 ± 0.56	2.76 ± 0.55	1.18
Brachiaria marandú	172.52 ± 19.69	2.44 ± 0.16	2.41 ± 0.16	1.33
Brachiaria xaraés	241.22 ± 38.32	2.27 ± 0.21	2.24 ± 0.22	1.52
Calopogonium	140.23 ± 3.56	2.60 ± 0.16	2.54 ± 0.16	2.31
Crotalaria breviflora	192.37 ± 27.13	3.12 ± 0.68	3.00 ± 0.66	3.79
Sunhemp	80.56 ± 5.61	2.54 ± 0.22	2.35 ± 0.23	7.46
Smooth rattlepod	184.08 ± 6.96	3.29 ± 0.13	3.23 ± 0.12	1.88
Crotalaria ochroleuca	140.07 ± 7.35	2.65 ± 0.09	2.62 ± 0.08	1.38
Showy rattlepod	135.92 ±1.30	2.63 ± 0.14	2.54 ± 0.13	3.65
Stylosanthes cv. Campo Grande	300.54 ± 44.88	4.55 ± 0.75	4.45 ± 0.73	2.04
Stylosanthes cv. Mineirão	1047.05 ± 34.79	4.77 ± 0.79	4.61 ± 0.80	3.44
Jack bean	10.43 ± 0.55	7.59 ± 0.29	2.35 ± 0.62	68.98
Pigeon pea	88.11 ± 1.53	3.05 ± 0.23	2.55 ± 0.20	16.53
Dwarf pigeon pea	75.53 ± 3.79	3.60 ± 0.53	3.28 ± 0.48	9.03
Sunflower cv. Iarama	93.14 ± 6.45	2.65 ± 0.20	2.29 ± 0.21	13.82
Sunflower cv. Uruguai	84.44 ± 2.89	2.84 ± 0.17	2.55 ± 0.12	10.37
Lab lab	84.34 ± 4.34	2.89 ± 0.16	2.16 ± 0.12	25.17
Millet	150.92 ± 9.08	2.24 ± 0.18	2.21 ± 0.17	1.29
Dwarf velvet bean	46.36 ± 2.26	3.76 ± 0.40	1.51 ± 0.13	59.85
Grey velvet bean	31.38 ± 2.87	4.94 ± 0.15	1.01 ± 0.44	79.61
Black velvet bean	19.65 ± 1.44	6.47 ± 0.24	1.70 ± 0.48	73.68

Green manure species were classified with respect to P uptake efficiency in the following five groups by the hierarchical cluster analysis with both variables SA and L-value (Fig. 2).

1st: highly efficient (black velvet bean and jack bean);
2nd: very efficient (grey velvet bean, *Stylosanthes* cv. Minerão and *Stylosanthes* cv. Campo Grande);
3rd: efficient (dwarf velvet bean, dwarf pigeon pea and *Crotalaria macronata*);
4th: moderately efficient (millet and *Brachiaria brizantha* cv. Xaraés);
5th: less efficient (lab lab, sunflower cv. Uruguai, pigeon pea, *Crotalaria breviflora*, sunhemp, *Brachiaria brizantha* cv. Marandú, *Crotalaria spectablis*, sunflower cv. Iarama, *Crotalaria ochroleuca*, *Calopogonium* and *Brachiaria ruziziensis*).

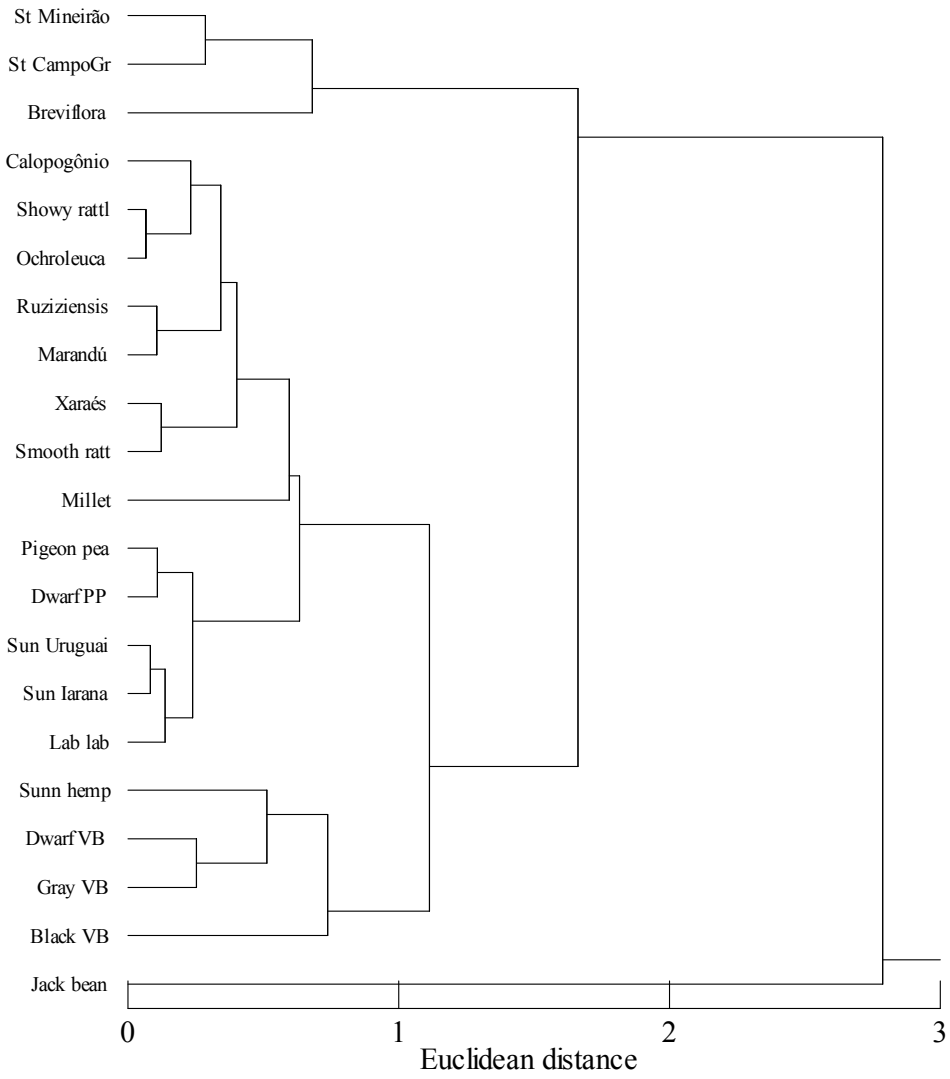

FIG. 1. Dendrogram obtained by hierarchical method of nearest neighbor based on distance of 0.5 for the green manure species. Hierarchical Cluster Analysis (HCA) - Average Method - for shoot dry matter (SDM), concentration and accumulation of P in SDM.

The cluster analysis with L-value discounting the P in plant derived from seed classified green manure species in five groups (Fig. 3).

1st: highly P uptake efficient (*Stylosanthes* cv. Minerão and *Stylosanthes* cv. Campo Grande);
2nd: very efficient (grey velvet bean);
3rd: efficient (black velvet bean and dwarf velvet bean);
4th: moderately efficient (dwarf pigeon pea, *Crotalaria macronata* and *Crotalaria breviflora*);
5th: less efficient (lab lab, millet, *Brachiaria brizantha* cv. Xaraés, sunflower cv. Iarama, jack bean, sunhemp, *Brachiaria brizantha* cv. Marandú, *Crotalaria ochroleuca*, sunflower cv. Uruguai, pigeon pea, *Crotalaria spectabilis, Calopogonium mucunoides* and *Brachiaria ruziziensis*).

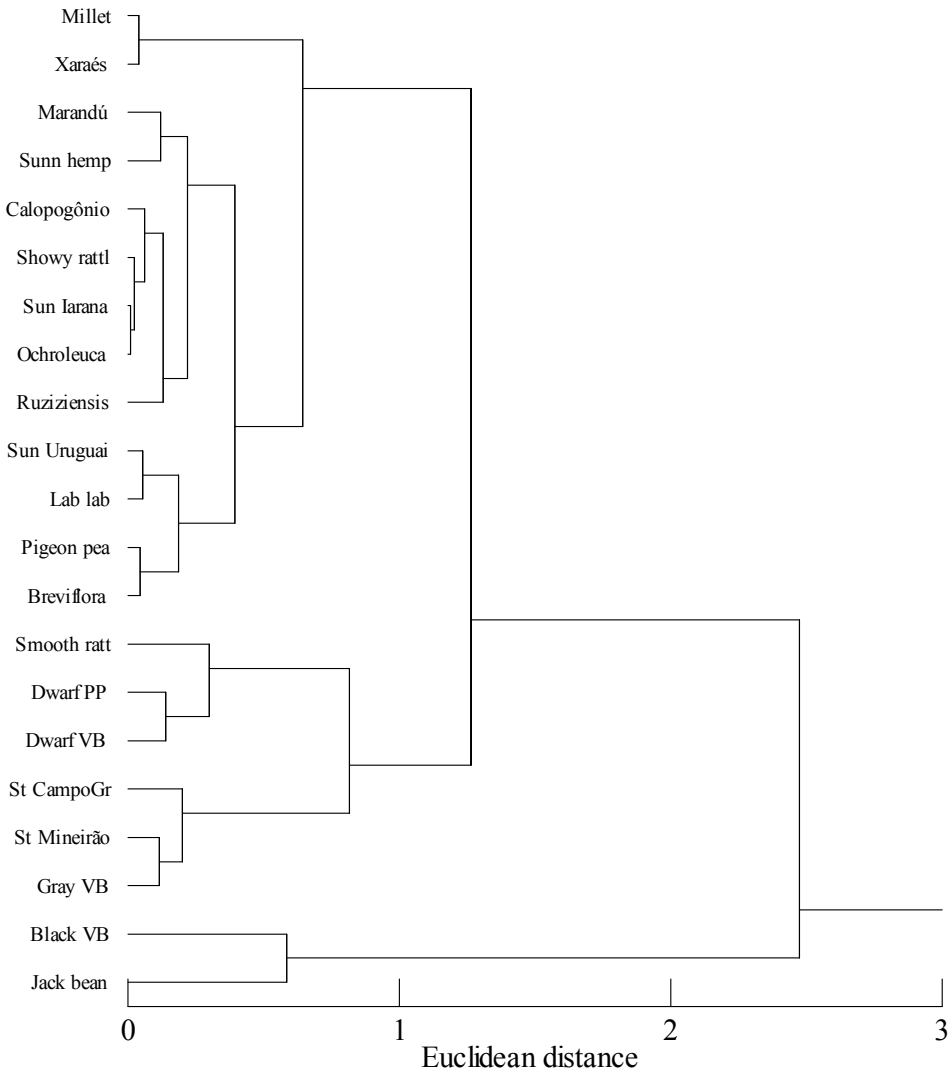

FIG. 2. Dendrogram obtained by hierarchical method of nearest neighbor based on distance of 0.5 for the green manure species. Hierarchical Cluster Analysis (HCA) - Average Method - for P specific activity (SA) and L-value.

The green manure plants may convert the relatively unavailable native P in the soil or residual fertilizer to more available chemical forms for the next crop. White lupin can take up more P from soil than alfalfa, clover, peas, vetch and wheat grown in P- deficient soil [5, 16]. With respect to the capacity of mobilization and recycling of nutrients between legumes and grasses used as green manure, pea and sunhemp showed better results compared to grass, due to higher biomass and higher nutrient concentrations in biomass [25].

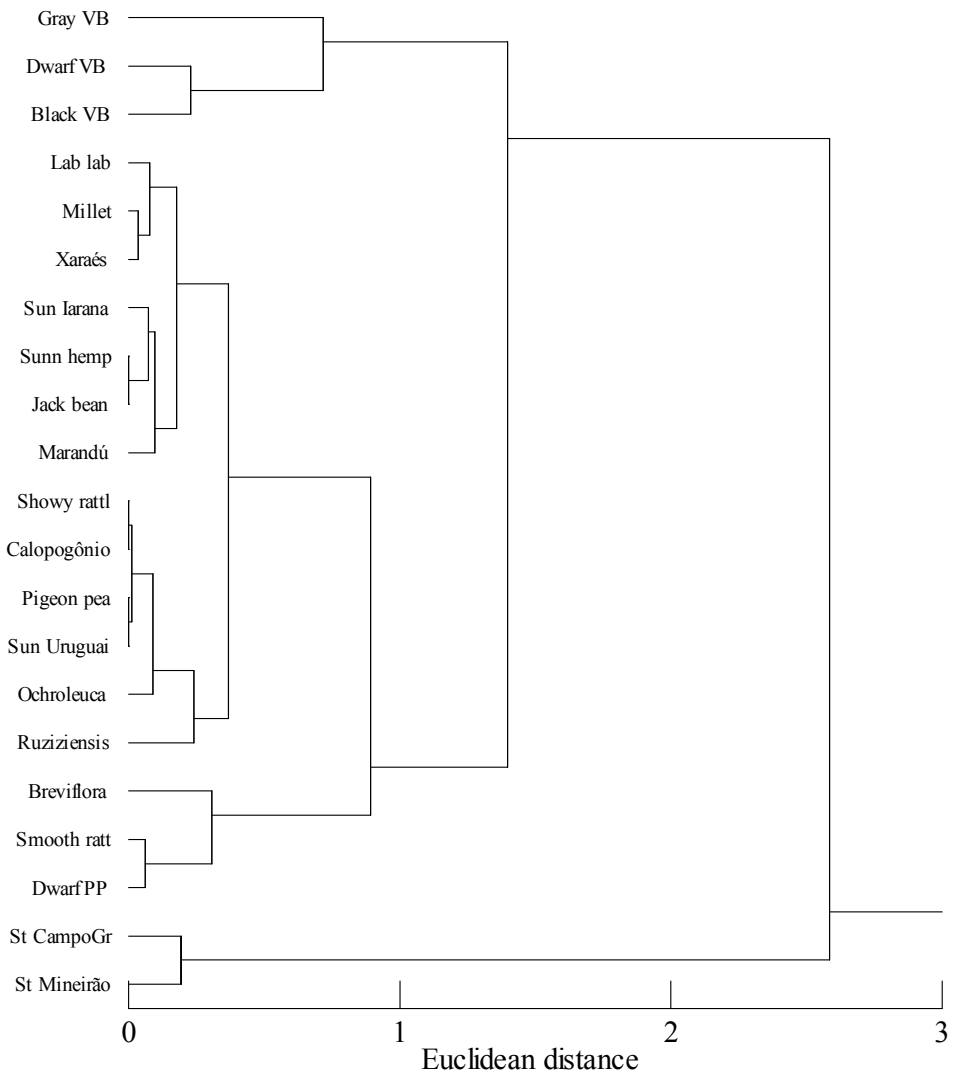

FIG. 3. Dendrogram obtained by hierarchical method of nearest neighbor based on distance of 0.5 for the green manure species. Hierarchical Cluster Analysis (HCA) - Average Method - for L-value discounting the P in the plant derived from the seed (L-s).

White lupin, pigeon pea and *Stylosanthes* are well adapted to acid soils deficient in P [3, 5, 26]. The white lupin roots secrete large amounts of citric acid, which solubilizes the fixed P [27–30], pigeon pea releases P from iron and aluminum phosphates through the secretion of piscidic, malonic and oxalic acids by roots [12, 31, 32]. *Stylosanthes* roots exude citrate and release P from Fe and Al phosphates. These organic acids solubilize the P, thus increasing P uptake by the plant subsequently [3, 33–35], making these species resistant to P deficiency in soil.

Legumes and grasses have been used as cover crops. However, they must have some desirable features, for their beneficial effects to succeed in the main crops, and facilitate the cultivation system [36]. The species to be used as green manures should provide enough biomass to cover the surface area and improve the yield of the main crops, such as providing nutrients as well as being compatible with the management of the main crop [37].

The L-values, considering or not the seed derived P, are illustrated in the Table 2. There was increasing variation in the differences between the L and L-s values of the green

manure species with higher values of seed derived P, varying form 1% for *Brachiaria ruziziensis* (lower seed P content, Table 1) up to 80% for grey velvet bean (Table 2), suggesting that the higher the seed P, the greater the influence on the correction of the seed P effect on the L-value (Tables 1 and 2). Reducing the L-value to more realistic levels by correcting the seed derived P were also shown in an experiment with ryegrass grown in three soils of very low, low and medium P status (soil I, II and III), containing 4, 14 and 37 mg initial $NaHCO_3$-soluble P kg^{-1} soil, and supplied with increasing amounts of added P. The L-values after correcting the seed-derived P were reduced by 69% in soil I, 18% in soil II and 10% in soil III. This effect was still much higher, when L-values were reduced by increasing levels of fertilizer P [22]. In a study on the dynamics of P uptake by wheat using radioisotopes ^{32}P and ^{33}P, the specific activities of the plants in the early development stage for both radioisotopes were much lower, presumably due to the high amount of seed- derived P in the plant [38]. Chickpea, faba bean, white lupin, canola and wheat were evaluated in the greenhouse for P uptake and growth in three soil types with low, medium and high P content, by labeling with ^{32}P. It was concluded that the L-value for faba bean grown in soils with low P was compromised by the large proportion of the seed P in relation to P taken up in shoots, making the calculation of L-value very sensitive to the estimated value of P derived from the seeds accumulated in the shoot [39].

Stylosanthes cv. Minerão and *Stylosanthes* cv. Campo Grande showed no difference between L-value and L-s value, probably because they have lower seed P contents (Table 1). Jack bean was classified in this study as the species that absorbed more P (Fig. 1), with higher SDM, P content and P uptake (Table 1), and highly efficient in P absorbing capacity (Fig. 2). However, when discounting the P from the seed (Table 1), it was the least efficient in P uptake (Fig. 3), despite its high SDM and P uptake in SDM (Table 1). The L-s value was the most trustworthy, because a fraction of the amount of P translocated from the seed is subtracted from the total P uptake by the plant [40, 41].

The specific activity which is inverse to the L-value showed no correlation between the L-s value, seed SA and L-value (-0.095^{ns}) and L-s value and L-value (-0.0012^{ns}), due to differences in the seed P contents of the green manures studied. Therefore, the results could be erroneous when the L-values are calculated without considering the P in the plant derived from the seed.

4. CONCLUSIONS

- Jack bean was more efficient in P utilization, i.e., the most productive under low soil available P conditions.

- *Stylosanthes*, regardless of cultivar Mineirão or Campo Grande, was the most efficient green manure species for P uptake.

- The seed-derived P in the green manures, when L-value is used, affects the identification and classification of P uptake efficiency

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ROOT TRAITS FOR BETTER NITROGEN ACQUSITION AND USE IN LOW-N SOILS

ROLE OF TRANSLOCTED SIGNALS IN REGULATING ROOT DEVELOPMENT AND NUTRIENT UPTAKE IN LEGUMES

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Abstract

Uptake of nutrients is achieved through the expression and activity of specific carrier/transporter mechanisms localized in the root system and distributed as a consequence of the development of the architecture of the system. Both root system development and the nutrient transport mechanisms are responsive to environmental factors that include nutrient supply and availability, water supply, salinity, soil acidity and compaction together with a wide range of biotic stresses. The response to each may be regulated at the molecular level by both local and systemic signals. These signals include the classical plant growth regulators but also low molecular weight compounds such as sugars and amino acids as well as macromolecules, including peptides, proteins and nucleic acids. Among the latter, recent research has shown that small RNA species and especially small interfering RNAs (siRNA) and microRNAs (miRNA) are potent and effective regulators of gene expression which, in the context of root development as well as nutrient uptake, have central and critical roles. Systemic (translocated) signals that specifically regulate root development and function are less well defined but analyses of phloem exudate in species of lupin (*Lupinus albus* and *L. angustifolius*) and species of *Brassica* and cucurbits have demonstrated that a wide range of macromolecules, including miRNAs, are present and potentially translocated from source organs (principally leaves) to sinks (shoot apical meristems, developing fruits and seeds, roots and nodules). While specific signaling roles for many of these macromolecules are yet to be discovered there are some that have been documented and their regulatory activity in organ development and functioning, as well as in nutrition, confirmed. The following article provides an up to date review and presents the results of recent research using lupin with emphasis on the analysis of small RNAs and their likely role(s) in regulation of root development and function.

1. ANALYSIS OF MACROMOLECULAR COMPOSITION OF PHLOEM EXUDATE IN LUPIN

Exudates were collected from incisions to the major vascular bundles associated with the sutures of developing fruits of *L. albus* and proteins separated using 2D PAGE. Proteomic analysis identified 83 proteins with the largest group comprising those involved in metabolism (24%), followed by redox regulation (8%), protein synthesis, turnover and sorting (9%), cell wall synthesis (6%). More prominent proteins were cyclophilin, ubiquitin, a glycine-rich RNA-binding protein, a group of proteins that comprise a glutathione/ascorbate-based mechanism to scavenge O_2 radicals, enzymes of glycolysis and other metabolism including methionine and ethylene synthesis [1]. Included was the 'florigenic' signal, flowering locus T-protein (FT). One third of the proteins sequenced remained unknown with some of these probably unique to lupin phloem. Both subunits of Rubisco and a chlorophyll a/b binding protein were also identified in exudate indicating some contamination with the contents of chloroplast-containing cells damaged by incision.

Analysis of a cDNA library constructed from lupin phloem exudate sequenced 1063 clones and identified 609 unique transcripts that were also classified into functional groups. Transcripts coding for proteins with unknown functions formed the largest category (280 sequences, 39% of all ESTs) while among those coding for proteins of known function were metabolism (15% of all ESTs; 11% general and 4% energy metabolism), protein modification/turnover (11%), and redox regulation, signaling and stress response and defense-related with around 5% of all ESTs in each of these groups. Potential signaling macromolecules included transcripts encoding proteins mediating Ca ion level and that for FT. For 31 of the transcripts that were identified their corresponding protein was also detected in phloem exudate. Although questions as to whether the many transcripts are translocated in phloem and, if indeed they act as signals as a consequence of their translocation, are unresolved. Their long distance movement is believed to occur as a ribonucleoprotein complex dependant on the presence of suitable RNA binding proteins (RBPs; [2]). As noted above, proteomic analysis of exudate from lupin identified a glycine-rich RNA-binding protein, and Ham et al. [2] have shown translocation of a 50kD RNA binding protein (RBP50) together with six different mRNA species in heterografting experiments with pumpkin stocks and cucumber scions.

A number of microRNAs (miRNA) were enriched in exudate and differed in exudate collected from different sites on the lupin plant. These data are discussed in detail below.

One group of molecules that lie between the low molecular weight solutes (sugars, amino acids, organic acids and alkaloids, etc) and macromolecules in phloem are the bioactive peptides. A recent review has highlighted the significance of an ever increasing list of these potentially powerful signals/regulators [3] and Hoffmann-Benning et al. [4] have shown that lupin exudates, for example, contains a large number of small peptides that to date have not been studied further. There is compelling evidence that a CLAVATA-like peptide is translocated in phloem from leaves to the root system in legumes where it exerts a regulatory role over the intensity of nodulation [5], and the possibility that there are other systemic signals among the peptides in phloem should not be overlooked.

These analyses from lupin, as do those reported by other researchers using exudates from *Brassica* species (*B. napus*) or cucurbits (e.g. melon, pumpkin and cucumber; reviewed in [6]), rely on incising the vasculature and collection of exudate from the site of the wound. While it is reasonable to assume that the contents of the sieve elements (SE) constitute the bulk of solutes and macromolecules in exudate there will necessarily be contamination from cells other than those of phloem that are damaged by the incision. The methods of collecting exudates and the likely levels of contamination have been recently reviewed [7]. Only the use of severed stylets from sap sucking insects (leaf hoppers and, mainly, aphids) offers the possibility of analyzing exudate that is minimally contaminated by cell contents other than SE. However, stylectomy *in situ* is technically difficult and is effective mainly with monocotyledon species yielding total volumes of a few microlitres at best. Nevertheless, confirmation of the macromolecular composition of exudate from severed vasculature using the few species that 'bleed' freely following a wound awaits comparative analysis of stylet exudate. These considerations emphasize the need for caution in interpreting data from species like lupin particularly in assigning translocated signaling roles for macromolecules that are present in very small amounts.

2. A CENTRAL REGULATING ROLE FOR MIRNAS

miRNAs function as post transcriptional regulators of gene expression both in plants and animals. They are small (18-26 nt) non-protein coding RNAs that are processed from

hairpin precursors by the ribonuclease III like enzyme, Dicer, and bind to cognate sequences in mRNAs. In plants, miRNAs bind to their targets through perfect or close to perfect complementarity causing degradation of the message or repression of translation. There is an ever increasing list of developmental processes that involve regulation by miRNAs including development of leaves, stems and roots (as well as legume nodules) together with flowering, fruit development and seed set. A recent review by Khan et al. [8] has assembled the growing evidence for miRNA regulation of gene expression as having a central role in root development and architecture. They have further identified plant response to a wide variety of stresses including both biotic and abiotic stresses as mediated through miRNA, in many cases in the root system.

Small RNAs ranging in size from 8 to 35 nt, with the majority 19 to 23 nt, were isolated from lupin phloem exudate. Those in the 18 to 26 nt size class were purified and used to construct a small RNA library, yielding sequences for 330. Seventeen sequences from the phloem library showed strong similarity to known miRNAs from seven different families, with 12 identified as probable miRNAs by homology with those from other species. While not all miRNAs identified in this study targeted transcription factors, the majority did, and their activity could potentially have a widespread impact on gene expression. Among the 11 miRNAs analyzed by northern blot and real time PCR in lupin tissues, some were prominent in phloem exudate while others were either absent or much lower than in the other tissues studied, suggesting that there is a specific spectrum of miRNAs in lupin phloem (Fig. 1). Furthermore, the miRNA composition in phloem exudates collected from the fruits or close to apical meristems differed markedly from the composition in exudate collected from the base of the stem close to the root system [1]. The most reasonable explanation for the presence of small RNAs in phloem is that they are transported from the companion cell (CC) to the SE. Thus, the fact that the pattern for five miRNAs in exudate collected at three different sites was not the same, suggests either their differential expression in the adjacent phloem CC at different sites and/or specificity in phloem loading. Interestingly, in another study [9] parallel analyses of miRNAs in stylet exudate and phloem tissue extracts of apple revealed that, while seven miRNAs were common, four that were amplified from the tissue (including CC) were not detected in exudate, lending further support to the idea that transfer to the SE is specific. If miRNAs serve as translocated signals it is not too surprising that the downward-moving (collected at the base of stem) and upward-moving phloem streams (collected from pods and axillary branches) showed distinct differences in their miRNA composition [1]. Thus lupins offer the possibility of sampling exudate from phloem translocating from ‘source’ organs of the shoot, including leaflet midribs and petioles, to ‘sinks’ such as fruits and apices, as well as to those of the root system separately [10]. It would be interesting to further exploit this ability by extending the range of miRNAs assayed to include the many that have now been identified in exudates (reviewed, [7]). MiRNA-guided post-transcriptional gene regulation is particularly interesting because miRNAs can regulate several protein-coding genes implicated in the same pathway, altering whole metabolic pathways and complex response networks in significant ways.

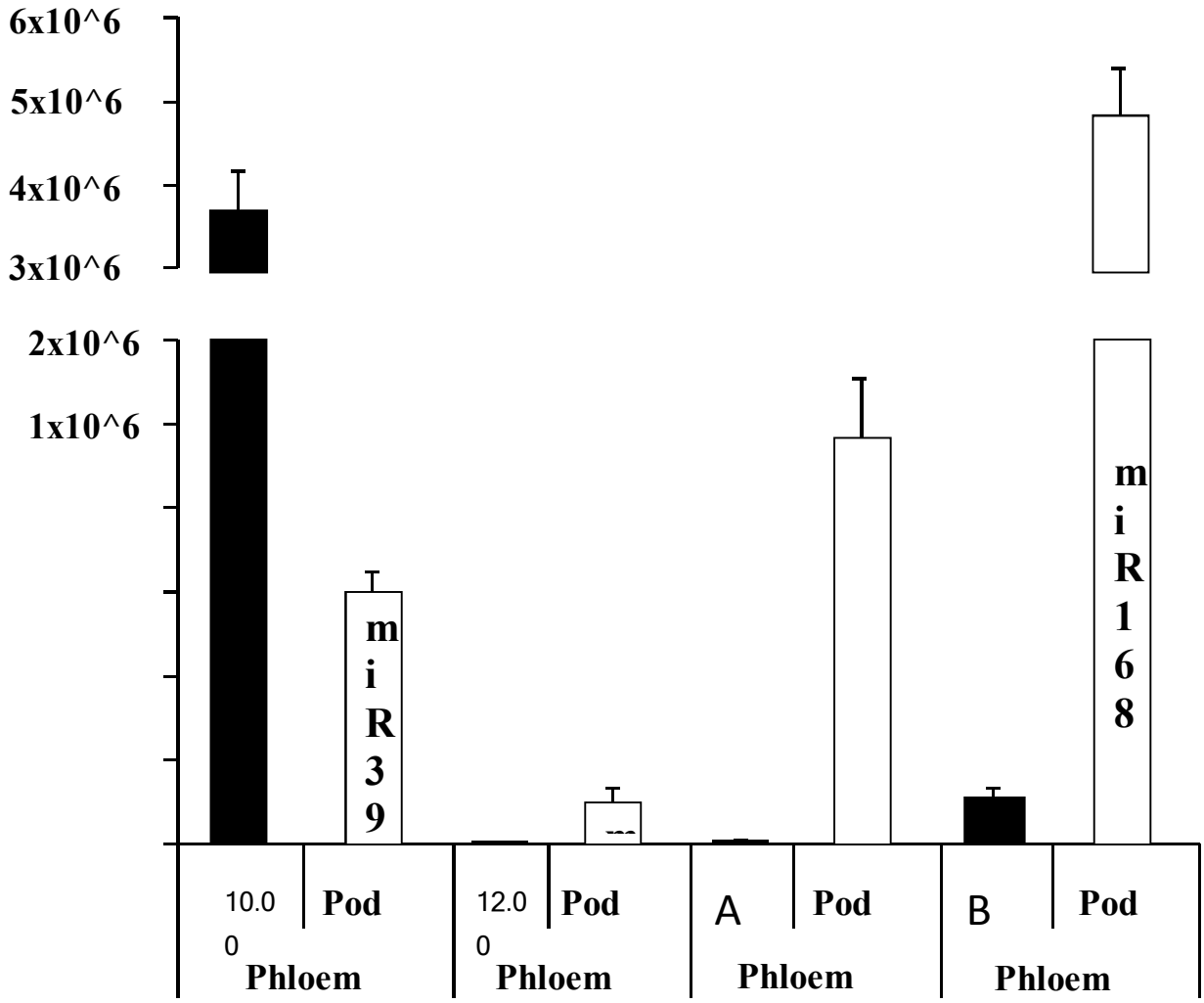

FIG. 1. Expression of miRNAs in phloem exudate (phloem) and extracts of tissue sampled from adjacent to the suture vasculature of lupin fruits (pod). The exudate assayed was collected from the incised vasculature of the same fruits. (Taken from Rodriguez-Medina et al. [1]). The vertical axis is expressed as molecules/ul template used in the PCR-based assays.

3. NUTRIENT UPTAKE AND HOMEOSTASIS

Physiological studies of plant mineral nutrition over many years have identified homeostasis as an important feature both in the uptake and accumulation of nutrients. Schachtman and Shin [11] reviewed the evidence for signaling events in relation to internal nutrient availability providing strong support for the idea that plant roots respond to fluctuations in the environment to enhance and optimize the acquisition of water and limiting nutrients. While self regulation of uptake by roots has been postulated, it was not until relatively recently that the tools of molecular biology have permitted the identification of ion transporters and their encoding genes with a range of affinities for the transported species. Thus, both high and low affinity transporters for Pi, NO_3^-, SO_4^{2-} and a range of cations have been described. Their differential expression in response to nutrient status has long been thought to involve both local and systemic signaling, the latter specifically through translocation in phloem, but until very recently these ideas remained speculative.

Perhaps the best documented relationship between a phloem-mobile miRNA and the regulation of nutrient uptake is that relating to Pi [12, 13]. The regulator in this case was identified as miR399. Also involved in the Pi response was miR398, which has been reported to participate in more abiotic and biotic stresses than any other miRNA [14]. In addition to regulating gene expression associated with Pi uptake, miR398 has a role in a number of stress responses including both bacterial and fungal pathogens, drought and salinity stress, UV-B radiation, responses involving abscisic acid (ABA) and responses associated with oxidative stress. The important target genes for miR398 are two closely related Cu/Zn superoxide dismutases (CSD1 and CSD2), a subunit of mitochondrial cytochome oxidase and the Cu chaperone for SOD. As a consequence Cu homeostasis in the plant is also regulated by this miRNA. In lupin phloem exudate miR399 was most prominent in samples of the downward moving stream collected at the base of the stem just above the root system, and was responsive to Pi nutrition, increasing significantly under limitation [1].

Huang et al. [15] have identified miR395 as a regulator of SO_4^{2-} uptake and as a mediator in regulating response of *Brassica napus* to heavy metal stress (Cd^{2+}). Transcriptional analysis showed 13 miRNAs, including miR395, differentially expressed at elevated levels in these two responses. Five genes, *BnSultr2;1* and *BnAPS1-4*, which encode a low-affinity SO_4^{2-} transporter and a family of ATP sulphurylases, respectively, were identified as the targets of miR395. SO_4^{2-} metabolism in roots through cysteine synthesis leads to the formation of glutathione, a central component of ROS metabolism, and in the formation of complexes with xenobiotic molecules entering the plant. Lupin phloem was found to contain miR395 as well as enzymes essential for redox regulation through ascorbate/glutathione and also glutathione-S-transferase that would mediate conjugation of xenobiotics [7]. That phloem exudate contains a high concentration of glutathione was discovered many years earlier in cucurbits [16].

It is also clear that systemic regulation of NO_3^- uptake by the N status of the plant is genetically regulated [17]. Split root experiments using transgenic *Arabidopsis (hni* mutants*)* have shown clearly that the overall plant N status systemically regulates NO_3^- uptake by the root system [17] through repression of the high affinity NO_3^- transporter gene, *NRT2.1*. The nature of the systemic signals have not been clearly identified but organic solutes of N (amino acids or amides) as well as micro RNAs, specifically miR167, have been suggested. MiR167 targets the auxin response factor gene (*ARF8*) as part of a regulatory mechanism modulating lateral root emergence, but further research is required to establish the relationship specifically for NO_3^- homeostasis. This miRNA was a prominent species in lupin phloem exudate [6] and occurred also in exudate from both *Brassica* and cucurbit species, as well as in stylet exudate from apple [9]. Vidal et al. [18] have also examined the regulation of N status in *Arabidopsis* and identified miR393 as a central controlling element. This micro RNA targets a transcription factor and one of the root expressed auxin receptors (*AFB3*) that provide a regulatory module integrating N status and auxin signaling to control root architecture. The picture is made more complex because cytokinins (CK) are increased in response to increased NO_3^- supply as a consequence of inducing one of the CK synthesis genes isopentenyltransferase (IPT3), leading in turn to changes in N uptake and in lateral root development (architecture). CK are translocated both in xylem and phloem [19] and as such could provide a further systemic signal that communicates N status from roots to shoots and vice versa. Ruffel et al. [20] have recently provided convincing evidence that NO_3^- sensing triggers long distance systemic signaling to report plant N demand through a NO_3^--CK relay system. They speculate further that indeed auxin may be part of the long distance signal as a means to inform the root system of the N status of the shoot. Clearly the signals that regulate N uptake, distribution and utilization in the plant are complex, and will require considerable

further research before the features are sufficiently defined to develop genetic markers that might assist in enhancing N economy in crop species.

A broad study of the differential expression of some 68 known miRNAs in common bean (*Phaseolus vulgaris*) using a macro-array hybridization approach identified 33 miRNAs of which five were only expressed under nutrient stress conditions [21]. The study used leaves, roots and nodules from plants suffering Pi or Fe deficiency, acidic pH or Mn toxicity. MiR157, miR156, miR167, miR319, and miR398 responded to all the nutrient stresses in each of the plant organs, and of this group all have been detected in phloem exudates from lupin, *Brassica* spp, cucurbits and importantly from stylet exudate of apple (reviewed in [7]). It is perhaps not surprising that miRNAs involved in nutrient status are at least in part among the translocated signals that traverse the distance between sources and sink tissues in a plant.

In the study by Valdez- Lopez [21] discussed above, most of the miRNAs that were responsive to the stresses in nodules were up regulated with the expression of some specific to this organ. A number of miRNAs have been found to be involved in the initiation and development of legume nodules in a wider range of legumes (reviewed in [21]) and definitive studies have used small RNA libraries from root tips and nodules of *Medicago truncatula* to reveal 100 putative new and 36 conserved miRNAs that are expressed. There seems little doubt that these powerful regulators of gene expression are involved in establishing and sustaining the symbiotic relationship.

4. CONCLUDING REMARKS

The functional significance of each of the proteins, transcripts and small RNAs in phloem provides new and exciting prospects to identify the many hypothetical 'signals' postulated by the wealth of physiological research that has described many of the processes involved in plant growth and development. However, the presence of this bewildering array of macromolecules in phloem also poses new questions about the biochemical features and maintenance of the SE itself. Not only does there appear to be specific metabolic components that might support the SE, there is also a wide range of proteins and transcripts that are, in theory at least, involved in defending the tissue against pathogens and predators (either for the plant generally or the phloem in particular). One of the prominent groups of mRNAs found in lupin phloem exudate was 10 transcripts (3% of defined ESTs) for the polyprotein of the bean yellow mosaic virus (BYMV) [1]. These were most likely due to the presence of feeding aphids but their presence and abundance in exudate indicates that the phloem stream is also a pathway for pathogen attack. How the 'protective' macromolecules/metabolism in phloem interact with a pathogen at the molecular level remains as another interesting question.

A recent compilation identified 13 miRNAs involved in plant responses to drought/salt stress [22]. Eight of these were present in lupin phloem exudate [1] and, importantly, six were also recovered from PCR amplification of apple stylet exudate [9]. There is thus a possibility that the responses in lupin to both drought and salinity are mediated through miRNAs translocated from sites where the stress is sensed to sites where a response is initiated. Participation of the root system in both sensing and responding to these stresses is clearly indicated. Both drought and salinity stresses are significant constraints to lupin production in Western Australian cropping systems and perhaps translocated miRNA signals can provide a basis for marker assisted crop improvement. Furthermore, sampling the phloem stream (as in lupin) for sensitive analysis of translocated low molecular weight solutes, transcripts and small RNAs, as well as specific proteins, is non-destructive and could conceivably be adapted for use as an effective selection tool in a breeding program.

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ASSESSMENT OF ROOT MORPHOLOGICAL TRAITS OF 16 TROPICAL AND FOUR TEMPERATE MAIZE CULTIVARS FOR NITROGEN EFFICIENCY IN SHORT-TERM NUTRIENT SOLUTION EXPERIMENTS WITH THE CIGAR ROLL AND GROWTH POUCH METHODS

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Abstract

Genotypic differences in N efficiency of maize have been reported by many authors. One of the reasons responsible for genotypic differences in N efficiency is differences in N uptake efficiency after anthesis. Continuous root growth and N uptake activity are responsible for the high N uptake efficiency of N-efficient genotypes. This study was conducted mainly to identify root parameters which could be used as secondary selection traits for genotypic differences in N efficiency of maize established in field experiments. The specific objective of the first experiment was to establish a relationship between root parameters with genotypic differences in N efficiency in the field, and to identify root traits to be used as secondary selection criteria for N efficiency. Four temperate and 16 tropical genotypes were grown in low-N nutrient solution with a cigar roll and a growth pouch culture for 9 and 10 days, respectively. In the cigar roll experiment individual root fractions (adventitious, seminal and primary root fractions) and in growth pouch experiment root distribution and root branching angle were of primary interest. Genotypic differences were found in most of the root traits, but the differences were not clear cut between N-efficient and inefficient genotypes with few exceptions. The N-efficient genotypes had the highest percentage of root length in the deepest (>20 cm) interval in the growth pouch, which also positively correlated with N uptake after anthesis and grain yield. The N-efficient genotypes also had a high percentage of roots in the root branching angle interval of 60-90^{o}. It was concluded that the high N uptake efficiency of N-efficient genotypes might be related to a higher percentage of roots growing downwards (high branching angle) and a high percentage of root length in deeper soil layers enabling them to exploit nitrate in the subsoil more efficiently. These two root parameters were found promising to use as selection criteria for N efficiency.

1. INTRODUCTION

Nitrogen (N) recovery efficiency in annual crops averages only about 42 and 29 % in developed and developing countries, respectively [1]. This low N recovery efficiency is associated with the loss of applied N by leaching, volatilization, denitrification and soil erosion [2]. In addition, use of inadequate crop management practices, as well as biotic and abiotic stresses are also responsible for low N use efficiency. Breeding of N-efficient cultivars is one of the strategies to improve the N efficiency of crops.

Selection for grain yield at low N seems to be necessary, but encounters some difficulties due to possible cultivar by N interactions [3]. With a decreasing level of N in the soil, non-genetic variation of yield is increasing due to greater soil variability. Therefore, selection based on additional plant traits contributing to N efficiency may be more precise and enhance the selection progress [4]. A better knowledge of morphological and physiological traits controlling N uptake and N utilization efficiencies in plants are essential for both selection of cultivars and well-defined breeding strategies to improve the N efficiency of crops [5]. Selection for root growth characteristics may provide a direct means for improving N uptake [4].

Nitrate-N is the most mobile nutrient in the soil. Plants develop different adaptations to exploit this temporally and spatially/differentially available nutrient. Roots in nutrient-rich zones can demonstrate both physiological and morphological alterations compared with those roots outside the zone [6]. Increasing the root system is an adaptation to increase the total volume of soil explored, and thereby increases the total amount of nitrate which the plant can access [7, 8]. A highly significant correlation between the size of the root system and grain yield at low N supply was observed [5]. Maize hybrids respond to N deficiency by increasing total root length [9]. The N-efficient maize line '478' showed higher values of total root length at low N compared to an inefficient cultivar [10].

Maize is a cereal and has a fibrous root system; however it can be divided into three distinct root fractions: primary root, seminal roots and adventitious roots. The primary root is the first single root that emerges from the seed, also called the radicle. Seminal roots also emerge from the seed, but later, and are numerous in number. Adventitious roots emerge from the stalk nodes. However, it is not well known how N supply affects these different components of the root system.

Nitrate supply has a pronounced influence on the total length of primary roots [10]. The primary root increased to the maximum at 0.4 mM level, and then declined sharply with increasing nitrate supply. It was confirmed that longer primary roots were the main reason for the enhanced root growth at low N [10]. When N supply is low uptake of N by maize plants depends on a deeper root system resulting from longer primary roots.

Roots respond to localized nitrate supplies by proliferating lateral roots within the nitrate-rich zone [11]. In barley, this ability is due to a combination of increased numbers of lateral roots and increased rates of lateral root elongation [12]. Low-input cultivars of maize develop more and longer seminal roots [13]. Since the seminal roots grow more in the downward direction than adventitious roots [14], they may particularly contribute to the exploration of the sub-soil during later stages of development. Maize hybrids respond to N deficiency by increasing the elongation of individual seminal roots and enhancing lateral root growth while reducing the number of seminal roots [9]. A negative correlation between yield and root number, particularly at low N supply, has been reported [15].

Nitrogen deficiency resulted in significant decreases both in shoot and root fresh and dry matter weight of young plants [16]. Low nitrogen generally suppresses shoot growth and increases root: shoot ratios with and without increasing the root biomass in maize [9]. Higher root dry matter at low N has been reported [13]. N deficiency induces higher dry matter allocation to the roots during the vegetative stage [17]. Deficiency in N results in a shift in dry matter allocation in favour of root growth [18]. In solution culture where N buffering ability is low, however, it is common that low N decreases root growth [10, 19–21].

A plant's ability to explore the soil and to compete effectively for soil resources is critically dependent on architecture of the root system [22]. Root architecture is determined by the pattern of root branching and by the rate of growth of individual roots [23]. These properties of a root system are not only under genetic control but are also highly plastic, being influenced by a wide range of physical, chemical and biological factors [11, 24, 25]. Simulation modelling revealed that deep-rooted architecture acquired more nitrate than a shallow-rooted structure [7]. Root architecture is mainly determined by the distribution of seminal roots, and seminal roots determine the architecture of the root system in the soil volume [9].

The fineness of a root is expressed as specific root length (length per unit weight of root). Roots with high specific root lengths (i.e., fine roots) are often found in plants grown under nutrient-deficient conditions [26]. Fine roots allow the root system to explore the soil volume effectively while minimizing the investment needed to construct and maintain the root system. On the other hand, thicker roots are more costly to produce, but have greater transport capacity and are less vulnerable to desiccation and physical damage, and thus are generally longer-lived [27]. The root diameter generally shows less plasticity than the stem diameter, and in many species specific root length does not change significantly as a function of nutrient availability [28]. In grasses, thicker roots and a high tissue density (dry matter per unit root volume) have also been associated with increased longevity. In nutrient-poor environments thicker roots with a longer lifespan may increase the residence time of nutrients in the plant, and provide an important means of nutrient conservation [29].

Many authors have discussed the response of root parameters under low-N conditions, but few reports have been published about the relationship of these root parameters to N efficiency at low-N. This study was designed to test the suitability of root morphological traits as secondary plant traits for N efficiency in short-term nutrient solution experiments.

2. MATERIALS AND METHODS

2.1. Experiment 1

Plants were grown in an environmentally controlled growth chamber of the Institute of Plant Nutrition, Leibniz Universität Hannover, Germany. The growth chamber was adjusted to 30 / 20^{o}C day / night temperature and 80 % relative humidity.

2.2. Plant material

A total of 4 temperate and 16 tropical maize (*Zea mays* L.) cultivars were chosen to study the relationship between root parameters and N efficiency. The experiment was conducted with seedlings grown on filter paper in nutrient solution. The temperate cultivars were obtained from KWS SAAT AG (Einbeck, Germany) and categorized according to N efficiency by the breeder (Tables 1). The tropical cultivars were obtained mainly from CIMMYT and categorized for their N efficiency by Worku [30] (Table 2).

TABLE 1. TEMPERATE MAIZE CULTIVARS USED IN THE STUDY

Number	Cultivar	Pedigree	Efficiency class
1	TH	TH x Tester	N-inefficient
2	NUE	NUE-	N-inefficient
3	ASK	ASKET	N-efficient
4	SL	SL x Tester	N-efficient

TABLE 2. TROPICAL MAIZE CULTIVARS USED IN THE STUDY

Cultivar code	Pedigree	Efficiency class	Source
C1	CML444/CML445//CML440	N-efficient	CIMMYT-Zimbabwe
C2	CML395/CML444//CML440	N-efficient	CIMMYT-Zimbabwe
C3	CML202/CML395//CML205	N-inefficient	CIMMYT-Zimbabwe
C4	SC515	N-inefficient	Seed-CO-Zimbabwe
C5	CML395/CML444//CML442	N-efficient	CIMMYT-Zimbabwe
C6	CML444/CML197//CML443	N-efficient	CIMMYT-Zimbabwe
C7	SC633	N-inefficient	Seed-CO-Zimbabwe
C8	CML181/CZL01005//CZL01006	N-inefficient	CIMMYT-Zimbabwe
C9	CML181/CML182//CML176	N-efficient	CIMMYT-Zimbabwe
C10	CML144/(16304/6303Q)-B-6-1-3-3-B*6	N-inefficient	CIMMYT-Mexico
C11	CML247/CML254	N-efficient	CIMMYT-Mexico
C12	CML78/CML373	N-efficient	CIMMYT-Mexico
C13	CML264/CML311//CML334	N-inefficient	CIMMYT-Mexico
C14	CML442/CML444//[MSRXPL9]C1F2-205-1(OSU23I)-1-1-X-1-X-B-B	N-efficient	CIMMYT-Kenya
C15	LPSC4F273-2-2-1-B-B-B/CML202//CML384	N-efficient	CIMMYT-Kenya
C16	CML312/CML247//CML78	N-efficient	CIMMYT-Kenya

2.3. The cigar roll experiment

Maize seeds were surface-sterilized with 10 % NaOCl solution for 2 minutes and then soaked in deionized water for 4 hours. Germination was carried out on moistened filter paper with the size of 23 x 35 cm. Seeds were placed at 1 cm below the top center of the filter paper and rolled like a cigar. The cigar rolls were placed upright in an 18 l capacity bucket containing 7 l of tap water and allowed to germinate. One seed placed per cigar roll represents one replication and a total of 8 replications were used. Germinated seedlings with an emerging radicle were transferred to a box filled with 5 l nutrient solution. The nutrient solution was composed of (μM): K_2SO_4 (500); KH_2PO_4 (100); $MgSO_4$ (325); NaCl (50); H_3BO_3 (8); $MnSO_4$ (1.0); $ZnSO_4$ (0.4); $CuSO_4$ (0.4); Na_2MoO_4 (0.1) and Fe-EDDHA (85). $Ca(NO_3)_2$ with a concentration of 100 μM was used as a low N source. The cigar rolls were placed randomly in the solution and the bucket was covered with aluminum foil to avoid drying. The experimental layout was a randomized complete block design, each bucket considered as a block. Plants were harvested 9 days after planting (DAP) for tropical and 10 DAP for temperate cultivars when they produced 3 visible leaves.

2.4. The growth pouch experiment

Growth pouches were made of filter paper (23 × 35 cm) placed in a randomly punctured plastic bag. Seeds were sterilized and soaked using the same procedure as for the cigar roll experiment.

Tropical cultivars were germinated in potato dextrose agar (PDA). The agar solution was prepared in the following way: 5 g agar was added to a 500 ml flask containing 200 ml tap water. The solution was placed in a microwave until it formed a clear solution and allowed to cool. 100 ml of 1mM $CaSO_4$ and 0.8 ml of 8 μM H_3BO_3 were added to the solution as a source of Ca and B for the germinating seeds. The solution was filled to the final volume of 500 ml with tap water. The agar solution was poured into plastic plates. Maize seeds were placed immediately into the agar solution before the agar solidified. The agar solution was added to the plate until the entire seeds were covered. One slice of agar per seed was cut and

placed in petri dishes. The petri dishes were then placed in the dark in a growth chamber in an upright position. Germinated seedlings together with the agar slices were fixed with pins on moist filter paper at the top center position 3 DAP and covered with randomly punctured plastic foil. A hole was made into the plastic pouch above the seeds for the shoots to emerge and the pouches were frequently sprayed with water to avoid drying. The pouches were hanged in a plastic box (42 × 30 × 27 cm size) containing 5 l nutrient solution using wooden sticks. The experimental layout was a randomized complete block design, each box considered as a block. The composition of the nutrient solution was the same as for the cigarroll experiment described in section 2.3.

Except for germination, the same procedure was followed for both tropical and temperate cultivars. Temperate cultivars were germinated in a sandwich experiment, because fungal growth was observed for the tropical cultivars in the agar. Seeds were placed on moistened filter paper at the top center position. The filter paper was covered with randomly punctured plastic foil and placed between foam and PVC on both sides. The PVC plates were fixed at the top and bottom side with plastic bands to avoid seed displacement. One seed per pouch representing one replication was placed upright in a plastic box containing 7 l tap water. After germination only the filter paper covered with plastic foil was transferred to a nutrient solution of the same concentration as described above. Treatments were replicated 6 times for both tropical and temperate cultivars. Harvesting and measurements were done at 10 DAP when plants had 3 visible leaves.

2.5. Measurements

After harvesting, shoots and roots were separated. In the cigar roll experiment, each root fraction (adventitious, seminal and primary roots) was counted and separated from the root system for scanning. Primary root length was measured manually with a ruler. Scanning was done by floating roots in a transparent plastic tray (30 × 20 × 2 cm size) filled with 1 cm layer of water. Larger root samples that could not fit into a tray were cut and dispersed to avoid overlapping. Images were analyzed for total root length, average root diameter, number of root tips and root volume using WinRHIZO image analysis software (WIN MAC, Regent Instruments, Inc.). Root tip diameter was measured on 1 cm root tips.

In addition to parameters taken in the cigar roll experiment, root branching angle from the horizontal surface and root distribution were measured for the plants grown in growth pouches. The branching angle of roots was measured in reference to the horizontal surface. Two vertical lines were marked 2 cm left and right from the kernel, and the distance between the roots crossing these lines and the horizontal layer at the height of the kernel was measured with a ruler. The branching angles were calculated according to the formula:

Tan (root branching angle) = distance from the horizontal layer (cm) / 2 cm

Root distribution was analyzed by dividing the whole length of the growth pouch into 6 different layers each of 4 cm length with the exception of the bottom layer. The intact root system was separated carefully from the filter paper by moistening the paper with water and scanned as a digital image using the WinRHIZO scanner. The root lengths of the 6 different layers were analyzed separately.

Other root parameters were derived from the measured root data; tips per length was calculated by dividing the number of root tips by the total root length (TRL); specific root length is the ratio of TRL and root dry weight; tissue density was calculated by dividing the root dry weight to the root volume. Root and shoot dry weights were measured after oven

drying the sample for 48 hr at 60 ^{o}C. Data on biomass N uptake at anthesis, post-anthesis, at harvest and grain yield were obtained from Worku [30]. The field experiments were carried out in Harare, Zimbabwe (2003 and 2004). The 16 maize cultivars were tested using three N treatments (severe N stress - N1, medium-N stress - N2, and optimum N supply - N3).

2.6. Statistical analysis

The results of the cigar roll and growth pouch experiments were analyzed as a randomly complete block design (RCBD). The General Linear Model Procedure (GLM) of the Statistical Analysis Systems (SAS) Package (SAS Institute Inc., 2002-2003) was used for statistical analysis of all root parameters. When significant genotypic differences (P<0.05) occurred as indicated by the ANOVA result, the Tukey test was used for comparing individual means. r^2 values were obtained from Sigma plot (Version 8.0). Finally simple correlation coefficients were calculated to assess the relationships between root parameters and N uptake and grain yield of the same tropical cultivars in field experiments conducted in Zimbabwe at low-N stress in 2003 and 2004.

3. RESULTS

3.1. Performance of tropical maize cultivars

3.1.1. Root parameters measured both in the cigar roll and growth pouch experiments

3.1.1.1. Shoot and root dry weight

Genotypic variations (P<0.001) were detected in shoot and root dry weight per plant in the cigar roll experiment (Fig. 1A). The N-efficient cultivars C5 and C1 accumulated the highest shoot dry weight in comparison to N-efficient C14, C15 and N-inefficient C4 and C10. Mean shoot dry weight of efficient cultivars was higher than for inefficient cultivars. The root dry weight of the efficient cultivars C5 and C2 was higher than that of the efficient cultivar C11 and the inefficient cultivar C10. Genotypic variation was also observed (P< 0.01) in shoot dry weight in the growth pouch experiment (Fig. 1A). N-efficient cultivars C6, C1 and C12 produced a higher shoot dry weight compared to the N-efficient cultivar C15. No variation was detected in root dry weight among the cultivars. However, N-efficient cultivars tended to produced higher root dry weight than inefficient cultivars. In all cultivars, higher shoot than root dry weight was observed in the cigar roll experiment, while in the growth pouch experiment shoot dry weight was lower than root dry weight.

3.1.1.2. Total root length

No genotypic variation was detected in total root length per plant in the cigar roll experiment (Fig. 1B). However, N-efficient cultivars tended to produce a higher mean total root length (307.5 cm) than inefficient cultivars (294.1 cm). However, cultivars differed in root length (P<0.001) in the growth pouch experiment (Fig.1B). Higher root length was achieved by N-inefficient C8 and N-efficient C1 cultivars in comparison to N-efficient cultivars C16, C14, C15 and N-inefficient cultivars C13 and C7. Comparing the two experiments, higher mean total root length (302.7 cm) was observed in the cigar roll experiment compared to growth pouch experiment (257.1 cm).

3.1.1.3. Primary root-axis length

Genotypic variation (P<0.01) was observed in the primary root-axis length in the cigar roll experiment (Fig. 1C). The N-efficient cultivar C1 produced the longest while another N-efficient cultivar C16 and N-inefficient cultivar C3 produced the shortest primary root. Genotypic variation was also detected in the growth pouch experiment (Fig. 1C). N-efficient cultivar C5 produced higher primary root length compared to another N-efficient cultivar C14. Comparing the two experiments, a higher primary root length (35.8 cm) was observed in the cigar roll compared to the growth pouch (30.5 cm) experiment.

3.1.1.4 . Number of adventitious and seminal roots

Genotypic variation (P<0.001) was observed in the number of adventitious and seminal roots per plant in the cigar roll experiment (Fig. 1D). The N-inefficient cultivars C7, C4, C8 and N-efficient cultivars C2, C6 and C12 produced more adventitious roots than the N-inefficient cultivar C10. The N-efficient cultivar C2 and N-inefficient cultivar C13 produced more seminal roots compared to the N-efficient cultivars C11, C9 and N-inefficient cultivars C8 and C4. The mean number of seminal roots produced by efficient cultivars was higher (5.4) than by inefficient cultivars (4.9). Genotypic variation was also detected (P< 0.01) in the growth pouch experiment (Fig. 1D). The highest number of adventitious roots was recorded by N-efficient cultivars C1 and C2 compared to the N-inefficient cultivar C13. The same efficient cultivars C1 and C2 also produced the highest number of seminal roots compared to the N-efficient cultivars C9, C14, C16 and N-inefficient cultivar C10.

3.1.1.5. Average root diameter

Genotypic variation was observed in average root diameter per plant in the cigar roll experiment (Fig. 2A). N-efficient cultivars C1, C9 and C2 produced the greatest root diameter compared to another efficient cultivar C14. Genotypic variation was also detected (P<0.001) in average root diameter per plant in the growth pouch experiment (Fig. 2A). The N-inefficient cultivar C13 and N-efficient cultivar C15 produced a higher root diameter than N-inefficient cultivars C7 and C8. Higher mean root diameter was recorded in growth pouches (0.647 mm) compared to the cigar rolls (0.584 mm).

3.1.1.6. Tips per length

Genotypic variation (P<0.01) was observed in tips per length in the cigar roll experiment (Fig. 2B). The N-efficient cultivar C1 produced the highest number of tips per root length in comparison to the other efficient cultivars C11, C5, C12 and C14. Genotypic variation (P<0.001) was also detected in the growth pouch experiment (Fig. 2B). Higher tips per root length were exhibited by the N-efficient cultivars C1, C9 and the N-inefficient cultivars C13, C10 and C3 compared to the N-efficient cultivar C16. Higher tips per length was observed in the growth pouch (3.77 tips cm^{-1}) compared to the cigar roll experiment (2.78 tips cm^{-1}).

3.1.1.7. Specific root length

Genotypic differences in specific root length existed in the cigar roll experiment (Fig. 2C). The N-efficient cultivar C11 had a higher specific root length compared to the other efficient cultivar C2. Genotypic variation (P<0.01) was also observed in the growth pouch experiment (Fig. 2C). Higher specific root length was achieved by the N-efficient cultivar C11 in comparison to another N-efficient cultivar C16 and N-inefficient cultivar C13. The mean specific root length of the growth pouch experiment (60.4 m g^{-1}) was higher than the cigar roll experiment (29.1 m g^{-1}).

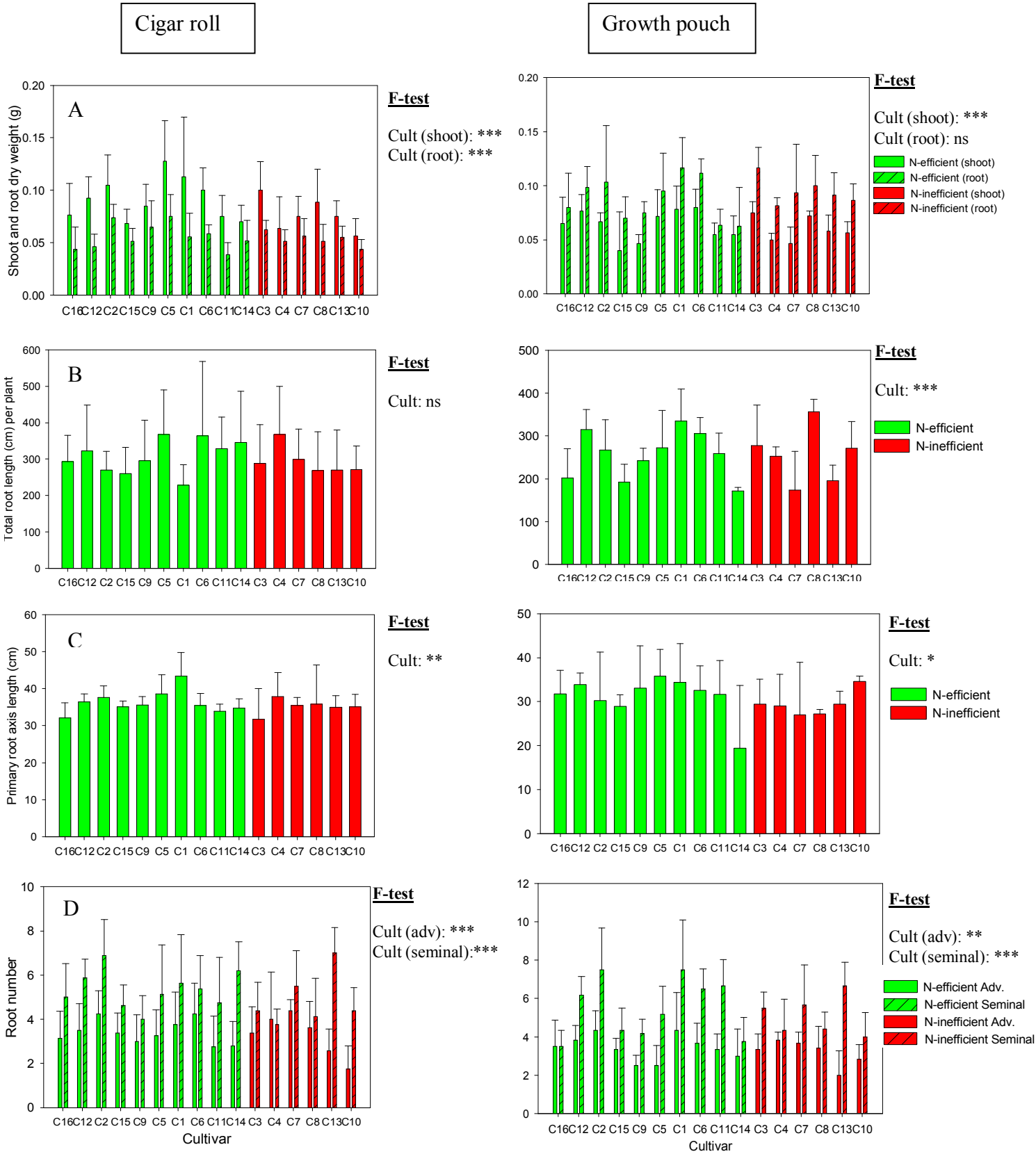

FIG. 1. Shoot and root dry weight (A), total root length (B), primary root-axis length (C) and adventitious and seminal root number (D) of 16 tropical maize cultivars grown in the cigar roll (n = 8) at 9 DAP and in the growth pouch experiment (n = 6) at 10 DAP at low N supply (100 µM N). Cultivars are arranged according to descending N efficiency in the field experiments from the left to the right side.

3.1.1.8. Tissue density

Genotypic variation in tissue density was not detected in the cigar roll experiment (Fig. 2D) due to the observed large standard deviation. However, the N-inefficient cultivars C4, C7 and C8 and efficient cultivars C5 and C9 tended to have a higher tissue density than other cultivars. Similarly, no genotypic variation was detected in the growth pouch experiment (Fig. 2D). Higher mean tissue density was recorded from plants grown in the growth pouch (0.12 g cm^{-3}) compared to those grown in the cigar roll experiment (0.07 g cm^{-3}).

3.1.2. Root parameters measured only in the cigar roll experiment

3.1.2.1. Relative root length of different root fractions

Cultivars were evaluated for the relative contribution of adventitious, seminal and primary roots to the total root length, but no genotypic differences were detected (Fig. 3A). However, N-inefficient cultivars tended to have higher mean percentages of adventitious and primary root length compared to N-inefficient cultivars. On the other hand, N-efficient cultivars tended to produce a higher percentage of seminal roots compared to N-inefficient cultivars.

3.1.2.2. Number of tips of the different root fractions

Genotypic differences ($P<0.01$) were detected in the number of adventitious, seminal and primary root tips per plant (Fig. 3B). The N-efficient cultivar C8 had more adventitious root tips than the other N-efficient cultivars C13 and C10. The N-efficient cultivars C2, C6 and C1 produced a higher number of seminal roots tips compared to the N-efficient cultivars C12, C14 and N-inefficient cultivar C8. A higher number of primary root tips was recorded by N-efficient cultivar C1 when compared to the other N-efficient cultivars C12, C15, C14 and the N-inefficient cultivar C13. The mean number of primary root tips of efficient cultivars were higher (266.7) than of the inefficient cultivars (236.1).

3.1.2.3. Average diameter of root fractions

Genotypic differences existed in mean diameter of adventitious and seminal roots, but no variation was detected in mean diameter of primary roots (Fig. 3C). The N-inefficient cultivar C3 had a higher diameter of adventitious roots than the N-efficient cultivar C15. Higher diameters of seminal roots were recorded by the efficient cultivars C2, C9, C1, C16, C6, C15, C5 and N-inefficient cultivars C3, C13, C10, C4, and a small diameter was measured for the N-efficient cultivar C14. In general, the mean diameters of primary roots were less than that of adventitious roots.

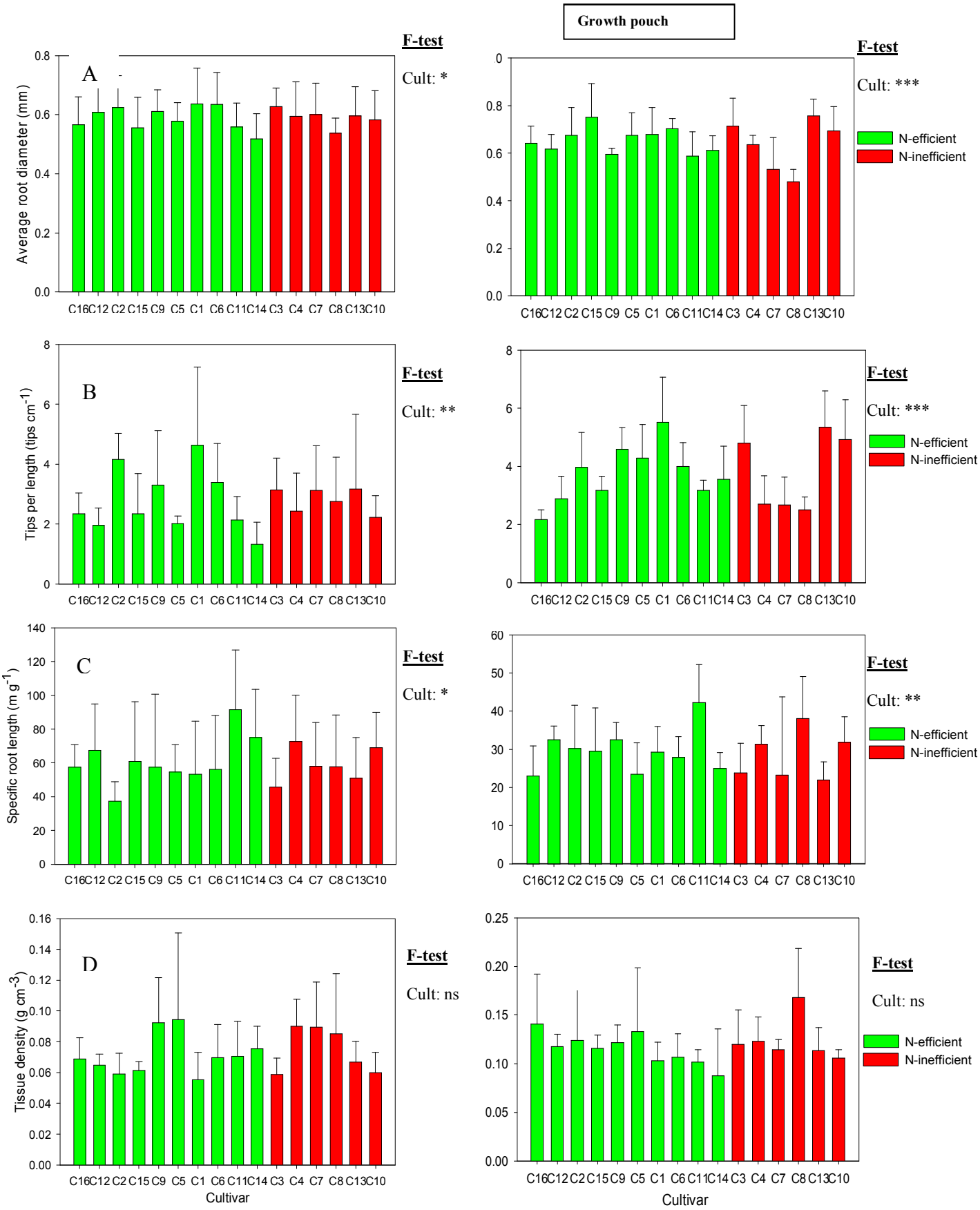

FIG. 2. Average root diameter (A), tips per length (B), specific root length (C) and tissue density (D) of 16 tropical maize cultivars grown in the cigar roll (n = 8) at 9 DAP and in the growth pouch experiment (n = 6) at 10 DAP at low N supply (100 µM N). Cultivars are arranged according to descending N efficiency in the field experiments from the left to the right side.

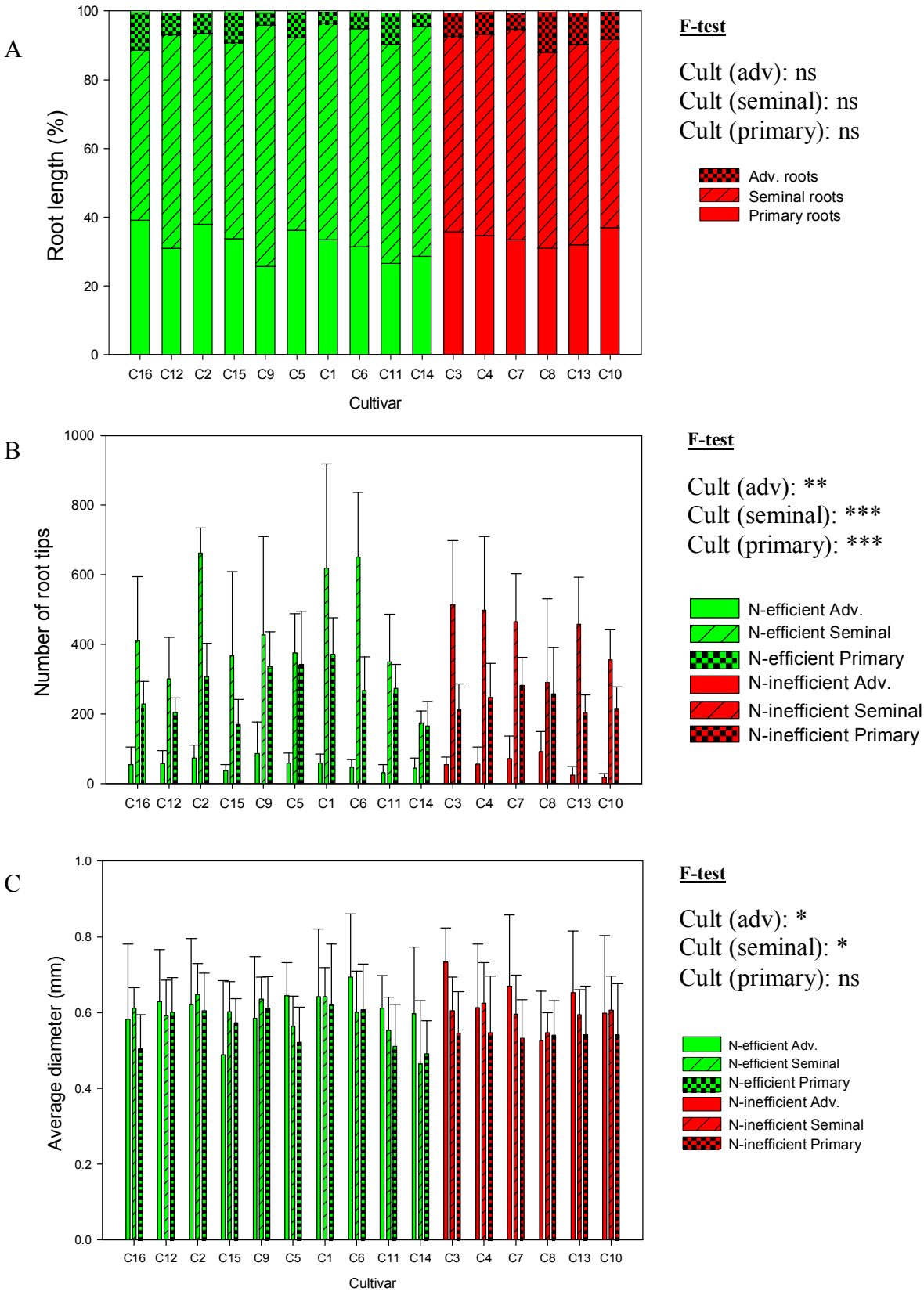

FIG. 3. Root length (%) of the total root length per plant of the different root fractions (A), number of root tips (B) and average diameter of adventitious, seminal and primary roots (C) of 16 tropical maize cultivars grown in the cigar roll experiment (n = 8) at 9 DAP at low N supply (100 µM N). Cultivars are arranged according to descending N efficiency in the field experiments from the left to the right side.

3.1.2.4. Root tip diameter of root fractions

Root tip diameter was analyzed from root tips of 1 cm length cut of individual root fractions. No genotypic variation was detected in root-tip diameter of adventitious roots while genotypic variation (P<0.01) was observed for seminal root-tip diameter (Fig. 4). A higher seminal root-tip diameter was recorded by N-efficient cultivar C12 in comparison to N-inefficient cultivars C10 and N-efficient cultivars C1, C16, C15, C14 and C9.

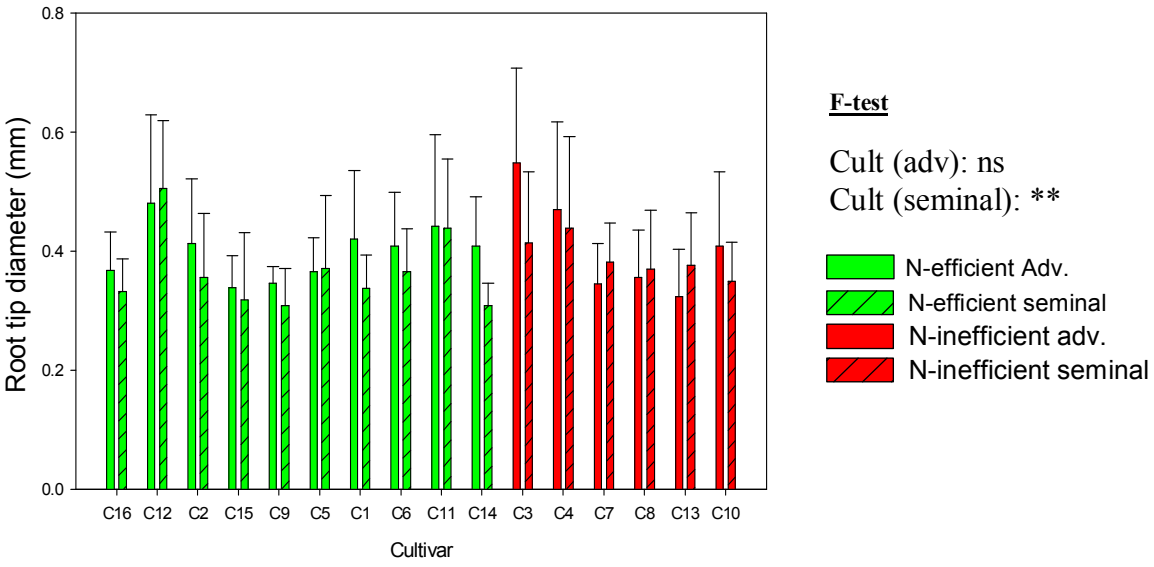

FIG. 4. Root tip diameter of adventitious and seminal roots of 16 tropical maize cultivars grown in the cigar roll experiment (n = 8) at 9 DAP at low N supply (100 μM N). Cultivars are arranged according to descending N efficiency in the field experiments from the left to the right side.

3.1.3. Root parameters measured only in the growth pouch experiment

3.1.3.1. Root length (%) distribution at different root-systems depth

Cultivars were evaluated in the percentage of root length produced in each layer of the growth pouch, which was partitioned into six layers with 4 cm interval from the surface. Genotypic variation was observed in all growth pouch layers except the fourth layer (12-16 cm from the surface) (Fig. 5A). In the first 0-4 cm interval, the N-inefficient cultivar C7 produced a higher (P<0.01) percentage of root length than the N-efficient cultivars C11, C5, C2, C6, C12, C1 and N-inefficient cultivars C13, C3 and C10. In the deepest growth pouch layer (>20 cm), the N-efficient cultivars C12, C6, C9 and C1 produced a higher (P<0.001) percentage of root length than the N-inefficient cultivar C8. Generally, N-inefficient cultivars tended to produce a higher percentage of root length in the upper layer and N-efficient cultivars in the lowest layer of the growth pouch.

3.1.3.2. Root tips (%) distribution at different root-systems depth

Cultivars were also evaluated for the percentage of root tips in each layer of the growth pouch. Genotypic variation was detected only in the first two layers (0-4 and 4-8 cm) (Fig. 5B). In the 0-4 cm interval, the N-inefficient cultivar C7 produced a higher (P<0.001) percentage of root tips than all other cultivars with the exception of cultivar C4. N-efficient cultivar C15 produced a higher (P<0.01) percentage of root tips in the growth pouch layer of 4-8 cm than N-inefficient cultivar C3 and N-efficient cultivar C1. No genotypic variation was

detected in the remaining growth pouch layers, but the mean percentage of root tips of the efficient cultivars tended to be higher than that of inefficient cultivars in the last two growth pouch layers. There were no differences in the remaining intervals.

3.1.3.3. Root length (%) in different diameter classes

Genotypic differences were detected (P<0.001) in percentage of root length of roots with a diameter class of 0-1 mm, 1-2 mm and >2mm (Fig. 5C). The dominant root types were fine roots with a diameter class of 0-1 mm comprising about 83.3% followed by roots in a diameter class of 1-2 mm (13.5%) and >2 mm (2.7%). In the diameter class of 0-1 mm, a higher percentage of roots were produced by the N-inefficient cultivars C8, C4 and N-efficient cultivars C16, C14, C15 and C11 in comparison to N-inefficient cultivars C13 and C3. In the diameter class of 1-2 mm, the N-inefficient cultivars C3 and C13 produced a higher percentage of roots than N-efficient cultivars C16, C5, C14 and N-inefficient cultivars C4 and C8. In the diameter class >2 mm diameter, the N-efficient cultivars C1 had a higher percentage of roots than N-efficient cultivars C16, C12 and N-inefficient cultivars C8 and C4.

3.1.3.4. Roots branching angle

Cultivars were evaluated for the percentage of roots they produced in a branching angle interval of 0-30^o, 30-60^o and 60-90^o (Fig. 6). No genotypic variation was detected in a root branching angle interval of 0-30^o, while genotypic variation was observed in the branching angle intervals of 30-60^o and 60-90^o. In the root branching interval of 30-60^o, a high percentage of roots was exhibited by the inefficient cultivar C7 compared to the efficient cultivar C16. The N-efficient cultivars C16 and C2 produced higher percentage of roots in a branching angle interval of 60-90^o compared to the inefficient cultivar C7.

3.3. Relationships between genotypic performances in short term nutrient solution in the cigar roll and field experiments

Correlation were calculated to observe the relationship between the cigar roll experiment parameters with N uptake and grain yield of this cultivars grown at low N in field experiments in Zimbabwe (Table 3). Total root length was positively and significantly correlated with N uptake at anthesis. Adventitious and seminal root numbers were not significantly correlated with N uptake at anthesis. A significant negative correlation was detected between average root diameter and N uptake at anthesis. None of the parameters showed correlations with N uptake after anthesis and grain yield.

No significant correlations were observed between root length in different layers of the growth pouch and N uptake at and after anthesis (Table 4). However, a trend for a positive correlation was detected between relative root length >20 cm and N uptake after anthesis and grain yield. No correlation was observed between roots within 0-30 ^{o} branching angle interval and N uptake after anthesis and grain yield. Percentage of roots within a branching angle interval of 30-60^o were significantly (P<0.1) and negatively correlated with N uptake after anthesis. A tendency of negative correlation was also observed between the percentage of roots within this branching angle interval and grain yield. A significant (P<0.1) positive correlation was observed between percentage of roots within the branching angle interval of 60-90^o and N uptake after anthesis. In addition to this, a trend for a positive correlation was observed between the percentage of roots within the branching angle interval of 60-90^o and grain yield.

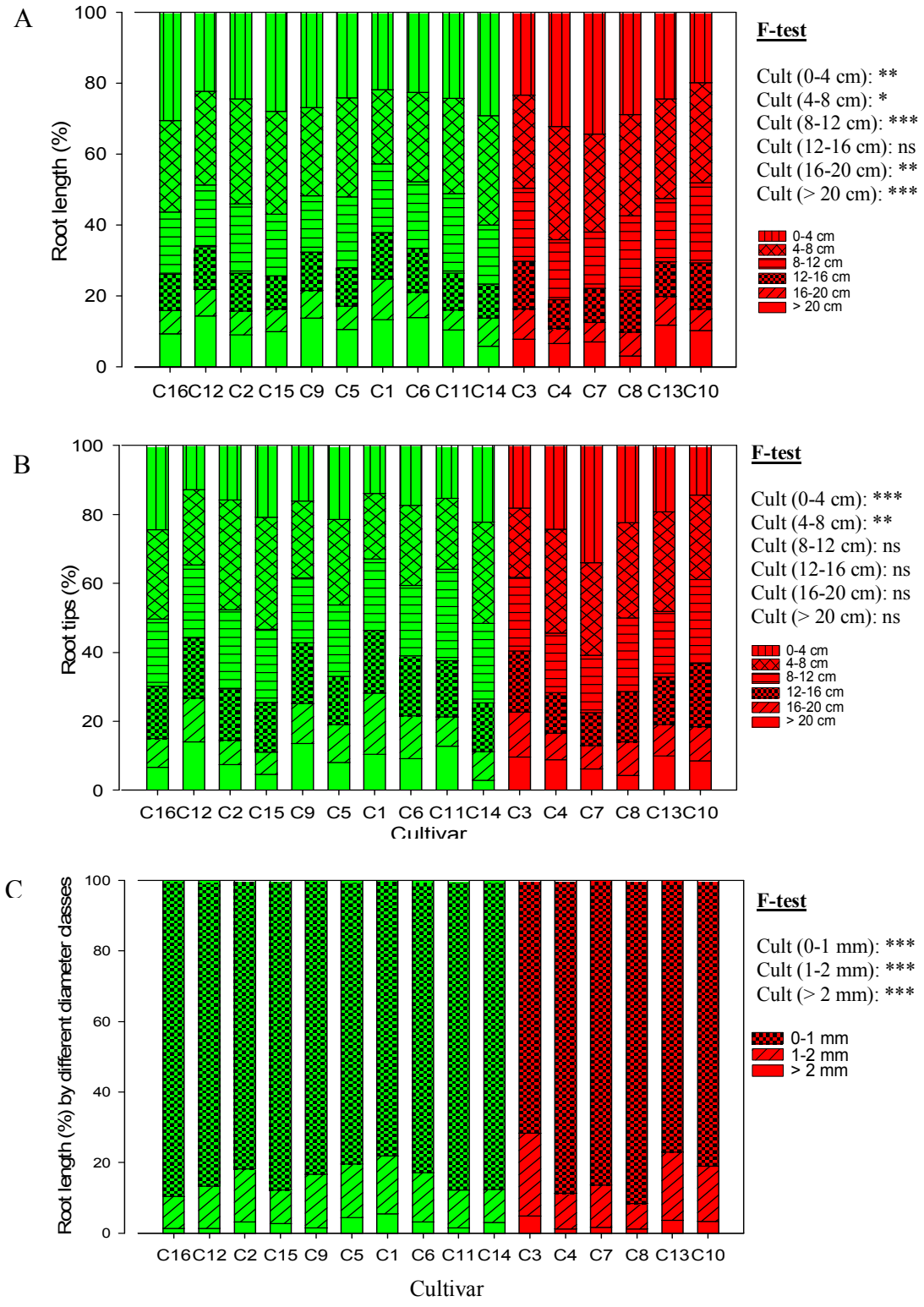

FIG. 5. Root length (%) of the total root length per plant (A), root tips (%) (B) at different root-systems depth from the surface and root length (%) in different diameter classes (C) of 16 tropical maize cultivars grown in growth pouches (n = 6) at 10 DAP at low N supply (100 µM N). Cultivars are arranged according to descending N efficiency in the field experiments from the left to the right side.

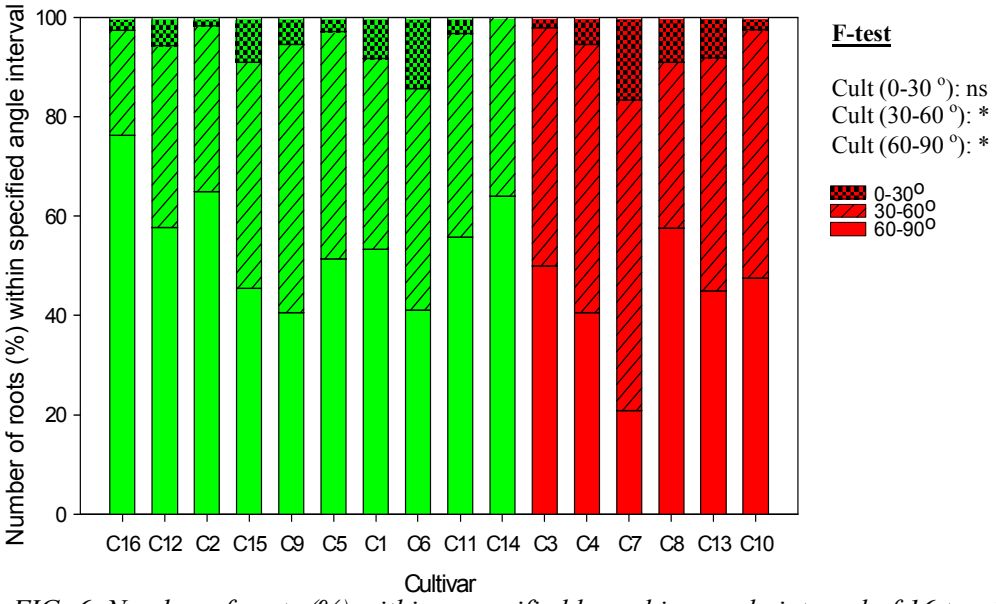

FIG. 6. Number of roots (%) within a specified branching angle interval of 16 tropical maize cultivars grown in growth pouch experiment (n=6) at 10 DAP at low N supply (100 μM N). Cultivars are arranged according to descending N efficiency in the field experiments from the left to the right side.

TABLE 3. CORRELATION COEFFICIENTS BETWEEN THE CIGAR ROLL ROOT-PARAMETERS AND N UPTAKE AND GRAIN YIELD OF THE SAME TROPICAL MAIZE CULTIVARS AT LOW N IN THE ZIMBABWE FIELD EXPERIMENTS

Parameter	N uptake			Grain yield
	Antheses	Post-antheses	Harvest	
Total root length (cm)	0.50*	–0.27	0.24	0.16
Adventitious root number	–0.15	0.11	–0.04	0.39
Seminal root number	–0.07	0.25	0.17	0.13
Average root diameter (mm)	–0.52*	0.23	–0.30	0.10

* denotes significance at $P<0.05$

TABLE 4. CORRELATION COEFFICIENTS (R) BETWEEN THE GROWTH POUCH ROOT-PARAMETERS AND N UPTAKE AND GRAIN YIELD OF THE SAME TROPICAL CULTIVARS AT LOW N IN THE ZIMBABWE FIELD EXPERIMENTS

Parameter	N uptake			
	Anthesis	Post-anthesis	Harvest	Grain yield
Relative root length (0-4 cm)	0.07	–0.10	–0.03	–0.03
Relative root length (4-8 cm)	0.29	–0.28	0.01	–0.22
Relative root length (8-12 cm)	–0.05	–0.22	–0.27	–0.30
Relative root length (12-16 cm)	–0.20	–0.08	–0.28	–0.08
Relative root length (16-20 cm)	–0.31	0.31	0.00	0.22
Relative root length (>20 cm)	–0.04	0.40	0.36	0.35
Relative root branching angle (0-30°)	0.09	–0.22	–0.13	–0.22
Relative root branching angle (30-60°)	0.16	–0.46+	–0.30	–0.42
Relative root branching angle (60-90°)	–0.16	0.45+	0.29	0.41

+ denotes significance at $P<0.1$

3.4. Performance of temperate maize cultivars

3.4.1. Root parameters measured in both the cigar roll and growth pouch experiments

3.4.1.1. Shoot and root dry weight

Genotypic variation ($P<0.01$) was observed in shoot dry weight per plant in the cigar roll experiment (Fig. 7A). The N-inefficient cultivar TH produced a higher shoot dry weight than the N-efficient cultivars ASK and SL and the N-inefficient cultivar NUE. No genotypic variation was observed in root dry weight, but the N-inefficient cultivars tended to produce a higher mean root dry weight than the N-efficient cultivars. Also, in the growth pouch experiment, genotypic variation was observed ($P<0.001$) in shoot dry weight (Fig. 7A). The N-inefficient cultivar TH produced higher shoot dry weight than the N-efficient cultivars ASK, SL and the N-inefficient cultivar NUE. No genotypic variation was detected in root dry weight. Similar to tropical cultivars, shoot dry weight of cultivars were higher than root dry weight in the cigar roll, but the opposite holds true for the growth pouches.

3.4.1.2. Total root length

No genotypic variation was observed in total root length per plant both in the cigar roll and growth pouch experiments (Fig. 7B). Comparing the two experiments, higher total root length was observed in the growth pouch experiment.

3.4.1.2. Primary root axis length

No genotypic variation was observed in primary root length in the cigar rolls while significant ($P<0.001$) variation between cultivars was observed in the growth pouch experiment (Fig. 7C). Longer primary roots were produced by the N-inefficient cultivar TH compared to the other three cultivars in growth pouches. Comparing the two experiments, a longer primary root length was observed in the growth pouch experiment (45.9 vs 31.7 cm).

3.4.1.3. Number of adventitious and seminal roots

Genotypic variation existed ($P<0.01$) in the number of adventitious roots per plant in the cigar roll experiment (Fig. 7D). The N-inefficient cultivar NUE and N-efficient cultivar SL had a higher number of adventitious roots than the N-efficient cultivar ASK. No variation was observed between cultivars in the number of seminal roots per plant in this experiment. Genotypic variation in the number of adventitious roots was detected in the growth pouch experiment (Fig. 7D). The two N-inefficient cultivars NUE and TH produced a higher number of adventitious roots than the N-efficient cultivars SL and ASK. No variation was observed between cultivars in the number of seminal roots per plant. On average, the N-inefficient cultivars produced a slightly higher number of seminal roots compared to the efficient cultivars. Comparing the two experiments, a higher mean number of adventitious roots was produced in the growth pouch experiment (3.5 roots) compared to the cigar rolls (2.8 roots), but the number of seminal roots was lower in the growth pouch (4.8 roots) than in the cigar roll experiment (5.7 roots).

3.4.1.4. Average root diameter

No genotypic variation existed in average root diameter in the cigar roll experiment (Fig. 8A). Genotypic variation ($P<0.01$) in this trait was observed in the growth pouch

experiment (Fig. 8A). The N-inefficient cultivar NUE produced thicker roots than the N-efficient cultivar ASK.

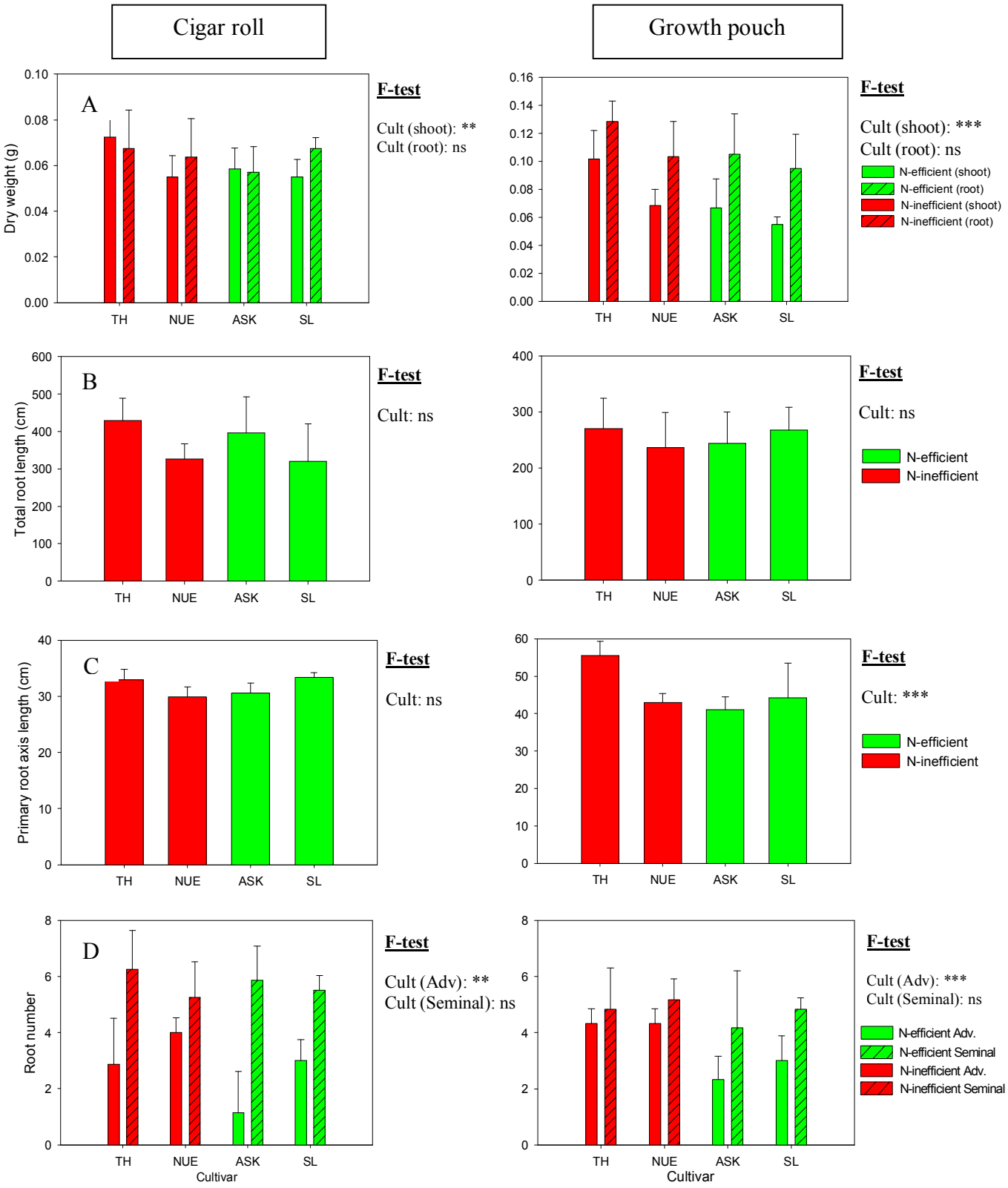

FIG. 7. Shoot and root dry weight (A), total root length (B), primary root-axis length (C) and adventitious and seminal root number (D) of 4 temperate maize cultivars grown in the cigar roll (left side) (n = 8) and growth pouch experiment (n = 6) at 10 DAP at low N supply (100 µM N).

3.4.1.5. Tips per root length

No genotypic variation was detected in tips per length in the cigar roll experiment (Fig. 8B). Genotypic variation was observed (P<0.001) in the growth pouch experiment (Fig. 8B). The N-inefficient cultivar NUE produced a higher number of tips per root length than the N-efficient cultivars ASK, SL and the N-inefficient cultivar TH. Generally, higher tip numbers per length were observed in the cigar roll (2.6 tips cm^{-1}) compared to the growth pouch experiment (1.6 tips cm^{-1}).

3.4.1.6. Specific root length

Specific root length is the total length of roots per unit of root dry weight. No genotypic variation was detected in specific root length in both the cigar roll and growth pouch experiments (Fig. 8C). Comparing the two experiments, a higher specific root length was exhibited in the cigar roll (40.9 m g^{-1}) compared to the growth pouch experiment (33.2 m g^{-1}).

3.4.1.7. Tissue density

Cultivars did not differ in tissue density in both the cigar roll and growth pouch experiments (Fig. 8D). Nevertheless, N-efficient cultivar SL tended to have higher tissue density than other cultivars in both experiments.

3.4.1.8. Root tip diameter of adventitious, seminal and primary roots

No variations were observed among cultivars in root tip diameter of adventitious, seminal and primary roots both in the cigar roll and growth pouch experiments (Figs. 9A and 9B).

3.4.2. Root parameters measured only in the cigar roll experiment

3.4.2.1. Relative root length of different root fractions

Cultivars were evaluated for the relative contribution of root fractions to the total root length in the cigar roll experiment (Fig. 10A). Genotypic variation (P<0.001) was detected in relative contribution of adventitious root length to the total root length. The N-inefficient cultivar NUE produced a higher percentage of adventitious root length than the N-efficient cultivars SL and ASK. No variation was detected in the relative contribution of seminal and primary root length to the total root length.

3.4.2.2. Number of tips of different root fractions

Genotypic variations existed in the number of adventitious root tips (Fig. 10C). The N-inefficient cultivar NUE produced a higher number of root tips than the N-efficient cultivar ASK. No variation was detected in the number of seminal and primary root tips.

3.4.2.3. Average diameter of root fractions

Cultivars were also evaluated for their thickness of adventitious, seminal and primary roots but no genotypic variation was detected (Fig. 10B).

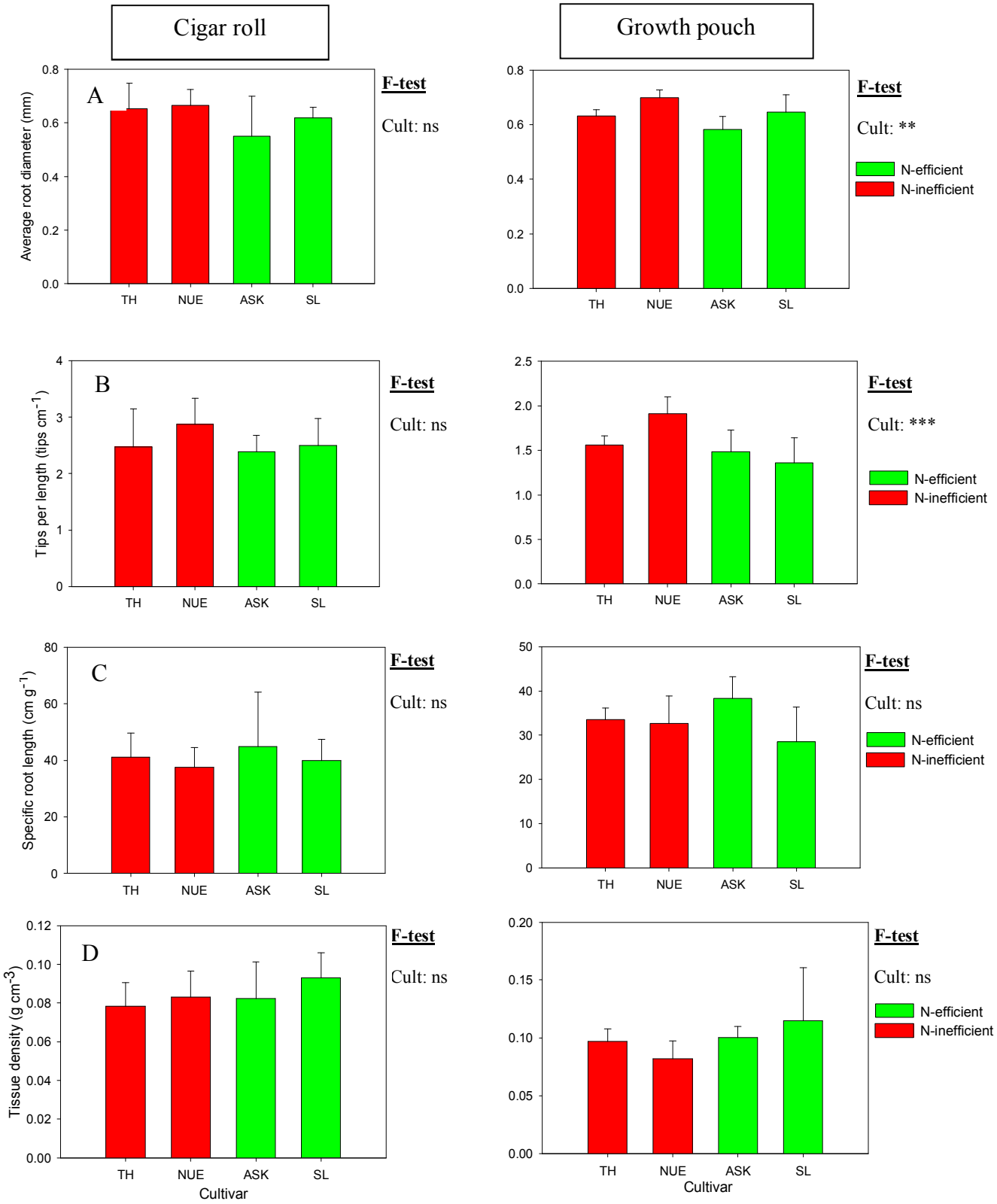

FIG. 8. Average root diameter (A), tips per length (B), specific root length (C) and tissue density (D) of 4 temperate maize cultivars grown in the cigar roll (left side) (n = 8) and growth pouch experiment (n = 6) at 10 DAP at low N supply (100 µM N).

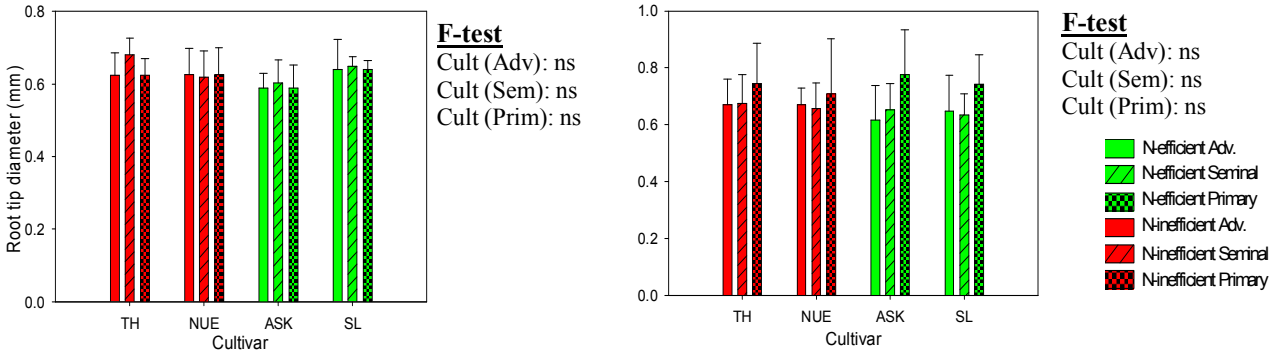

FIG. 9. Root tip diameter of 4 temperate maize cultivars grown in the cigar roll (A) (n = 8) and growth pouch experiments (B) (n = 6) at 10 DAP at low N supply (100 μM N).

3.4.3. Root parameters measured only in the growth pouch experiment

3.4.3.1. Root length (%) distribution at different depth intervals from the surface

Cultivars were evaluated on the percentage of root length produced in each layer of the growth pouch, which was partitioned into six layers with 4 cm intervals. Genotypic variation was found in the first two layers (0–4 and 4–8 cm) from the surface (Fig. 11A). In the 0–4 cm interval, the N-inefficient cultivar NUE and the N-efficient cultivar SL produced a higher percentage of root length than the N-inefficient cultivar TH. In the 4–8 cm interval, the N-efficient cultivars ASK and SL produced a higher percentage of root length ($P<0.001$) than the N-inefficient cultivar TH. No genotypic variation existed in the remaining growth pouch layers.

3.4.3.2. Root tips (%) distribution at different root-systems depth from the surface

Genotypic variation was observed ($P<0.01$) in the relative number of root tips in the growth pouch layer of 0–4 cm (Fig. 11B). The N-inefficient cultivar NUE produced a higher percentage of root tips than the N-efficient cultivar ASK and the N-inefficient cultivar TH. The N-efficient cultivar ASK produced a higher percentage of root tips than the N-inefficient cultivar TH in the growth pouch layer of 4–8 cm. No genotypic variation was detected in the growth pouch layers of 8–12, 12–16 and 16–20 cm. In the last growth pouch layer (>20 cm) N-inefficient cultivar TH produced a higher percentage of root tips than the other N-inefficient cultivar NUE.

3.4.3.3. Root length (%) in different diameter classes

No genotypic variation existed in the percentage of roots in the diameter classes of 0–1, 1–2 and >2 mm (Fig. 11C). More than 88 % of the total root length consisted of roots within the 0–1 mm diameter class, followed by the diameter classes of 1–2 mm and >2 mm with 10.5 and 0.5%, respectively.

3.4.3.4. Root branching angle

Cultivars were evaluated for the relative number of roots within the root branching angle interval of 0–30, 30–60 and 60–90^{o} from the surface (Fig. 11D). No genotypic variation was observed in any root branching interval. However, N-inefficient cultivars tended to produce a higher percentage of roots in a root branching interval of 0–30^{o} and 30–60^{o}, while

N-efficient cultivars produced a higher percentage of roots in the root branching interval of 60–90°.

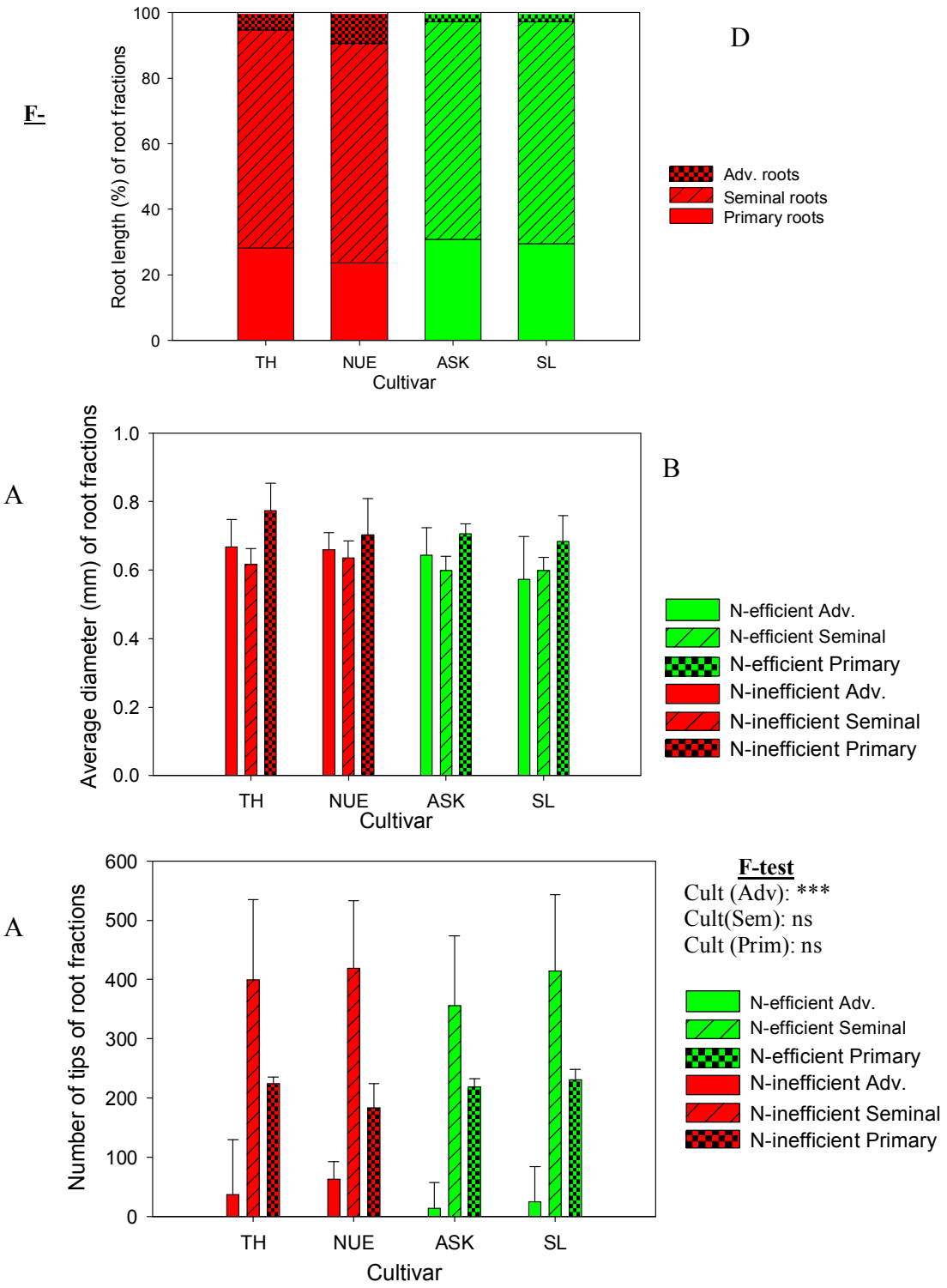

FIG. 10. Root length (%) of root fractions (A), average diameter of root fractions (B) and number of root tips of the different root fractions (C) of 4 tropical maize cultivars grown in the cigar roll experiment (n = 8) for 9 DAP at low N supply (100 µM N).

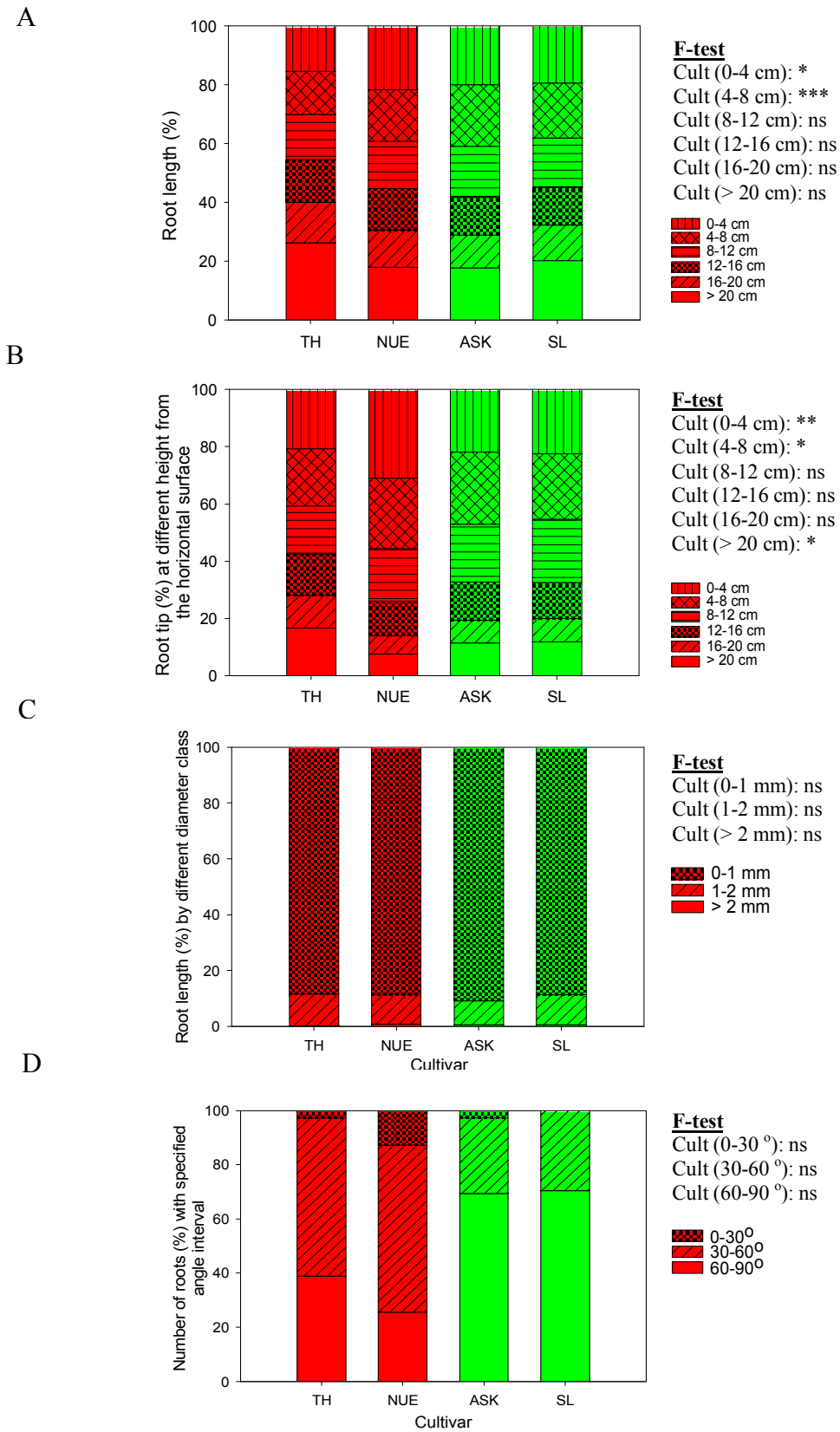

FIG. 11. Root length (%) (A), root tips (%) (B) at different depth intervals from the surface, root length (%) by different diameter classes and number of roots (%) within a specified root branching angle from the horizontal surface of 4 temperate maize cultivars grown in the growth pouch experiment (n = 6) at 10 DAP at low N supply (100 μM N).

4. DISCUSSION

The present study was designed to assess the relationship between root parameters in short-term experiments and the N-efficiency of tropical and temperate maize cultivars in field experiments.

Due to the existence of large genotypic difference in carbon assimilation under low N, it is quite possible that root growth may be enhanced, unchanged, or even suppressed [9]. Nitrogen deficiencies resulted in significant decreases both in shoot and root dry matter weight of young plants [16]. On the other hand, after investigating a range of tropical cultivars, no correlation was found between root dry matter of a nutrient solution experiment and N uptake in the field [31]. It was concluded that shoot dry matter at early growth stages in nutrient solution is not related to shoot biomass at anthesis and grain yield, and therefore, could not be used as a selection parameter for N efficiency [31]. Other authors also found no correlation between root dry matter of seedlings and root parameters or important agronomic traits in the field [4, 32], and concluded that root dry matter is not a useful criterion for indirect selection. In our experiments we have found inconsistent results for the two sources of maize cultivars. High shoot dry weights were observed by N-efficient tropical cultivars compared to inefficient cultivars (Fig. 1A), while efficient temperate cultivars produced lower shoot dry weight than inefficient cultivars (Fig. 7A). The root dry weight results were also inconsistent. No significant differences were observed between cultivars in root dry weight of tropical and temperate cultivars in the cigar roll experiment, but higher root dry weights of inefficient tropical and temperate cultivars were observed in the growth pouch experiment (Fig. 7A). This implies that the shoot and root dry weight of cultivars at the seedling stage might not be a reliable parameter for selection for N efficiency.

Genotypic variation in root length of maize seedlings was reported [13], and a highly significant correlation was found between the size of the root system and grain yield at low N supply in field experiments [5]. Maize hybrids respond to N deficiency by increasing total root length (TRL) [9]. However, in our experiment no genotypic differences were observed in this trait. Other authors reported no correlation between root length in nutrient solution and total shoot nitrogen uptake [31, 32]. However, in our experiment we found positive and significant correlations between root lengths of 16 tropical cultivars in the cigar roll experiment and N uptake up to anthesis (Table 3). Nevertheless, no correlation was found with total root length after anthesis, which is very important for N efficiency. Therefore, it seems that a high root length at the early growth stage is not a suitable selection parameter for N efficiency.

Maize hybrids respond to N deficiency by elongation of individual seminal roots while reducing the number of seminal roots [9]. Since seminal roots grow more in a downward direction than adventitious roots, they may particularly contribute to the exploration of the sub-soil during later stage of development [14]. In our study, significant differences were observed among tropical cultivars in the number of seminal roots both in the cigar roll and growth pouch experiments; moreover the mean seminal root number of N-efficient cultivars was higher than inefficient cultivars. Significant genotypic differences were also observed among temperate cultivars in the cigar rolls, but the N-inefficient cultivars had a higher number of seminal roots than the efficient cultivar ASK (Figs. 1 and 7D). Low-input cultivars of maize developed more and longer seminal roots than the high-input cultivars [13]. A higher seminal root number which was found for some N-efficient tropical cultivars is beneficial, because an efficient cultivar seems to be able to exploit the available N in deeper soil layers. On the other hand, Heuberger [4] concluded that the number of seminal roots

might not be a good predictor for early root growth in the field, but a high length of seminal roots seemed to be more promising to identify cultivars with high penetration capacity into the subsoil. We also observed no correlation between the number of seminal roots in the cigar rolls and N uptake and grain yield of tropical cultivars (Table 3). Taken into consideration the inconsistency of the result and literature findings it is possible to conclude that this parameter is not a suitable selection parameter for N efficiency at low N.

Heuberger [4] reported significant genotypic variation in the number of adventitious roots of maize in one pot experiment, but also found inconsistent correlations between this fraction of the root system and root parameters in the field. Genotypic variation in this parameter was also observed for maize [33] and other cereals [34]. In our study the number of adventitious roots showed a similar trend as the number of seminal roots i.e. N-efficient tropical cultivars produced more adventitious roots while efficient temperate cultivars had fewer adventitious roots (Figs. 1 and 7D). We also observed no correlation between the numbers of adventitious roots of tropical cultivars in the cigar rolls and N uptake and grain yield (Table 3). Heuberger) [4] stated that the number of adventitious roots of seedlings may only be a weak indicator for later development, since the bulk of adventitious roots are formed at later growth stages, and cultivars may have different patterns of development. It was also suggested that the number of adventitious roots is less important for a deep rooting system [35]. Taken together, the inconsistence of the result and literature findings, the number of adventitious roots at early growth stages cannot be used as a selection criterion for N efficiency.

It has been mentioned that at later growth stages (after flowering), the newly developed roots are characterized by a reduced diameter associated with increased root length in the deeper soil layers maintaining N uptake [36]. In our study we found significant negative correlation between the average root diameter and N uptake up to anthesis in the cigar rolls (Table 3), suggesting that thinner roots are more important for high N uptake at low N.

The fineness of roots is expressed as specific root length (length per unit dry weight), and roots with high specific root length (fine roots) are found in plants grown under nutrient deficient conditions [26]. In our experiment, significant differences were observed in this trait among tropical cultivars both in cigar roll and growth pouch experiments. However, this difference could be found only among a limited number of cultivars (Fig. 2C). No genotypic differences were detected in this trait among temperate cultivars (Fig. 8C).) It has been proposed that fine roots (high specific root length) allow the root system to explore the soil volume effectively while minimizing the investment needed to construct and maintain the root system [27]. However, our results do not support this hypothesis, and therefore this trait is not suitable to be used as a selection parameter for N efficiency.

Low tissue density enables a fast resource acquisition as a plant can rapidly expand its root system with low investment in dry matter; however, the low dry-matter produced by such a fast growing plants is likely to have a short life span [37]. In grasses, thicker roots and a high tissue density have been associated with increased longevity [29]. In our experiment, no genotypic differences were observed among tropical and temperate cultivars in tissue density (Figs. 2 and 8D). However, some N-efficient cultivars tended to have higher tissue density than inefficient cultivars. High tissue density is a result mostly of a high amount of cell wall material and lignin [38, 39]. A lower percentage of air space may also contribute to a high density [40]. Nevertheless, in the absence of genotypic difference it would be difficult to generalize that high N uptake efficiency is due to the presence of high tissue density.

Therefore, this trait in young seedlings also appears to have no relevance for screening of cultivars for N efficiency.

Lynch [22] proposed that studies of the root distribution and root architecture are important because soil resources are unevenly distributed, or subject to localized accumulation or depletion, so that the spatial deployment of the root system will measure the ability of a plant to exploit those resources. He stated that, since they are difficult to observe and to quantify we know little about these parameters. In our study, we used growth pouches to study the root distribution of cultivars in the pouch and correlate them with the field result. Some N-inefficient tropical cultivars possessed a relatively higher percentage of root length in the 0-4 and 4-8 cm layers; on the other hand, N-efficient tropical cultivars possessed a relatively higher percentage of root length in the deepest >20 cm layer (Fig. 5A). Though non-significant, a tendency for a positive correlation was also observed between the percentage of root length in the lower layer of the pouch and N uptake after anthesis and grain yield (Table 4). This result is in agreement with Worku [30] who reported that an N-efficient cultivar developed relatively more roots in the subsoil (60-90 cm) than an N-inefficient cultivar. Also, the N-efficient cultivar developed more vertically oriented fine roots and exploited more mineral-N in the soil layer below 60 cm. Similar trends were observed in the percentage of root tips in the upper 2 and the lowest grow-pouch layers for the tropical cultivars. However, the result obtained for the temperate cultivars did not fit this trend: the N-inefficient cultivar possessed a higher percentage of root length both in the first and the deepest layer (Fig. 10B). Worku [30] stated that the root-length density determined for tropical maize cultivars was much lower than has been previously reported for temperate cultivars, implying that differences in root-length densities and distribution in the subsoil between cultivars may be of greater importance in tropical than in temperate environments.

The angle of root growth has been reported as an important factor in determining the distribution of the root system in the soil [41]. In our study, N-inefficient tropical and temperate cultivars possessed high percentages of roots at a branching angle interval of 0-30 and 30-60^{o}, while N-efficient cultivars produced high percentage of roots at a branching angle interval of 60-90^{o} (Figs. 6 and 11D). This suggests that N-efficient cultivars produce more vertically grown roots than inefficient cultivars. Moreover, the percentage of roots in a branching angle interval of 60-90^{o} was positively correlated (P<0.1) with N uptake after anthesis and grain yield (Table 4). Dunbabin and colleagues [7, 25] concluded on the basis of their simulation model that plants with a deep-rooted architectures acquire more nitrate than shallow-rooted plants. The high N uptake efficiency of N-efficient cultivars might be related to this deep rooted architecture.

It is concluded form our work presented here, that among the many shoot and particularly root parameters assessed in the growth pouch and cigar roll experiments with seedlings, the only parameters which might be suitable to be used as a selection parameter for N efficiency of tropical maize cultivars might be the percentage of root-length distribution in a horizontal layer and the percentage of roots with a specified branching-angle interval measured in the growth pouch experiment.

ACKNOWLEDGMENTS

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EVALUATION AND SELECTION OF MAIZE (*ZEA MAYS* L.) GENOTYPES TOLERANT TO LOW N SOIL

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Abstract

The identification and/or the development of germplasm with traits which enhance N uptake and N use efficiency in low N soil could significantly sustain maize production on stress environments. The use of secondary traits highly correlated with grain yield and high heritability, could speed up the development of genotypes adapted to low N environments. Arbuscular mycorrhiza fungi are known to enhance P uptake, but its role on plant N nutrition has not been extensively studied. The study aimed to (i) identify tolerant and/or low N responsive genotypes (ii) measure the correlated response of grain yield with some agronomic plant characteristic under low N and under mycorrhiza inoculation (iii) measure the combining ability and the gene effects of the lines under low and high N and (iv) to identify stable and high yielding hybrids adapted to low and high N condition. Initial screening of 99 genotypes for two years identified 30 inbred lines that were evaluated in split plots for: grain yield, root volume, chlorophyll content, leaf area index, and mycorrhizal colonization. Significant genotype x soil N level interactions were obtained among the tested inbreds for all measured traits, except for chlorophyll content which exhibited similar ranking from one soil N level to another. In addition to selection for grain yield, 5 lines were retained for their good root volume, 4 for their chlorophyll content and stay green traits, 3 for their leaf area index and the last 3 for their mycorrhizal colonization. Diallel crosses among the 15 selected lines yielded 105 F1 hybrids evaluated in split plots, with 3 soil treatment levels (20 kg–N ha^{-1}, 20 kg–N ha^{-1} + mycorrhiza and 100 kg–N ha^{-1}). Significant differences were detected among the 3 soil treatments as well as for genotypes x soil interaction for all measured traits. On 20 N plots, 10 hybrids yielded at least as good as the check hybrid: $Expl_{24}$ x 87036 (3.0 t ha^{-1}). Among the 20 parents involved in these crosses, 8 had been retained for their chlorophyll content, six for their root volume, four for their leaf area index and two for their mycorrhizal colonization. On plots with 20 N, in addition to mycorrhizal inoculation, 8 hybrids yielded more than the check (3.2 t ha^{-1}). Among the 16 inbreds involved, 5 were retained for their root volume, 4 for their mycorrhizal colonization and leaf area index, respectively, and 3 for their chlorophyll content. On 100 N plots, 13 hybrids yielded more than the check (5.4 t ha^{-1}). Among the 26 inbreds involved, 13 (50%) were selected for their root volume, six (25%) for their mycorrhizal colonization, four (15.4%) for their leaf area index, and three (11.5%) for high chlorophyll content. It was concluded that on low N soil, selection for root volume was correlated with grain yield. However, maximum benefit was obtained in hybrids when their inbred parents exhibited complementary plant characteristics such as the efficient colonization by mycorrhiza, good leaf area index and high chlorophyll content.

1. INTRODUCTION

Low nitrogen (N) stress in the soil is one of the most important abiotic stresses causing yield reduction in maize production in tropical soils [1]. In these soils, 90% of maize is grown by resource poor farmers who cannot afford expensive N fertilizers. Since 2007, the price of N fertilizer has increased in developing countries mostly, making it inaccessible to

poor farmers. To alleviate this constraint, the identification and/ or the development of germplasm with traits which enhance N uptake and utilization in poor soils could significantly contribute to maize production on these stress environments. Several studies have indicated that useful genetic potential for the improvement of N use efficiency exists in maize [2]. Therefore, the application of efficient farming techniques and/or the use of plant varieties that have a better N use efficiency could reduce the use of N fertilizer [3]. Breeding strategies to develop stress tolerant maize inbred lines include screening and selection of inbred lines under managed stress conditions, multi-location testing of hybrids in a representation sample of the target environment, and selection under high plant populations [4]. However, the *per se* performance of maize inbred lines doesn't predict the performance of hybrids for grain yield or any other traits [5]. Given the low heritability of yield under stress conditions, it has been suggested that the use of the secondary traits that possess high heritability and are correlated with tolerance to low-N could help to speed up the development of high yielding, stable and adapted varieties [6].

The ability of plants to access soil N varies among species and genotypes within species and is affected by interactions of plants and microbes [7]. In addition to the inherent genetic potential of a plant to extract N from soil, the ability to interact with microbes can also be a major determinant of a plant's ability to grow in soils with low levels of bioavailable N. Arbuscular mycorrhizal (AM) fungi are plant symbionts that are known to enhance P uptake in numerous plant species. The role of AM fungi on plant N nutrition has not been studied as extensively as that of P, but it is now receiving more attention. It has been shown that AM fungi directly affect N absorption and N assimilation [8] particularly in neutral soils [9]. Cruz et al. [10] reported that AM fungi may improve plant N nutrition when grown in nutrient-poor soil, and Mäder et al. [11] suggested that AM fungal hyphae may contribute substantially to plant N nutrition.

The knowledge of the correlated response of plant characteristics (especially root systems and leaf chlorophyll concentration) and grain yield on low-N soils, as well as the combining ability and gene action of selected genotypes could help to speed up the development of high yielding, stable and adapted cultivars. The potential use of nuclear technique and the studies on rhizosphere characteristics, (root exudates, genotype x mycorrhiza interaction, genotype x cultural system) will enhance the attainment of the expected results. The advantages of N use efficiency would be the reduction of the quantity of N fertilizer below the recommended rate of 100 kg–N ha^{-1} for the humid forest zone of Cameroon. This would significantly contribute to sustainable maize production both economically and environmentally. The overall objective of this study was to increase food security in developing countries by sustaining maize production using low-N tolerant germplasm and/or mycorrhizal (AM) fungi. The specific objectives were to (i) identify, through intensive evaluation, maize germplasm tolerant and/or responsive to low N soils of the tropics (ii) measure the correlated responses of maize grain yield with some plant agronomic characteristics under low N and under mycorrhizal inoculation (iii) measure the combining ability and the gene effects of selected lines exhibiting various levels of production under low N.

2. MATERIALS AND METHODS

2.1. Site description

The experiment was conducted at Nkolbisson located at 11°36′ E and 3°44′ N in the humid forest zone, 5 km from the capital city, Yaounde. The altitude is 650 masl. The annual rainfall is 1560 mm, with a bimodal distribution. The average temperature is 23.5°C, the soil

is a sandy clay with pH (water) of 4.5, CEC (cmol (+) kg^{-1} of 4.79) and Al (cmol (+) kg^{-1} of 0.30). The main farming system is maize/groundnut/cassava as monocultures or intercropped.

Site preparation started in April 2007 at Nkolbisson with soil N depletion (Fig. 1). This consisted of planting 90 000 plants of maize ha^{-1} in 2006 and early 2007. Soil liming was carried out in 2008 by applying "dolomite" (35% CaO, 20% MgO) at the rate of 2000 kg ha^{-1} to remove acidity effect. Soil samples were collected before liming, and were analysed at the Soil, Plant and Fertilizer laboratory of IRAD at Nkolbisson (Table 1).

TABLE 1. SOME OF THE SOIL CHARACTERISTICS AT NKOLBISSON

Soil property	Value
Organic matter (%)	4.06
Organic carbon (%)	2.36
Total nitrogen (%)	0.10
C: N	23.6
CEC (cmol kg^{-1})	7.27
pH	4.8

FIG. 1. Soil depletion at Nkolbisson.

2.2. Mycorrhizal substrate and inoculum preparation

Sand from the Wouri sea (Littoral zone of Cameroon) was collected, sieved (<2 mm) and washed with distilled water, resulting in a pH of 6.8, and an N content of 0.6 g kg^{-1}, with 0.2 mg NO_3^- kg^{-1}. This substrate was autoclaved at 121°C for 20 min on three consecutive occasions, and then inoculated with soil extract (20 ml pot^{-1} of sand: water mixture, 1: 1 v/v) filtered through a Whatman No. 1 paper, to reintroduce the native microbial community, with the exception of propagules of AM fungi. Arbuscular mycorrhizal fungi was provided by the Regional Laboratory of Biological Control and Applied Microbiology of IRAD, Cameroon. The mixed culture was composed of three AM fungi, *Acaulospora tuberculata, Gigaspora margarita* and *Glomus intraradices*. All of these AM have demonstrated enhancement of plant growth, phosphorus and P concentration in plants and high % colonization of maize and sorghum roots in acid soils [12]. Therefore, the inoculum used consisted of soil, mycorrhizal root fragments and spores and mycelia of the mixed species. Inoculation was carried out by adding 20 g of inoculum in one plastic pot of 2 liters [12].

2.3. Plant material

Plant materials used in the 2009 experiment included 30 inbred lines selected from the original 99 lines evaluated in 2007 and 2008. Their origin and characteristics are shown in Table 2. Eleven (11) of these line originated from IRAD, Cameroon, twelve (12) were obtained from IITA, five were obtained from CIMMYT, Mexico, and 2 were from CIMMYT, Colombia. The 30 inbred lines were evaluated in 2009 at Nkolbisson on 20 N plots as well as on 100 N plots. Three replications were used, one of which was for destructive sampling for data collection on root characteristics.

TABLE 2. THE ORIGIN, AND PLANT CHARACTERISTICS OF INBRED LINES USED IN THE 2008 AND 2009 SCREENING EXPERIMENTS

Code	Inbred lines	Origin†	Color	Characteristics and heterotic pattern
L1	TZSTR 133	IITA	White	Striga Tolerant
L2	Ku 1409	IITA	Yellow	low N soil Tolerant
L3	M 131	IRAD	White	low N soil tolerant
L4	Exp1 24	IRAD	White	High yielding
L5	Cla 17	CIMMYT	Yellow	Tolerant to Al toxic soil
L6	88069	IRAD	Yellow	Good root volume
L7	TZSTR 131	IITA	Yellow	Striga tolerant
L8	TZSTR 137	IRAD	White	Good root lenght
L9	88094	IRAD	White	Mycorrhiza use
L10	1368	IRAD	Yellow	Good leaf area
L11	87036	IRAD	Yellow	Root volume
L12	CML 358	CIMMYT	Yellow	High chlorophyll
L13	CML 254	CIMMYT	Yellow	Good mycorrhize colonization
L14	TZSTR 140	IITA	Yellow	Good root volume
L15	ATP S425W	IRAD	White	Tolerant to acid soil
L16	0114077	IITA	White	mycoorhize efficient
L17	0114079	IITA	White	Mycoorhize efficient
L18	0114075	IITA	White	Mycoorhize efficient
L19	ATP S7 33Y-2	IRAD	Yellow	Root volume
L20	ATP S5 31Y-1	IRAD	Yellow	Tolerant to acid soil
L21	ATP S6 22Y-2	IRAD	Yellow	Tolerant to acid soil
L22	ATP S8 26Y-3	IRAD	Yellow	Tolerant to acid soil
L23	ATP S9 30Y-3	IRAD	Yellow	Tolerant to acid soil
L24	ATP S5 26Y-1	IRAD	Yellow	Tolerant to acid soil
L25	CML 365	CIMMYT	White	Tolerant to acid soil
L26	9450	IITA	Yellow	Temperate adapted
L27	9071	IITA	White	Temperate adapted
L28	5012	IITA	White	Temperate adapted
L29	Entrada 3	CIMMYT	White	Tolerant to acid soil
L30	Entrada 29	CIMMYT	White	Tolerant to acid soil

†CIMMYT, Colombia

In 2010, 15 of these lines were selected based on their plants characteristics when evaluated under low N (Table 3). Five (5) of these line were selected for their root characteristics, four (4) were retained for their stay green character at 20 N as measured by their chlorophyll content, three (3) lines each were retained for their mycorrhizal colonization and for their leaf area index. These lines were crossed in a diallel to obtain 105 F_1 hybrids.

TABLE 3. INBRED LINES, ORIGIN, PEDIGREE, HETEROTIC PATTERN, ADAPTATION AND CHARACTERISTICS

Inbred line	Origin†	Pedigree	Heterotic pattern	Adaptation	Characteristics‡
87036	IRAD	TZMSR x pop32	Eto	Mid-altitude	High root volume
M 131	IRAD	TZMSR x POP32	Eto	Mid-altitude	High myc. colon.
ATP-S9-30-Y-1	IRAD	Acid tolerant pop	-	Lowland	High leaf area index
9450	IITA	B73	Temperate	Lowland	High root volume
CML 358	CIMMYT	Pop SA3	Eto	Lowland	High chlorophyll
88069	IRAD	-	-	Mid-altitude	High root volume
88094	IRAD	TZMSR x pop43	Tuxpeño	Mid-altitude	Myc. colon.
1368	IITA	Pop21	Tuxpeño	Lowland	Leaf area
ATP-S6-20-Y-1	IRAD	Acid tolerant pop	-	Lowland	Chlorophyll content
9848	IITA	-	Temperate	Lowland	Leaf area
CAM Inb gr1 17	IRAD	SuwanI	Tuxpeño	Lowland	Root volume
ATP-S5-31-Y-1	IRAD	Acid tolerant pop	-	Lowland	Chlorophyll content
CML 254	CIMMYT	-	-	Lowland	Myc. colon
9071	IITA	N 28	Temperate	Lowland	Root volume
5012	IITA	-	Temperate	Lowland	Chlorophyll content

†CIMMYT: Centro International de Mejoramiento de Maíz y Trigo; IRAD: Institut de Recherche Agricole pour le Développement; IITA: International Institute of Tropical Agriculture
‡Myc. colon., mycorrhizal colonization

2.4. Data collected

For all experiments, the following data were collected:

- Number of days from planting to 50% silking (DTS)
- Number of days from planting to 50% anthesis (DTA)
- Anthesis Silking Interval (ASI)
- Leaf width(LWIDTH)
- Leaf length(LLENGTH)
- Stem diameter(STEMDIA)
- Leaf area in cm^2 (LAREA)
- Chlorophyll concentration (with a SPAD 502chlorophyll meter) (CHLOROCONIC)
- Root weight (ROOTWT)
- Plant height (PHT)
- Ear height (EHT)
- Plant aspect (PA)
- Plant stand at harvest (PAH)
- Number of ears at harvest (EAH)
- Mycorrhizal colonization
- Grain yield (YIELD)

2.5. Root characteristics

Root weight was measured on a destructive replication. Data were recorded on six consecutive plants per plot at anthesis.

2.6. Experimental design and data analysis

The preliminary field evaluation of the 30 inbreds retained was carried out in 2009. These lines were evaluated at Nkolbisson using a RCBD with 2 replications. For the

mycorrhiza experiment in pots, the screening consisted of a factorial arrangement; the factors were: 2 mycorrhizal treatments: M0 = plot without mycorrhiza, M1= plot with mycorrhiza, two N levels (N1 = 20 N and N2 = 100 N), and the factors consisted of the 30 maize inbred lines evaluated in three replications. Fourteen days after sowing, a single application of N in distilled water was made. Plants were watered every three days for 2 months. Rorison's nutrient solution without N was applied to supply deficient nutrients when observed. Chlorophyll rate, plant height (cm) and leaf number were collected one month after planting. Additional parameters collected included: root colonization [13], root length (cm), root dry weight (g), plant fresh weight (g). Mycorrhizal colonization data were arc sine transformed to normalize their distribution. Data were analyzed using the statistical SAS package v. 9.1, and significant differences between means were made using the Student-Newman-Keuls test.

In 2010, the 15 inbred lines retained as described above were crossed in diallel to obtain 105 F_1. During the second rainy season, these hybrids were evaluated in a field experiment. The experimental design consisted of a split plot with the main plot consisting of 3 soil treatments: 20 N, 20 N + mycorrhiza, 100 N. The sub-plots consisted of the tested 105 genotypes arranged in an alpha lattice. For all experiments, three seeds were planted per hill and later on was thinned to two plants per hill. Row and hill spacing were 0.75 and 0.50 m, respectively. The total plant density was 53 333 plants ha^{-1} and the experimental unit consisted of a single row per plot, 5 m long.

ANOVA was performed for each soil correction using alpha-lattice. The percentage grain yield reduction under different N levels was computed for all genotypes. Genotypes with small difference were considered resistant to low N soil. Efficient genotypes on low N soil were those which exhibited high grain yield on both low N and high N. The top 20% for grain yield at each soil N level were identified, and high yielding common genotypes for all soil treatments were determined. In addition, parents with high appearance frequency were determined. This gave an indication of parents most adapted to low N as well as high N soil. The GCA and the SCA of the parental line were estimated using the Griffing method. Finally, the correlations between grain yield and measured plant characteristics at each soil correction level were calculated. This suggested plant characteristics to be used when breeding and selecting for low N tolerant and efficient genotypes

3. RESULTS AND DISCUSSION

3.1. Preliminary evaluation and selection of inbred lines

The combined analysis for the 30 lines indicated significant differences between the two N levels for all measured characteristics (Table 4). All genotype performed differently for all the measured traits at the two N levels. Significant genotypes x soil N levels interactions were obtained for all the measured traits except chlorophyll concentration, indicating that the tested genotypes had different relative performance from one soil nitrogen level to another.

3.2. Grain yield

Grain yield of the 30 inbreds used in the preliminary evaluation are shown on Table 5. At 20 kg–N ha^{-1}, 7 lines produced more than 2.0 t ha^{-1}. These were 88069 (3.41 t ha^{-1}), 114075 (3.02 t ha^{-1}), 87036 (2.84 t ha^{-1}), ATP S8 30 Y–3 (2.54 t ha^{-1}), M 131 (2.48 t ha^{-1}), CML 365 (2.43 t ha^{-1}), and ATP S9 30 Y–1 (2.11 t ha^{-1}). Five lines yielded between 1.5 and 2.0 t ha^{-1}: ATP S5 26 Y–1, Ku 1409, 9071, 114079, Cla 17. At 100 kg–N ha^{-1}, 16 lines yielded at least 2.2 t ha^{-1}. The best ones included: 88069 (4.98 t ha^{-1}), 87036 (4.92 t ha^{-1}), 114075 (4.04 t ha^{-}

^{1}), 114077 (3.74 t ha^{-1}), CML 365 (3.54 t ha^{-1}), M 131 (3.26 t ha^{-1}), ATP S_8 30 Y–3 (2.97 t ha^{-1}) and Cla 17 (2.8 t ha^{-1}).

TABLE 4. MEAN SQUARES FROM THE COMBINED ANALYSIS OF THE 30 INBRED PARENTAL CROSSES AT THE TWO N LEVEL FOR ALL MEASURED TRAITS AT 20 KG–N HA^{-1} DURING THE 2009 AND 2010 GROWING SEASONS

Traits	Mean squares†		
	N level (N)	Varieties (V)	N x V
DTA	42.1**	5.9**	3.8**
DTS	115.2**	7.05**	6.2**
ASI	11.25**	2.14**	1.46*
LAREA	480288.5**	40898**	4663.1*
STEMDIA	4.89**	0.27**	0.06*
CHLOROCONC	1445.9**	52.1**	0.13ns
ROOTWT	135766**	21363.3**	9750.2**
PHT	14.4**	2793.4**	1120.7**
EHT	523.6**	823.3**	486.8**
YIELD	9.7**	7.6**	2.1**

†Degrees of freedom for: N level = 1; Varieties = 29; N x V = 29;
* and ** denote significance at $P<0.05$ and $P<0.01$, respectively

The percent grain yield reduction due to fertilisation varied from 3.70 (TZSTR 137) to 87.5% (TZSTR 133). Thirteen lines showed grain yield reduction due to N fertilization of less than 25%. Among these lines, 88069, ATP S9 30 Y–1 (6.2%), ATP S8 30 Y–3 (14.5%), Ku 1409 (15.8%) ATP S5 26 Y–1 (20.6%), 9071 (21.6%) and M 131 (23.9%) exhibited more than 1.5 t ha^{-1} at 20 kg–N ha^{-1}and were among the 10 best yielding at 100 kg–N ha^{-1}.

3.3. Leaf area

Leaf area index at 20 N ranged from 160.3 (TZ–STR 137) to 508.4 (CML 365) (Table 5). The average of the trial was 319.9. Eight genotypes exhibited leaf area index of more than 352.4. They were: CML 365, 1368, ATP S9 30 y–1, 88069, 114075, 87036, M131, ATP S6–22–Y–2. At 100 N, leaf area index varied from 311.0 (TZ–STR 133) to 682.5 (88069), with a mean value of 423.2. Thirteen genotypes exhibited leaf area index of more than 425. These were: 88069, ATP S9 30 y–1, CML 365, 87036, 114075, 00114077, M131, ATP–S6–20–y–1, ATP–S6–22–y–2, Entrada 29, CML 358, 1368. Genotypes retained for best leaf area index at both 20 N and 100 N included: 88069, 87036, 9848, 1368, 114075, ATP–S6–22–y–2, M131, CML 365, ATP–S9–30–y–1.

3.4. Chlorophyll content

The chlorophyll contents of the 30 genotypes at 20 N and 100 N are shown in Table 5. In general, mean chlorophyll concentration was higher at 100 N than 20 N. At 20 N, chlorophyll content varied from 26.9 for 88094 to 41.0 for CML 358, with a mean of 34.8. Nine inbreds exhibited chlorophyll content of at least 36.0. These were: 114 075, ATP S5 26 y-1, KU1409, ATP-S6-20 y-1, ATP-S6-22-y-2, ATP-S5-31-y-2, Entrada 29, CML 358, 5012. At 100N, chlorophyll content varied from 35.9 (Entrada 3) to 49.2 (CML 358), with a mean of 40.5. Twelve inbreds had chlorophyll content of at least 42.0. Among these, 6 had high chlorophyll content at 20 N and those selected were: CML 358, ATP-S5-26-y-1, 5012, Ku 1409, ATP-S6-20-y-1, ATP-S6-22 y-2.

TABLE 5. GRAIN YIELD AND PLANT CHARACTERISTICS OF THE ORIGINAL 30 MAIZE INBRED LINES AT 20 AND 100 KG–N HA^{-1}

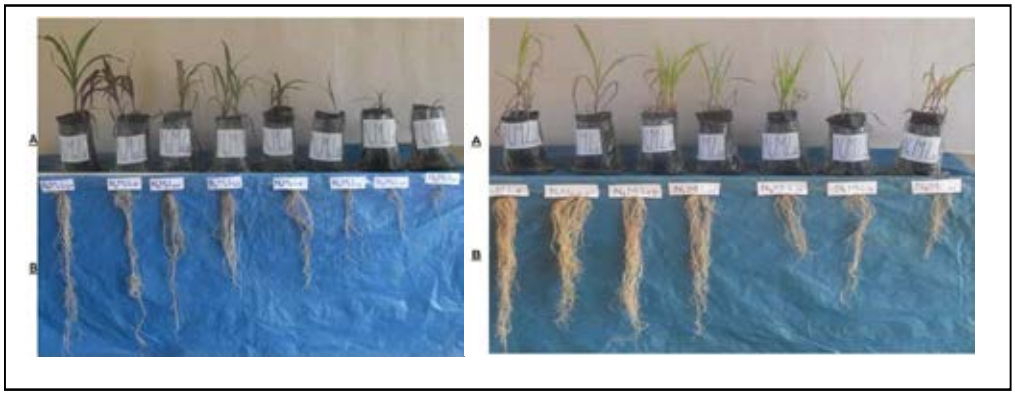
Varieties	Yield		% yield	Leaf area index		Chlorophyll content		Root weight	
	20 N	100 N	loss	20 N	100 N	20 N	100 N	20 N	100 N
88069	3.4	5.0	19.7	471.1	682.5	35.2	39.7	161.2	206.3
114075	3.0	4.0	25.3	456.5	502.3	36.3	39.2	79.3	246.5
87036	2.8	4.9	42.3	397.3	556.8	35.7	42.2	74.0	291.1
ATP S8 30 Y-3	2.5	3.0	14.5	292.0	411.5	32.7	40.6	14.7	16.7
M 131	2.5	3.3	23.9	391.0	455.5	35.3	42.9	39.5	92.0
CML 365	2.4	3.5	31.4	508.4	567.0	32.5	42.0	59.3	177.6
ATP S9 30 Y-1	2.1	2.3	6.2	504.1	621.1	35.2	41.6	28.3	34.4
ATP S5 26 Y-1	1.9	2.4	20.6	323.5	410.0	37.7	45.2	38.3	31.7
Ku 1409	1.9	2.3	15.8	235.6	325.5	37.1	45.5	23.6	87.6
9071	1.7	2.2	21.6	345.9	387.1	35.9	38.1	55.2	92.0
114079	1.7	2.4	28.4	327.4	385.7	33.6	40.5	40.4	246.5
Cla 17	1.6	2.8	44.9	256.9	426.8	32.5	40.7	28.8	61.2
TZSTR 140	1.5	1.8	17.0	310.0	352.7	34.9	39.7	13.7	15.0
CML 254	1.5	1.6	7.1	311.4	415.3	31.9	42.6	38.3	43.3
114077	1.4	3.7	62.3	354.9	482.7	30.3	42.9	138.0	140.0
ATP S6 20 Y-1	1.3	1.4	5.7	313.6	425.5	38.3	44.0	39.5	43.0
Entrada 3	1.3	1.8	28.6	294.4	367.9	31.2	35.9	21.0	32.3
ATP S6 22 Y-2	1.2	2.1	42.7	352.4	445.2	37.2	47.3	43.0	138.4
9450	1.1	1.4	23.2	268.8	341.3	34.4	34.7	38.3	41.0
1368	1.0	2.0	46.7	278.7	406.4	34.8	37.8	18.0	33.0
ATP S5 31 Y-2	0.8	1.8	53.9	251.4	323.7	38.0	40.2	13.7	47.7
Entrada 29	0.7	2.0	65.2	220.6	488.4	36.7	37.4	8.7	39.0
4001STR	0.7	2.5	71.4	248.0	397.2	35.0	43.0	18.0	20.0
CML 358	0.6	0.8	23.2	307.2	427.1	41.0	49.2	20.0	92.0
Exp1 24	0.6	2.2	72.0	275.7	397.0	32.1	38.9	12.0	77.0
5012	0.5	1.7	69.0	226.4	323.0	37.7	42.1	39.5	48.0
TZSTR 131	0.5	2.1	73.9	345.9	387.1	35.9	37.0	54.5	39.4
TZSTR 137	0.5	0.6	3.7	160.3	337.3	33.5	34.7	6.5	20.0
88094	0.5	1.1	53.3	262.8	336.2	26.9	36.5	30.0	30.0
TZSTR 133	0.3	2.2	87.5	305.6	311.8	31.9	32.1	12.3	16.7
MEAN	1.7	2.2		319.9	423.2	34.8	40.5	40.0	94.9
CV (%)	55.2	32.4		15.9	12.4	3.2	7.8	0.5	0.0
Lsd	1.6	1.2		83.2	85.9	5.2	5.2	0.3	0.0
S.E	1.2	1.7		93.0	99.4	4.0	4.8	34.8	94.5

3.5. Root weight

Root weight of the 30 tested inbred lines taken at 20 N and 100 N are presented in Table 5. At 20 N, genotype root weight varied from 6.5 g (TZ-STR-137) to 161.2 g (88069), with a mean of 40.0 g. Nine inbreds exhibited root weights of at least 40.4 g. These were: 88069, 114077, 114075, 87036, CML 365, 9071, TZ-STR 131,114079, and Cam inb gp1.17. At 100 N, genotype root weights ranged from 15.0 to 291.1 g, with an average of 94.9 g. Eight genotypes exhibited root weights of at least 130.0 g. These were; 88069, 114075, 87036, CML 365, 114079, 114077, Cam inb gp1.17 and ATP-S6-22-y-2. It was noted that 7 lines ranked among the best at 20 N as well as for 100 N. These were: 88069, 114075, 87036, CML 365, Cam inb gp1.17, 114079 and 114077.

3.6. Shoot weight and root weight response to mycorrhizal inoculation

In neutral sand with low nitrogen (20 kg–N ha^{-1}) and without the addition of AM fungi, maize inbred lines differed for shoot and root length (Fig. 2). The best lines were: 0114077, 88069, AT S7 33Y-2, ATP S4 25W, 87036 and TZSTR 131. The average shoot and root length ratio was around 4.7 g.



A **B**

FIG. 2. Growth variation and root density of some maize inbred lines on sand (A) 20 N fertilizer (B) 20 N fertilizer with addition of mycorrhizae.

With the addition of AM fungi, SFW and root length increased significantly with 20 kg–N ha^{-1}. Varieties CML 365, ATP S5 26 Y-1, CML 254, M 131, 88094, TZSTR133 exhibited high general performance (Fig. 2B). The addition of AM fungi to 100 kg–N ha^{-1}, improved SFW and RL of maize inbred lines except for 88069, ATP S6 22 Y-2 and CML 365. The selected inbred lines based on their plant characteristics are presented in Table 6.

TABLE 6. SIX BEST INBRED LINES FOR SELECTED PLANT CHARACTERISTICS

Rank	Grain yield	Root volume	Leaf area index	Chlorophyll content	Mycorrhizal
1	88069	88069	1368	CML.358	CML 254
2	ATP-S9-30Y-1	87036	ATP-S9-30-Y-1	ATP-S6-20-Y-1	88094
3	87036	Cam Inb gp1 17	9848	5012	M131
4	M131	9450	88069	ATP-S9-30-Y-1	87036
5	9071	9071	87036	M 131	ATP-S9-30-Y-1
6	ATP-S5-26Y-1	CML 365	CML 365	ATP-S5-31Y-1	CML 365

Mean grain yield ranged from 0.2 t ha^{-1} for 5012 x 9450 to 4.3 t ha^{-1} for CML 254 x 1368 on 20 N. Twenty (20) hybrids yielded more than 2.5 t ha^{-1}, with 8 yielding more than the check variety 87036 x Exp1.24 (3.0 t ha^{-1}). The top 20% for grain yield on 20 N plots are shown in Table 7. Grain yield varied between 2.4 t ha^{-1} for 9450 x Cam inb to 4.3 t ha^{-1} for 1368 x CML 254. The frequency of appearance of individual inbred parents showed that ATP SR S6 20 Y-1 retained for its high chlorophyll content appeared 8 times, CML 254 selected for its mycorrhizal efficiency appeared 5 times, 9071, Cam inb gp1.17 and 87036 selected for their root volume appeared 4 times each, as well as ATP-SR S5 31 Y retained for its chlorophyll content. Inbreds 5012, ATP SR S9 30 Y-1 and 1368 appeared 3 times. They were retained for their chlorophyll content and for their leaf area index, respectively. M131 and CML 358 did not appear as parents among the 20% top hybrids.

Ten (10) hybrids yielded as much as or better than the check hybrids (Expl.24 x 87036: 3.0 t ha^{-1}). Four had 23% grain superiority. Among the twenty parents involved in the ten top hybrids, 8 were retained for their chlorophyll content, 6 for their root volume, 4 for their leaf area index and 2 for their mycorrhizal colonisation.

TABLE 7. GRAIN YIELD OF THE 20 TOPS % ON 20 N, AS COMPARED TO PERFORMANCE ON 100 N AND 20 N + MYCORRHIZA

Rank	Hybrids	Criteria†	Yield 20 N	% check
1	1368 x CML 254	Leaf x myc	4.3	143.3
2	1368 x 9848	Leaf x leaf	3.7	123.3
3	ATP S6-20-Y-1 x 5012	Chlo x chlo	3.7	127.3
4	ATP S6-20-Y-1 x ATPS5-31-Y-1	Chlo x chlo	3.7	123.3
5	ATP S6-20-Y-1 x 9071	Chlo x root	3.6	120.0
6	ATP S6-20-Y-1 x CML 254	Chlo x myc	3.6	120.0
7	CAM INB GR1 17 x 9071	Root x root	3.2	106.7
8	88094 x ATP S6-20-Y-1	Myc x chlo	3.1	103.3
9	87036 x ATP S9-30-Y-1	Root x leaf	3.0	100.0
10	9450 x ATP S6-20-Y-1	Root x chlo	3.0	100.0
11	87036 x ATP S6-20-Y-1	Root x chlo	2.9	96.6
12	CML 254 x 9071	Myc x root	2.8	93.3
13	87036 x ATP S5-31-Y-1	Root x chlo	2.7	90.0
14	ATP S5-31-Y-1 x 9071	Chlo x root	2.7	90.0
15	88094 x 5012	Myc x chlo	2.7	90.0
16	1368 x CAM INB GR1 17	Leaf x root	2.6	86.7
17	5012 X ATP S5-31-Y-1	Chlo x chlo	2.5	83.3
18	87036 X CAM INB GR1 17	Root x root	2.5	83.3
19	ATP S9-30-Y-1 X 88069	Leaf x root	2.5	83.3
20	ATP S9-30-Y-1 X CML 254	Leaf x myc	2.5	83.3
21	ATP S6-20-Y-1 X CML 254	Chlo x myc	2.4	80.0
22	9450 x CAM INB GR1 17	Root x root	2.4	80.0

† Chlo, chlorophyll; Root, root volume; myc, Mycorrhiza; Leaf, leaf area index

Results obtained on plots which received 20N and were inoculated with AM showed that grain yield varied from 1.8 t ha^{-1} for 9848 x Cam inb gp1.17 to 4.5 t ha^{-1} for 1368 x CML 254. The check variety yielded 3.3 t ha^{-1}, which represented a 7% increase over its value on 20 N plots. Eight hybrids yielded at least 3% better than the check, and 2 of them exhibited at least 38 % grain yield superiority.

The top 20% yielding hybrids, presented in Table 8, exhibited a mean of 3.04 t ha^{-1}. This represented a 35% grain yield increase over their performance on 20 N. Thirteen hybrids had at least 25% grain yield superiority over their performance on 20 N. CML 254, retained for its mycorrhizal colonization, appeared 7 times, followed by 9848, 87036 and ATP-S5-31-Y-1 which appeared four time each. These lines were selected for their leaf area index, root volume, and for their chlorophyll content, respectively. Eight (8) hybrids yielded more than the check (3.2 t ha^{-1}). Among the 16 parents involved in these crosses, 5 were selected for their root volume, four (4) parents for their mycorrhizal colonization and leaf area index, respectively, and three (3) for their chlorophyll content. In fact, 29.5% of the crosses involved at least one inbred selected for mycorrhizal colonization. Twenty seven percent involved at least one line retained for its root volume, 25% had at least one line with good chlorophyll content, and 18% involved lines retained for their leaf area index.

TABLE 8. GRAIN YIELD OF THE TOP 20% ON 20 N + MYCORRHIZA AS COMPARED TO THEIR PERFORMANCE ON 20 N AND 100 N

Genotypes	Criteria†	Yield myc.	% check	Yield 20 N	% of 20 N
1368 X CML 254	Leaf x myc	4.5	136	4.3	105
ATP S9-30-Y-1 X 9450	Leaf x root	4.4	133	1.6	275
88069 X CML 254	Root x myc	3.7	112	0.9	411
CML 358 X CML 254	Chlo x myc	3.6	109	1.0	260
5012 X CAM Inb gr1 17	Chlo x root	3.5	106	1.8	194
88094 X 1368	Myc x leaf	3.4	103	1.5	227
9848 X 9071	Leaf x root	3.3	100	0.8	413
87036 X ATP S5 31 Y-1	Root x chlo	3.3	100	2.7	246
M131 X 5012	Myc x chlo	3.2	97	1.3	246
CML 358 X 88069	Chlo x root	3.2	97	1.9	356
87036 X ATP S9 30 Y-1	Root x leaf	3.1	94	3.0	103
88094 x ATP S5-31-Y-1	Myc x chlo	2.7	82	1.5	180
87036 X CML 254	Root x myc	2.7	79	2.0	135
ATP S5-31-Y-1 X CML 254	Chlo x myc	2.7	79	3.7	73
M131 X 9848	Myc x leaf	2.7	79	1.9	137
87036 X CML 358	Root x chlo	2.5	76	2.0	125
9450 X CML 254	Root x myc	2.5	76	1.2	208
1368 X 9848	Leaf x leaf	2.4	73	3.7	65
M131 x ATP-S5-31-Y-1	Myc x chlo	2.4	73	2.3	104
88094 X 5012	Myc x chlo	2.4	73	2.7	89
CML 254 X 9071	Myc x root	2.4	73	2.8	86
9450 X ATP S6-20-Y-1	Root x chlo	2.3	70	3.0	77
9848 X CAM Inb gr1 17	Leaf x root	2.3	70	1.5	153

†Chlo, chlorophyll; Root, root volume; myc, Mycorrhiza; Leaf, leaf area index

The top 20% of hybrids on 100 N are shown on Table 9. Grain yield ranged from 5.0 t ha^{-1} for M131 x 9071 to 9.5 t ha^{-1} for ATP-SR-30-Y-1 x CML 254. Average grain yield was 5.9 t ha^{-1} as compared to 2.3 t ha^{-1} on 20 N. This represented a 257% yield superiority, ranging from 123% for 1368 x CML 254 to 560% for 88069 x Cam inb gp1.17. The inbred CML 254, appeared 7 times, followed by 9071 which appeared 6 times. Inbreds 88069 and ATP-SR-30-Y-1 appeared 5 times. Inbred 88094 was involved in 4 crosses. Inbreds 87036, M131 and ATP-SR-20-Y-1 appeared 3 times each. ATP-SR-31-Y-1, 9450 and Cam inb gp1.17 appeared 2 times each. Finally, 1368 and 5012 were represented only once each. CML 358 and 9848 did not appear among the top 20%. The frequency of appearance of plant characteristics indicated that on 100 N, 18 F1 crosses (41%), had at least one parent selected for its root volume. Fourteen F1 (31.8%) involved at least one inbred selected for its mycorrhizal colonization. Six F1 (13.6%) used at least one parent selected for its leaf area index and chlorophyll content, respectively.

3.7. Combining ability of the lines

Significant mean square values for the orthogonal partition of the hybrid sum of squares indicated general combining ability (GCA). Differences were obtained at the two soil correction levels (20 N and 100 N) for chlorophyll content and grain yield. GCA for leaf area index was significant only at 100 N. This indicated that these parameters were controlled by additive gene action. Significant specific combining ability (SCA) effects were detected only for grain yield at 100 N, indicating the presence of non additive gene action for this trait.

TABLE 9. GRAIN YIELD OF THE 20 TOP HYBRIDS ON 100 N COMPARED TO THEIR PERFORMANCE AT 20 N

Rank	Hybrids	Criteria†	Yield 100 N	% check 100N	Yield 100 N	% grain yield increase/20 N
1	ATP S9-30-Y-1 x CML 254	Leaf x myc	9.5	176	2.5	380
2	ATP S9-30-y-1 x 88094	Leaf x myc	7.2	133	1.6	450
3	CML 254 x 9071	Myc x root	8.0	148	2.8	286
4	ATP S5-31-Y-1 x CML 254	Chlo x myc	6.7	124	3.6	186
5	ATP S9-30-Y-1 x 88069	Leaf x root	6.4	119	2.5	256
6	ATP S6-20-Y-1 x 9071	Chlo x root	6.1	113	3.6	169
7	9450 x CAM INB GR1 17	Root x root	6.0	111	2.4	250
8	88069 x 9071	Root x root	6.0	111	1.4	429
9	88069 x 88094	Root x myc	5.8	107	1.9	305
10	87036 x ATP S9-30-Y-1	Root x leaf	5.8	107	3.0	193
11	88069 x CAM INB GR1 17	Root x root	5.6	103	1.0	560
12	87036 x CML 254	Root x myc	5.6	103	2.0	280
13	ATP S5-31-Y-1 x 9071	Chlo x root	5.5	102	2.7	204
14	1368 x CML 254	Leaf x myc	5.3	98	4.3	123
15	ATP S9-30-Y-1 x ATP S6-20-Y-1	Leaf x chlo	5.3	98	1.9	279
16	M131 X 9450	Myc x root	5.2	96	1.2	433
17	87036 x ATP S6-20-Y-1	Root x chlo	5.1	94	2.9	176
18	88069 X CML254	Root x myc	5.1	94	0.9	286
19	88094 x 5012	Myc x chlo	5.1	94	2.7	189
20	88094 x 9071	Myc x root	5.1	94	1.8	283
21	M131 X CML 254	Myc x myc	5.0	93	1.8	278
22	M131 X 9071	Myc x root	5.0	93	1.1	455

† Chlo, chlorophyll; Root, root volume; myc, Mycorrhiza; Leaf, leaf area index

The combining ability of the 15 lines used in the F1 evaluation is shown in Table 10. On 20 N soil, the best combiners were: ATP-SR-20-Y-1 (0.6), 87036 (0.4), ATP-SR-31-Y-1 (0.4), CML 254 (0.4), 1368 (0.2), 9071 (0.2) and ATP S9-30-Y-1 (0.1). On 20 N added to mycorrhize, the best positive combiners were: ATP S9-30-Y-1 (0.7), M131 (0.4), 87036 (0.02) and CML 254 (0.0). On 100 N soil, the best combiners were: CML 254 (1.3), ATP-SR S9-30-Y-1 (1.1), 88069 (0.4), 9071 (0.70), M131 (0.3), 88094 (0.2), ATP-S6-20-Y-1 (0.2). Good combiners on 20 N and 100 N included: ATP SR S9 30 y-1, ATP-SR-20-Y-1, CML 254. Good combiners on 20 N plus mycorrhiza and 100 N included: M131, ATP-SR S9-30Y-1 and CML 254 (0.0). Good combiners on 20 N and 20 N plus mycorrhiza included: 87036 , ATP-SR-30Y-1and CML 254. Finally the best combiners on the 3 soil types were only ATP-SR-S9-30Y-1 and CML 254.

The five best specific combiners on 20 N included: 1368 x CML 254, 1368 x 9848, ATP SR S9 30 Y-1 x ATP-SR S5 31 Y-1, 1368 x ATP-SR S6 20 Y-1 and CAM INB gp1.17 x CML 254. The five best specific combiners on 100 N included: 9450 x CAM INB gp1.17, 9450 x 9071, CAM INB gp1.17 x CML 254, ATP SR S9 30 Y-1 x CML 254 and ATP SR S9 30 Y-1 x ATP SR S5 31 Y-1.

3.8. Correlation between grain yield and plant characteristics

The correlation values between grain yield and some measured plant characteristics are shown in Table 11. At 20 N, all measured characteristic were positively correlated with

grain yield. The highest values were obtained for days to silk, chlorophyll content and root volume. At 100 N, only number of green leaves, total number of leaves, and leaf area index showed significant correlations with grain yield. It could be suggested that indirect selection criteria for grain yield at 20 N, which could have a correlated response at 100 N included: green leaves, total leaves and leaf area index.

TABLE 10. GRAIN COMBINING ABILITY ACROSS THE THREE ENVIRONMENTS

Inbred lines	Yield (t ha^{-1})		
	20 N	20 N + mycorrhiza	100 N
87036	0.42	0.02	–0.01
M131	–0.40	0.40	0.30
ATP S9-30-Y-1	0.10	0.70	1.10
9450	–0.10	–0.50	–0.50
CML 358	–0.70	–0.01	–0.80
88069	–0.40	–0.60	0.40
88094	–0.10	–0.10	0.20
1368	0.20	–0.04	–0.40
ATP S6-20-Y-1	0.60	–0.01	0.20
9848	–0.40	–0.20	–0.80
5012	0.00	–0.20	–0.90
cam inb gp1 17	–0.10	–0.30	–0.90
ATP-S5-31-Y-1	0.40	–0.30	0.00
CML 254	0.40	0.00	1.30
9071	0.20	–0.10	0.70

TABLE 11. CORRELATION COEFFICIENTS BETWEEN GRAIN YIELD AND SELECTED PLANT CHARACTERISTICS

Plant characteristics	Grain yield	
	20 N	100 N
Days to anthesis	0.3**	0.15 ns
Days to silk	0.4***	0.13 ns
A.S.I	0.3***	0.02 ns
Green leaves 2	0.2*	0.35***
Total leaves	0.3***	0.29***
Leaf area	0.3***	0.26***
Root Volume	0.4***	0.12 ns
Chlorophyl 2	0.4***	0.03 ns
Chlorophyl 3	0.4***	0.14 ns
Leave Senescence 1	0.2*	0.03 ns
Leave Senescence 2	0.2*	0.08 ns

*, **, *** denote $P<0.05$, $P<0.01$, $P<0.001$, respectively; ns, not significant

4. CONCLUSIONS

This study revealed that four F1 crosses were high yielding on the 3 types of soil N correction (20 N, 20 N + mycorrhiza, 100 N). These were: 1368 x CML 254; 87036 x ATP-SR-S9-30-Y-1; CML 254 x 9071; and 88094 x 5012. Three out of the 4 crosses involved at least one parent retained for its root volume. Three hybrids performed well on 20 N as well as 20 N + mycorrhizal inoculation. These were: 1368 x 9848; 9450 x ATP-S6-20-Y-1 and 87037 x ATP-S5-31-Y-1. These hybrids included parents selected for root volume, chlorophyll content and for leaf area index. Based on performance between 20 N and 100 N, 6 hybrids were identified: ATP-SR-S6-20-Y-1 x 9071; 87036 x ATP-SR-S6-20-Y-1; ATP-SR-S5-31-Y-

1 x 9071; ATP-S9-30-Y-1 x 88069; ATP-SR-S9-30-Y-1 x CML 254 and 9450 x Cam inb gp1.17. These genotypes involved 6 parents selected for their root volume, 3 parents retained for their leaf area index, 2 parents for their chlorophyll content and one parent with mycorrhizal use efficiency. Finally, when comparing performance on 20 N with mycorrhiza and 100 N, three hybrids were as good in the one treatment as in the other: 88069 x CML 254; 87036 x CML 254 and ATP-SR-S5-31-Y-1 x CML 254. These involved 2 inbreds with good root volume crossed to inbreds with good mycorrhizal use efficiency. These findings suggested that inbred selection for root volume was important. However, for maximum utilisation in a breeding programme, those lines must be crossed to another line having complementary characteristics such as mycorrhizal use efficiency, good leaf area index, or good stay green character as evidenced by high chlorophyll content.

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PRODUCTIVITY OF UPLAND RICE GENOTYPES UNDER DIFFERENT NITROGEN DOSES

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Abstract

Nitrogen (N) deficiency is one of the most yield-limiting nutrients in upland rice growing area in Burkina Faso. A field experiment was carried out from 2008 to 2010 in Farakoba research center with the objective to evaluate 200 upland rice (*Oryza sativa* L.) genotypes from WAB, NERICA, CNA, CNAX, IRAT and IR lines for N use efficiency. The treatments consisted of three levels of N: low, medium and high at 20, 60 and 100 kg–N ha^{-1}, respectively. Both grain and straw yield increased with N application. The yields were highest for NERICA and WAB lines compared to the other lines, and this was consistent over the N doses. A large variability was found among the genotypes. Three groups of genotypes were identified according to N use efficiency. The high N use efficiency genotypes were found in WAB and NERICA lines. The N concentration in the shoot at flowering significantly increased with N doses and this was similar for N taken up by genotypes.

1. INTRODUCTION

The demand for rice in sub-Saharan Africa is growing faster (by 5.6% per year) than for any other major food staple. Urbanization and changes in employment patterns are driving changing consumer preferences, such that rice is no longer a luxury food. West Africa currently imports 40 to 60 % of the total rice consumed. Urgent action is needed to ensure that African countries will have enough rice to feed their rural and urban populations. Rice is a strategic crop in West Africa; it constitutes an important source of food and farm income for many rural households in the region. In the specific case of Burkina Faso, the average annual paddy production is 100 000 t which is far below the need of the country, estimated to be 304 986 t. Eighty-two % of the demand in rice is imported, corresponding to 40 billion F CFA. However, in the most appropriate ecological zones, farmers are increasingly involved in producing upland rice. For instance, the area cropped with upland rice was increased by 50% in the south Sudanian zone of Burkina Faso during the past 10 years [1]. The current level of upland rice production can be significantly increased by using improved and adapted rice cultivars in combination with low-cost agronomic practices.

The possibility to increase total rice production in Burkina is possible through the improvement of the upland system. However, yields are very low under this system (around 800 kg ha^{-1}) due mainly to inappropriate farming methods and low inherent soil fertility. Phosphorus (P) and nitrogen (N) are the most limiting nutrients for crop production in the Sahel. Nitrogen is one of the most important nutrients for rice production because it contributes to chlorophyll, amino acid and protein synthesis [2]. Nitrogen is necessary during the early growth stages of rice as it gives vigour and increases tillering. The development of rice varieties tolerant to low soil nutrients and efficient in using N is important for the many thousands of poor farmers in the Sahel. The current study was carried out to evaluate the N

use efficiency by selected upland rice cultivars developed by the Burkina National Institute for Agricultural Research (INERA) in partnership with the West African Rice Network (WARDA). These varieties are high yielding and are efficient in N and P recovery under poor soil conditions. We assume that genotypes which are efficient in using N are also tolerant to low N conditions.

2. MATERIAL AND METHODS

2.1. Sites characterization

The experiment was carried out from 2008 to 2010 in Farako-Bâ research center in Burkina Faso, West Africa. The geographic coordinates of the centre are 11°06' latitude north, 4°20' longitude west and 405m altitude. The climate is south Soudanian [3] characterized by a long dry season (October to April) and a short wet season (May to September). Average annual rainfall (1995-2005) in the area is 950 mm with a cropping season of 5 months (May to September). Rainfall is characterized by large annual viability. Average minimum and maximum temperatures are also variable and depend on the season. During the dry season minimum and maximum temperatures vary between 17 and 37°C, and between 10 and 32°C during the rainy season. Evaporation is also a function of the season and varies from 8.7 mm d^{-1} in January-February to 3.7 mm d^{-1} in August. The main soil types in the Farako-Bâ research center are Ferrasols (FAO classification) [4]. These soils are acidic and very poor in organic matter, available P and total N (Table 1).

TABLE 1. SOME CHEMICAL PROPERTIES OF THE SOIL AT FARAKOBA IN 2007

Soil property	Average values†
Total C (%)	0.29 (0.05)
Total N (%)	0.03 (0.004)
C: N ratio	8.6 (0.74)
Organic matter (%)	0.50 (0.08)
Total P (mg kg^{-1})	84 (10.9)
Available P (mg kg^{-1})	3.6 (0.79)
pH_{H2O}	5.6 (0.15)
pH_{KCl}	4.3 (0.17)

†Standard deviations are given in parentheses

2.2. Genotypes

Six (6) lines and 200 accessions of upland rice were used for the experiment (Table 2).

TABLE 2. LINES AND NUMBER OF ACCESSIONS

Line†	Number of accessions
WAB	175
NERICA	18
CT	2
CNA	2
CNAX	1
IRAT	1
IR	1
Total	200

†Provided by AfricaRice, Senegal (the Sahelian center)

2.3. Experimental design and data collection

A pre-cropping with maize to deplete soil and homogenize soil N content was carried out. The maize was planted in early May and was harvested 3 weeks later. The experimental design was randomized blocks with 3 treatments, consisting of 3 doses of N as urea at low, intermediate and high rates (20, 60 and 120 kg–N ha^{-1}, respectively), with a split application at 15 and 45 days after sowing (DAS):

The size of blocks was 60 × 3 m = 180 m^2. The blocks were separated by a 1 m interval. There were four blocks. The distance between genotypes in the same block was 40 cm. The 200 genotypes were sown on continuous lines of 3 m. The field was amended with 400 kg ha^{-1} Burkina rock phosphate and 100 kg ha^{-1} of KCl to correct P and K deficiencies, respectively. The Burkina rock phosphate and KCl were incorporated in the soil during land preparation.

The following data were collected:

- Germination rate
- Number of tillers
- Day to flowering
- Day to maturity
- Panicle weight
- Grain weight
- Straw weight

Soil samples were taken before sowing at 0-20cm. Plant samples were collected at flowering in each treatment (genotype × N).The samples (soil and plants) were pre-dried in direct sun light and subsequently oven-dried at 60^{o}C for 48 h. The samples were ground and sieved to 2 mm.

2.4. Laboratory and statistical analysis

The soil and plant samples were analyzed for their N status in the soil-water-plant laboratory at Farakoba research center. The N concentrations of samples were determined by Kjeldahl digestion. Excel was used for primary data processing. The analysis of variance (ANOVA) was performed using GENSTAT 7th Edition. The means were separated using the least significant difference (LSD) at P<0.05.

3. RESULTS AND DISCUSSION

3.1. Environmental conditions

Rainfall conditions varied during the 3 years of the experiment. In 2008, the total rainfall received was 1100 mm in 3 months with drought spells at the end of June and early July. In 2009, 950 mm of rain was received at the site with an early ending (end of September). Rainfall was adequate in 2010 (1172 mm) with a drought spell at the beginning of the season (in June). This erratic rainfall affected the planting date which probably affected the yield.

3.2. Yields

The effect of N dose on yield (grain and straw) was different for genotypes and lines (Table 3). N application increased both grain and straw yields for all the genotypes (P<0001), and the higher the N dose, the higher the grain and straw yields (Table 3). These results are consistent with those reported in the literature [5, 6] where significant yield differences among upland rice genotypes under low, medium and high fertility levels were found in Brazilian Oxisols under field conditions. The results are also supported by other findings [4, 7–9] where the most growth limiting nutrient was N. Over all the genotypes, the intermediate dose generated an 11% grain yield increase compared with the low dose, while the high dose gave a 29% increase compared with the low dose. However, there was a very large variability among genotypes from the same line and between lines (P<0001). Some genotypes showed very low harvest indices. In fact, lines CNA and CNAX produced twice as much straw as grain. The genotypes from IR and IRAT produced >6 times more straw than grain (Table 3).

TABLE 3. UPLAND RICE GRAIN AND STRAW YIELDS

Line	Grain yield (kg ha^{-1})			Straw yield (kg ha^{-1})		
	N20	N60	N100	N20	N60	N100
CNA	681	915	972	1086	1417	1812
CNAX	692	746	974	1726	1948	2505
CT	692	746	974	1434	1607	1933
IR	156	282	417	1449	1881	2830
IRAT	44	77	283	1156	1208	1630
NERICA	889	1036	1231	1366	1577	1744
WAB	787	868	995	1337	1500	1816
Statistics	Line: P<0.001, LSD = 78 N treatment : P<0.001, LSD = 452 Line x N treatment: P = 0.999, LSD = 783			Line: P = 0.17 N treatment : P<0.001, LSD = 87 Line x N treatment: P = 0.957		

Under the low N conditions NERICA and WAB lines performed better compared to the other lines (Table 3). The lowest yield was obtained with the IRAT genotypes and this was consistent over the N treatments. The yields varied from 149 to 1743 kg ha^{-1} for NERICA genotypes, but N application always increased grain yield (P<0.001) for all genotypes. The response to N application was very high for NERICA 10, 11 and 13. Yields were lower for NERICA 6, 7 and 15 independent of N treatments.

The yield increment from the intermediate to the highest N dose was only about 20% (Fig. 1). The overall productivity of NERICA genotypes was very low compared to their potential yield of around 2 t ha^{-1}. Furthermore grain yields were comparables for NERICA 17 and NERICA 5 at N60 and N120. Two genotypes generated very low yields (Nerica 7 and 15). The low and intermediate doses were comparable for some genotypes. Except for Nerica 7 and 15, the grain yield was always above 500 kg ha^{-1} without N application. The genotypic response to N doses allowed ranking in three N efficiency index groups (Fig. 1, Table 4): N efficient genotypes (Efficiency index >0.26), medium N efficient genotypes (Efficiency index between –0.224 and 0.014) and N inefficient genotypes (<–0.224). The efficient N genotypes are those generating high yield under both low and high N dose.

Upland grain yields of CNA genotypes were lower than found for NERICA. The yields for CNA genotypes varied from 400 to 1243 kg ha^{-1}. N treatments significantly increased upland grain yield. Again, the yield benefit was low except for the CNAX genotype (Fig. 2). For the latter genotype, comparable yields were obtained for N20 and N60. The

response of WAB genotypes to N treatments varied the most compared to NERICA and CNA genotypes, from 11 to 3023 kg ha^{-1}. The following genotypes generated more than 2000 kg ha^{-1}: WAB 36-54, WAB 56-77, WAB 881-10-37-18-14-P1-HB, WAB 907-12-3-1-1-1-HB, WAB 963-3A1.1, WAB711-B-2A1.1. The highest yield was obtained with the WAB 881-10-37-18-14-P1-HB (3023 kg ha^{-1}). These good yields were obtained with the high N dose. The genotypes WAB788-58-1-2-HB and WAB963-B-12A1.2 and WAB775-21-5-2-HB showed very low grain yield. The high dose of N generated the highest grain yield for all the genotypes.

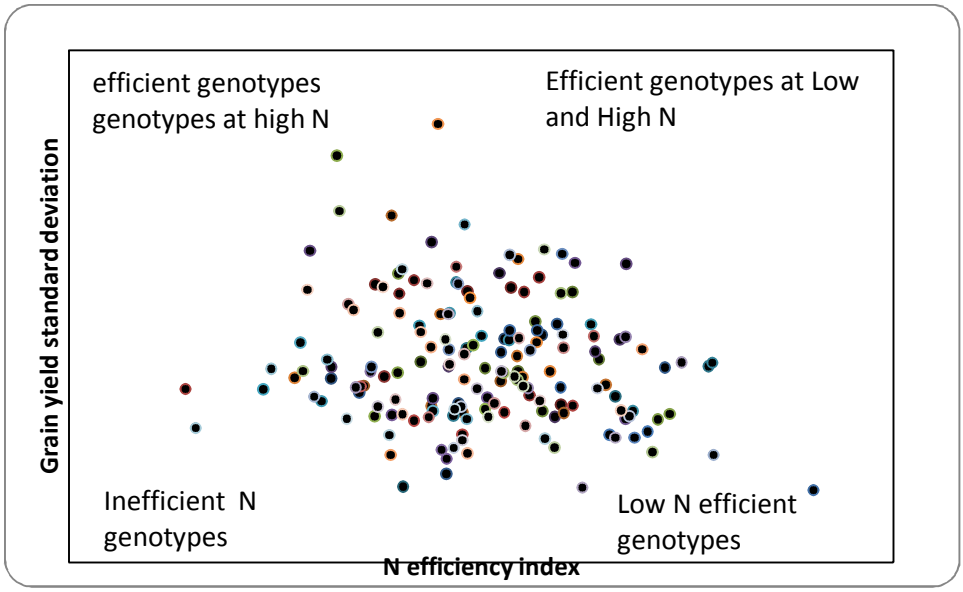

FIG. 1. N efficiency index vs. grain yield standard deviation

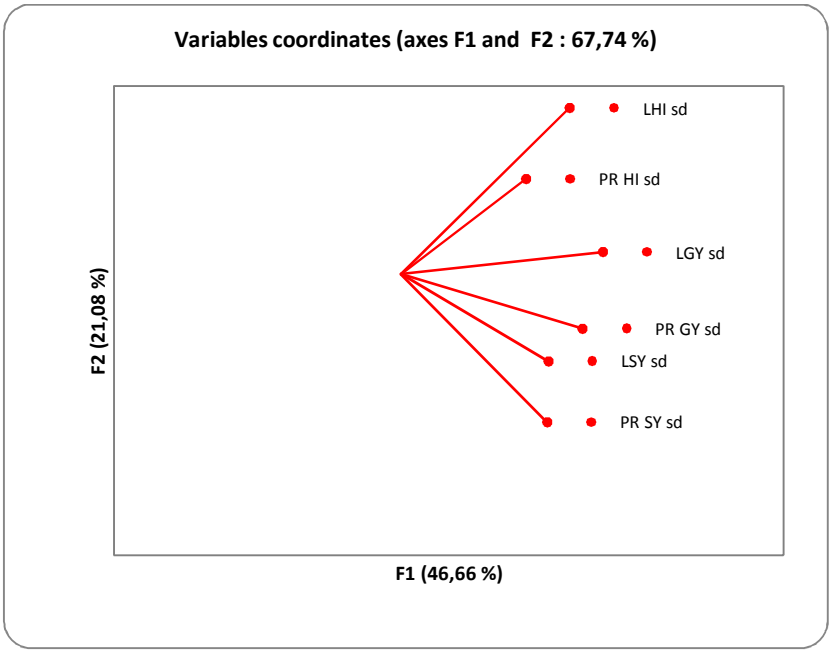

FIG. 2. Potential contribution for each factor

The yields for the efficient genotypes were around 1000 kg ha^{-1}, and were almost double with the high N dose, mainly for WAB 767 and WAB 881 (Fig. 3). These two genotypes are potential genotypes for high grain production.

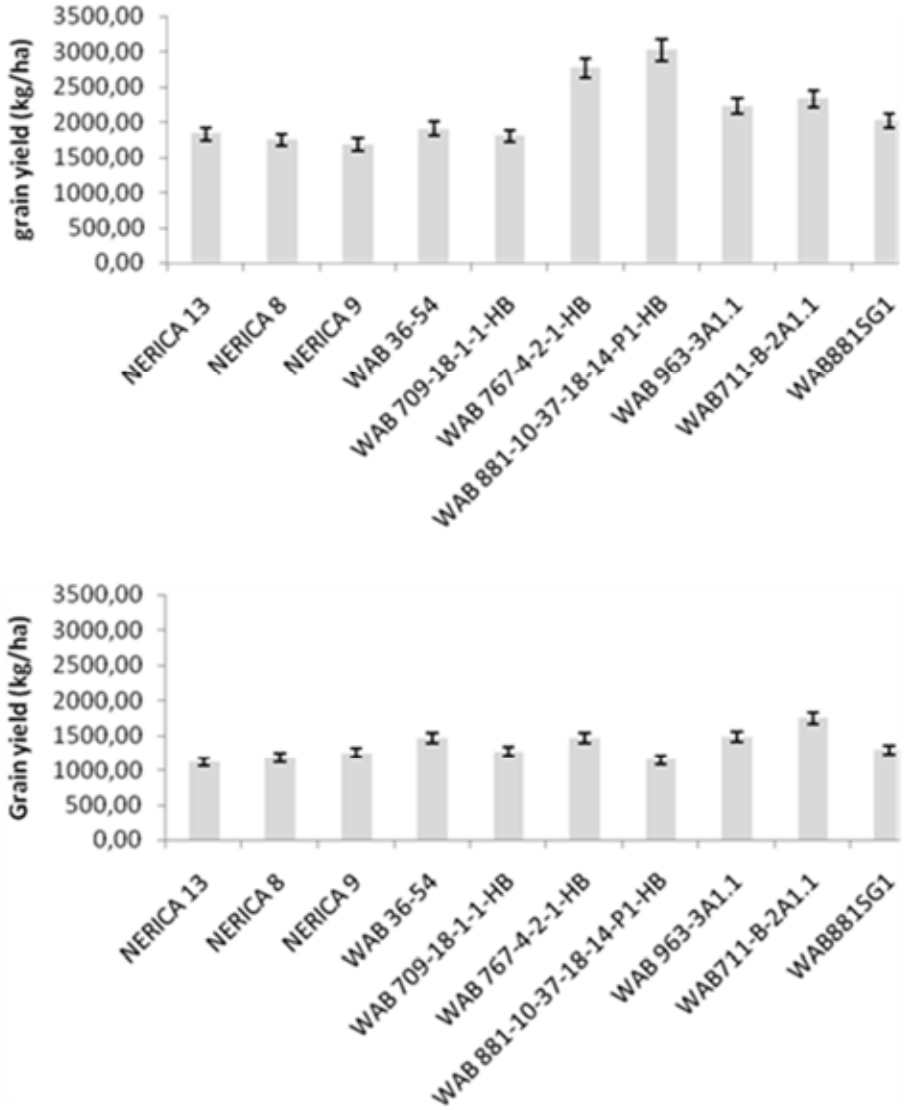

FIG. 3. Upland grain yield for N efficient genotypes at low and at high N doses.

3.3. N concentration

Genotypic N concentrations varied widely and were highest for the WAB and NERICA genotypes. The concentration of N in the shoots varied from 0.7 to 1.6% for the WAB genotypes (Fig. 4), and from 0.8 to 1.4 for the NERICA genotypes (Fig. 5). The concentration of N in shoots was highest for WAB 340 BB 2H2 and lowest for WAB 711 132A1.1.5 (Fig. 4). For the NERICA genotypes the concentration of N in shoots was stable, and NERICA 5 and NERICA 8 had a higher concentration of N in shoots with the high N dose compared to the other genotypes from the same group (Fig. 5).

TABLE 4. CLASSIFICATION OF GENOTYPES NITROGEN USE EFFICIENCY INDEX

Highly Efficient	Moderately Efficient	Inefficient
NERICA 13, NERICA 9, NERICA5, WAB 1645-11A1.1 WAB 1645-4AB.1, WAB 340-B-B-2-H2, WAB 36-54, WAB 515-B-16A1.2 WAB 709-18-1-1-HB, WAB 711-137-3, WAB 718-27-2-1-HB, WAB 880 SG6 WAB 880-1-38-18-20-P3-HB, WAB 907-12-3-1-1-1-HB, WAB 963-3A1.1, WAB537-14-4-1-1-1-HB, WAB711-B-2A1.1 WAB759-34-5-1-HB, WAB788-51-3-2-HB, WAB881-10-37-18-14-P2-HB, WAB905-B-2A1.1	CNA 6675, CNA 6675, CNA 6680, CNAX 17625J-48-B-1, IR 47686-13-2-2 NERICA 1, NERICA 10, NERICA 11, NERICA 12, NERICA 14, NERICA 17, NERICA 18, NERICA 2, NERICA 3, NERICA 4, NERICA 6, NERICA 7, NERICA 8, WAB 1022-3-2-1-1-1-HB, WAB 1087-B-37A1.2, WAB 1092-B-40AB.1, WAB 1095-B-1A1.1, WAB 1275-6AB.1, WAB 1618-321-8-2A1.1, WAB 1645-3A1.1, WAB 1645-3A1.2, WAB 1645-4A4.1, WAB 1645-5A1.2, WAB 1645-5A6.1, WAB 1645-6AB.1, WAB 1645-7A13.2, WAB 1645-7A3.1, WAB 1645-7A5.2, WAB 1645-8A3.1, WAB 1645-8AB.1, WAB 272-B-B-2-H3, WAB 450-12-2-BL1-DR3, WAB 502-12-2-1, WAB 502-18-4-1, WAB 56-77 WAB 616-30-3-2, WAB 704-17-4-HB, WAB 707-32-3-1-HB, WAB 709-26-4-1-HB, WAB 709-73-3-2-HB, WAB 711-136-2, WAB 718-26-1-1-HB, WAB 748-13-2-HB, WAB 748-14-2-HB, WAB 767-4-2-1-HB, WAB 775-84-3-2-HB, WAB 781-47-4-2-2-1-1-HB, WAB 788-18-2-1-HB, WAB 788-25-1-1-1-1-1-HB, WAB 788-58-2-1-HB, WAB 801-27-1-1-HB, WAB 854-B-50A1.1 WAB 878 SG1, WAB 878 SG35, WAB 878 SG36, WAB 878 SG43, WAB 878-6-37-8-3-P1-HB, WAB 880 SG34, WAB 880 SG37, WAB 880 SG62, WAB 880-1-131-1-16-P1-HB, WAB 880-1-32-1-2-P1-HB, WAB 880-1-38-13-1-P1-HB, WAB 880-1-38-19-26-P2-HB, WAB 880-1-38-20-14-P1-HB, WAB 881-10-37-18-12-P3-HB, WAB 881-10-37-18-14-P1-HB, WAB 881-10-37-18-15-P1-HB, WAB 881-10-37-18-5-P1-HB, WAB 881-10-37-18-7-P3-HB WAB 881-10-37-18-7-P4-HB, WAB 891 SG12, WAB 891 SG31, WAB 894-B-3A1.2, WAB 897-B-B-B-B-24, WAB 950-B-93A1.2, WAB 995-6A1.1 WAB 99-7, WAB1094-B-58AB.1, WAB375-B-12-H3-1, WAB450-11-1-1-P28-4-HB, WAB450-24-2-2-P33-HB, WAB450-I-B-P-422-HB, WAB570-32-2-1-1-1-HB, WAB570-35-2-1-HB, WAB616-53-4-3-1-1-HB, WAB721-13-1-1-1-3-HB, WAB757-17-1-HB, WAB767-2-5-1-HB, WAB775-104-2-1-HB WAB775-49-1-1-HB, WAB781-75-3-1-HB, WAB788-19-1-1-2-HB, WAB804-23-1-1-2-HB, WAB880-1-38-20-15-P2-HB, WAB880-1-38-20-16-P2-HB, WAB880SG37, WAB880SG42, WAB880SG50, WAB881-10-37-18-3-P1-HB, WAB881-10-37-18-8-P2-HB, WAB881SG1, WAB901-1A1.1, WAB901-1A2.1, WAB901-7A2.1, WAB902-B-14A1.1, WAB902-B-16A1.1, WAB903-5-1-1-HB, WAB905-B-12AB.1, WAB910-B-3A1.1, WAB910-B-4AB.1, WAB910-B-5AB.1, WAB915-B-3A1.1, WAB919-72-4-1-HB, WAB925-B-3A1.1, WAB951-B-181AB.1, WAB952-B-47AB.1, WAB954-B-51AB.1, WAB963-B-12A1.2, WAB964-B-3A1.2, WAB969-40-1-HB	IRAT 136, NERICA 15, WAB 1234-1A9.2, WAB 1645-5A4.1, WAB 564-8-1-2, WAB 712-56-4-1-HB, WAB 725-27-3-1-1-2-HB, WAB 748-11-2-HB, WAB 767-2-4-1-HB, WAB 880-1-38-12-2-P1-HB, WAB 880-1-38-19-23-P1-HB, WAB 880-1-38-20-26-P2-HB, WAB1079-B-39A1.1, WAB569-35-1-2-1-HB, WAB569-36-1-1-1-HB, WAB616-54-11-1-1-1-HB, WAB759-55-1-2-HB, WAB775-21-5-2-HB, WAB775-49-2-3-HB WAB788-58-1-2-HB, WAB880SG73 WAB923-B-6A1.1, WAB960-B-11A1.1

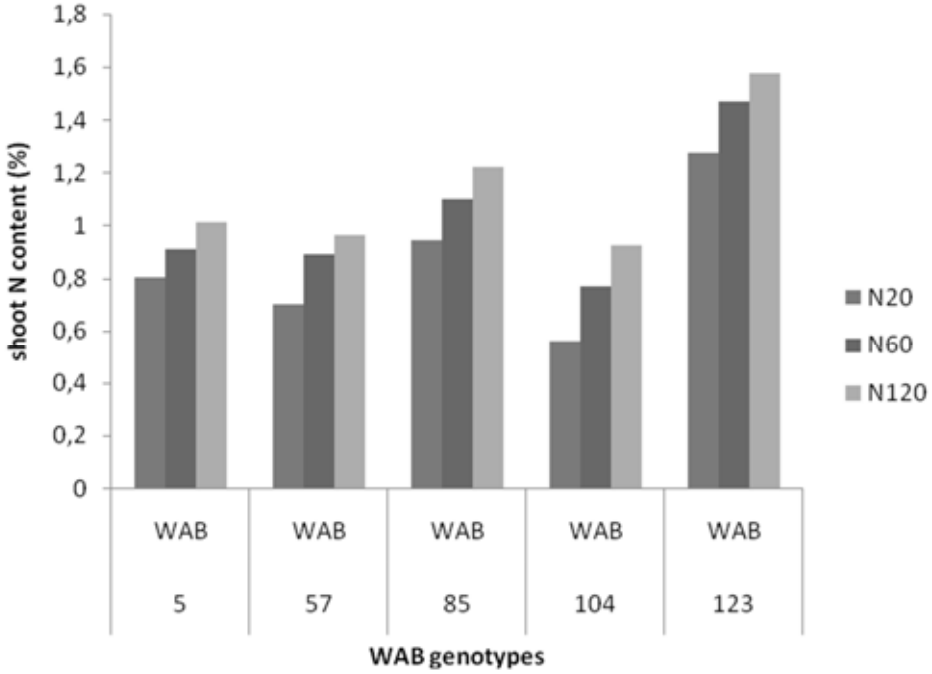

FIG. 4. Shoot N concentration (%) of WAB genotypes as a function of N treatments.

At lower N doses the N concentration was higher for NERICA genotypes than for the other genotypes. The higher N taken up with increased N dose is related to deep root development allowing the genotypes to explore more soil depth [10, 11]. N application improves the use of carbohydrate by the plants and stimulates the development of the rooting system, which increases the uptake of other nutrients necessary for plant growth [12]. Nitrogen is also essential for enzyme synthesis and photosynthesis [13]. The effect of N nutrition is therefore an indirect effect through other processes.

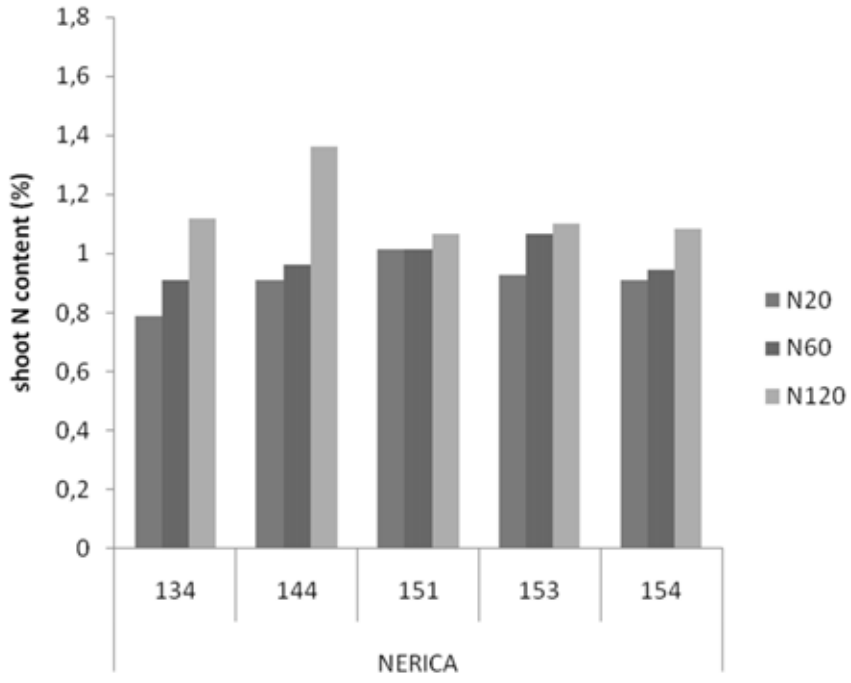

FIG. 5. Shoot N concentration (%) of NERICA genotypes as a function of N treatments.

3.4. N uptake

Flowering is an important growth stage for rice because it's when the translocation of nutrients occurs from shoot to grain. The quantity of N taken up by upland rice shoots at flowering showed highly significant differences between N treatments ($P<0.001$) and genotypes ($P = 0.030$) (Table 5). No significant correlation was found between N treatments and genotypes. N application increased N taken in the shoot at flowering this was consistent for all genotypes and lines (Table 5). The highest quantity of N was taken up by genotype WAB 340-B-B-2-H2 for all N treatments. The lowest quantity of N was taken up by NERICA 17. The variability between genotypes was lower for the NERICA compared to WAB genotypes.

TABLE 5. NITROGEN TAKEN UP IN SHOOT BY GENOTYPES AT FLOWERING

Genotype	N taken up in the shoot (kg–N ha^{-1})		
	N20	N60	N120
NERICA 15	10.8	16.0	31.8
NERICA 17	6.3	11.7	18.6
NERICA 18	12.1	19.7	27.5
NERICA 8	10.2	13.6	23.4
NERICA5	11.2	15.0	19.2
WAB 340-B-B-2-H2	16.2	24.7	48.8
WAB 748-11-2-HB	7.9	14.2	25.1
WAB 881-10-37-18-7-P4-HB	8.6	21.3	28.1
WAB450-24-2-2-P33-HB	13.3	18.8	25.2
WAB711-B-2A1.1	8.8	14.2	19.2
Statistics	N treatment: $P<0.001$, LSD = 4.9 Genotype: $P = 0.030$, LSD = 9.0 N treatment x genotype: ns		

4. CONCLUSIONS

N application significantly affected upland rice grain and straw yields; the higher the N dose the higher the grain and straw yield. However, the level of yield increment was different for genotypes and lines. The increment was lower for the NERICA than for the WAB genotypes. At low N supply some genotypes from WAB and NERICA lines generated sustainable upland grain yields. The harvest index of genotypes was only affected for IRAT, and IR lines. With N application these genotypes accumulated more shoot than grain. A very large variability was observed within the WAB and NERICA genotypes. The yields were higher for the efficient WAB compared to the NERICA genotypes. N doses also affected the N concentration and N uptake of genotypes. N concentration was higher at low N dose for NERICA than for the WAB genotypes. The opposite situation was found with the WAB genotypes. The high dose generated higher yields with WAB genotypes than with the other lines. This study allowed the selection of nutrient use efficient genotypes, which will be integrated into the farming system to boost productivity.

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USING UPLAND RICE ROOT TRAITS TO IDENTIFY N USE EFFICIENT GENOTYPES FOR LIMITED SOIL NUTRIENT CONDITIONS

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Abstract

Crop production in the Sahelian countries of Africa is limited by many factors. The most important are low potential yields of local varieties, low inherent soil fertility and low applications of external inputs (organic and mineral fertilizers). A field experiment was conducted from 2007 to 2008 with the objective to develop and validate screening protocols for plant traits that enhance N acquisition and utilization in upland rice grown in low N soils of two hundred (200) upland rice (*Oryza sativa* L.) genotypes from WAB, NERICA, CNA, CNAX, IRAT and IR lines. An experiment in small pots was carried out in a greenhouse of Farakoba research center. The pots were filled with a sandy soil and upland rice genotypes were grown during three weeks, harvested and studied for their root characteristics (seminal root length, adventitious root number, lateral root length and number and roots hair density). The small pot method was reliable for root trait characterisation at the seedling stage. A large variability among genotypes was exhibited for the root characteristics. The variability was larger within the NERICA and WAB lines compared to the other lines. The length of the seminal roots varied from 10 to 40 cm, the lateral root number ranged between 3 and 15 and the number of adventitious roots varied between 2 and 7. The selected root traits can be used to identify high nutrients and water use efficient genotypes.

1. INTRODUCTION

Crop production in the Sahelian countries of Africa is limited by many factors [1]. The most important are low potential yields of local varieties, low inherent soil fertility and low applications of external inputs (organic and mineral fertilizers) [2–5]. The upland soils in the area are deficient in N and P [6], although considerable efforts were devoted to the improvement of soil conditions for crop production through the design and diffusion of best soil-nutrient management practices. The adoption rate of these technologies remained low due to mainly limited incomes of small poor farmers and socio-economic constraints. The design and the use of crop genotypes adapted to these hard environments and the use of organic amendments combined with small quantities of mineral fertilizers are of importance for thousands of smallholders in the Sahel. The current level of upland rice production can be significantly increased by using improved and adapted rice cultivars in combination with low-cost agronomic practices. Recently, many varieties of upland rice were developed by the Burkina National Institute for Agriculture Researches (INERA) in partnership with the West African Rice Network (WARDA). These varieties are high yielding and are efficient in N and P recovery under poor soil conditions. In most cases, the agronomic performances of these new upland rice genotypes were proved by previous studies. But, specific traits that could justify the high performances of these new genotypes are less studied. Furthermore, information on appropriate fertilizer doses or optimal combination of agronomic practices for optimum performances of these new genotypes is not available.

Plant roots play an important role in water and nutrient acquisition. Both constitutive and adaptive root growth have been implicated in the improved performance of rice under rainfed conditions [7]. For the genetic improvement of the root system, the information on genotypic diversity is required on the traits related to the size and distribution of the root system [8]. This study aimed to clarify the magnitude of the effect of genotype on the variations in root traits related to the size and distribution of the upland rice root systems. The main objective was to identify the best upland rice varieties able to improve the smallholders' cropping system productivity through a better use of nutrients, particularly N from soil and fertilizers. The study aimed specifically to develop and validate screening protocols for plant traits that enhance N acquisition and utilization in upland rice grown in low fertility soils.

2. MATERIAL AND METHODS

The experiment was carried out in Farako-Bâ research center in the south-Sudanian zone of Burkina Faso from 2007 to 2008.

2.1. Plant material

Six (6 lines) and 200 genotypes of upland rice were used in the experiments (Table 1). The upland lines were received from AfricaRice in 2007.

TABLE 1. LINES AND NUMBER OF ACCESSIONS

Line	Number of accessions
WAB	175
NERICA	18
CT	2
CNA	2
CNAX	1
IRAT	1
IR	1
Total	200

2.2. Pot experiments

Two screening experiments were carried out. The first one started on May 21, 2007 and ended on June 30, 2008. The second screening was from January 8, 2008 to February 14, 2008. The soil used to grow the genotypes was a sandy soil collected in the center (bulk density, 1.5 g cm^{-3}, organic matter, 7%; total P, 323 mg kg^{-1}, available P, 16 mg kg^{-1}, pH_{KCl}, 6.0 and pH_{water}, 6.7). The soil was thoroughly mixed to make it uniform. Cylindrical PVC pots of l liter capacity were filled with 700 g of sandy soil, the pots were watered to field capacity and allowed to equilibrate before rice was panted. The pots were perforated at their base to allow adequate water and air circulation. Five (5) grains of each of the 200 upland rice genotypes were planted in each pot. The pots were thinned after emergence to 3 plants per pot. The pots were randomly arranged in a screen house at ambient temperature in three blocks corresponding to repetitions. Pots were watered once a day (morning).

2.3. Root parameter measurements

Fourteen days after sowing, the soil was carefully washed with tap water and roots were sampled. The following parameters were measured:

- plant height

- number of leaves
- seminal root length (SRL) was measured as the length of the longest root
- adventitious root numbers (ARN) were counted for each plant
- the lateral root length (LRL) and lateral root number (LRN)
- root hair density
- the above and below ground biomass of each genotype was separated, sun dried for 2 days and oven dried at 70°C during 24 h

3. RESULTS AND DISCUSSION

3.1. Screening at the seedling stage

Genotypes were significantly different for all variables except for the straw dry weight (Table 2). Positive correlations were found between plant height and the number of lateral roots, the number of leaves and genotype straw dry weight (Table 3), and between seminal root length and lateral root length. The straw dry weight and root dry weight were also positively correlated (Table 3).

TABLE 2. ANOVA ON GENOTYPE ROOT CHARACTERISTICS

Variables	Probability	LSD
Plant height (cm)	<0.001	7.60
Number of leaves	<0.001	0.82
Seminal root length (cm)	<0.001	6.18
Number of lateral roots	<0.001	3.87
Number of adventitious roots	<0.001	1.41
Lateral root length (cm)	<0.001	6.12
Number of root hairs	<0.001	0.68
Straw dry weight (mg)	0.075	75.2
Root dry weight (mg)	<0.001	48.7

The genotypes were ranked in 6 groups according to the root characteristics (Fig. 1).

3.2. Rooting system and physiological characteristics of upland rice genotypes

3.2.1. Plant height

Genotype heights ranged between 25 and 55 cm (Fig. 2). Most of the genotypes showed plant height around 40 cm. Only one genotype was higher than 50 cm and two genotypes showed plant height below 30 cm. The genotype WAB 537 144 11 HB was the shortest one while 12 genotypes from the WAB line showed a plant height above 50 cm. Genotypes WAB 5677 and WAB 788 18 2 1 HB were the highest.

TABLE 3. CORRELATION COEFFICIENTS AMONG ROOT CHARACTERISTICS

Parameter	Plant height	Seminal root length	Lateral root length	Number of leaves	Number of adventitious roots	Number of lateral roots	Straw dry weight	Root dry weight	Roots hair density
Plant height	1								
Seminal root length	0.196	1							
Lateral root length	–0.251	**0.436****	1						
Number of leaves	0.310*	0.289	0.168	1					
Number of adventitious roots	0.218	0.087	–0.053	0.172	1				
Number of lateral roots	**0.461****	0.01	–0.341	0.168	0.169	1			
Straw dry weight	0.308*	0.069	–0.092	0.139	0.077	0.175	1		
Root dry weight	–0.007	0.110	0.084	0.035	0.035	0.040	**0.410****	1	
Roots hair density	0.116	0.163	0.194	0.092	0.107	0.110	0.109	0.137	1

*, $P<0.05$; **, $P<0.01$

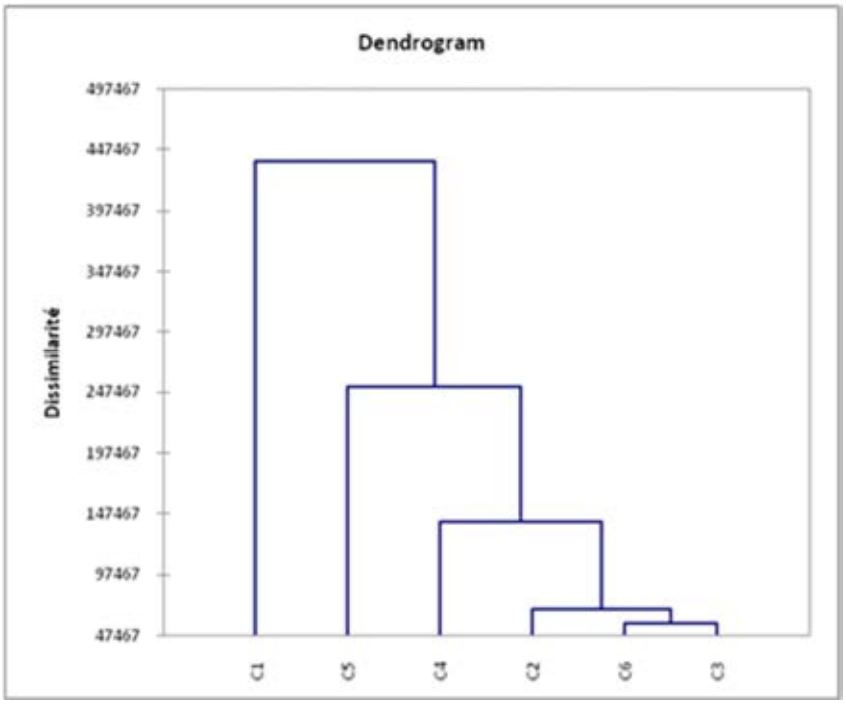

FIG. 1 . Classification of genotypes according to root characteristics.

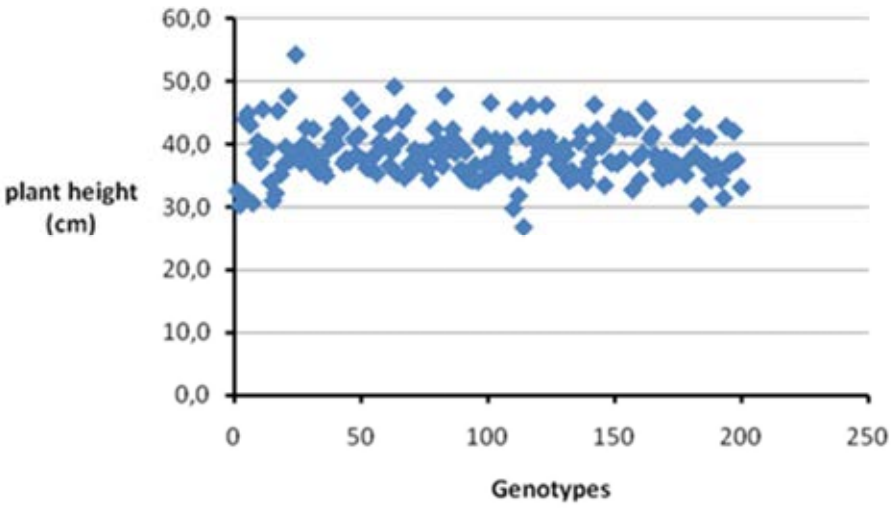

FIG. 2. Heights (cm) of genotypes 3 weeks after sowing.

3.2.2. Root length

The seminal root length showed different pattern for genotypes (Fig. 3). The data are scattered and ranged between 10 and 40 cm. This root trait could be a potential index for difference between genotypes. Deep rooting has been emphasized as an important adaptation to stress in rice [9], and it was also reported that upland rice genotypes have seminal root lengths above 10 cm in Burkina Faso [10]. Long seminal roots mean future deep rooting, which is necessary for water uptake [11–13].

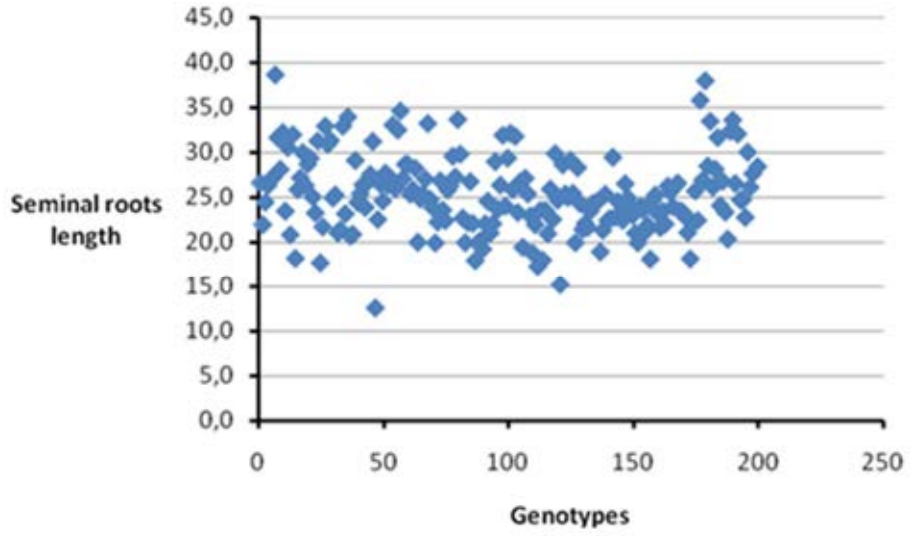

FIG. 3. Seminal root length (cm) of genotypes 3 weeks after sowing.

3.2.3. Number of lateral roots

The number of lateral roots varied from 3 to 15 (Fig. 4). This character seems to be an important trait marking difference between genotypes that will probably be important for nutrient uptake and accumulation by genotypes. The lateral roots show the possibility of the genotype to explore the upper layer of the soil. The number of lateral roots was lower than the number indicated in the literature. This is due to the fact that the plants were very young and this was also observed previously [14, 15]. Genotypes with a high number of lateral roots will develop a shallow rooting system which is not very good for N and P uptake. A higher lateral root number can be also a characteristic for adaptation to drought [16].

3.2.4. Lateral root length

Lateral root length showed a less scattered pattern compared to the number of lateral roots. The number varied between 9 and 25 cm (Fig. 5). Most of the genotypes showed a length between 15 and 20 cm. Only one genotype showed a lateral root length around 30 cm.

3.2.5. Number of adventitious roots

The adventitious roots are precursors of lateral and seminal roots, and are therefore important for the crop during the vegetative and the reproductive phases. Adventitious root numbers differed among genotypes and varied between 2 and 7. Most of the genotypes had 2 to 5 adventitious roots. One genotype had more than 6 adventitious roots (Fig. 6).

3.2.6. Number of leaves

The number of leaves per genotype varied between 3 and 7. Most of the genotypes had 4 to 6 leaves. This physiological character was not different between genotypes (Fig. 7). These results are in line with those from [17] who found 5 leaves 20 days after sowing. The number is therefore a genotype dependent trait.

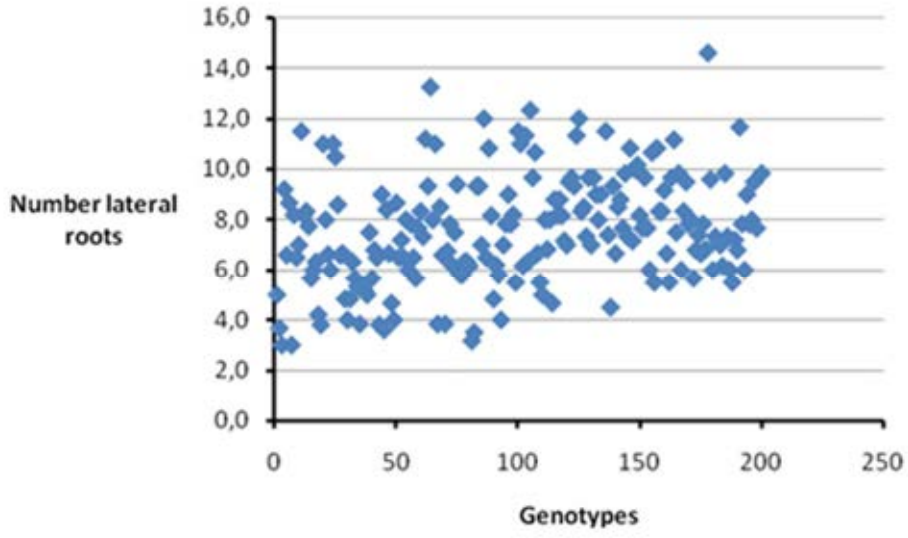

FIG. 4. Number of lateral roots of genotypes.

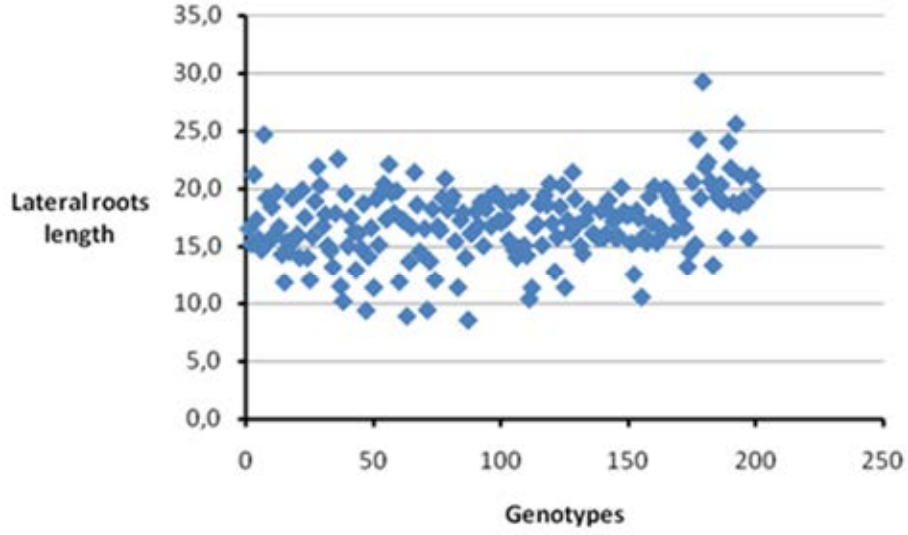

FIG. 5. Lateral root lengths (cm) of genotypes.

3.2.7. Roots hair density

Roots hair density differed highly among genotypes and varied between 1 and 3 (Fig. 8). Based on this root trait the genotypes can be ranked in different groups. This character is also important for water and nutrient uptake from the soil. Root hairs form from root epidermal cells. It is presumed that root hairs contribute to the adhesion of the growing root to the rhizosphere and assist in the uptake of nutrients and water from the soil by increasing the absorptive surface area [18]. This character can be of importance for plant N uptake.

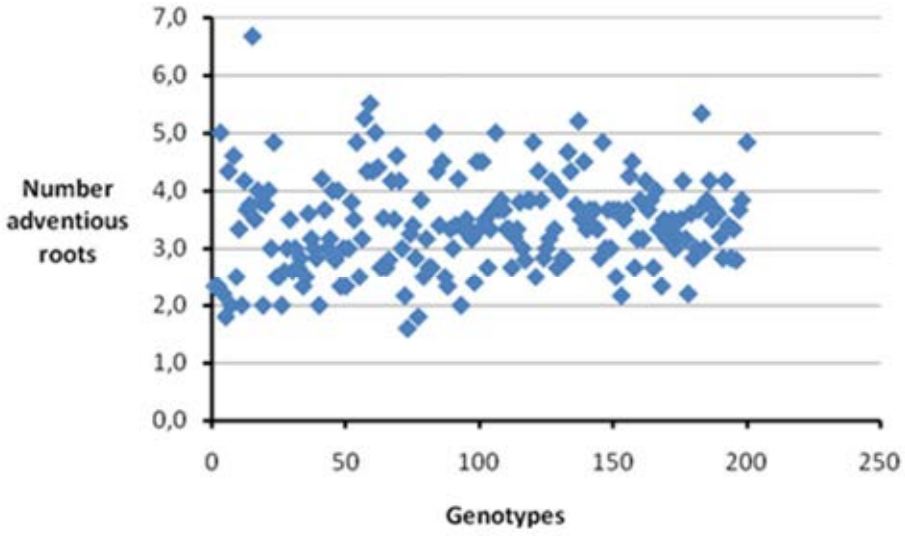

FIG. 6. Number of adventitious roots of genotypes.

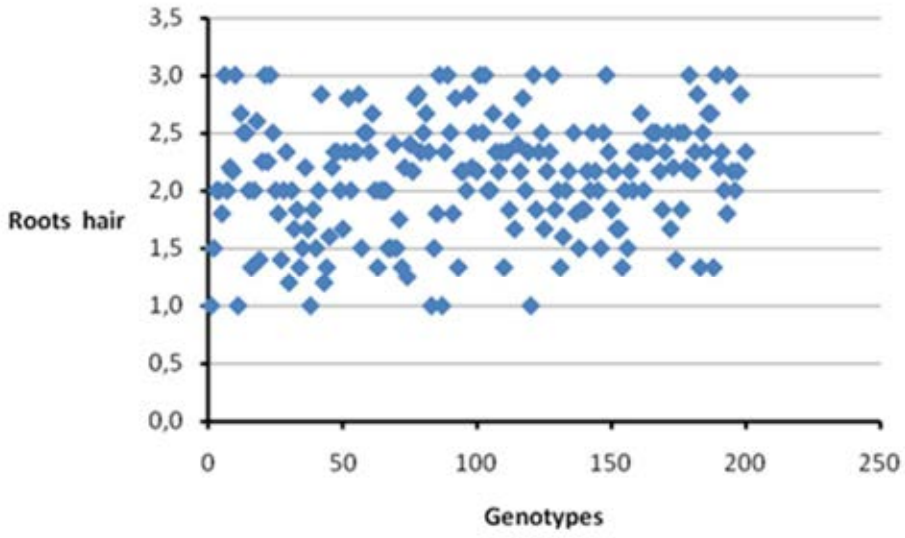

FIG. 7. Number of leaves of genotypes.

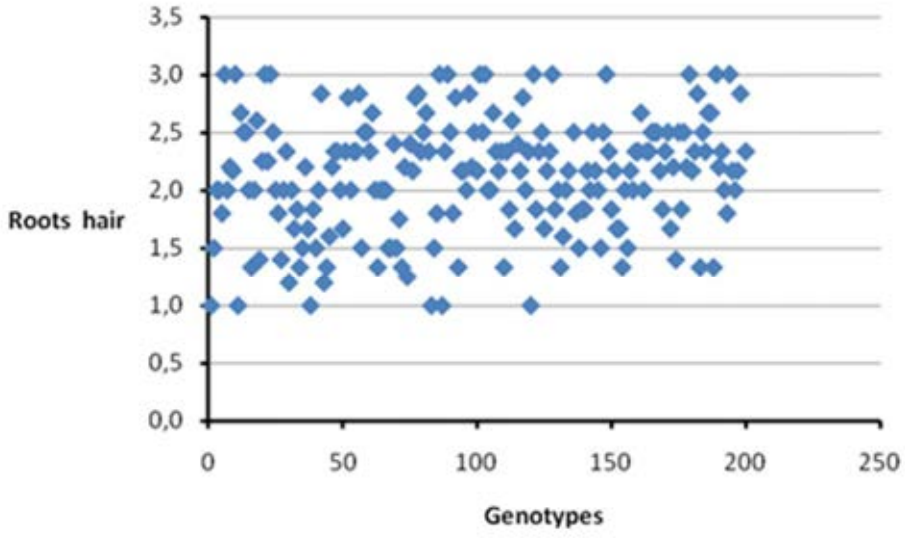

FIG. 8. Root hair density of genotypes.

3.3. Rooting system for upland rice lines

The lines differed for all variables except for plant height, number of leaves and root hair density (Table 4). The lowest plant height was obtained for the CNA line and plant heights were comparable for the other lines. For lateral roots and seminal root lengths, the lowest value was found for the CNAX line. The CNA line had a very long seminal root. The lengths of seminal roots were comparable for the other lines.

All of the genotypes presented on average 4 well developed leaves, and this character was not significantly different for lines. The CNAX line showed a very high number of adventitious roots while the CNA and IR lines showed the lowest number. The number of lateral roots was comparable for CNA, CNAX, NERICA and WAB, but lower compared to the other lines. Straw and roots dry weight showed the same pattern. The values were lowest for CNAX and CT and highest for NERICA.

The data showed also large variability among lines, especially for WAB and NERICA, with respect to minimum and maximum values (Table 5). The variability was consistent over all variables and was very high for lateral and seminal root lengths, the number of lateral roots and straw and roots dry weight.

4. CONCLUSIONS

From the current study we concluded that the small pots experiment is a reliable method for root trait characteristics for seedlings. The results showed that:

- Upland rice genotypes differed for root traits, and adventitious roots, lateral root number, and root hair density are potential traits to characterize nutrients and water uptake from the soil which is very important for low inherent fertility soils in the Sahel.

- Very large variability among root characteristics within the upland rice lines. The variability was much higher for WAB and NERICA genotypes compared to the other lines, and this was consistent over all the measured variables.

- The correlations between yield components and roots traits showed no clear pattern even though positive correlations were found between yields and seminal root length and number of leaves.

- Further investigation should be carried out on the selected root traits and also on plant physiological characteristics (nutrient uptake, nutrient use efficiency and nutrient content in the shoots and the roots) for possible significant correlation with root traits.

TABLE 4. ROOT CHARACTERISTICS OF UPLAND RICE GENOTYPES AT THE SEEDLING STAGE 3 WEEKS AFTER EMERGENCE

Line	Plant Height (cm)	Lateral root length (cm)	Seminal root length (cm)	Number of leaves	Number of adventitious roots	Number of lateral roots	Shoot dry weight (mg)	Roots weight (mg)	Roots hair density
CNA	41.10	22.01	31.01	4.16	2.92	6.67	193.3	99.3	2.25
CNAX	37.02	11.80	18.25	5.00	6.67	5.67	116.8	55.2	2.00
CT	39.08	14.67	26.77	5.00	3.67	12.33	167.7	100.7	2.00
IR	41.03	20.27	25.03	4.50	2.83	11.33	207.0	138.5	2.50
IRAT	40.27	16.10	24.60	4.25	3.75	11.50	214.2	139.0	2.50
NERICA	38.69	17.10	23.74	4.31	3.47	8.23	227.2	111.1	2.01
WAB	38.27	17.31	25.56	4.77	3.38	7.34	195.0	111.8	2.13
F pr.	0.24	0.037	0.002	0.071	<.001	0.003	0.028	0.120	0.57
LSD	7.94	6.38	7.01	0.89	1.52	4.12	98.63	55.83	1.18

TABLE 5. MINIMUM (MIN) AND MAXIMUM (MAX) VALUES FOR LINES SCREENED AT THE SEEDLING STAGE

Line	Plant height (cm)		Root length (cm)				Number of								Dry weight plant^{-1} (mg)			
			Lateral		Seminal		Leaves		Adventitious roots		Lateral roots		Root hairs		Straw		Roots	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
CNA	25.0	48.0	12.0	33.6	23.0	43.5	3	5	1	4	0	12	1	3	100	300	53	128
CNAX	24.0	39.5	3.0	21.3	7.0	31.0	4	6	3	12	4	7	1	3	54	200	4	100
CT	30.5	46.5	12.0	19.0	19.0	32.3	4	6	2	5	7	17	2	2	43	233	29	138
IR	39.7	42.0	14.0	25.0	19.5	32.0	4	6	2	4	10	13	1	3	100	300	100	171
IRAT	28.0	50.3	11.5	25.9	19.9	32.0	4	5	3	5	9	14	1	9	100	280	95	171
NERICA	20.0	54.0	1.5	37.0	11.5	48.0	2	6	0	6	0	17	1	3	40	442	12	200
WAB	14.5	69.5	1.5	42.6	4.4	52.5	2	9	0	13.5	0	22	1	3	21	560	7	740

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EVALUATION OF UPLAND RICE GENOTYPES FOR EFFICIENT UPTAKE OF NITROGEN AND PHOSPHORUS

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Abstract

Upland rice grown by subsistence farmers in the tropics and subtropics is known to produce very low yields due to it being planted on low fertility soils and under drought-prone conditions. Little information is available on upland rice cultivar differences in response to N and P fertilization in Asia, thus screening for P (PUE) and N use efficiency (NUE) of upland rice genotypes is a necessary first step. The objectives of the study were: (i) to identify upland rice genotypes with root characteristics favorable for efficient N and P uptake and utilization, (ii) to evaluate the selected genotypes for their grain yield, and (iii) to assess the variability of N and P use efficiency in upland rice genotypes grown under field conditions. Several laboratory, glasshouse and field experiments were carried out from 2007 to 2011 at Universiti Putra Malaysia to achieve the above objectives. Fifteen local and 15 upland rice genotypes from WARDA were identified to have long roots, and it was observed that some of the WARDA lines showed longer root length than the local landraces. This is a good trait since it is known that longer root length will enhance the absorption of easily mobile nutrients such as nitrate and potassium. Glasshouse and field evaluation of N use efficiency by these upland rice genotypes showed that high N is utilized (40–80% of applied N), with good grain yield, and P use efficiency is similar to other crops (4–8%).

1. INTRODUCTION

Upland rice is grown as a staple food by subsistence farmers on approximately 17 million hectares worldwide [1]. One of the key constraints to food production under these conditions is N and P deficiency, often associated with low organic matter, high P fixation and severe soil acidity. Although indigenous upland rice cultivars appear well suited to these conditions, being both low-P and high-acidity tolerant relative to other cereals, repeated cropping without fertilizer addition can only be sustained by shifting cultivation and long fallow periods. A key objective for upland rice research is therefore to improve cultivar yield potential under the prevailing conditions [2].

Average yields of rain fed upland rice are 1.1 t ha^{-1} but this varies according to soil type, fertilizer use, rainfall and agronomic practices [1]. Increased root length density in upland rice is important for maintaining plant water status. Deeper and thicker roots are hypothesized to improve performance of rain-fed rice under drought by more efficient water extraction from deep soil layers. Drought-adapted varieties tend to have longer roots [3]. The low yield of upland rice is largely a consequence of its production being limited to infertile or drought-prone uplands, and to low harvest index of traditional cultivars [4]. Traditional cultivars are generally tall, have few tillers, and produce low but stable yields under unfavorable environments. They tend to lodge under favorable conditions and are thus not suited to high-input management.

Genetic selection and plant breeding techniques helped to develop rice varieties that are resistant to pests, diseases, and adverse environmental conditions such as drought, nutrient deficiencies, toxicities, and salinity. However, genetic selection to improve the rice crop's nutrient-use efficiency has received little attention. The need for developing and identifying superior nutrient efficient genotypes, especially for N and P is evident because of the low

availability of these nutrients in tropical soils. For P, it was found that total root length of winter wheat was positively correlated to grain yield [5]. However, few breeders have paid attention to the possibility of manipulating root length for making crop genotypes P efficient. This may be partly due to the technical difficulties and expense involved in studying root systems. Model simulations of plant root systems for predicting optimum root architecture for P acquisition in low P conditions [6] are promising. However, validation of such models through fioun experiments still remains a problem as development of root systems is influenced by a large number of soil and climatic factors [7],

Another limitation to plant growth in many of the highly weathered tropical and sub-tropical humid soils (Ultisols and Oxisols) is the availability of phosphorus (P) due to inherently low P content which is exacerbated by high P fixing capacities of added P fertilizers. Malaysia is no exception, and therefore P deficiency is the major constraint to upland rice production resulting in low yields. Conventional amelioration of P deficiency by application of fertilizer is becoming increasingly uneconomical and ecologically unsound as the efficiency of added fertilizer is low ≈ 10% [8]. Consequently, improvement of P uptake and P use efficiency (PUE) by crops is critical as an economic, environmental-friendly and sustainable strategy. The key to increase P recovery from less accessible forms is using crop cultivars that are more efficient in P use. High PUE is achieved through efficient internal P utilization and/or increased acquisition of more P from soil. The mechanisms by which these are achieved include: (i) Exudation of chemical compounds such as phosphatase into the rhizosphere to increase the solubility of P in the soil [8]. (ii) Changes of root morphology under P deficiency to permit the plant to explore a greater volume of soil [9]. (iii) Increase in the P uptake kinetics via activating special high affinity root transporters in P deficient conditions. Little information is available on upland rice cultivar differences in response to N and P fertilization in Asia, thus screening for high P and N use efficiency of upland rice genotypes is necessary to develop varieties with high PUE and NUE. The objectives of the study were:

(i) to identify upland rice genotypes with root characteristics favorable for efficient N and P uptake and utilization
(ii) to evaluate the selected genotypes for their N and P response on grain yield
(iii) to assess the variability of N and P use efficiency in upland rice genotypes grown under field conditions.

2. MATERIALS AND METHODS

2.1. Gigar roll method

Upland rice seeds from each land race (30 local and 200 from WARDA) were soaked in water and allowed to germinate using the cigar-roll method. Roots that developed were measured after 14 d using the WINRhizo Image Scanner EPSON Perfection V700 PHOTO. Root characteristics such as length, diameter, surface area, volume and number of root tips were measured.

2.2. Nitrogen uptake and N use efficiency

2.2.1. Glasshouse experiments

Two glasshouse experiments determined the N use efficiency of the upland rice genotypes. The first batch was planted on 3rd September 2007. The following genotypes were used in four replications:

1. WAB788-56-2-1-2-HB (WARDA No. 17)
2. WAB767-2-5-1-HB (WARDA No. 51)
3. WAB915-B-3A1.1 (WARDA No. 69)
4. NERICA 7 (WARDA No. 143)
5. KENINGAU AN749
6. BATU GARAM AN1249
7. NABAWAN AN773
8. BECOR
9. PULUT PETAI
10. SELAYANG

The first fertilizer application was made on 12th Sept 2007 using 8 g TSP; 2 g MOP; 2 g urea with 2.03 atom % ^{15}N excess. The second fertilizer application was on 29th Oct 2007 with 1g MOP and 1 g ^{15}N labeled urea, and the third fertilizer application was on 12th November 2007 using 1g MOP and 1 g ^{15}N-labeled urea. The total N added was (4 × 0.46 g) = 1.84 g pot^{-1}. Harvesting was done on 8th January, 2008.

A second glasshouse experiment was planted using another 15 genotypes on 5th November 2007 in four replications. They were:

1. WAB 878 SG 43 (WARDA No. 192)
2. WAB 748-11-2-HB (WARDA No. 57)
3. WAB 880-1-38-20-15-P2-HB (WARDA No. 113)
4. WAB 905-B-2A 1.1 (WARDA No. 26)
5. WAB 801-23-2-2-HB (WARDA No. 61)
6. WAB 788-58-2-1-HB (WARDA No. 56)
7. WAB 709-26-4-1-HB (WARDA No. 80)
8. Bukit Garam AN1334
9. Bukit Garam AN 753
10. Bukit Garam AN 582
11. Kinabatanagn AN1084
12. Bertih
13. Satang
14. Wangi
15. Lebar Dukong

The soil used in the glasshouse experiment was a Munchong series soil (Oxisol) having a pH of 4.38, Bray-1P of 4.16 mg kg^{-1} and total N of 0.16%. The fertilizers applied were the same as the first experiment for P and K, while N was doubled due to the first experiment which did not perform well. The nitrogen applied was urea with 5 atom % ^{15}N excess. The plants were harvested at the end of March 2008 except for variety Wangi and Lebar Dukong which were still at the booting stage. The parameters recorded for the harvested plants were plant height, panicle length, panicle number and grain weight. All data obtained were analyzed statistically using SAS package version 9. Tukey Studentized Range test (HSD) was used for comparison of means.

2.2.2. Field experiment

This experiment was conducted on a Bungor series soil (Typic Kadiudult) at Ladang Puchong, University Putra Malaysia. Sixteen soil samples were taken randomly in each block using an auger, and were thoroughly mixed and sub-sampled. The subsamples was air-dried, ground and sieved to pass through a 2.0 mm sieve size and kept for analysis. Plots 2.1 × 2.1m were marked and arranged in a complete randomized block design, with 8 plots in each block. Eight upland rice genotypes were planted on 13th March, 2008. For each plot, a subplot measuring 1.5 × 1.5 m in the centre of the main plot was marked for the application of ^{15}N-labelled fertilizer. The remaining area was fertilizer with unlabelled ammonium sulphate . Upland rice seeds were planted at a spacing of 30 x 30cm. First application of fertilizers was made at 20 days after planting (60 kg–N ha^{-1},100 kg–P ha^{-1}, 60 kg–K ha^{-1}) and second application was at 40 days after planting (60 kg–N ha^{-1}, 60 kg–K ha^{-1}).

The genotypes used for this field experiment were:

Nabawan, Tenom, WAB804-23-1-1-20HB (WARDA No. 20), WAB 880 SG37 (WARDA No. 99), Sintok, Pulut Petai, Merah dan Kuku Belang

Plant samples were taken at harvest. Parameters collected were total grain yield, plant height, panicle number (no/clump),1000 grain weight (g), filled grains (%), growth duration (days to maturity) and tiller number. The central six plants from each sub-plot were used for N-15 analysis.

The chemical properties of the original soil used were: $pH_{(water)}$, 4.75; Bray-1 extractable P, 6.70 mg kg^{-1}; total N, 0.18% ; organic C, 1.025%; exchangeable cations (cmol (+) kg^{-1} soil), K, 0.16, Mg, 2.25, Ca, 0.65 and CEC, 6.7 cmol kg^{-1} soil.

2.3. Phosphorus uptake and P use efficiency

Ten genotypes, Tenom, Nabawan, Keningau, Kinabatangan, Kuku Belang, Merah, Wangi, BG582, Pulut petai and BG1334A, were grown in 25 kg polybags in the glasshouse. The soil was an Ultisol labelled with 100 µCi ^{32}P containing 1 mg P as KH_2PO_4 prior to planting of the seeds. P fertilizer at 50 kg P ha^{-1} was applied to the treated polybags. The other half was not applied with P fertilizer. N and K was added as urea and muriate of potash at 150 kg–N and 200 kg K ha^{-1}, respectively, to all polybags at 30, 60 and 90 d after planting in three split-applications. The response of these plants under glasshouse condition were not satisfactory, thus another six genotypes were replanted in polybags with the same treatment, but were placed under field conditions.

3. RESULTS

3.1. Root characteristics (cigar roll method)

Distinct differences were observed from these land races of upland rice. Principal Component Analysis carried out on the data collected for all the 220 genotypes of upland rice collected showed that the two components (root region, and root length) showing the highest eigen values of >1.0 and accounted for 81.6% of the standardized variance. FACTOR retains the two components based on the eigen values >1.0 rule. The first component (root region) has large positive loadings for all variables except average root diameter. The second factor (root length) only correlated well with root volume.

Greater root length has been found to relate to the ability of roots to penetrate deep into the soil to absorb nutrients such as nitrate which is easily leached into the soil, especially in tropical areas which have heavy rainfall of >2000 mm rain per year. This "deep root" characteristic would also help in the plants being able to withstand low rainfall (drought) periods. The top 15 local landraces that showed good root length characteristics are shown in Table 1.

TABLE 1. ROOT LENGTH AMONG LOCAL RICE LANDRACES GROWN UNDER UPLAND CONDITIONS

No.	Landrace	Origin	Root length (mm)
1	Pulut Petai	Pahang	155 a
2	Bukit Garam 1249	Sabah	143 ab
3	Lebar Dukong	Pahang	132 bc
4	Wangi	Pahang	122 c
5	Kuku Belang	Kelantan	121 c
6	Padi Burung	Pahang	118 c
7	Satang	Pahang	115 c
8	Tenom AN1214	Sabah	114 c
9	Merah	Kelantan	113 c
10	Pulut Galah	Pahang	110 c
11	Keningau AN763	Sabah	108 c
12	Bertih	Pahang	103 c
13	Bukit Garam AN753	Sabah	97 c
14	Nabawan AN773	Sabah	97 c
15	Pungop	Sarawak	97 c

Data within a column followed by the same lower case letter are not significantly different (P<0.05)

The top 15 lines obtained from WARDA that showed good root length characteristics are shown in Table 2. It can be seen that the roots produced by WARDA lines were significantly longer than those of the local landraces except for Pulut Petai and Bukit Garam 1249 (Table 1).

TABLE 2. ROOT LENGTH AMONG WARDA RICE LINES GROWN UNDER UPLAND CONDITIONS

No.	Line	Number in WARDA List	Root length (mm)
1	NERICA 14	150	206 a
2	WAB1094-B-58AB-1	100	197 ab
3	WAB326-B-B-19-H1-H1-HB	68	162 bc
4	WAB707-32-3-1-HB	74	156 cd
5	WAB716-27-2-1-HB	33	156 cd
6	CNA6675	180	149 d
7	WAB781-75-31-HB	18	144. d
8	WAB995-6A1.1	184	143 d
9	NERICA 5	134	140 d
10	WAB880-1-38-19-33-P1-HB	188	139 d
11	WAB 165	137	139 d
12	WAB340-B-B-2-H2	123	138 d
13	WAB880-1-38-18-20-P3-HB	198	138 d
14	WAB564-8-1-2	79	137 d
15	WAB503-18-4-1	179	136 d

Data within a column followed by the same lower case letter are not significantly different (P<0.05)

3.2. Nitrogen characteristics of upland rice

3.2.1. Glasshouse experiment 1

The average dry matter, N concentration and total N uptake together with the mean ^{15}N enrichment in the genotypes are shown in Table 3.

TABLE 3. TOTAL DRY WEIGHT, N CONCENTRATION, TOTAL N UPTAKE AND MEAN ^{15}N ENRICHMENT OF GENOTYPES

Genotypes	Total DW (g pot^{-1})	% N	Total N (mg pot^{-1})	^{15}N (atom % excess)
WAB788-56-2-1-2-HB (17)	18.36 ab	2.83	531 ab	0.464
WAB767-2-5-1-HB (51)	10.56 bcd	2.89	309 abc	0.485
WAB915-B-3A1.1 (69)	3.69 d	2.78	103 c	0.404
NERICA 7 (143)	8.03 cd	3.14	257 bc	0.400
KENINGAU 749	4.88 d	2.85	131 c	0.465
BATU GARAM 1249	13.14 abcd	3.55	469 ab	0.421
NABAWAN 773	15.00 abc	3.51	504 ab	0.461
BECOR	20.85 a	2.54	521 ab	0.454
PULOT PETAI	20.41 a	2.76	565 a	0.471
SELAYANG	17.62 ab	2.86	506 ab	0.455
		ns		ns

Data within a column followed by the same lower case letter are not significantly different (P<0.05); ns, not significant (P<0.05)

The highest dry matter produced was from Becor and Pulot Petai (2 local landraces). No significant difference in the N concentration among the 10 lines was observed. Also the ^{15}N enrichment did not show any significant difference. The % and amount of N derived from the fertilizer are given in Table 4.

TABLE 4. PERCENTAGE N DERIVED FROM FERTILIZER AND N TAKEN UP FROM FERTILIZER

Genotypes	NdfF (%)	NdfF (mg pot^{-1})
WAB788-56-2-1-2-HB (17)	21.7	116 a
WAB767-2-5-1-HB (51)	22.7	94 ab
WAB915-B-3A1.1 (69)	18.9	34 b
NERICA 7 (143)	18.7	69 ab
KENINGAU 749	21.8	33 b
BATU GARAM 1249	19.7	93 ab
NABAWAN 773	21.6	121 a
BECOR	21.3	113 a
PULOT PETAI	22.0	122 a
SELAYANG	21.3	108a
	ns	

Data within a column followed by the same lower case letter are not significantly different (P<0.05); ns, not significant (P<0.05)

On average, 20% of the N in rice came from the applied fertilizer, with Pulot Petai showing the highest percentage. This is expected since this landrace did show good root length and good root branching habit.

3.2.2. Glasshouse experiment 2

The dry matter yield and N concentration of straw and grain and given in Table 5.

TABLE 5. DRY MATTER AND N CONCENTRATION OF STRAW AND GRAIN

Genotypes	Straw DW (g pot^{-1})	Straw (% N)	Grain DW (g pot^{-1})	Grain (% N)
WAB 878 SG 43	42.93 cde	2.96 d	12.84 ab	3.78 ab
WAB 748-11-2-HB	52.93 cd	3.04 bcd	23.08 a	3.53 ab
WAB 880-1-38-20-15-P2-HB	28.70 de	3.27 abc	18.72 ab	4.05 ab
WAB 905-B-2A 1.1	23.90 e	3.46 a	15.07 ab	3.59 ab
WAB 801-23-2-2-HB	55.80 cd	3.05 bcd	17.29 ab	3.76 ab
WAB 788-58-2-1-HB	53.16 cd	2.70 d	24.23 a	3.29 b
WAB 709-26-4-1-HB	63.28 bc	3.10 bcd	15.03 ab	3.65 ab
Bukit Garam AN1334	38.88 cde	3.27 abc	11.27 ab	3.61 ab
Bukit Garam AN 753	51.33 cde	2.99 cd	15.96 ab	3.99 ab
Bukit Garam AN 582	35.02 de	3.47 a	11.68 ab	3.75 ab
Kinabatanagn AN1084	29.82 de	3.27 abc	12.26 ab	3.79 ab
Bertih	88.27 ab	2.84 d	14.41 ab	4.21 a
Satang	111.96 a	3.31 abc	7.04 b	3.73 ab

Data within a column followed by the same lower case letter are not significantly different (P<0.05)

Straw DW was highest in Satang while grain DW was highest in WAB 788-58-2-1-HB. This experiment showed a higher N concentration due to higher rate of N applied. The ^{15}N enrichment of tissue, % N derived from the fertilizer and % fertilizer use efficiency are given in Table 6.

TABLE 6. ^{15}N ENRICHMENT OF TISSUE, PERCENTAGE N DERIVED FROM FERTILIZER AND PERCENTAGE N USE EFFICIENCY

Genotypes	Straw	Grain	Straw + grain	
	^{15}N (atom % excess)		NdfF (%)	NUE (%)
WAB 878 SG 43	2.473	2.193 ab	26.6 a	12.0
WAB 748-11-2-HB	2.475	2.073 ab	20.2 a	19.5
WAB 880-1-38-20-15-P2-HB	2.325	1.943 b	31.7 a	16.5
WAB 905-B-2A 1.1	2.320	2.260 ab	32.7 a	12.4
WAB 801-23-2-2-HB	2.523	2.245 ab	26.7 a	15.8
WAB 788-58-2-1-HB	2.435	2.243 ab	31.6 a	18.6
WAB 709-26-4-1-HB	2.103	2.278 ab	18.7 ab	11.9
Bukit Garam AN1334	2.115	2.078 ab	19.6 ab	9.3
Bukit Garam AN 753	2.313	2.395 a	26.7 a	12.4
Bukit Garam AN 582	2.000	2.185 ab	21.4 ab	9.8
Kinabatangan AN1084	2.225	2.330 ab	27.6 a	11.0
Bertih	2.053	2.075 ab	16.3 ab	14.5
Satang	2.383	2.208 ab	6.2 b	6.4
	ns			ns

Data within a column followed by the same lower case letter are not significantly different (P<0.05); ns, not significant (P<0.05)

The percentages of N derived from fertilizer were higher in this experiment, with up to 32% of the plant N from the fertilizer. Phenotypic differences among the genotypes are given in Table 7.

TABLE 7. AGRONOMIC CHARACTERISTICS OF THE RICE PLANTS

Genotypes	Plant height (cm)	Numbers of				1000 grain weight (g)
		Tillers	Panicles pot^{-1}	Spikelets panicle^{-1}	Grains panicle^{-1}	
WAB 878 SG 43	104.3 a	14.3 ab	17.0 ab	292.7 a	116.6 a	35.62 ab
WAB 748-11-2-HB	118.3 a	9.3 bc	13.3 ab	174.5 a	174.9 a	33.93 ab
WAB880-1-38-20-15-P2-HB	113.7 a	11.5 abc	12.0 b	102.2 a	122.7 a	38.48 a
WAB 905-B-2A 1.1	100.3 a	5.3 bc	9.8 b	218.4 a	128.1 a	24.63 abc
WAB 801-23-2-2-HB	115.0 a	10.3 bc	12.8 ab	425.8 a	114.4 a	10.20 c
WAB 788-58-2-1-HB	103.1 a	19.8 a	21.0 a	113.5 a	198.5 a	27.12 abc
WAB 709-26-4-1-HB	118.9 a	10.5 bc	15.0 ab	112.3 a	93.1 a	31.27 ab
Bukit Garam AN1334	151.7 a	9.3 bc	12.5 ab	104.0 a	119.6 a	16.20 bc
Bukit Garam AN 753	151.0 a	8.3 bc	12.5 ab	124.0 a	44.2 a	25.45 abc
Bukit Garam AN 582	151.8 a	9.8 bc	10.3 b	254.1 a	142.2 a	23.15 abc
Kinabatangan AN1084	133.4 a	9.3 bc	13.0 ab	251.9 a	177.4 a	21.61 abc
Bertih	134.9 a	7.8 bc	10.8 b	136.4 a	136.2 a	24.96 abc
Satang	142.3 a	4.3 c	8.3 b	361.0 a	159.4 a	29.03 abc

Data within a column followed by the same lower case letter are not significantly different (P<0.05)

The average height of the rice plants was between 1 m and 1.5 m. The highest number of tillers produced was observed from the lines supplied by WARDA. The local landraces were quite tall (between 1.3-1.5 m), but the tillers produced were not as many as the West African lines. The number of panicles produced per plant varied among the lines, with the African lines showing the highest number. The weight per1000 grains showed almost similar values amongst the 13 lines tested. Correlations carried out between the agronomic characteristics showed positive correlation between grain weight and %NdfF, with Pearson correlation coefficient (r) of 0.607. Correlations on log of root length and all the agronomic characteristics did not show any significant relationships.

3.2.3. Field experiment

The seed dry weight ranged from 204 g to 816 g plot^{-1} and the highest seed dry weight was obtained from WARDA99 to 816 g plot^{-1}, the lowest seed dry weight was obtained from Merah with 204 g plot^{-1} (Table 8). The highest plant dry weight was obtained from Merah with 2735 g plot^{-1} while the lowest plant dry matter yield was obtained from WARDA20 with 794 g plot^{-1}. The plant height ranged from 130.5 to 194.5 cm. The highest plant height was obtained from Merah with 194.5 cm, with WARDA20 being the shortest at 130.5 cm. The length of panicle varied from 23.6 to 29.3 cm. Pulut Petai had the longest panicle with 29.3 cm, while Nabawan had the shortest at 23.6 cm. Seed lengths (1.0 cm) were not significantly different in all genotypes tested.

TABLE 8. RICE PLANT CHARACTERISTICS AT HARVEST

Genotypes	Grain DW (g plot^{-1})†	DMY (g plot^{-1})†	Panicle length (cm)	No. tillers hill^{-1}	Plant height (cm)
Nabawan	518 bc	949 de	23.6 e	9.5 ab	155.5 cd
Tenom	665 ab	863 de	24.8 de	6.4 b	139.6 de
WARDA20	729 ab	794 e	27.7 ab	7.8 b	130.5 e
WARDA99	816 a	1199 cd	27.8 ab	6.9 b	144.9 de
Sintok	727 ab	1492 c	25.7 cd	7.4 b	166.0 bc
Pulut Petai	806 a	2271b	29.3 a	7.1 b	190.3 a
Merah	204 d	2735 a	26.4 bc	11.8 a	194.5 a
Kuku Belang	371 cd	2170 b	28.7 a	8.1 b	178.5 ab

†DW, dry weight; DMY, dry matter yield; plot size was 2.25 m^2; Data within a column followed by the same lower case letter are not significantly different (P<0.05)

There was no significant difference (P<0.05) in N concentration in the plant between the upland rice genotypes used (Table 9). N concentration ranged between 2.59 to 3.05%. The P concentration ranged from 0.31 to 0.43%. Kuku Belang showed the highest P concentration of 0.43%. WR20 showed significantly higher K concentration (3.76%) than WR99, Tenom, Nabawan, Sintok, Merah, Kuku Belang and Pulut Petai, while Kuku Belang and Pulut Petai showed similar K concentrations. Ca concentration in plants ranged from 443 to 653 mg kg^{-1} and Mg concentration ranged between 270 to 480 mg kg^{-1}. The Cu concentration ranged from 98 to 106 mg kg^{-1}; Fe ranged from 255 to 379 mg kg^{-1}; Mn from 804 to 946 mg kg^{-1}; and Zn from155 to 186 mg kg^{-1} (Table 9).

TABLE 9. NUTRIENT CONCENTRATIONS IN RICE PLANTS

Variety	Concentration (%)			Nutrient concentration (mg kg^{-1})					
	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Nabawan	2.96 a	0.37 abc	2.93 bc	443 b	372 bc	101 a	378 a	905 a	168 abc
Tenom	2.65 a	0.31 c	2.94 bc	489 ab	300 cd	103 a	365 a	930 a	166 abc
WARDA20	2.99 a	0.35 bc	3.76 a	626 a	480 a	106 a	365 a	935 a	168 abc
WARDA99	3.02 a	0.33 c	3.12 b	493 ab	343 bcd	101 a	379 a	946 a	167 abc
Sintok	3.05 a	0.33 c	2.84 bc	550 ab	376 bc	98 a	261 a	826 a	155 c
Pulut Petai	2.59 a	0.35 bc	2.41 c	574 ab	298 cd	97 a	255 a	918 a	161 bc
Merah	2.90 a	0.41 ab	2.67 bc	633 a	388 b	100 a	285 a	804 a	182 ab
Kuku Belang	2.83 a	0.43 a	2.47 c	595 ab	270 d	103 a	308 a	936 a	186 a

Data within a column followed by the same lower case letter are not significantly different (P<0.05)

The ^{15}N enrichment in plants ranged between 0.629 to 0.753 atom % excess (Table 10), while the ^{15}N enrichment in upland rice seeds ranged from 0.553 to 0.757 atom % excess. The total NdfF in upland rice ranged from 61.0 to 121.0 kg ha^{-1} (Table 10). Merah showed the highest NdfF with 121.0 kg ha^{-1} and the lowest was obtained from variety Tenom with 61.0 kg ha^{-1}. Variety Merah showed the highest nitrogen use efficiency in upland rice with 80.7% and the lowest nitrogen use efficiency in upland rice was obtained from variety Tenom with 40.7%.

TABLE 10. ^{15}N ENRICHMENT, AMOUNT OF NITROGEN DERIVED FROM FERTILIZER AND PERCENTAGE NITROGEN USE EFFICIENCY OF UPLAND RICE

Variety	^{15}N (atom % excess)		N derived from fertilizer (kg ha^{-1})			% N use efficiency
	Plant	Grain	Plant	Grain	Total	
Nabawan	0.671 a	0.553 a	40.2 de	23.0 bc	63.2 bc	42.1 bc
Tenom	0.721 a	0.614 a	31.3 e	29.7 abc	61.0 c	40.7 c
WRDA 20	0.629 a	0.684 a	27.7 e	41.5 ab	69.2 bc	46.2 bc
WRDA 99	0.731 a	0.696 a	48.3 cde	51.8 a	100.1 abc	66.7 abc
Sintok	0.711 a	0.654 a	64.0 bcd	38.8 ab	102.7 ab	68.5 ab
Pulut Petai	0.770 a	0.758 a	70.6 bc	43.0 ab	113.6 a	75.7 a
Merah	0.729 a	0.714 a	109.3 a	11.7 c	121.0 a	80.7 a
Kuku Belang	0.753 a	0.685 a	78.6 b	21.1 bc	99.7 abc	66.4 abc

Data within a column followed by the same lower case letter are not significantly different (P<0.05)

3.3. P uptake efficiency of upland rice

Out of the 10 genotypes used, only 5 genotypes germinated and survived. The five were Tenom, Nabawan, Keningau, Kinabatangan, and BG1334A. Most of the fertilizer P was found in the grain (Table 11) and between 3-8% of the applied P fertilizer was utilized by the rice plants (Table 12).

TABLE 11. DRY MATTER YIELD, P UPTAKE AND PERCENTAGE OF P DERIVED FROM FERTILIZER IN STRAW AND GRAIN OF RICE PLANTS

Genotypes	Dry matter (g pot^{-1})		P (mg pot^{-1})		PdfF (%	
	Straw	Grain	In straw	In grain	In straw	In grain
Tenom	36.04	17.15	42.4 a	47.24 a	0.00 b	39.40 b
Nabawan	24.26	15.48	23.2 c	43.03 a	9.90 b	72.69 a
Keningau	40.97	23.12	37.6 b	45.05 a	0.00 b	22.08 c
Kinabatangan	40.02	19.52	46.8 a	44.00 a	22.64 a	40.98 b
BG1334A	29.95	10.07	39.3 ab	24.24 b	35.51 a	47.47 b
	ns	ns				

Data within a column followed by the same lower case letter are not significantly different (P<0.05), ns, not significant (P<0.05)

TABLE 12. AMOUNT OF P DERIVED FROM FERTILIZER IN STRAW AND GRAIN OF RICE AND PERCENTAGE OF FERTILIZER P UTILIZED BY THE RICE PLANTS

Genotypes	PdfF (mg pot^{-1})			P use efficiency
	In straw	In grain	Total	(%)
Tenom	0.0 b	18.6 b	18.6 b	7.7 ab
Nabawan	2.4 b	31.3 a	33.7 a	8.0 a
Keningau	0.0 b	10.0 c	10.0 c	2.4 b
Kinabatangan	10.6 a	18.0 b	28.6 a	6.8 a
BG1334A	14.0 a	11.5 bc	25.5 a	6.1 a

Data within a column followed by the same lower case letter are not significantly different (P<0.05)

No significant difference in straw yield between genotypes were measured (Table 13). WARDA 100 gave the highest seed yield of 13.28 g $polybag^{-1}$ (Table 13). Most of the P taken up was found in the straw. The highest percentage and amount of P derived from fertilizer was obtained from grains of WARDA100 (Table 14). No difference in percentage P use efficiency was observed.

TABLE 13. DRY MATTER YIELD, P UPTAKE AND PERCENTAGE OF P DERIVED FROM FERTILIZER IN STRAW AND GRAIN OF RICE PLANTS GROWN UNDER FIELD CONDITIONS

Genotypes	Dry matter (g polybag^{-1})		P (mg pot^{-1})		PdfF (%)	
	Straw	Grain	Straw	Grain	Straw	Grain
BG1334A	63.94 a	9.10 b	60.4 a	12.1 bc	31.4 ab	17.3 b
WR100	51.66 a	13.28 a	47.7 ab	21.6 a	42.6 ab	51.9 ab
BG582	55.61 a	7.46 b	50.2 ab	11.6 bc	31.5 ab	30.4 ab
NB773	57.90 a	8.34 b	49.6 ab	12.9 bc	42.1 ab	34.1 ab
KB1084	55.93 a	9.55 b	46.3 ab	15.0 b	40.9 ab	42.1 ab
KN749	48.37 a	8.15 b	41.7 ab	12.3 bc	21.5 b	30.7 ab
TE1211	57.88 a	9.63 b	45.6 ab	12.7 bc	42.3 ab	49.8 ab
BG753	53.83 a	7.72 b	42.6 ab	8.9 c	51.8 ab	30.3 ab
BG1249	54.66 a	8.30 b	33.7 b	10.6 bc	60.6 a	58.7 a

Data within a column followed by the same lower case letter are not significantly different (P<0.05)

TABLE 14. AMOUNT OF P DERIVED FROM FERTILIZER IN STRAW AND GRAIN AND P USE EFFICIENCY OF RICE PLANTS GRPOWN UNDER FIELD CONDITIONS

Genotypes	PdfF (mg pot^{-1})			P use efficiency
	Straw	Grain	Total	(%)
BG1334A	17.2 a	2.4 b	20.4 a	2.45 a
WR100	19.5 a	11.4 a	30.9 a	3.71 a
BG582	173 a	3.6 b	20.9 a	2.50 a
NB773	23.8 a	4.4 b	29.0 a	3.49 a
KB1084	19.3 a	6.8 ab	26.2 a	3.14 a
KN749	12.1 a	5.0 b	17.1 a	2.05 a
TE1211	26.1 a	7.1 ab	33.2 a	4.00 a
BG753	28.3 a	2.0 b	30.8 a	3.70 a
BG1249	28.6 a	5.7 b	34.3 a	4.12 a

Data within a column followed by the same lower case letter are not significantly different (P<0.05)

4. DISCUSSION

Plant root systems are highly plastic in their development and can adapt their architecture in response to prevailing environmental conditions [10, 11]. In *Arabidopsis*, it has been shown that uniformly high nitrate (10 mM) suppresses lateral development, while plants grown at low levels of nitrate (10 μM), and when a section of the primary root was exposed to high nitrate levels, lateral root production was stimulated specifically in that area. The main effect of nitrate appears to be on the rate of lateral root elongation rather than on lateral root initiation, while the elongation rate of the primary root is identical on 10 μM and 10 mM nitrate, and the metabolism of nitrate is apparently not necessary for the architectural changes [10]. Since metabolism of nitrate is not required for the root architectural changes, the differences observed in the root length of the upland rice landraces shown must be due to their genetic differences.

The N content in grain and straw obtained in our studies were similar to those obtained in upland rice varieties in Brazil [12]. Harvest indices (HI) (grain yield / total biomass yield) were also similar to the traditional upland rice variety from Northern Laos [13]. Fertilizer nitrogen use efficiency for these varieties tested ranged between 40 and 80%, which is very high, considering that efficiency value between 23-30% was reported for upland rice when 300 kg–N ha^{-1} was applied [14].

It has been mentioned that rooting depth is one of the root characteristics that determines the ability of a crop to intercept N, particularly NO_3^- during periods of leaching [15]. Since upland rice is grown under aerobic conditions, most of the N will be in the form of NO_3^-, and is liable to be leached under heavy rainfall conditions of the tropics. Thus varieties capable of producing long seminal roots will be potentially capable of producing deep roots. In this study, we did not find any positive relationships between root characteristics taken at two weeks of age with all the agronomic characteristics of the plants grown in the greenhouse or in the field.

5. CONCLUSION

WARDA lines showed significantly longer root length compared to local upland land races at 2 weeks old using the cigar-roll method, but no relationships were found between these characteristics and the agronomic characteristics of the plants when they were grown in the greenhouse or field conditions. Nitrogen fertilizer use was high in the lines tested, while P use was similar to other crops.

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BIOLOGICAL NITROGEN FIXATION EFFICIENCY IN BRAZILIAN COMMON BEAN GENOTYPES AS MEASURED BY ^{15}N METHODOLOGY

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Abstract

Common bean (*Phaseolus vulgaris* L.) represents the main source of protein for the Brazilian and other Latin-American populations. Unlike soybean, which is very efficient in fixing atmospheric N_2 symbiotically, common bean does not dispense with the need for N fertilizer application, as the biologically fixed N (BNF) seems incapable to supplement the total N required by the crop. A experiment under controlled conditions was conducted in Piracicaba, Brazil, to assess N_2 fixation of 25 genotypes of common bean (*Phaseolus vulgaris* L.). BNF was measured by ^{15}N isotope dilution using a non-N_2 fixing bean genotype as a reference crop. The common bean genotypes were grown in low (2.2 mg N kg^{-1} soil) or high N content soil (200 mg N kg^{-1} soil), through N fertilizer application, as urea-^{15}N (31.20 and 1.4 atom % ^{15}N, respectively). The bean seeds were inoculated with *Rhizobium tropici* CIAT 899 strain and the plants were harvested at grain maturity stage. The contribution of BNF was on average 75% of total plant N content, and there were differences in N fixing capacity among the bean genotypes. The most efficient genotypes were Horizonte, Roxo 90, Grafite, Aporé and Vereda, when grown in high N soil. None of the genotypes grown in low N soil was efficient in producing grains compared to those grown in high N soil, and therefore the BNF was not able to supply the total N demand of the bean crop.

1. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the main source of protein for the Brazilian population. Nitrogen (N) is the nutrient taken up in larger amounts by the bean plant, and N supplied as fertilizer is expensive and easily lost by volatilization or leaching [1, 2]. Approximately 50% of total N uptake is exported in the grain and the remainder stays in the soil in the form of crop residues [1, 3].

Maximizing the use of N by bean is important because of the economic and environmental aspects, as this nutrient presents risk to the environment as potentially contaminating groundwater [4] due to leaching of nitrate. Moreover, it is observed in field that it is possible to achieve bean yields above 2500 kg ha^{-1} according to the BNF process without N addition [5, 6]. However, inoculants are used in only 2–3% of the acreage of common bean [7]. As the ability for nodulation varies with the common bean genotypes and

Rhizobium strain, the nodulation efficiency is highly dependent on the genotype of common bean [8].

The objective of this study was to compare the common bean's ability for effective BNF, evaluated using the ^{15}N dilution technique, for 25 common bean genotypes grown under controlled conditions with no limitation to BNF (light, temperature, water, nutrients, and efficient rhizobium strain association with common bean).

2. MATERIAL AND METHODS

2.1. Experimental

The experiment was conducted in the greenhouse at the Center for Nuclear Energy in Agriculture (CENA / USP), located at latitude 22°42'30" S, longitude 47°38'01" W and 554 m altitude, in Piracicaba, Sao Paulo, Brazil.

The study were performed in 3.0 l plastic pots, containing 2.5 kg of air-dried soil, collected from the 0 to 0.20 m layer of a dystrophic Typic Haplustox [9]. The soil had 280, 70 and 650 g kg^{-1}content of clay, silt and sand, respectively, and the following chemical characteristics: pH (0.01 mol l^{-1} $CaCl_2$), 4.5; organic matter, 18.0 g dm^{-3}; P extracted by resin, 5 mg dm^{-3}; K, 0.6 $mmol_c$ dm^{-3}; Ca, 11.5 $mmol_c$ dm^{-3}; Mg, 5.2 $mmol_c$ dm^{-3}; H + Al, 35.4 $mmol_c$ dm^{-3}; CEC, 52.7 $mmol_c$ dm^{-3}; sum of bases, 17.3 $mmol_c$ dm^{-3}; base saturation, 32.8%, according to methodology described by [10] and P by Mehlich-1, 3 mg dm^{-3} [11].

After application of lime (calcium carbonate equivalent = 110%) to raise the base saturation to 70% for the common bean, according to the official recommendation of the Bulletin 100 [12], the soil was incubated for 30 days and the moisture content was maintained at approximately 70% of water holding capacity.

The experimental design was a randomized complete block with four replications. The experiment consisted of 52 treatments, which were divided into two groups. Each group had 26 treatments, which consisted of 25 common bean genotypes (Aporé, BRS Grafite, BRS Horizonte, BRS Pitanga, BRS Vereda, BRSMG Pioneiro, Carioca 80, CNF 10, FT Nobre, IAC Tybatã, IAC UNA, Jalo precoce, LP 01-38, Ônix, Ouro Negro, Pontal, Rosinha, Roxão EEP, Roxinho, Roxo 90, Rubi, Rudá, Sangue de Boi, Thayú e Timbó) and a control plant (non-nodulating common bean).

The first treatment (group of 25 common bean genotypes and one standard plant species) was carried out to compare the BNF between genotypes in the absence of mineral-N. As the ^{15}N isotopic dilution method requires the labeling of soil N, a low rate of mineral N (2.20 mg kg^{-1} soil as ^{15}N-urea with high enrichment of 31.20 atom % ^{15}N was used. The second treatment was to compare the BNF by the same common bean genotypes used in the first group in the presence of mineral-N. N was applied at 200 mg kg^{-1} soil as ^{15}N-urea (1.41 atom % ^{15}N). To avoid inhibiting the nodulation of plants, the mineral N dose was split in two applications, 1/3 at sowing and the remaining portion at the V4 stage (issuance of the third trifoliate leaf).

The P and K fertilization was performed with application of 200 mg kg^{-1} soil of P as triple superphosphate and 200 mg kg^{-1} soil of K as potassium sulfate in all pots. Micronutrient fertilization was performed by application of a nutrient solution in all treatments at rates of 0.5 mg kg^{-1} of B, 1.5 mg kg^{-1} of Cu, 3.0 mg kg^{-1} of Fe, 2.0 mg kg^{-1} of Mn, 3.0 mg kg^{-1} of Zn and 0.1 mg kg^{-1} of Mo.

The experiment was conducted in summer under conditions of high intensity of light and temperature, in order not to limit the BNF. The seeds of the bean genotypes were inoculated with the strain of *Rhizobium tropici* CIAT 899 (SEMIA 4077), which is recommended commercially for common bean in Brazil. The inoculation was done three hours before sowing, at a rate of 500 grams of inoculants (109 viable cells g^{-1} peat) to 50 kg of seed plus 300 ml of 10% sugar solution (w: v) to improve its adherence to the seeds.

Five common bean seeds from each genotype and standard plant (non-nodulating bean genotype - NORH 54) were sown in each experimental, thinned to one plant per pot. Soil moisture was maintained at approximately 70% of water retention capacity during the experiment. Plants were harvested after the pod maturity stage and separated into roots, shoots (stems, leaves and bark of legumes) and grain. At harvest, the number of nodules per plant and fresh weight of nodules per plant were measured.

Plant samples were dried and ground in Wiley-type mill, passed through 10 mesh sieve and weighed on a precision analytical balance (five decimal) for ^{15}N isotope (atom %) and total N determination in a mass spectrometer (IRMS) interfaced with an elemental analyzer, according to methodology described by [13].

2.1. N calculations

N uptake was calculated according to the following equation:

TN = N x Sdm

Where:

TN = plant total N content (mg $plant^{-1}$);
N = plant N concentration (g kg^{-1});
Sdm = shoot dry mater (g $plant^{-1}$).

The equation of symbiotic fixed N is presented below [14].

$$\%\text{Npdfix} = 1 - \left[\frac{(\%\text{A}^{15}Nexcess)_{genotype}}{(\%\text{A}^{15}Nexcess)_{control}} \right]$$

Where:

% Npdfix: % N in the plant derived from the symbiotic fixation;
$(\%A\ ^{15}N\ excess)_{genotype}$: atom % ^{15}N excess in the nodulated common bean genotype;
$(\%A\ ^{15}N\ excess)_{control}$: atom % ^{15}N excess in the control plant (non-nodulating bean);

With the total N content in the SDM of common bean genotypes (TN, mg $plant^{-1}$), the amount of N in the plant derived from symbiotic fixation (QNpdfix) was calculated.

$$\text{QNpdfix} = \frac{(\%\text{Npdfix}).(\text{NA})_{\text{genotype}}.}{100}$$

Where:

QNpdfix: amount of N in the plant derived from symbiotic fixation (mg);
% Npdfix: % of N in the plant derived from symbiotic fixation;
$(TN)_{genotype}$: total N content in the Sdm of the common bean genotype (mg).

2.2. Statistical analysis

Cluster analysis of common bean genotypes was carried out with the SAS 9.1 - "Statistical Analysis System" [15] and SYSTAT version 10.2 software programs, using the UPGMA (unweighted pair group arithmetic average clustering). The cluster analysis was preceded by the standardization of data before the Euclidian distances calculation, as the studied variables presented different scales. After standardization all the variables were equally important in the determination of these distances. Final results of the groups were presented as dendrograms. Within the text, the symbol * preceding a genotype denotes the treatment fertilized with the higher rate of urea.

3. RESULTS AND DISCUSSION

With high urea application, the number of nodules = 0; shoot DM = 54.24 g plant^{-1}; N uptake in shoots = 307 mg plant^{-1}; N derived from fertilizer in shoots = 69.0%; N derived from BNF in shoots = 0%; N derived from soil in shoots = 31.0%; Recovery of urea N in shoots = 42.4%; Root DM = 15.76 g plant^{-1}; Grain DM = 3.83 g plant^{-1}; N uptake in grain = 141 mg plant^{-1}; number of pods = 20.5; number of grains = 40.3; N derived from fertilizer in grain = 69.4%; N derived from BNF in grain = 0%; N derived from soil in grain = 30.6%; Recovery of urea-N in grain = 19.7%; total shoot DM = Shoot DM + Grain DM = 58.07 g plant^{-1}; N uptake by total shoot DM = 449 mg plant^{-1}; N derived from BNF in total shoot DM = 0%; N derived from fertilizer in total shoot DM = 69.1%; Recovery of urea-N in total shoot DM = 62.1%; and N derived from soil in total shoot DM = 30.9%.

With low urea application, the number of nodules = 0; shoot DM = 12.40 g plant^{-1}; N uptake in shoots = 80.5 mg plant^{-1}; N derived from fertilizer in shoots = 2.5%; N derived from BNF in shoots = 0%; N derived from soil in shoots = 97.5%; Recovery of urea N in shoots = 36.4%; Root DM = 5.37 g plant^{-1}; Grain DM = 0.96 g plant^{-1}; N uptake in grain = 31.6 mg plant^{-1}; number of pods = 9.0; number of grains = 16,5; N derived from fertilizer in grain = 1.6%; N derived from BNF in grain = 0%; N derived from soil in grain = 98.4%; Recovery of urea-N in grain = 9.4%; total shoot DM = Shoot DM + Grain DM = 13.36 g plant^{-1}; N uptake by total shoot DM = 112 mg plant^{-1}; N derived from BNF in total shoot DM = 0%; N derived from fertilizer in total shoot DM = 0.5%; Recovery of urea-N in total shoot DM = 45.9%; and N derived from soil in total shoot DM = 99.5%.

The N uptake from BNF in total shoot DM of 25 common bean genotypes at low or high application of urea, correlated significantly and positively with shoot DM (0.656 ***), grain DM (0.699 ***) and root DM (0.493 ***). This increase in production of DM of shoots, roots and grain indicates that plants responded both in grain production as well as shoots and roots biomass to N supplied by the BNF. Considering these three variables, the level of homogeneity of 1.0 in the Euclidean distance, we observed the formation of the following seven distinct homogeneous groups as shown in Fig. 1.

1st: Jalo Precoce, Grafite, Roxão EEP and Rubi;
2nd: *Roxão EEP and *Jalo Precoce;
3rd: Sangue de Boi, CNF 10, Ônix, Pontal, Roxo 90, Horizonte, Thayú, Pitanga, IAC UNA, FT Nobre, Aporé, LP 01-38, Ouro Negro, Pioneiro, Rudá, Carioca 80, Rosinha, Tybatã, Roxinho and Vereda;
4th: Timbó;
5th: *Carioca 80, *FT Nobre, *Roxo 90, *Ouro Negro, *Rosinha, *Pontal, *Pioneiro, *CNF 10, *Rudá, *Rubi, *Pitanga, *Aporé, *Horizonte, *Grafite, *Sangue de Boi, *LP01-38, *Ônix and *Tybatã;

6th: Vereda, *Roxinho, *IAC UNA and *Thayú; and
7th: *Timbó.

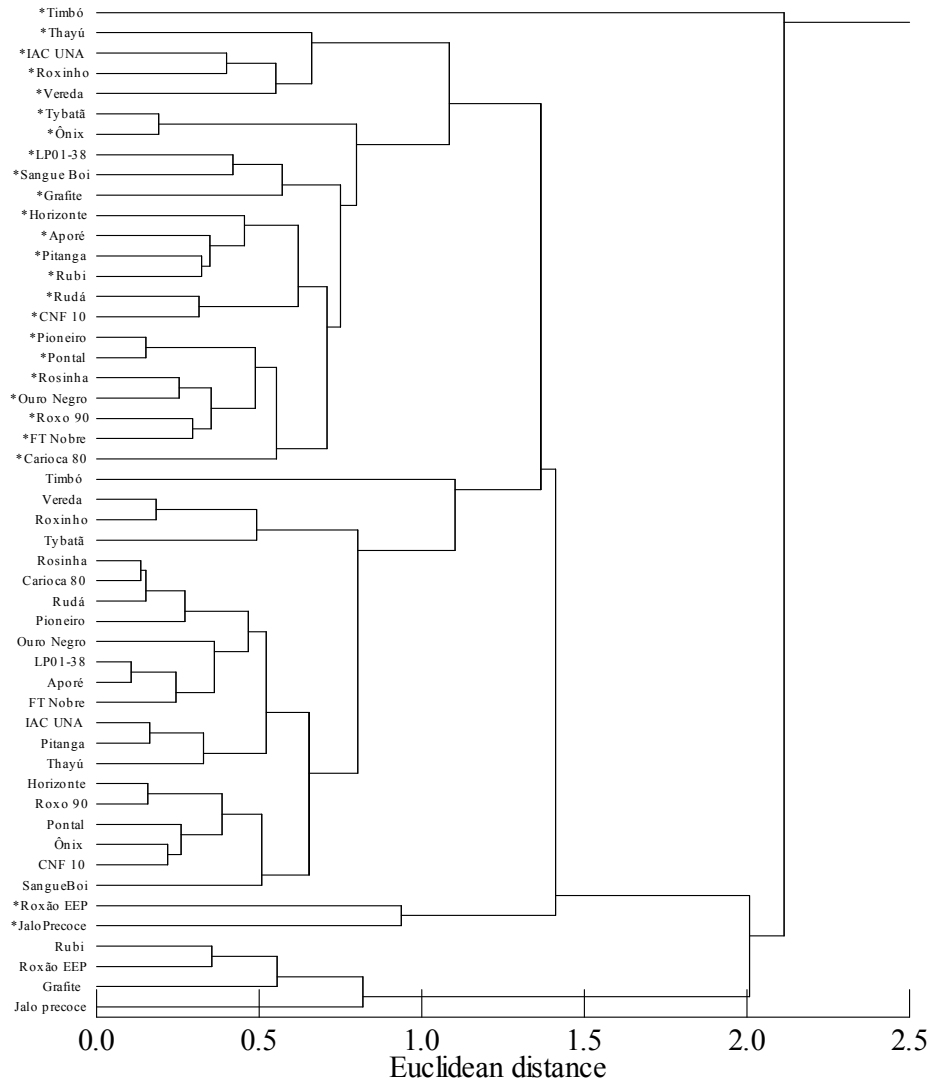

*FIG. 1. Dendrogram resulting from hierarchical cluster analysis of 25 genotypes of common bean, based on DM (g) of shoots, roots and grain and N in total shoot DM derived from BNF. * denotes genotypes that received high urea fertilization.*

There was a response in grain production, and shoot and root DM to BNF, even in genotypes fertilized with the higher rate of urea, and this productive response varied among genotypes (Fig. 1). The genotypes, in association with rhizobium, which most benefited from BNF and produced more DM of shoots roots and grain were Vereda, Roxinho, IAC UNA and Thayú, in the treatment with higher rate of N fertilization (6th group). The BNF contributed around 70% of total N in these genotypes, even with the higher addition of urea-N; it shows that, depending on the bean genotype, BNF can contribute with high amounts of N_2 fixation, even in N fertilized plants. Moreover, considering the seven groups of genotypes, there was no genotype in the treatments with low and high fertilizer N classified in the same group. Therefore, the genotypes that depended only on soil N and BNF as N sources for development were not as efficient in terms of shoot, root and grain DM production, as those receiving a higher rate of N fertilizer. On average, considering the 25 bean genotypes, fertilized with low or high urea, BNF contributed approximately 75% of total N absorbed by the plants.

The number of plant nodules correlated positively with the fresh weight of nodules (0.704 ***), the total N content in shoot DM (0.367***) and with the total plant N derived from BNF (0.350***). Although significant, the correlation coefficients of the number and fresh weight of nodules with total plant N and N uptake in the plant from BNF were relatively low. The number and fresh weight of nodules had coefficients of variation of 18.2 and 20.2%, respectively (Table 1).

TABLE 1. NODULE FRESH WEIGHT AND NUMBER, N UPTAKE OF SHOOTS AND N DERIVED FROM BNF OF 25 GENOTYPES OF COMMON BEAN AT LOW (+) OR HIGH (++) UREA APPLICATION

Genotype	Nodule fresh weight (mg plant^{-1})		Nodule number		N uptake (mg)			
					Total in shoots		Derived from BNF	
	+ urea	++ urea	+ urea	++ urea	+ urea	++ urea	+ urea	++ urea
Carioca 80	0.59	0.52	162	173	968	1412	855	1048
Rudá	1.03	0.66	171	107	955	1271	840	859
Aporé	1.34	2.22	139	227	853	1405	755	914
Pontal	1.82	1.28	195	199	855	1358	729	885
Horizonte	1.83	3.09	191	242	844	1403	720	907
Pioneiro	0.76	0.95	114	130	909	1401	792	889
Rosinha	0.84	1.05	155	212	954	1358	854	909
Rubi	0.95	1.62	136	163	680	1321	565	828
Vereda	1.51	2.11	173	248	1158	1560	1028	1129
Tybatã	1.40	1.53	158	155	944	1456	837	936
CNF 10	1.04	0.81	157	145	894	1276	771	742
Roxão EEP	1.03	0.97	141	113	571	1005	446	539
Sangue de Boi	0.84	0.73	126	115	700	1332	584	838
Roxinho	1.25	0.86	135	135	1096	1628	967	1097
Timbó	0.66	0.68	108	102	1048	1387	935	969
Roxo 90	1.49	2.02	147	301	837	1399	723	906
Pitanga	1.47	1.43	158	149	1050	1369	928	925
Ouro Negro	1.53	1.30	171	149	969	1424	838	905
Ônix	1.41	1.08	142	137	851	1415	736	944
IAC UNA	0.80	1.06	153	168	1045	1628	888	1159
Jalo precoce	0.77	1.19	133	171	404	1238	256	678
Grafite	1.21	1.93	139	246	540	1251	433	834
Thayú	1.27	1.72	157	268	1128	1821	1006	1351
FT Nobre	1.36	1.14	124	145	930	1532	772	1015
LP01-38	1.45	1.56	154	157	873	1273	740	741
Average	1.26		162		1140		839	
CV (%)	20.2		18.2		12.7		13.6	

Considering these four variables, the level of homogeneity in the Euclidean distance of 1.0 (Fig. 2), seven homogeneous groups were obtained:

1st: Jalo Precoce, Grafite, Roxão EEP, Rubi, Sangue de Boi and *Roxão EEP;
2nd: Horizonte and Pontal;
3rd: Pitanga, Tybatã, Ouro Negro, LP01-38, Roxo 90, Ônix, Aporé, FT Nobre, CNF 10, Rudá, IAC UNA, Rosinha, Carioca 80, *CNF 10, *Jalo Precoce, *Rosinha, *Pontal, *LP01-38, *Rubi, Roxinho, Thayú, Vereda, *Tybatã, *Pitanga, *Ouro Negro, *FT Nobre, *Ônix, *Pioneiro, *Timbó, *Rudá, *Sangue de Boi, Timbó and Pioneiro;
4th: *Carioca 80, *Roxinho and *IAC UNA;
5th: *Thayú;
6th: *Roxo 90, *Grafite, *Aporé and *Vereda; and

7th: *Horizonte.

Among these groups, the 6th and 7th groups, formed by the genotypes *Horizonte, *Roxo 90, *Grafite, *Aporé and *Vereda stood out, especially on nodulation and N accumulation (Table 1). These five common bean genotypes with highest nodulation were grown with the high N rate.

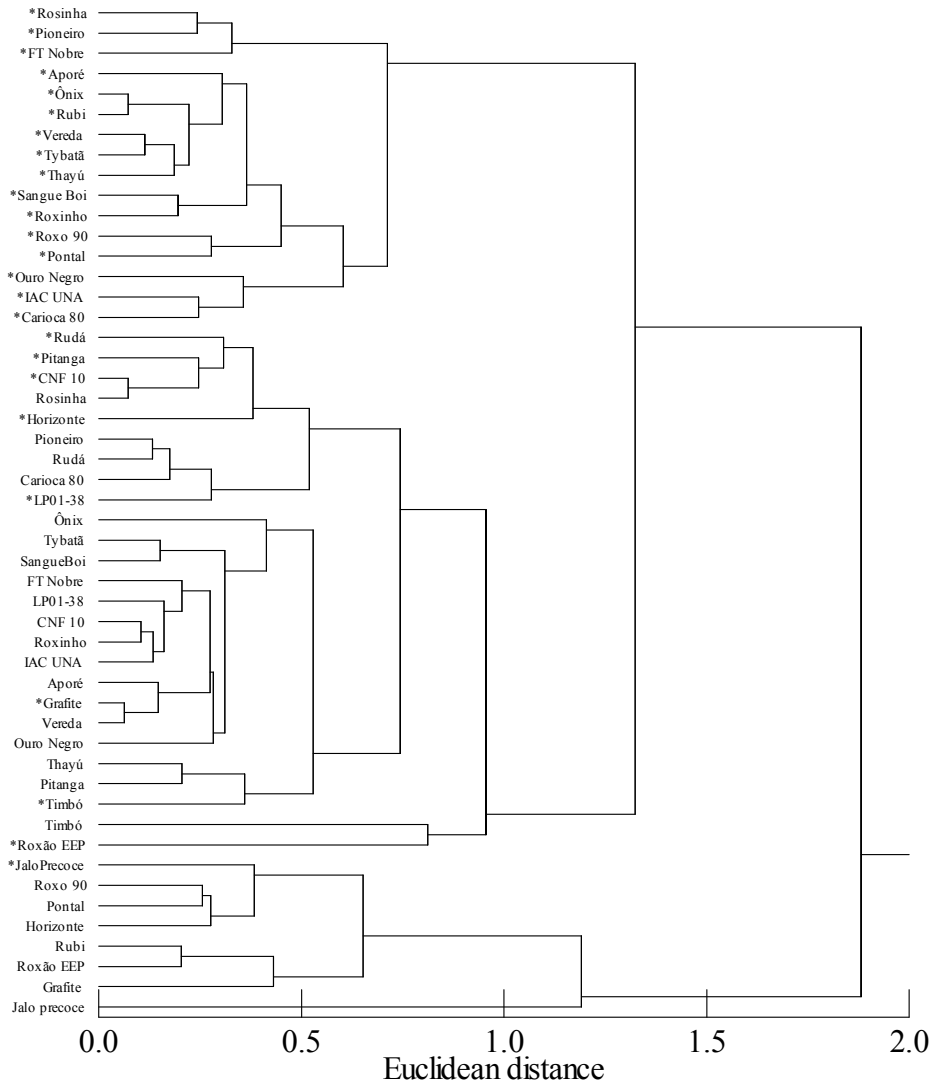

*FIG. 2. Dendrogram resulting from hierarchical cluster analysis of 25 genotypes of common bean based on number and fresh weight of nodules, accumulation of N (mg) in total shoot dry matter and N in total shoot DM derived from BNF. * denotes genotypes that received high urea fertilization.*

Considering the edible portion and economic interest of the crop, it was observed that the grain DM of common bean genotypes, fertilized with a low or high rate of urea-N, correlated significantly and positively with the number of pods (0.824***) and the number of grains (0.878***). The number of pods and seeds per plant were highly and positively correlated with grain production. The number of pods is one of the most important in increasing the production of common bean [16].

1st: (low productive group) formed by treatment Jalo Precoce;
2nd: (moderately productive group) by treatments Grafite, Roxão EEP, Rubi, Horizonte, Pontal, Roxo 90 and *Jalo Precoce;
3rd: (moderately to very productive group) by treatments *Roxão EEP, Timbó, *Timbó, Pitanga, Thayú, Ouro Negro, Vereda, *Grafite, Aporé, IAC UNA, Roxinho, CNF 10, LP01-38, FT Nobre, Sangue de Boi, Tybatã, Ônix, *LP01-38, Carioca 80, Rudá, Pioneiro, *Horizonte, Rosinha, *CNF10, *Pitanga and *Rudá; and the
4th: (very productive group) by the treatments *Carioca 80, *IAC UNA, *Ouro Negro, *Pontal, *Roxo 90, *Roxinho, *Sangue de Boi, *Thayú, *Tybatã, *Vereda, *Rubi, *Onix, *Aporé, *FT Nobre, *Pioneiro and *Rosinha.

From grain DM, number of pods and number of grains (Table 2), in the level of homogeneity of 1.0 in the Euclidean distance dendrogram (Fig. 3), we observed the formation of four homogeneous groups:

TABLE 2. MEAN DRY MATTER YIELD OF SHOOTS, ROOTS AND GRAIN, NUMBER OF PODS AND GRAINS OF 25 GENOTYPES OF COMMON BEAN AT LOW (+) OR HIGH (++) UREA APPLICATION

Genotype	Dry matter yield (g plant^{-1})						Number plant^{-1}			
	Shoot		Root		Grain		Pods		Grain	
	+ urea	++ urea	+ urea	++ urea	+ urea	++ urea	+ urea	++ urea	+ urea	++ urea
Carioca 80	20.0	29.4	7.3	9.6	28.3	32.7	20.0	24.3	108.0	122.3
Rudá	18.7	26.1	6.5	8.3	27.8	29.4	19.0	23.3	115.7	150.3
Aporé	17.6	35.5	5.4	11.1	24.5	28.1	19.0	27.7	95.7	172.7
Pontal	17.0	35.3	7.8	9.5	20.1	33.7	11.7	24.0	74.7	160.7
Horizonte	20.0	38.9	6.0	9.2	17.3	25.5	12.0	21.3	66.7	132.3
Pioneiro	18.8	34.7	5.3	8.8	28.4	35.2	20.0	31.3	121.3	174.3
Rosinha	20.8	34.5	6.6	11.3	29.3	35.9	22.7	32.3	127.7	189.0
Rubi	14.6	32.8	4.8	9.5	15.0	29.7	11.0	28.7	59.0	158.7
Vereda	23.7	42.8	10.6	16.4	24.8	30.1	18.0	27.0	105.7	160.0
Tybatã	21.2	45.2	9.3	12.4	22.4	29.5	17.7	26.3	104.0	154.7
CNF 10	16.8	26.3	7.2	8.0	23.4	28.9	16.0	22.7	94.3	131.7
Roxão EEP	13.2	27.2	5.3	10.8	13.3	21.0	11.7	26.7	50.7	117.7
Sangue de Boi	14.5	30.6	7.1	14.1	20.7	31.0	17.3	30.0	103.3	161.0
Roxinho	23.2	36.3	10.5	15.3	23.7	32.2	16.7	30.3	99.7	172.0
Timbó	31.9	43.8	9.3	21.2	15.7	19.8	22.3	21.7	81.7	100.3
Roxo 90	19.8	33.5	5.6	13.2	19.2	33.2	14.3	27.0	72.7	161.7
Pitanga	21.3	30.7	5.6	9.6	22.6	27.6	21.7	24.7	106.3	136.0
Ouro Negro	18.9	30.1	3.8	11.9	26.2	35.5	17.0	26.7	87.7	135.0
Ônix	19.6	41.9	6.9	12.2	21.8	28.8	13.3	28.7	94.3	159.3
IAC UNA	21.4	35.6	6.1	12.6	24.0	32.3	17.3	24.7	92.0	138.7
Jalo precoce	10.3	33.8	5.3	16.4	7.0	18.2	5.3	15.7	21.3	56.0
Grafite	14.8	35.4	8.1	14.6	10.4	24.9	8.7	18.3	48.0	102.0
Thayú	21.7	38.5	6.9	14.4	24.9	30.5	21.7	27.0	107.3	146.3
FT Nobre	19.6	33.4	5.4	12.7	21.9	33.5	15.7	29.3	93.0	187.3
LP01-38	17.9	30.9	4.7	11.9	24.5	29.2	15.3	18.7	93.0	99.0
Average	26.8		9.4		25.5		20.9		116.1	
CV (%)	11.9		15.2		12.9		13.7		14.5	

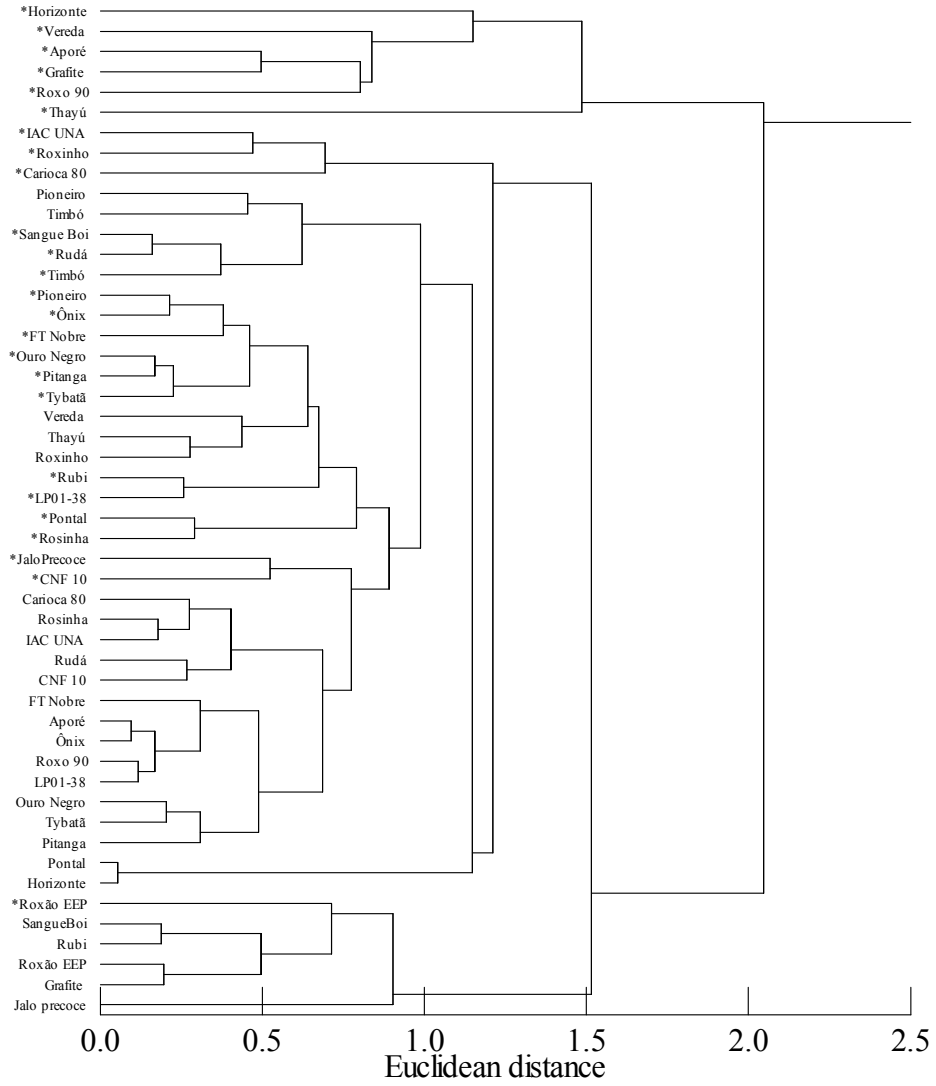

*FIG. 3. Dendrogram resulting from hierarchical cluster analysis of 25 genotypes of common bean based on grain dry matter, number of pods and number of grains. * denotes genotypes that received high urea fertilization.*

Among the 25 common bean genotypes in the treatment with low N fertilizer, none were classified in the group of very grain productive. This indicates that BNF was not able to meet all the N demands of the plant N to achieve equivalent grain yields of the same genotypes that received urea-N fertilization. Common bean is considered to be a species with a low capacity for nodulation and BNF compared to other grain legumes [17]. Nevertheless, the BNF has contributed up to 90 kg–N ha^{-1} in various bean crops, which represented 40 to 50% of the demand of this crop [18]; in seven field experiments the observed average and maximum values were, respectively, 35 and 70% of N in the plant from the atmosphere in common bean genotypes [19].

The efficiency of bean BNF depends, among other factors, on the genotype. The selection of genotypes more efficient in symbiosis with rhizobia is an alternative to reduce N fertilization. For example, in field studies, fertilization with 20 kg–N ha^{-1}, together with inoculant strain of *R. tropici* CIAT 899 permitted common bean to yield higher than 3000 kg ha^{-1}, equivalent to the application of 160 kg–N ha^{-1} [6]. In addition, one must consider that the success of inoculation with strains of bean rhizobia with high efficiency is associated with

competitive ability of such strains and adaptation to environmental conditions [20]. Under appropriate environmental conditions, the atmospheric N_2 fixed by symbiosis can meet most of the N needs of common bean [21]. However, some soil conditions such as low pH and high concentrations of Al often limit all stages of root infection, nodule formation and assimilation of N by the plant [22].

Studies under field conditions have shown that it is possible to achieve bean yields above 2500 kg ha^{-1} by the BNF process without addition of mineral N fertilizer [5, 6]. It is noteworthy, however, that the energy used in BNF is ATP; the photosynthates are important to the process of BNF for the N_2-fixing organisms, because they generate reducing power and ATP for the nitrogenase system, are substrates for growth and maintenance of microbial cells and supply carbon skeletons, ATP and reducing power for the assimilation of NH_3 [23]. Therefore, environmental conditions or management practices that influence the availability of photosynthates also affect BNF. The bean growing season (winter/summer), for example, influences the availability of photosynthates, and thus the BNF.

4. CONCLUSIONS

- Common bean genotypes differ in their ability to fix N_2 from the atmosphere through BNF.

- Among 25 common genotypes, Horizonte, Roxo 90, Grafite, Aporé and Vereda were the most efficient in BNF, when grown in the presence of urea fertilizer;

- Under controlled conditions (no limitation of temperature, water and nutrients), only with BNF and soil as sources of N for plants (without fertilization with urea), common bean genotypes cannot produce grain, shoot or root dry matter equal to those fertilized with urea.

ACKNOWLEDGMENTS

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GENOTYPIC VARIATION OF EARLY MATURING SOYBEAN GENOTYPES FOR PHOSPHORUS UTILIZATION EFFICIENCY UNDER FIELD GROWN CONDITIONS

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Abstract

Variability in the utilization of phosphorus (P) by 64 early-maturing soybean (*Glycine max* L. Merr.) genotypes under low-P soil conditions were evaluated in 2009 and 2010 at Shika, Nigeria. Fifteen phenotypic variables; number of nodules, nodule dry weight, grain yield, plant biomass, total biomass, biomass N and P content, Phosphorus Utilization Index (PUI), shoot P Utilization efficiency (PUIS), grain P Utilization efficiency (PUIG), Harvest Index (HI), Biological N fixed (BNF), total N fixed and N and P uptake were measured. The four clusters revealed by cluster analysis were basically divided along (1) plant biomass and uptake, (2) nutrient acquisition and utilization and (3) nodulation components. Three early maturing genotypes, TGx1842-14E, TGx1912-11F and TGx1913-5F, were identified as having high P utilization index and low P uptake. These genotypes could be a potential source for breeding for P use efficiency in early maturing soybean genotypes.

1. INTRODUCTION

Soybean (*Glycine max* L. Merr.) is an important source of protein to many households in West Africa. It is an emerging grain legume in the farming systems in the West African savanna region, and is considered to contribute to the N economy of the soil through biological N_2 fixation (BNF) with indigenous rhizobia populations. It has been stated that more than 25% of its total shoot N content can come from BNF [1]. Critical to the optimal growth of soybean and BNF is the adequate supply of P to the crop, but P is one of the most limiting nutrients in the West African savanna region because of the kaolinitic soil properties of the area. This problem results in reduced plant growth and final grain yield [2]. It has been observed that soybean response to low P condition varies with different genotypes [3], and some mechanisms have been attributed to the differential response exhibited by various genotypes. In alleviating the problem of the low P condition in the soil, maximum potential of

adaptation of the plants with a larger relative capacity in the absorption and use efficiency of the P will be a good alternative.

Most breeding programmes for P efficiency have had their major focus on P acquisition efficiency. Most crops have relatively efficient P uptake capacity but low P translocation and remobilization, and hence PUE becomes a significant bottleneck for further improvements in crop P efficiency [4]. It is therefore imperative that research programmes are initiated and implemented with the objective of identifying P utilization efficient genotypes within existing gemplasm.

Because of the problem associated with P availability in the soils of the tropical region and the high cost of P fertilizers, it is pertinent to develop varieties that are early maturing and are able to efficiently use P even under low P soil conditions. Screening of large accessions of soybean for P use efficiency is one of the activities of the IITA in Nigeria. The screening can help to identify various genotypes that have better P use efficiency and use them in the breeding program. The objective of this study was to evaluate the genetic variation of 65 early maturing soybean genotypes for P utilization efficiency.

2. MATERIALS AND METHODS

2.1. Location

Field experiments were conducted at Shika (11^{o} 13' N, 7^{o} 12'E) in 2009 and 2010. Shika is located in the Northern Guinea savanna and has a unimodal rainfall pattern with about 1100 mm rainfall per annum. The soil type is a ferric lixisol [5]. Fields were cleared, ploughed, and ridges spaced 75 cm apart were constructed with a tractor-mounted ridge. The plot size was 2.25 m^2 (three rows per plot, a 1.0 m alley between plots, 1.5 m between replications). The plots were supplied with P at the rate of 20 kg P ha^{-1} TSP which was applied by broadcasting, and incorporated into the soil with hand hoes before planting of the 64 soybean genotypes (Table 1) two days after in both years. The experimental design was Randomized Complete Block (RCB). Each soybean genotype treatment was replicated three times. The soybean genotypes were obtained from the germplasm collection of IITA, Nigeria.

2.2. Planting

Soybean was sown along the ridges and thinned to two plants per hole at a spacing of 12.5 cm with 0.75 cm between rows, 2 weeks after planting (WAP). The plots were weeded manually using hoes at 3, 6 and 10 WAP. Sampling was carried out at 50% flowering for BNF, at pod-filling for P-use efficiency, and at harvest maturity for grain yield. At the first sampling (50% flowering), four soybean plants were carefully dug out from the 0.5 m portion of the two central rows. To minimize damage to the root system, the soil around the plant was loosened using forks. Plant samples were processed for number and dry weight of nodules, and measurement of N_2-fixation using the hot water extraction method [6–8]. The ground stem and petiole of the shoot was passed through 1 mm sieve, after which the sample was extracted with 25 ml boiling water for 2 min. The extract was filtered, made up to volume (50 ml), and stored at -15^{o}C to be analysed later for ureides and % N derived from the atmosphere (% Ndfa).

Harvested plant samples were chopped into 10- to 20-mm pieces and sub-sampled with about 200 g fresh weight being oven-dried at 70 C before grinding to pass through a 0.5 mm sieve for measurement of N and P concentrations. For the last harvest, plant samples were separated into reproductive (grains) and vegetative parts (shoots) after oven drying. The

grains were threshed from the pods and further dried. Plant shoots, nodules and straw were dried in the oven at 70 ºC to a constant weight before weighing. Total N in the grains, shoots and straw was determined by the Kjeldahl procedure [9]. Phosphorus concentration in tissues was determined using the procedures described by [10].

2.3. P utilization efficiency

Phosphorus Utilization Index (PUI) was determined as the inverse of nutrient (P) concentration in the biomass. Grain P utilization efficiency (PUIG) was determined as expressed by [11, 12), while shoot P utilization efficiency (PUIS) were determined as expressed by [13].

PUIG = Grain yield x PUI

PUIS = Harvest index x PUI

2.4. Statistical analysis

Combined analyses of variance were conducted for eleven variables measured in 2009 and 2010 using the Generalized Linear Model (GLM) Procedure of the statistical analysis system (14). The cluster analysis was performed by the CLUSTER analysis of the GENSTAT package using the standardized data of the complete linkage method, which explained a greater proportion of the variation in the set of data than other methods. Data were subjected to Genotype × Trait (GT) analysis [14, 15] to identify cultivars that were superior with respect to selected traits. The data were not transformed ('Transform = 0'), standardized (Scale = 1) and were trait centered (Centering = 2). The trait values were standard deviation-standardized because traits were measured in different units. The polygon and the vector views of the GT biplot were constructed using all measured traits, and were based on genotype-focused singular partitioning ('SVP = 2), which rendered them appropriate for visualizing the relationship among traits. The GT biplot analysis was carried out using GGE Biplot, a Windows application that fully automates biplot analysis [15, www.ggebiplot.com). The GGE bipplot model 2 equation used is

$$(\hat{Y}_{ij} - \mu - \beta_j)/ d_j = \lambda_1 g_{i1} e_{1j} + \lambda_2 g_{i2} e_{2j} + \varepsilon_{ij}$$

where:

Y_{ij} is the genetic value of the combination between inbred i and trait j;

μ is the mean of all combinations involving trait j;

βj is the main effect of trait j;

$\lambda 1$and $\lambda 2$ are the singular values for PC1 and PC2;

gi1 and gi2 are the PC1 and PC2 eigenvectors, respectively, for inbred i;

e1j and e2j are the PC1 and PC2 eigenvectors, respectively, for trait j;

dj is the phenotypic standard deviation; and

εij is the residual of the model associated with the combination of inbred i and trait j.

TABLE 1. THE 64 GENOTYPES OF SOYBEAN EVALUATED AT SHIKA BETWEEN 2009 AND 2010

Genotype	Genotype
TGx1019-2EN	TGx1911-9F
TGx1485-1D	TGx1912-11F
TGx1740-2F	TGx1912-12F
TGx1805-8F	TGx1912-1F
TGx1830-20E	TGx1912-2F
TGx1834-1E	TGx1912-3F
TGx1835-10E	TGx1912-6F
TGx1842-14E	TGx1912-9F
TGx1871-12E	TGx1913-5F
TGx1876-4E	TGx1914-11F
TGx1878-12E	TGx1914-17F
TGx1880-3F	TGx1914-2F
TGx1888-29F	TGx1914-4F
TGx1892-10F	TGx1917-1F
TGx1893-10F	TGx1918-1F
TGx1895-19F	TGx1918-2F
TGx1895-22F	TGx1918-3F
TGx1895-23F	TGx1918-5F
TGx1895-33F	TGx1920-1F
TGx1895-50F	TGx1921-2F
TGx1895-6F	TGx1921-6F
TGx1902-1F	TGx1921-7F
TGx1903-11F	TGx1922-1F
TGx1903-13F	TGx1923-4F
TGx1903-1F	TGx1904-4F
TGx1903-2F	TGx1904-5F
TGx1903-3F	TGx1904-6F
TGx1903-4F	TGx1908-6F
TGx1903-7F	TGx1909-2F
TGx1903-8F	TGx1909-3F
TGx1911-7F	TGx1911-2F
TGx1911-8F	TGx1911-3F

3. RESULTS

Significant (P<0.05) genotypic variation was observed in grain yield, HI, total biomass at harvest, PUIG and PUIS (Table 2). Plant biomass, biomass P concentration, P uptake and PUI were, however, not significantly affected by the genotype. Grain yield ranged between 377 kg ha^{-1} in TGx 1985-19F and 1798 kg ha^{-1} in TGx 1880-3F. The PUI, PUIG, PUIS and HI ranged between 3.54 in TGx 1895-33 and 5.97 in TGx 1913-5F; 1561.35 in TGx 1895-19F and 8197.32 in TGx 1880-3F: 0.89 in TGx 1888-29F and 2.01 in TGx 1913-5F and 0.20 in TGx 1871-12E and 0.42 in TGx 1922-1F, respectively (Table 2). TGx 1880-3F was significantly (P<0.05) higher in grain yield and PUIG than 75% of other genotypes.

TABLE 2. SUMMARY OF THE STATISTICAL ANALYSIS OF BIOMASS AND GRAIN YIELD, P ACCUMULATION AND USE EFFICIENCY OF 64 GENOTYPES OF SOYBEANS EVALUATED AT SHIKA BETWEEN 2007 AND 2008

Statistics	Plant biomass (kg ha^{-1})	Grain yield (kg ha^{-1})	Total biomass (kg ha^{-1})	HI	P conc. (%)	P uptake (kg ha^{-1})	PUI	PUIG	PUIS
Mean	5300.1	1001.7	6301.7	0.31	0.24	13.0	4.35	4327.1	1.38
Minimum	3303.7	377.7	3987.1	0.20	0.19	7.40	3.54	1561.4	0.89
Maximum	7816.1	1797.9	8736.1	0.42	0.29	21.7	5.97	8197.3	2.01
SE	2022	247.6	2075	0.1	0.04	6.4	0.74	1282	0.39
F statistics for Genotype (G)	ns	***	*	***	ns	ns	ns	***	***
F statistics for Year (Y)	***	***	***	***	ns	***	ns	***	***
F statistics for G × Y	ns	***	ns	***	ns	ns	ns	ns	ns

Symbols *, **, *** denote P<0.05, P<0.01, P< 0.001, respectively; ns, not significant

The soybean genotypes did not significantly (P<0.05) vary in % Ndfa (Table 3). However, number of nodules, nodule dry weight and total N fixed were significantly (P< 0.05) influenced by the genotype. The %Ndfa ranged from 51.4% in TGx 1918-2F to 62.2% in TGx 1805-8F (Table 3). TGx 1740-2F had significantly (P<0.05) higher number of nodules than other genotypes except TGx 1911-9F, 1911-3F and 1903-3F. TGx 1911-9F, 1917-1F, 1921-2F and 1912-9F were the top four genotypes for total N fixed and N uptake among the 64 genotypes (Table 3).

TABLE 3. SUMMARY OF THE STATISTICAL ANALYSIS OF NODULATION, N ACCUMULATION AND FIXATION OF THE 64 GENOTYPES OF SOYBEAN EVALUATED AT SHIKA BETWEEN 2009 AND 2010

Statistics	No. of nodules (×10^7)	Nodule dry weight (kg ha^{-1})	Biomas N conc (%)	%Ndfa	Total N fixed (kg ha^{-1})	N uptake (kg ha^{-1})
Mean	1.24	97.0	2.82	55.9	82.7	144.5
Minimum	0.38	33.7	2.16	51.4	48.0	88.2
Maximum	3.09	158.9	3.34	62.2	135.5	224.1
SE	0.44	33.4	0.32	3.6	31.7	54.3
F statistics for Genotype (G)	***	***	**	ns	*	*
F statistics for Year (Y)	ns	***	***	***	***	***
F statistics for G × Y	***	***	ns	ns	ns	ns

Symbols *, **, *** denote P<0.05, P<0.01, P< 0.001, respectively; ns, not significant

The G × T biplot with a polygon view was used to identify genotypes that were superior with respect to some traits. The genotypes at each vertex of the polygon (vertex cultivar) possessed the highest values for traits found within its sector. The principal components PC1 and PC2 accounted for 58.8% of the total variation among the measured traits of the genotypes (Fig. 1). In the biplot view, TGx1918-5F was the vertex genotype in the sector that contained traits HI, grain yield, PUI, PUIS, PUIG and BNF, indicating that this genotype had the highest values for these traits among the 64 genotypes (Fig. 1). The genotype, TGx 1921-2F had the highest values for the number of nodules, total biomass and plant biomass, while TGx 1912-9F was the vertex genotypes for the sector that had nodule dry weight, N uptake and total N fixed. The plot showed that TGX1903-2F was the best cultivar for biomass P content and TGX 1892-10F for biomass N content (Fig. 1).

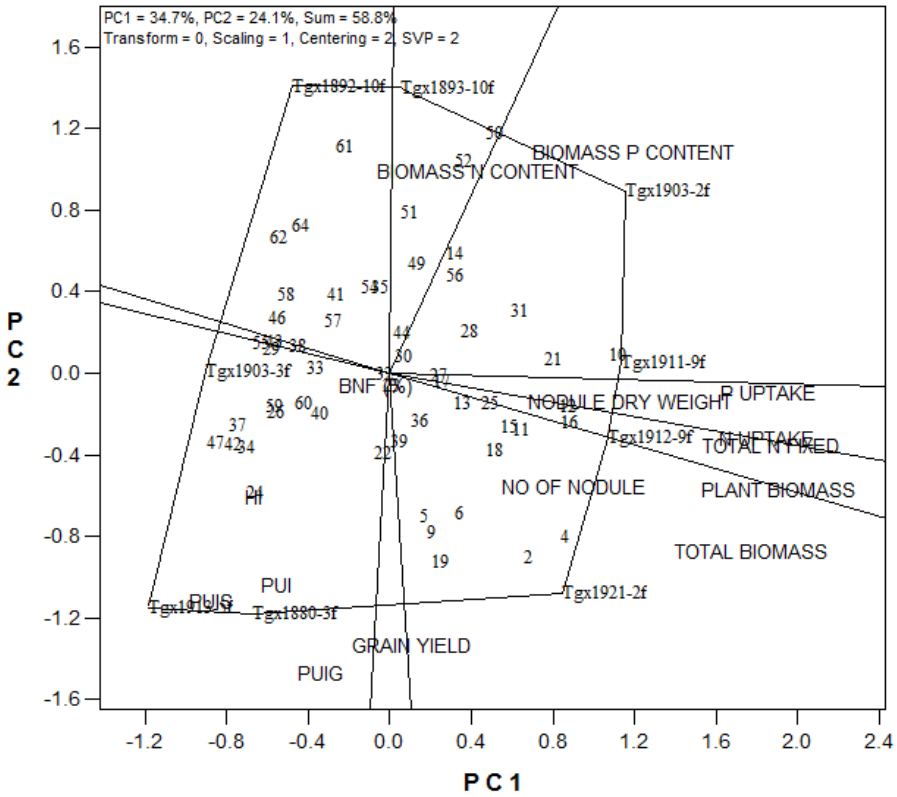

FIG. 1. Genotype × trait biplot of all traits of 64 early maturing soybean genoypes evaluated in Nigeria between 2009 and 2010. PUI = Phosphorus Utilization Index; PUIG = Grain P utilization efficiency; PUIS = shoot P utilization efficiency.

The interrelationships among the measured traits were displayed in a vector view biplot (Fig. 2), the rays connecting the traits to the biplot origin are referred to as trait vectors and the cosine of the angle between the vectors of any two traits measures the similarity between them. However, traits with shorter vectors are considered not strongly correlated with those with longer vectors and also were probably not strongly correlated with other short vector traits [14]. Therefore, even though BNF had acute angle (<90°) with HI, PUIS, PUI, PUIG and grain yield, its short vector suggested that it had weak positive correlation with those traits (HI, PUIS, PUI, PUIG and grain yield). The vector lengths of number of nodule and nodule dry weight were also relatively shorter than those of total biomass, plant biomass, total N fixed, N and P uptake and grain yield which had acute angles with it. Biomass N and P contents had longer vectors and were at angles >90° to PUI, PUIS, PUIG, HI and grain yield, indicating that they were negatively correlated with PUI and grain yield. Grain yield, PUIG and PUIS had acute angles between them with longer vectors, indicating they were positively correlated. Similarly, total biomass, plant biomass, N and P uptake and total N fixed were also positively correlated, except that PUIG, PUI and PUIS had angles approximately 90° with total N fixed, indicating a non significant correlation between PUI and total N fixed. PUI and biomass P content had an angle that is approximately 180^0 between their vectors, indicating a high negative correlation between the two variables. Similar high negative correlations were observed between the following trait pairs; Grain yield and biomass N concentration, PUIG and biomass P concentration, and PUIS and biomass P concentration. In general, the pattern of relationships among these traits is such that it is easy to classify them into three groups; Total biomass, plant biomass, N uptake, P uptake, and total N fixed all in one group; Biomass N concentration and biomass P concentration in the second group; and grain yield, PUI, PUIS

and PUIG in the third trait group. Other traits could be grouped into any other of the three groups because of the relatively short vectors, indicating that they are unique in characterizing the soybean genotypes.

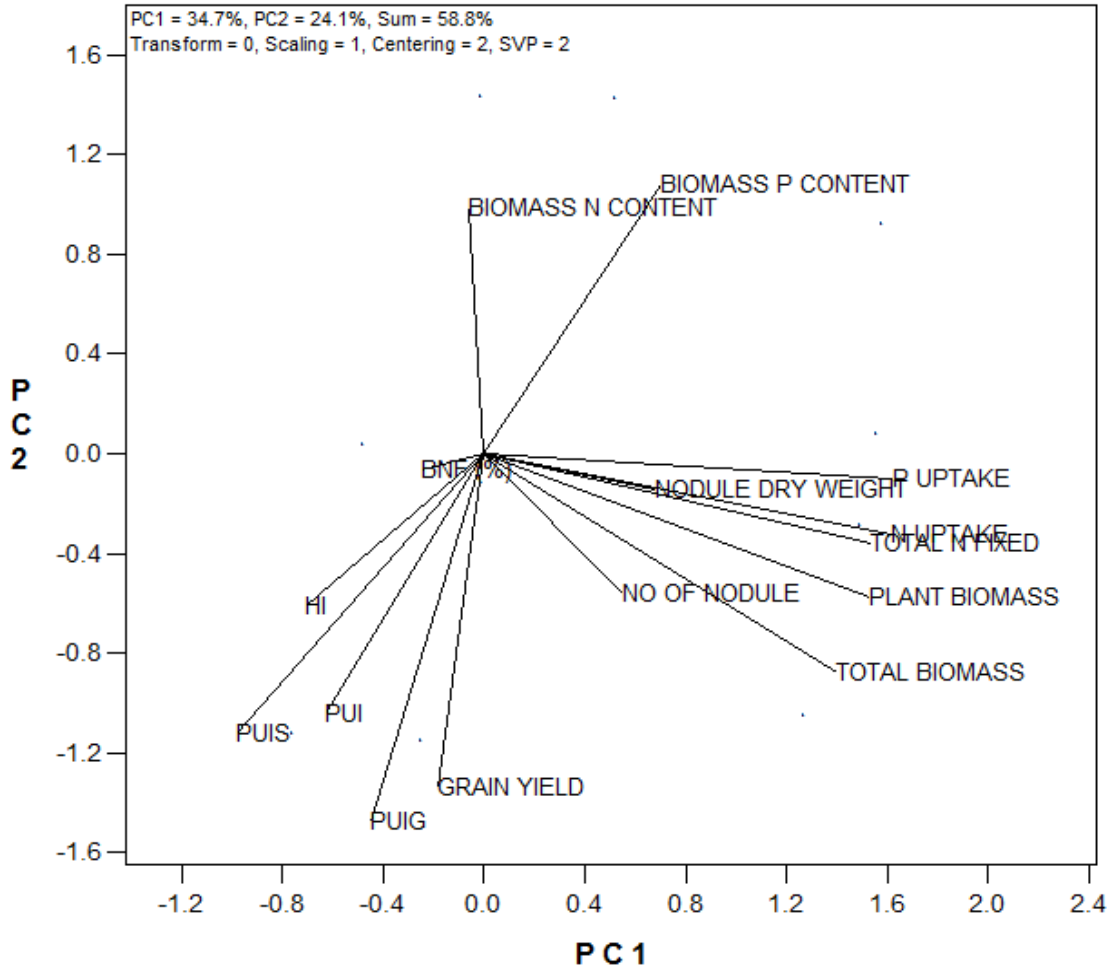

FIG. 2. A vector view of the genotype × trait biplot showing interrelationship among all traits of the 64 early maturing genotypes evaluated in Nigeria between 2009 and 2010. PUI = Phosphorus Utilization Index; PUIG = Grain P utilization efficiency; PUIS = shoot P utilization efficiency.

Clustering of the soybean genotypes based on the eleven variables produced four major clusters at a reversed distance of 0.8 which showed a remarkable correspondence with the PCA axes (Fig. 3). Cluster one had nine genotypes and were loaded with genotypes that had high plant biomass N and P uptake and total N fixed (e.g. TGx1911-9F, TGx 1914-2F and TGx 1921-2F). Cluster two had 25 genotypes that included genotypes TGx 1893-10F and 1895-33F, which were high in biomass N and P content. Cluster three was composed of three genotypes, TGX1842-14E, TGX1912-11F and TGX1913-5F, which generally had high P utilization efficiency but low P uptake and N fixation. TGx1912-11F and TGx1913-5F had the highest P utilization index among the 64 genotypes. Cluster four comprised 27 genotypes. The grain yield, BNF and nodule dry weight were high for most of the genotypes in this cluster. Among the genotypes found in this cluster were TGX1880-3F, TGX1903-7E and TGX1917-1F, TGX1911-3F and TGX 1805-8F.

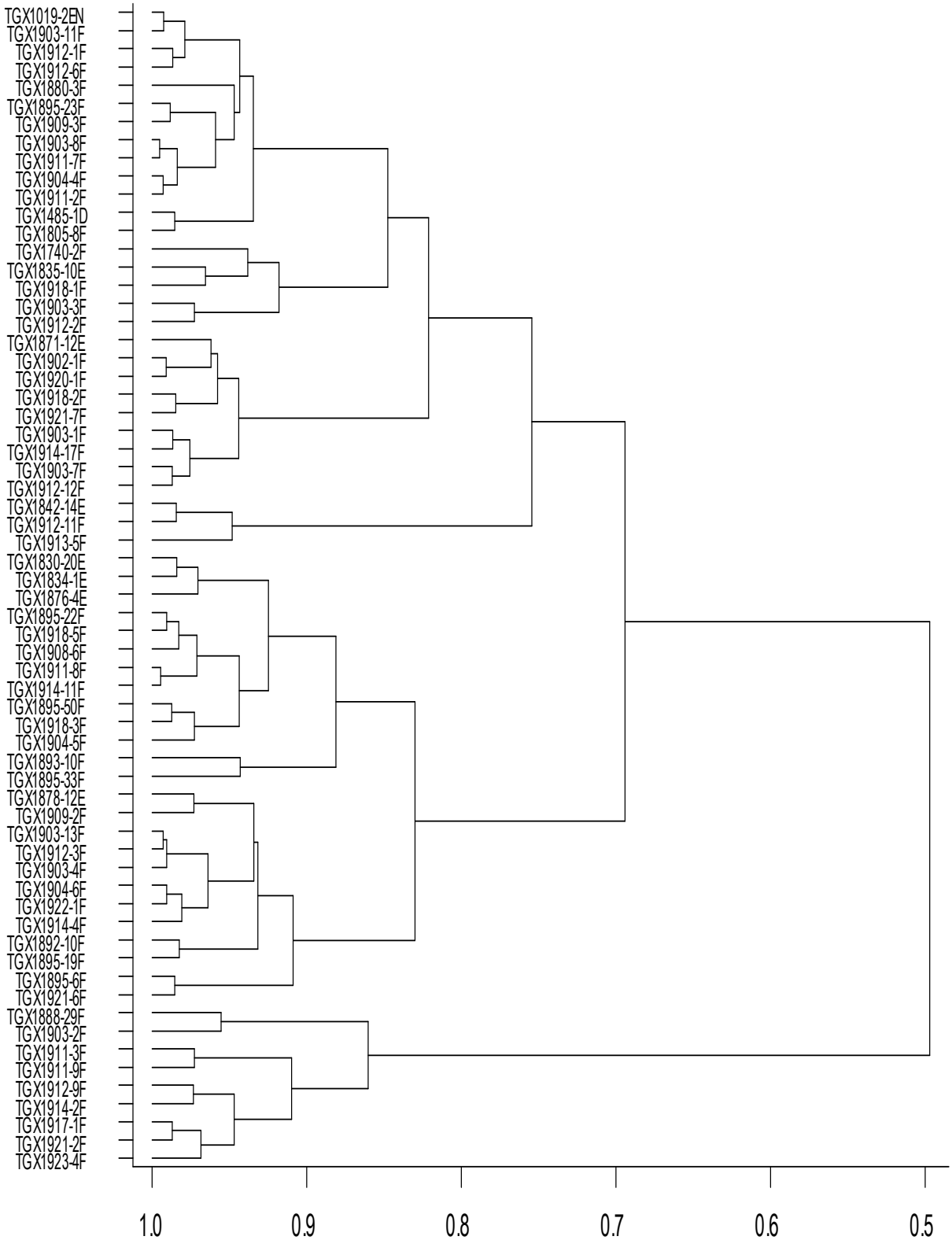

FIG. 3. Dendrogram of 64 early maturing soybean genotypes derived from quantitative traits using first four principal components and complete linkage cluster analysis.

4. DISCUSSION

The significant positive and negative correlation of grain yield with PUI and biomass N concentration, respectively, is an indication that P concentration of the plant may not increase the grain yield of soybean, but how efficiently the P accumulated in the biomass is

utilized. There had been a contention on whether P acquisition or utilization should be the main focus of P use efficiency in breeding programmes. Breeding P efficient genotypes has focussed more on P acquisition efficiency (PAE) [17]. The result of the present study is an indication that PAE may not only be the best solution but also the P utilization efficiency. It has been speculated from soybean studies that enhancement of P utilization efficiency might become a potentially powerful strategy for increasing P efficiency in modern crops grown in intensive cropping systems [5].

Although the P Utilization Index of the 64 early maturing genotypes was not significantly affected by genotype, the P utilization for grain development measured as PUIG, and for harvest index measured as PUIS, was significantly influenced by genotype. This was probably due to the significant genotypic variation for grain production which could have influenced the P sink during grain formation. Therefore breeding of genotypes for grain production on low P soil can be enhanced by selecting those genotypes with high PUIG and PUIS. Genotypes identified with high PUIG and PUIS in this study included TGx 1880-3F, 1913-5F and 1485-1D. Screening for the P utilization efficient early maturing soybean genotypes in this study as revealed by the GT biplot produced a result that was probably more desirable to farmers in low P soil. The sector that had the trait PUI, PUIG and PUIS also had the grain yield and HI. The indication for this was that the genotypes that fell into this sector were the best for the traits in the sector. Therefore, selection of a genotype within this sector for further breeding programmes can produce high P utilization efficient and high grain yield genotypes for low P soil.

The cluster analysis was consistent with the GT biplot analysis. Like the GT biplot, the four clusters can be grouped along three major components which were biomass accumulation, nutrient accumulation and nodulation components. Cluster 1 was defined more by high plant biomass, N and P uptake and total N fixed. Cluster 2 was defined more by the high nutrient concentrations (N and P) in the biomass whereas cluster 3 was defined by their high PUI and low P uptake. Cluster 4 was defined by the nodulation variables and grain yield. Phosphorus utilization efficiency (PUE) is the ability to produce biomass or yield using the acquired P [17].

Most crops have relatively efficient P uptake capacity but low P translocation and remobilization, and hence PUE becomes a significant bottleneck for further improvements in crop P efficiency [5]. The genotypes in cluster 2 displayed a higher propensity to accumulate N and P. However it was the genotypes in cluster 3 that were able to efficiently use P better than other genotypes. The interesting finding of this study was that just three genotypes, TGX1842-14E, TGX1912-11F and TGX1913-5F were selected for PUI which was used as a measure for P utilization efficiency. It means that the three genotypes could be good candidates for further breeding work.

It was expected that PUI will influence BNF because P is a component of ATP which provides the energy that helps the symbiont to fix N in soil. Nitrogenase makes use of ATP for the reduction of N_2. However, there was no significant relationship between the PUI and BNF. The probable explanation could be that competition from the plant for energy for biomass accumulation and grain filling exceeded that of the symbiont that fixes N in the root nodules of these genotypes. Therefore, more of the P in the genotypes was utilized for biomass formation.

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ABBREVIATIONS AND ACRONYMS

ARC (WARDA)	Africa Rice Center (West Africa Rice Development Association)
AM	Arbuscular mycorrhizal
ARB	Adventitious Root Branching
ARL	Adventitious Root Length
ARN	Adventitious Roots Number
BNF	Biological Nitrogen Fixation
BRB	Basal Root Branching
BRD	Basal Root Depth
BRGA	Basal Root Growth Angle
BRL	Basal Root Length
BRN	Basal Root Number
BRWN	Basal Root Whorls number
CENA/USP	Center for Nuclear Energy in Agriculture/University of Sao Paulo
CGIAR	Consultative Group on International Agricultural Research
CIAT	Centro Internacional de Agricultura Tropical (International Center for Tropical Agriculture), Colombia
CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo (International Maize and Wheat Improvement Center), Mexico
CRP	Coordinated Research Project
ENR	Efficient and non-responsive
ER	Efficient and responsive
FAO	Food and Agriculture Organization, Rome
GY	Grain Yield
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics, India
IITA	International Institute of Tropical Agriculture, Nigeria
INRAN	Institut National de Recherche Agronomique du Niger (INRAN)
IRAD	Institute of Agricultural Research for Development
IRRI	International Rice Research Institute, Philippines
LAI	Leaf Area Index
LCHL	Leaf Chlorophyll
LRL	Lateral Root Length
LRN	Lateral Root Number
NARS	National Agricultural Research Systems
NENR	Non-efficient and non-responsive
NER	Non-efficient and responsive,
NERICA	New Rice for Africa
NODWT	Nodule weight
NUE	Nitrogen use efficiency
PAE	Phosphorus acquisition efficiency
PCA	Principal Component Analysis
PEI	Phosphorus efficiency index
PLHT	Plant Height
PRB	Primary Root Branching
PRD	Primary Root Depth
PUE	Phosphorus utilization efficiency
PUI	Phosphorus utilization index
RA	Root Angle
RAMC	Root Arbuscular Mycorrhiza Colonization
RB	Root Branching

RCA	Root cortical aerenchyma
RDIA	Root Diameter
RGY	Relative Grain Yield
RHLD	Root Hairs Length Density
RILs	recombinant inbred lines
RL	Root Length
RLD	Root Length Density
RSA	Root Surface Area
RTV	Root Volume
SA	Specific activity
SARI	Savannah Agricultural Research Institute
SHB	Shoot Biomass
SNF	Symbiotic nitrogen fixation
SRE	Seminal Root Elongation
SRL	Seminal Root Length
STDIA	Stem Diameter
UNESCO	United Nations Educational, Scientific and Cultural Organization

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