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Improving yield and nitrogen fixation of grain legumes in the tropics and sub-tropics of Asia

Results of a co-ordinated research programme organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

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FOREWORD

Although grain legumes are a major source of dietary protein and oil for the peoples of Asia, yields are generally poor, and diminishing farmer-interest in their cultivation has caused scarcities. This situation requires redress, particularly since the achievement of full yield potential of legumes is an important aspect of sustainable agricultural production. Pulses possess the ability to symbiose with soil-inhabiting rhizobia bacteria in the fixation of N_2 within root nodules. The bacteria convert atmospheric N_2 to forms that the legume host can use and thus yield well in soils in which non-leguminous crops would need generous applications of fertilizer N. Furthermore, the return of vegetative legume residues to the soil after grain harvest can constitute a significant input of N for the potential benefit of subsequent cereal and other non-leguminous crops – hence the importance of legumes for cropping-system sustainability.

The N_2-fixing symbiosis is characterized by legume x rhizobium specificity. Some host genotypes are superior to others in the ability to fix N_2, and, in turn, some rhizobial strains have similar superior capability. This diversity in the plant and rhizobial gene pools offers a means of improving grain yields, especially in developing countries of Asia in which locally important legumes and their micro-symbionts have been little studied.

Within this context, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture initiated a Co-ordinated Research Project on The Use of Isotopes in Studies to Improve Yield and N_2 Fixation of Grain Legumes with the Aim of Increasing Food Production and Saving N-fertilizer in the Tropics and Sub-Tropics of Asia that was operational from 1990 to 1995. This Project was underpinned by extensive experience in the use of ^{15}N-labelled fertilizer in quantifying N_2 fixation by food and pasture legumes; the isotope-dilution technique, recognized as the most accurate mode of quantifying fixation, was developed at the IAEA and has been used profitably for over 20 years in co-ordinated research projects that were focused on aspects relevant to the sustainability of agriculture in developing countries in which food security is most under threat.

This effort to improve N_2 fixation by food legumes in Asia, and in so doing to increase productivity of cereal-based farming systems as a whole, was timely in terms of regional needs. It was complemented by an overlapping Co-ordinated Research Project entitled "The Use of Nuclear and Related Techniques in Management of Nitrogen Fixation by Trees for Enhancing Soil Fertility and Soil Conservation in Fragile Tropical Soils". The project involved scientists from Australia, Bangladesh, China, India, Malaysia, Pakistan, the Philippines, Sri Lanka, Thailand and Viet Nam. S.K.A. Danso was the Project Officer.

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture thanks the United Nations Development Programme for its generous financial support of this project. The IAEA officer responsible for this publication is G. Keerthisinghe of the Soil and Water Management and Crop Nutrition Section of the Joint FAO/IAEA Division. This publication was edited by A.R.J. Eaglesham.

EDITORIAL NOTE

CONTENTS

SUMMARY

The restorative effects of legumes on agricultural soil are documented in Greek and Roman literature, and have been exploited by farmers since antiquity. However, the swellings or nodules on the roots were not linked with those benefits but thought to be storage organs or of pathological origin, until the pioneering research of Drs. Hellreigel and Wilfarth in Germany a little over a century ago.

Hellreigel and Wilfarth's experiments compared oat, buckwheat and rape, with pea, serradella and lupin, grown in sand culture with N-free nutrient solution. A water extract of soil, containing an insignificant amount of N, was added to each pot. The non-legumes remained N-deficient, whereas the legumes after a time recovered from "N-hunger," became dark green and grew luxuriantly. It was concluded that the *Papilionaceae* obtained the N from the atmosphere with the assistance of bacteria, from the soil extract, in the root nodules. Within a few months of publication of these results, the great Russian microbiologist Beijerinck isolated nodule bacteria, which he designated *Bacillus radicicola*, applied them back to legumes and satisfied Koch's postulates. A new arena of scientific endeavour was born.

Bacillus radicicola has been replaced by several genera and many species of soil-borne bacteria, the rhizobia, that infect, with varying degrees of specificity, certain groups of legume species. The rhizobia proliferate in the rhizosphere of the potential host plant then penetrate the root epidermis, either directly or via root hairs, and invade cortical cells. Host-cell division takes place, and highly differentiated nodules develop within which atmospheric N_2 is converted to ammonia by the microsymbiont and released to the host legume. Thus, an effectively nodulated legume can thrive in a soil that is deficient in mineral N.

Prior to the mid-1970s, research interest in N_2 fixation was restricted to a few groups of scientists in Europe, USA and Australia. The energy crisis of 1973-1974 caused sudden, rapid increases in the costs of nitrogenous fertilizers and stimulated interest in legume N_2 fixation and the possibility of replacing chemical fertilizers with organic alternatives. Many developing countries were particularly adversely affected by the increased costs of crop production, which stimulated research on tropical legumes and their role in tropical agriculture.

Concomitant with this new interest in the biological fixation of atmospheric N_2 in the 1970s and the possibility of its exploitation for improved agricultural production in the developing world, the isotope-dilution methodology was developed at the Agency, as a way of precisely measuring fixation by field-grown legumes. A small quantity of ^{15}N-enriched fertilizer is applied to the soil and the legume assimilates atmospheric N_2 supplied by the root-nodule symbionts, in addition to the isotope-enriched N from the soil. Since non-fixing plants do not have access directly to atmospheric N_2, the resulting $^{15}N/^{14}N$ ratio of tissue in any two species differs in proportion with the amount of N fixed. The isotope-dilution technique has been used extensively over the past 20 years in co-ordinated research projects sponsored by the IAEA.

A limitation of the isotope-dilution methodology is uncertainty in the choice of suitable non-N_2-fixing reference species. The $^{15}N/^{14}N$ absorbed by the fixing plant changes significantly with time, therefore, for a non-fixing species to be a suitable reference, it must absorb $^{15}N/^{14}N$ with the same temporal pattern, and both should obtain their mineral N from similar soil-N pools. Occasionally, estimates of the amount of N fixed are negative as a result of the non-fixing check having a different pattern of usage of the ^{14}N and ^{15}N pools; on the other hand, cereal species have

been shown to be acceptable non-fixing checks for some legumes. Of course where data appear to be meaningful, they may not be so; it is advisable to use more than one non-fixing species to provide comparisons of determinations of fixed N.

Although the energy crisis is long past, food security is increasingly under threat in many developing countries of the tropics in which traditional agricultural systems are becoming unsustainable as a result of demographic pressures. The global population is expected to double by 2040, and much of that increase will occur in developing countries in which hunger is already a threat. Local needs to make arable land satisfy present food demands are resulting in over-exploitation that results in ever-less productivity per unit area. When soil goes into a phase of rapid decline in fertility, erosion often increases with far-reaching deleterious environmental consequences that may be impossible to reverse. In such circumstances, crop productivity may not be significantly improved by even heavy applications of synthetic fertilizers, in the rare situation where such are available to the subsistence farmer, because levels of organic matter that define fertility have reached critically low values.

The pressing needs to reverse these trends demand that land-use systems be developed that sustain fertility and avoid mining the soil of its nutrient resources. These new approaches must be sustainable both biologically and economically, with appropriate inputs such that fertility is not sacrificed for the short-term production of crops. A fundamental component that must be included is the recycling of renewable resources so that the factor that defines fertility is maintained in the soil: the organic matter content. Furthermore, the nutrients that are removed from the system in the harvested crop must be replaced. Where regenerable, biological sources of nutrients are available, new systems must be designed and developed to exploit them. And in one regard we are fortunate: the mineral nutrient that is most easily lost from the soil and which is needed in greatest supply by crops is N, which has the inexhaustible biological source described above. In symbiosis with soil-borne rhizobial bacteria, legumes are able to "fix" atmospheric N_2 in root nodules and thrive in soil that is low in available N. Therefore, for example, cowpea [*Vigna unguiculata* (L.) Walp.], a legume important for human nutrition in Africa, Asia and South and Central America, can yield optimally in soil that is impoverished in N in which a cereal crop, such as corn (*Zea mays* L.), would fail. Moreover, in returning the vegetative residues from a cowpea crop to the soil, the system may become enriched in N, as well as in organic matter, for the potential benefit of a subsequently planted cereal, for example. It is important to understand that symbiotic N_2 fixation by legumes will occur in useful amounts only when N is the factor most limiting to growth; if moisture, or insect predation, or some other nutrient is growth-limiting, or if soil mineral N is plentiful, then symbiotic N_2 fixation will occur only minimally if at all.

Legume residues are known to improve crop productivity by two modes of action: a direct effect on nutrient supply and indirect effects on soil structure. Legume crop residues are low in C:N, lignin and polyphenol, characteristics that result in rapid decomposition, particularly under warm, moist tropical conditions. This rapidity of decomposition is inherently problematical in making it difficult to synchronize the release of nutrients by mineralization with the period during which nutrient needs are most pressing in the arable crop of interest. To optimize the utilization of organic sources of nutrients, it is essential to develop management options that ensure that organic-nutrient release occurs when the crop may exploit it efficiently, otherwise significant losses may occur.

The precise quantitative contributions of N from grain legumes to the soil and to subsequent crops are not well understood. There is a need for quantitative assessment of symbiotic N_2 fixation

under field conditions, particularly in tropical agriculture. Even if all of the vegetative residues are returned to the soil after grain harvest, there is not necessarily a gain in N: if the N removed in the grain is greater than that fixed in the root nodules, then the system is depleted of N. Influencing factors in this equation are the N availability in the soil and the N-harvest index, i.e. the fraction of plant N at physiological maturity that is sequestered in the grain. The greater the availability of mineral N to the legume crop the less will be the amount of N fixed, and the greater the N in the grain the less will be the amount of N returned in the stover to the soil. For example, although soybean is a high fixer of N when the soil is low in mineral N, many cultivars move more than 80% of the total plant N into the grain with a resultant depletion of N from the system after grain harvest, notwithstanding the return of all vegetative residues to the soil. Nevertheless, a soybean break crop often has beneficial effects on the yield of a subsequently planted cereal – benefits that are often assumed to be from a N contribution but instead result from breaking disease cycles, inhibitory effects on predatory insect populations and from inputs of organic matter of a quality superior to that from a cereal. From the point of view of exploiting legumes optimally, it is important to separate N-benefit effects from non-N effects, and clearly this requires precise determination of the amount of N fixed by a legume crop, the amount of N removed in the grain, the amount of N applied to the soil as vegetative residue and the amount of that N taken up by a subsequent crop. It is generally accepted that the best way of measuring fixation is with the isotope-dilution technique. Furthermore, the application of ^{15}N-labelled residues to the soil allows a precise determination of how much of that N is later assimilated by a subsequent crop.

Australia provides a fine example of how symbiotic N_2 fixation by legumes may be exploited for the benefit of agricultural productivity in general. Legumes, both pasture and grain, have been important in the western and southern regions of the Australian cereal belt for the past 50 years. The pasture legumes have a dual role, sustaining animal production and supplying N to the soil for use by subsequent cereal crops. Prior to the early 1950s, plant-available N was conserved in the soil through bare fallowing, a practice that depletes organic matter and damages soil structure, which led to large-scale soil erosion; wheat yields were static at around 0.8 t/ha.

With the introduction of legume-based pastures, yields increased and stabilized at around 1.3 t/ha, due almost entirely to N benefit from legume N_2-fixation. Net increments in soil N under the pasture commonly ranged from 35 to 100 kg/ha depending on productivity of the legume. Soil structure also benefitted, with enhancement in water infiltration and root penetration.

The N_2 fixed by legume crops in Australia has an economic value, in terms of both the N itself and rotational benefits. The value of fixed N in a legume crop can be calculated using an average value for %Ndfa (the proportion of legume N derived from N_2 fixation) and the average yield data. The annual value of N_2 fixed by grain legumes in Australia has been calculated at US $43/ha. When the economic impact of legume effects on subsequent cereal yields is included, e.g. the benefits of chickpea to wheat production in the northern cereal belt, using yields from five rotation experiments, an 85% increase in the annual gross margin is obtained, from US $120/ha to US $222/ha. Thus, legume N_2 fixation is a valuable process in Australian agriculture, and with appropriate management may be similarly valuable in other countries.

Within the above context, the Co-operative Research Project, "Improving yield and nitrogen fixation of grain legumes in the tropics and sub-tropics of Asia" was initiated by the IAEA in 1990. Much of Asia's population is dependent on rice as the chief source of sustenance, and it has been estimated that, within the next 30 years, a 65% increase in production will be necessary to

meet the requirements of the growing population. This need forces the development of higher-yielding varieties that will require increased fertilizer inputs. In the face of prices that are currently prohibitive for growers in much of the developing world, alternatives to synthetic chemical N-fertilizers are needed urgently. Hence, new attention must be paid to exploiting biological sources of N to meet the requirements of rice and the other important cereal crops. Not only do grain legumes (pulses) provide a potential source of N in rice-consuming Asia, they are already important as sources of dietary protein. However, for economic and other reasons, the acreage planted to pulses in that region has been declining in recent years; the production of cereals is more lucrative for many farmers, hence legume cultivation is increasingly marginalized to poorly productive soils. Given the potential importance of the contribution of N as well as being a significant source of food protein, it is important that legumes be integrated into farming systems, and management practices adopted to maximize fixation and to optimize the utilization of organic N in stovers applied to the soil for benefits to the production of rice and other cereals.

This co-ordinated research project involved scientists from Australia, Bangladesh, China, India, Malaysia, Pakistan, Philippines, Sri Lanka, Thailand and Viet Nam. The research had three broad components:

- To increase N_2 fixation and grain yields of target legumes.
- To investigate possible benefits from the incorporation of grain-legume stover on subsequent cereal yields.
- To transfer isotope technology to participants.

Increases in N_2 fixation and grain yields by selection and breeding may be obtained only if there exists genetic diversity for these components. Data from several countries confirmed that such genetic diversity exists in pulses that are of dietary importance in Asia. In Viet Nam, for example, varietal differences were found in greenhouse-grown plants in terms of nitrogenase activity, and %Ndfa values ranged from 11 to 63% for groundnut and from 9 to 79% for soybean. Field experiments also revealed significant varietal differences: %Ndfa values ranged from 36 to 56% for groundnut and from 28 to 58% for soybean.

The possibility of accentuating this genetic diversity with mutational breeding was demonstrated. In Bangladesh, the commercial chickpea varieties Nabin and Hyprosola were inferior to advanced mutant lines. In Malaysia, Matjan mutant MJ/40/42 consistently produced the highest pod yields, at above 4 t ha^{-1}, 14-22% higher yields than its parent. However, the mutant lines did not show consistent agronomic performance from year to year. Total dry matter yield did not correlate with pod yield, and pod yield did not correlate with amount of N fixed; clearly further research is needed to develop consistent superior characteristics. In Viet Nam, γ-irradiated seeds of groundnut and soybean were propagated in bulk from M_1 to M_4. Five high-yielding mutant lines of both species, selected from the M_5 populations, had %Ndfa values of 55 and 57%, significant improvements over the parent-cultivar values of 25 and 29% for soybean and groundnut, respectively. Of course, it is important to verify that higher %Ndfa values occur in lines of superior growth and total-N accumulation.

In the case of mung bean, as shown in Pakistan, Philippines and Thailand, N_2 fixation was not the chief yield-determinant, and selection for crop vigour and high yield would also select for fixation. These findings illustrate the importance of not *assuming* that low legume yields result from a lesion in the root-nodule symbiosis. The determination of %N values in the leaves is essential when appraising

the reason for poor legume growth; if the symbiosis is poorly effective, %N values of <2 are likely, and, as a corollary, %N values of >2.5 would normally indicate that a factor other than N is growth limiting. In the absence of facilities with which to rapidly obtain N-concentration values, leaf color can be indicative; yellowing is likely to indicate N deficiency, but care must be exerted so as not to misinterpret Fe or Mg deficiency.

In other cases, such as with lentil in Pakistan and groundnut in Malaysia, N_2 fixation *per se* was the chief yield-determinant, raising the question of the specifics of the constraint to maximum expression of symbiotic potential. In Sri Lanka, positive growth responses to applied N were obtained with cowpea, indicating the potential to improve N_2 fixation and yields by combining compatible genotypes and rhizobial strains. With pigeon pea in Australia, lack of Fe was found to be a critical factor in some soils; therefore, the possibility must be kept in mind of nutritional deficiencies (easily remedied) impinging directly on the legume's ability to foster the symbiosis or to utilize fixed N.

Strong responses to inoculation with rhizobia shown by lentil in Pakistan serve as an apt reminder that incompatibility with, or sparse populations of, indigenous rhizobia may explain a legume's poor growth; in such cases, inoculation with an effective rhizobium can transform the poorly fixing genotype into a vigorous, productive crop. In Pakistan, inoculation of lentil had significant positive effects on nodulation, total biomass, grain yield, N yield, %Ndfa and total Ndfa. Moreover, there were statistically significant differences amongst the four rhizobial strains used as inoculants, in nodule number, nodule dry weight, plant biomass, grain yield, N yield, %Ndfa and total Ndfa, illustrating the importance of host-cultivar/rhizobial-strain compatibility for obtaining maximum N_2 fixation. The locally isolated rhizobial strain Lc 26 out-classed the exotic TAL 1397, producing the highest number of nodules and the largest nodule dry weight, biomass, grain yield, total N and Ndfa, at 150, 200, 78, 57, 76 and 242%, respectively, higher than the uninoculated control. These data show that indigenous strains may be the best available, their numbers in the soil needing to be boosted by re-application as inoculants.

In Bangladesh, inoculation of groundnut increased average nodule number by 77%, 99% and 148% at three locations; the increases in nodule dry weight, plant dry weight, pod and stover yields due to inoculation ranged from 93 to 146%, 55 to 77%, 43 to 50% and 29 to 80%, respectively. Inoculated cv. G-97 recorded a seed yield of about 1.5 t/ha at Ishurdi, 47% higher than that produced by Nabin, a variety widely cultivated in Bangladesh. Total-N yield and the amount of N fixed by G-97 with inoculant were also higher than for Hyprosola, which is known for high yield and protein content. In India, inoculant application to chickpea resulted in at least a doubling of nodule number, a three-fold increase in nodule mass, and seed-yield increases ranging from 24 to 50%.

On the other hand, negative effects of inoculant application, as with black gram in Pakistan, show that circumspection is needed in choosing rhizobial strains for use in inoculants – if more than one legume is grown in a cropping system, the possibility of adverse consequences of inoculant application must be considered. Two black-gram cultivars showed decreases in biomass, total N, Ndfa (amount of N derived from fixation), and grain yield of 31-85% in response to inoculation. And in Australia, yield reductions of common bean with rhizobial inoculation suggested that the native rhizobia were more effective at fixing N_2 than was the strain used in the inoculant.

The nodulation-variants of chickpea discovered in India demonstrate that, within a cultivar, there may be diversity in compatibility with a rhizobium strain. The relative nodulation differences were consistent across locations; the S4 and S5 (high nodulating) lines were generally superior to the

S1 and S2 (low nodulating) variants for nodulation, N_2-fixation, total dry matter and grain yield, i.e. nodule number and mass correlated significantly with total dry matter, grain yield, total N and quantity of fixed N. The preponderance of chickpea plants had high-nodulation capability, but the exciting possibility exists, with other cultivars of chickpea and within cultivars of other legume species, of selecting for improved nodulation and thus obtaining increased fixation and yield.

Soybean screening work in Australia revealed cultivars with superior nodulation and N_2 fixation in the presence of high concentrations of nitrate. These genotypes were of Korean origin and had shoot yields similar to commercial cultivars Bragg and Davis, suggesting that increased N_2 fixation reduced their use of soil N. Post-harvest measurements of soil nitrate confirmed this; immediately after grain harvest, up to 34 kg/ha additional N was recovered from the Korean plots compared with Bragg plots. Seed yields of the Korean lines were, on average, 30% less than that of Bragg, due to a combination of shattering, early maturity and poor agronomic type. Correlation matrices among the indices of nodulation and N_2 fixation and plant growth and grain yield revealed independence between the symbiotic- and yield-related characters. Therefore, the Korean lines appear to be suitable for use as high-fixing donor parents in a breeding programme with selection for both grain yield and N_2 fixation. The differences in nodulation between the Korean genotypes and commercial cultivars were found to occur only when symbiosis was stressed, i.e. with moderate to high nitrate levels in glasshouse sand-culture or in the field, or with low numbers of soybean rhizobia in the field. In the absence of stress, nodulation of the two groups was similar, therefore, enhanced nodulation of the Korean genotypes likely results from more-efficient rhizobial infection and/or nodule initiation. It remains to be determined whether genotypes of other legume species have similar superior characteristics.

Experiments with mung bean in the Philippines demonstrated genetic diversity for improvement of subsequent maize growth and yield. In Sri Lanka, beneficial residual effects on growth of subsequent maize could not be related to N_2 fixation by the preceding cowpea, and although there was no evidence of direct transfer of N from cowpea to intercropped maize, there was greater efficiency of use of N for total crop production during intercropping. It is fundamentally important that we gain better understanding of the various sources of benefit that accrue from incorporating legume stovers into the soil, in addition to N: breaks in the cycles of disease that affect non-legumes, suppression of predatory insects that infest non-legumes, improvements and increases in the soil organic matter fractions, and possibly other unknown factors.

Precise quantification of fixed N is a useful aid in the assessment of genetic diversity for N_2 fixation. The utilization of fertilizers enriched in ^{15}N for this purpose is a unifying aspect of the work described in this document. Therefore, the transfer of this technology has clearly been successful. The use of any method brings understanding of its limitations, and efforts in Pakistan and India with chickpea showed that less precise, and thus less expensive, methods have utility for large-scale breeding and selection for N_2 fixation; however, the superiority of genotypes thus identified should be checked using the isotope-dilution technique. Contractors in Thailand investigated the usefulness of the ^{15}N natural-abundance technique for estimating fixation; they were able to apply the method to a screening of over 400 genotypes. But, anomalous aspects of their data indicate that adjustment is needed before the method can be applied with complete confidence.

Improving the yields of legumes while maintaining optimum N_2-fixing capability, and increasing legume productivity by removing constraints for the symbiosis, are simple conceptually but not so in practice. The complexity of the problems involved are indicated in this document. Moreover,

pulses in Asia, despite their nutritional importance, are grown largely on marginal, impoverished soils. In such conditions, yield potentials are low and it is likely that N is not a chief growth-limiting factor. Therefore, adopting management practices to improve the root-nodule symbiosis, e.g. by application as inoculant of a rhizobial strain found to be superior to indigenous types in greenhouse trials, will have no utility. Only by growing legumes in fertile conditions, possibly with inputs to raise yield ceilings, then inoculants may have an optimizing role and only if N is the chief growth-limiting factor. This 5-year programme has shown that there is a wealth of genetic diversity in the food legume species of importance in Asia in their capacity to fix N_2 and to contribute N for the benefit of non-fixing crops in the farming system. The data herein demonstrate that there is significant potential to exploit the root-nodule symbiosis for better yields of all components of the cropping systems of the region. There is need to research the various contributions of legumes within those cropping systems, *with long-term experiments so that cumulative effects may be detected and documented. It is hoped* that this document will serve as a foundation for future work on improving productivity of cropping systems as a whole by maximizing N_2 fixation by its component grain legumes.

SELECTION AND BREEDING OF GRAIN LEGUMES IN AUSTRALIA FOR ENHANCED NODULATION AND N_2 FIXATION

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Abstract

SELECTION AND BREEDING OF GRAIN LEGUMES IN AUSTRALIA FOR ENHANCED NODULATION AND N_2 FIXATION

During the period 1980-87, the areas sown to grain legumes in Australia increased dramatically, from 0.25 Mha to 1.65 Mha. These increases occurred in the western and southern cereal belts, but not in the north in which N continued to be supplied by the mineralization of soil organic matter. Therefore, there was a need to promote the use of N_2-fixing legumes in the cereal-dominated northern cropping belt.

Certain problems had to be addressed before farmers would accept legumes and change established patterns of cropping. Here we describe our efforts to improve N_2 fixation by soybean, common bean and pigeon pea. Selection and breeding for enhanced N_2 fixation of soybean commenced at Tamworth in 1980 after surveys of commercial crops indicated that nodulation was sometimes inadequate, particularly on new land, and that the levels of fixed-N inputs were variable and often low. Similar programmes were established in 1985 (common bean) and 1988 (pigeon pea). Progress was made in increasing N_2 fixation by these legumes towards obtaining economic yields without fertilizer N and contributing organic N for the benefit of subsequent cereal crops.

1. INTRODUCTION

In Australia, the total area for agriculture is around 470 Mha, with pastures of native species on 90% (420 Mha) and improved grass and legume pastures on 6% (26 Mha). Just under 4% (16 Mha) is used for cropping, most of which occurs in Western Australia (32% of total) and in New South Wales (24%). The other states, in order of importance, are South Australia, Queensland and Victoria.

In all states, most of the cropped area is used for cereal production; legumes are of secondary importance. The cereal:legume ratios are low in WA and Vic. (around 6:1), intermediate in SA and Qld. (around 12:1) and highest in NSW (25:1). The ratio for the whole of Australia is 10:1. In the northern cereal belt of NSW, the area of particular interest to scientists involved in legume N_2- fixation projects, the ratio of cereal:legume is 33:1.

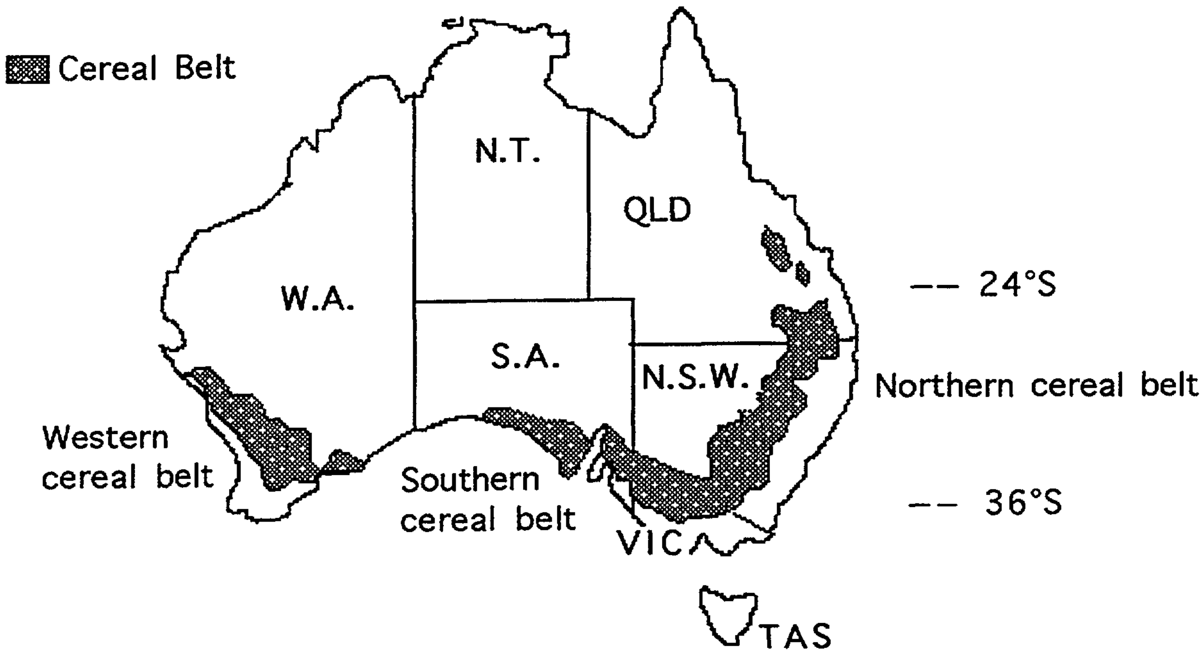

FIG 1. Map of Australia, showing the six states and two territories, and the three major divisions of the cropping belt.

Not included in the above calculations are areas sown to pasture legumes, which are widely used in mixed-farming systems in the western (WA) and southern (SA, Vic. and southern NSW) regions of the cropping belt. Their inclusion would widen the gap between those states and northern NSW and Qld.

2. CROPS GROWN

Cropping in Australia is dominated by wheat (Table I), which accounts for 63% of all cereal production and around 60% of total crop production. Almost all is grown as a rain-fed crop. Barley accounts for a further 20% of total crop production. Average yields for cereals are low (range 1.3 - 1.8 t/ha), except for irrigated rice. Yields of the rain-fed legumes are even lower, ranging from 1.1 to 1.3 t/ha. Soybean, for the most part, is irrigated. The principal legume crops are lupin and field pea, together accounting for almost 80% of legume production.

2.1. Role of legumes

Legumes, both pasture and crop, are important in the western and southern regions of the Australian cereal belt [1]. The pasture legumes have a dual role in these farming systems, sustaining animal production and supplying N to the soil for use by subsequent cereal crops. Such systems are only relatively recent, however. Prior to the early 1950s, plant-available N was conserved in the soil through bare fallowing (Fig. 2), a practice that depleted organic matter and damaged soil structure, and led to large-scale soil erosion. Average wheat yields during this time remained static at around 0.8 t/ha.

TABLE I. AREA AND PRODUCTION FIGURES FOR CEREAL AND PULSE LEGUME CROPS IN AUSTRALIA FOR 1992-93 (CROP REPORT PROJECT, ABARE, 1994)

Crop	Major producer	Total area (Mha)	Production (Mt)	Av. yield (t/ha)
Wheat	WA, NSW	9.10	16.2	1.78
Barley	SA, WA	2.96	5.40	1.82
Oats	NSW, WA	1.15	1.94	1.70
Sorghum	Qld., NSW	0.43	0.55	1.30
Rice	NSW	0.13	0.96	7.38
All cereals	WA, NSW	13.9	25.5	1.83
Lupin	WA	1.03	1.20	1.17
Field pea	SA. Vic.	0.38	0.46	1.21
Chickpea	Qld., NSW	0.15	0.17	1.13
Faba bean	SA, Vic.	0.08	0.10	1.25
Soybean	Qld., NSW	0.03	0.05	1.67
All legumes[a]	WA, SA, Vic.	1.83	2.12	1.16

[a]Includes mung bean, navy bean, cowpea, peanut and pigeon pea.

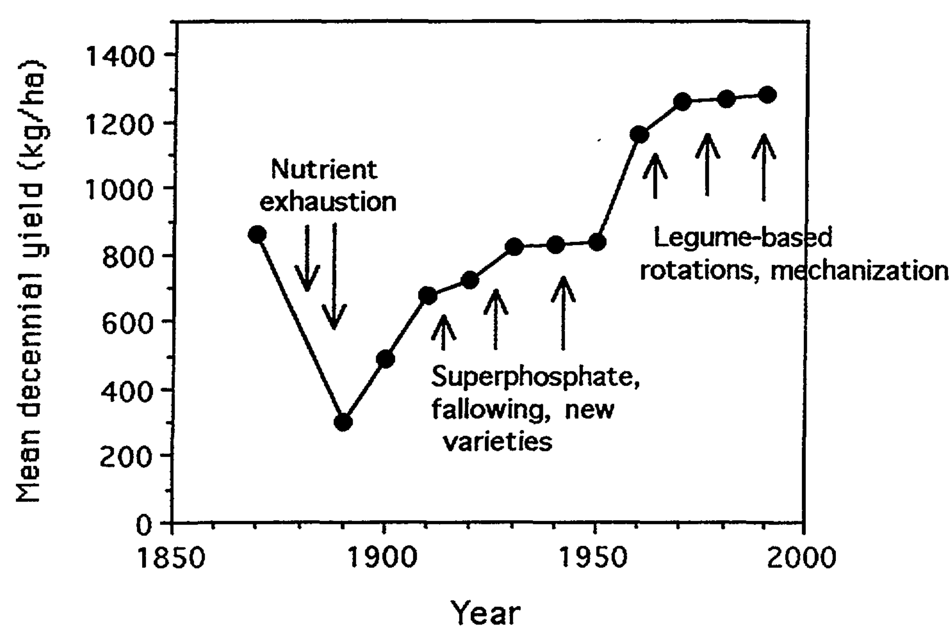

FIG. 2. Average wheat yields for Australia, 1860-1980 (unweighted means in 10-year intervals).

Following the introduction of legume-based pastures in the 1950s, yields increased and stabilized at around 1.3 t/ha, due almost entirely to N benefit from legume N_2-fixation. Net increments in soil N under the pasture commonly ranged from 35 to 100 kg/ha and reflected the productivity of the legume [1]. Soil structure also benefitted from the pasture phase, resulting in enhancements in water infiltration and plant-root penetration.

During the late 1970s (i.e. almost 30 years after the introduction of pasture leys), a number of factors combined to again change the basic cereal-production system, from the pasture ley-cereal to a more flexible combination of pasture ley-cereal and grain legume-cereal. These factors included concern for the decline in thrift of pasture legumes, soil problems (acidity and salinity), increasing cereal disease, particularly root and crown rots, and more favourable returns from cropping compared with livestock production. It is again noteworthy that these legume systems (both pasture and crop) were used only in the western and southern cereal belts. Legumes were not grown in the northern cereal belt, where N was supplied through the mineralization of native organic matter.

During the period 1980-87, the areas sown to grain legumes increased dramatically from 0.25 Mha to 1.65 Mha, an increase of 560%. The initial expansion was entirely because of increased sowing of lupin in WA. During the mid 1980s, the area sown to pea also increased, whereas between 1980 and 1990 cereals actually declined from 15.6 Mha to 13.5 Mha, after reaching a peak of 18.7 M ha in 1983. Thus, the ratio of cereal:legume decreased almost linearly from 60:1 in 1980 to 10:1 in 1990.

2.2. Economic value of legume N_2 fixation

The N_2 fixed by legume crops in Australia has an economic value, in terms of both the N itself and rotational benefits. The value of fixed N in a legume crop can be calculated using an average value for Pfix (the proportion of legume N derived from N_2 fixation) and the average yield data (Table II). The values used for Pfix, harvest index (HI) for total dry matter (DM), and %N of DM in the calculations were derived from the literature. The Pfix value of 80% reflects the low N status of most agricultural soils in the country, but may be excessive for some of the better-quality soils.

The annual value of N_2 fixed by grain legumes in Australia of US$94 million compares favourably with the estimated annual value of N_2 fixed by pasture legumes of US$1100 million [1]. The total (legume-based) pasture area is 26 Mha, therefore US$1100 million per 26 Mha is equivalent to US$43/ha, similar to the figure of $51/ha derived for grain legumes (Table II). Thus, legume N_2 fixation is a valuable process in Australian agriculture.

The economic value of legume-effects on subsequent cereal yields is not included in Table II, and would add considerably to the totals. An economic analysis of the benefits of chickpea to wheat production in the northern cereal belt, using yields from five rotation experiments, indicated an 85% increase in the annual gross margin, from US$120/ha to US$222/ha (Table III).

For a farmer in the northern region, replacement of every third crop with chickpea, e.g. growing 134 ha wheat and 66 ha chickpea rather than 200 ha wheat, would result in the overall farm gross margin increasing from US$24,000 to US$44,880. On the same basis, increased value of production for the region would be about US$250 million.

TABLE II. ECONOMIC VALUE OF N_2 FIXATION BY GRAIN LEGUMES

Item	Australia
Area sown (ha)	1.83 x 10^6
Production (t)	2.12 x 10^6
Average yield (t/ha)	1.16
Total DM yield[a] (t/ha)	3.87
Total N yield[b] (kg/ha)	97
Total N_2 fixed[c] (kg/ha)	77
Value[d] of N_2 fixation (US$/ha)	51
Total value (US$)	$94 x 10^6

[a]Assuming a harvest index of 0.3.
[b]Assuming 2.5% N for DM.
[c]Assuming an average Pfix of 80%.
[d]N valued at US$0.66/kg.

TABLE III. ECONOMIC ANALYSES OF THREE CROPPING SYSTEMS IN AUSTRALIA'S NORTHERN CEREAL BELT. YIELDS ARE AVERAGES OF FIVE EXPERIMENTS, CONDUCTED 1987-92 (W. FELTON, H. MARCELLOS, D. HERRIDGE, UNPUBLISHED)

Item	Wheat (W-W-W)		Chickpea (CP-W-W)		Fallow (F-W-W)	
	Yld (t/ha)	US$	Yld (t/ha)	US$	Yld (t/ha)	US$
Year 1	2.33	252	2.02	465	-	-
2	2.24	242	3.18	343	3.37	364
3	2.47	267	2.51	272	2.59	280
Total		761		1080		644
Less fixed costs[a]		77		77		77
Less variable costs[b]		325		338		217
Gross margin/3 yrs		359		665		350
Gross margin/yr		120		222		117

Wheat yields: 1.43 - 4.34 t/ha, chickpea yields: 1.29 - 2.90 t/ha. On-farm prices: US$110/t for wheat, US$230/t for chickpea.
[a]Calculated at US$26/ha/yr. [b]US$108/ha/yr for wheat; US$121/ha/yr for chickpea.

Although similar wheat yields to those following chickpea could be achieved in a wheat-only system with applications of 60-80 kg N/ha as fertilizer, the net financial return would fall short of the chickpea-wheat rotation, principally because of the high value of chickpea grain and the added cost of the

fertilizer N. Furthermore, other rotational benefits of the chickpea phase such as disease and weed management and improved soil structure provide sustainable, long-term benefits.

3. ENHANCING LEGUME N_2 FIXATION THROUGH SELECTION AND BREEDING

We saw a need to promote the use of N_2-fixing legumes in the cereal-dominated agriculture of Australia's northern cropping belt. Summer and winter legumes could be grown, although some species were more adapted and had better market potential (e.g. soybean, mung bean, pigeon pea, chickpea, faba bean) than others. However, there existed certain problems that had to be addressed before farmers would accept the legumes and change established patterns of cropping.

We report three programmes that addressed the N_2-fixation problems of soybean, common bean (also known as navy bean) and pigeon pea. Selection and breeding for enhanced N_2 fixation of soybean commenced at Tamworth in 1980 after surveys of commercial crops indicated that nodulation was sometimes inadequate, particularly on new land, and that the levels of N_2 fixation were variable and often low. Similar programmes were established in 1985 with common bean and in 1988 with pigeon pea (Table IV).

TABLE IV. PROGRAMMES AND COLLABORATING PERSONNEL AT THE NSW AGRICULTURE RESEARCH CENTRE, TAMWORTH

Species	Definition of problem	Research activity	Collaborating personnel
Soybean *(Glycine max)*	• Poor nodulation on new land, low grain yields. • Low and variable N_2 fix'n in commercial crops.	***Selection*** of improved N_2-fixing genotypes; commenced 1980. ***Breeding*** for enhanced nodul'n and fixation; commenced 1986	Dr IA Rose Breeder NSW Ag.
Pigeon pea (*Cajanus cajan)*	• Poor nodulation and N_2 fixation, reduced grain yields on alkaline vertisols.	***Selection*** for high nodulating, high-fixing genotypes with improved grain yields in vertisols. ***Research*** on effects of applied Fe and N.	Mr JF Holland Agronomist NSW Ag.
Common bean (*Phaseolus vulgaris)*	• Commercial crops don't fix sufficient N for high yields. Require fertilizer N	***Selection*** from potentially useful genotypes on basis of nodul'n and N_2 fixation. ***Evaluation*** of rhizobia.	Dr RJ Redden Breeder, QDPI Mr J Brockwell, Dr MB Peoples CSIRO

3.1. Soybean

The rhizobia that nodulate soybean do not occur naturally in Australian soils. Consequently, seed must be inoculated at sowing. Nodulation failures, particularly in land sown for the first time to soybean, resulting in yellowing of foliage, poor crop growth and reduced grain yield, have been a feature of the industry since its inception, and have caused a great deal of concern. Experiments at the NSW Agriculture Field Station on the Liverpool Plains, Breeza, indicated that nodulation failures in first-year crops of soybean resulted from insufficient numbers of rhizobia in the seedling rhizosphere. Suggested strategies to overcome the problem were improved inoculants and inoculation procedures. On the other hand, selection and breeding for more-vigorously-nodulating cultivars would provide a more satisfactory, long-term solution to the problem.

Even when crop yield was not affected by poor nodulation, research indicated that less than 60% of the N required by the average crop was derived from N_2 fixation [2]. The remainder was taken up as nitrate from the soil and the net effect of soybean cropping was loss of soil N fertility. Thus, some of the advantage of growing a legume was lost. We concluded that development of cultivars of soybean capable of fixing large amounts of N_2 even at moderate to high levels of soil nitrate, would provide an economic bonus to the farmer and make soybean a far more valuable and attractive crop. The potential saving of soil and fertilizer N in Australia was 5,000 t/annum, valued at $3 million. Globally, the potential saving was 5 million tonnes of N, valued at $3 billion.

In response to these challenges, we commenced a programme in 1980 with objectives to improve the N_2-fixation capacity of soybean and, at the same time, solve the problem of poor nodulation and low yields of crops sown on 'new' land. The first step was to screen a large number of genotypes for nodulation and symbiotic tolerance of nitrate. Plants were assessed for growth, nodulation and N_2 fixation (relative ureide-N in xylem sap and plant parts; see *3.3.1.*). The first two cycles of screening involved growing plants in sand-filled pots in a glasshouse, supplied with either nitrate-free nutrients or nutrients containing 2.5 m*M* nitrate. A further two cycles were conducted in high-nitrate field soils.

There were large variations in responses to nitrate (Table V; [3]). From the original 489 genotypes, 66 'nitrate-tolerant' lines were identified on the basis of an overall index that combined the three ureide values and the nodulation index. The second screening was similar to the first in identifying variation, and confirmed the consistency of 32 of the original 66 'tolerant' lines. Genotypes of Korean origin displayed higher than average levels of nodulation and N_2 fixation in the presence of nitrate.

Of the original 19 Korean lines, 15 (80%) were included in the second screening, and nine (47%) were selected for the third round of (field) screening. Only 5% of the remaining 470 genotypes were selected as high-fixing after the two glasshouse screenings. It became apparent also that substantial differences in tolerance to nitrate occurred among the commercial cultivars, e.g. Davis and Lee had greater tolerance than Bragg.

In the third year, 40 genotypes were sown into a high-nitrate soil in the field [4]. Genotypes of Korean origin showed the highest levels of nodulation and N_2 fixation (Table VI). They had shoot yields similar to commercial cultivars Bragg and Davis, suggesting that increased N_2 fixation reduced their use of soil N. Post-harvest measurements of soil nitrate confirmed this; immediately after grain harvest, up to 34 kg/ha additional N was recovered from the Korean plots compared with the Bragg plots. Seed yields of the Korean lines were, on average, 30% less than that of Bragg, due to a combination of shattering, early maturity and poor agronomic type. Correlation matrices among the indices of nodulation and N_2 fixation and plant growth and grain yield revealed independence between the symbiotic- and yield-related characters. Therefore, the Korean lines appeared to be suitable for use as high-fixing donor parents in a breeding programme with selection for both grain yield and N_2 fixation.

TABLE V. MEAN VALUES FOR UREIDE INDICES OF N_2 FIXATION AND FOR NODULATION OF GENOTYPES OF SOYBEAN SCREENED FOR NITRATE TOLERANCE (2.5 m*M* NITRATE-N, SUPPLIED WITH NUTRIENTS)

	Relative ureide-N values Xylem sap (%)	Shoots (%)	Roots (%)	Nodulation index[a] (%)
First glasshouse screening				
High N_2-fixing lines (66)[b]	43	39	35	3.3
Low N_2-fixing lines (9)	10	5	3	1.2
Second glasshouse screening				
High N_2-fixing lines (32)	38	20	29	3.7
Low N_2-fixing lines (2)	15	4	10	1.8
Bragg	16	6	8	1.8

[a] (nodule mass/shoot mass) x 100. [b]A total of 489 genotypes in the first screening and 87 in the second. Data show selected groups of high- and low-fixing lines [3].

TABLE VI. ASSESSMENTS OF NODULATION, N_2 FIXATION AND YIELD OF SELECTED 'NITRATE TOLERANT' KOREAN GENOTYPES OF SOYBEAN AND COMMERCIAL CULTIVARS IN A HIGH NITRATE SOIL AT BREEZA, NSW, 1985 [4, 5]

Genotype	Nodulation Wt. (mg/plant)	Nodulation No/plant	Pfix (%)[a]	Shoot DM (g/plant)	Grain yield (t/ha)
Nitrate tolerant					
Korean 466	376	34.5	31	45.9	1.6
Korean 468	254	16.8	18	43.3	1.7
Korean 469	176	19.5	22	41.6	1.4
Korean 464	319	16.5	11	48.1	1.5
Commercial					
Bragg	24	2.0	0	39.7	2.2
Davis	40	1.3	0	48.5	2.2

[a]Proportion of N obtained from N_2 fixation, assessed during mid pod-fill using the xylem ureide technique [6].

Subsequent comparisons of Korean genotypes 466 and 468 with commercial cultivars, Bragg and Davis, and mutants of Bragg, nts1007 and nts1116 [7], at five field sites showed that the Korean genotypes nodulated better than did Bragg, Davis and nts1116, and were about equal to nts1007 [8]. Values for Pfix, estimated using xylem ureide and natural ^{15}N abundance methods, were similar for the two Korean genotypes, nts1007 and Davis. Bragg had the lowest values for Pfix, with nts1116 intermediate between Bragg and the other four. These high levels of symbiotic activity of the Korean genotypes were in spite of low seed yields and early maturity.

Results from the four years of screening indicated that differences in nodulation between the Korean genotypes and commercial cultivars occurred only when symbiosis was stressed, i.e. with moderate to high nitrate supply in glasshouse sand-culture or in the field, or with low numbers of soybean rhizobia in the field. In the absence of stress, nodulation of the two groups was similar. Thus, enhanced nodulation of the Korean genotypes was not mediated through a loss of the autoregulatory processes that limit nodulation, as with nts mutants [9, 10], or through an altered ability to assimilate and metabolize nitrate [3], but likely resulted from more-efficient rhizobial infection and/or nodule initiation.

The four Korean genotypes, 464, 466, 468 and 469 were used as high-fixing parents in crosses with commercial cultivars, Valder (maturity group [MG] IV), Reynolds (MG VI), Forrest (MG VI) and Bossier (MG VIII) [11]. The breeding protocol differed in a number of ways from that used in a successful programme with common bean (F. Bliss and co-workers, University of Wisconsin, USA): material was screened for the most part in high-nitrate rather than low-nitrate soils; the xylem-ureide method was used to assess N_2 fixation; initial assessments of N_2 fixation were with individual F_2 plants, although later assessments (F_6 and F_7) involved populations of plants. A summary of activities is presented in Table VII.

Nitrogen fixation was assessed on individual F_2 plants using the xylem-ureide method. Sap was extracted from the top half of each plant leaving the lower half to continue growth and to produce seed for harvest [11, 12]. The relative abundance of ureide-N of the F_2 plants varied between 2 and 55%, indicating segregation for N_2-fixation activity (Fig. 3). There was no evidence of heterosis, in contrast to results reported before for alfalfa [13], pea [14] and soybean [15]. Average relative ureide-N values for the 11 F_2 populations were surprisingly constant (24 - 29%, equivalent to Pfix values of 13 - 20%) and were between the lower values of the commercial parents (17 - 28%; Pfix values 2 - 19%) and the higher values of three of the four Korean parents (39 - 42%; Pfix values 36 - 40%). The relative ureide-N value for the fourth Korean parent, 464, was 27%, about the same as for Reynolds, the best commercial parent. Although average N_2 fixation activities of the F_2 populations were below the best Korean lines, 35 individual F_2 plants had equally high N_2 fixation, i.e. relative ureide-N $>40\%$.

The F_2 populations were culled on the basis of N_2 fixation (xylem relative ureide-N value $>31\%$) (Fig. 3), plant type (agronomic rating >2 on a scale of 1 to 6) and seed colour (yellow, green or yellow-green). Evidence of linkages between N_2 fixation and other more easily determined plant characters was also sought. Correlation matrices of these characters showed no such linkages. Problems could have occurred if N_2 fixation was found to be linked to certain traits of the Korean genotypes, e.g. black, brown seeds, poor agronomic type. On the other hand, linkage to other more benign traits could have led to simpler procedures for selecting material.

At the commencement of this study, the genetic control of enhanced N_2 fixation in the Korean genotypes was unknown. Major genes had been identified that influence *Bradyrhizobium* compatibility [16, 17, 18] and hypernodulation [19] of soybean. Frequency distributions of relative ureide-N values in each of the 11 F_2 populations were normal, with no evidence of discontinuities to suggest a major gene segregation.

TABLE VII. PEDIGREES OF 11 POPULATIONS OF SOYBEAN AND NUMBERS OF SINGLE PLANTS OR LINES ASSESSED AT EACH GENERATION FOR EITHER PLANT AND SEED TRAITS (F_2-F_7 GENERATIONS), YIELD (F_4-F_7 GENERATIONS) OR N_2 FIXATION (F_2, F_6 AND F_7 GENERATIONS)

Population/ pedigree	F_2 single plants 1986-87	F_2-derived in F_3 gener'n 1987	F_3-derived in F_4 gener'n 1987-88	F_3-derived in F_5 gener'n 1988-89	F_3-der'd in F_6, F_7 1989-91
	(number)				
A. 464 x Valder	161	22	144	36	3
B. Valder x 464	61	6	45	5	0
C. Valder x 466	87	16	89	26	1
D. Valder x 468	49	8	58	14	1
E. Reynolds x 466	72	9	73	25	8
F. Reynolds x 464	14	1	11	1	0
G. 464 x Forrest	38	5	49	10	3
H. Forrest x 469	119	14	112	37	6
J. Bossier x 464	121	6	46	10	0
K. 468 x Bossier	93	12	109	29	10
L. Bossier x 469	34	5	47	7	1
Total	849	104	783	200	33

Culling of F_3-derived lines in the F_4 and F_5 generations was based on yield and agronomic traits. Nitrogen fixation was again assessed in the F_6 and F_7 generations. A number of the F_3-derived lines were clearly superior and, importantly, stable in N_2 fixation (Fig. 4). In both the F_6 and F_7 generations, these lines had relative ureide-N values of around 40%, compared with consistently lower or more variable values for other lines. A number of the consistently high-fixing lines (A82-3, D22-8, K78-1 and E68-5) were subsequently used as parents in a backcrossing programme (I.A. Rose and D.F. Herridge, unpublished data).

The values for yield and N_2 fixation presented in Table VIII summarize the progress made in the first cycle of selection. At the high-nitrate sites in the F_6 and F_7 trials, Forrest was obtaining 27 and 33% of its N from N_2 fixation at the time of sampling. By contrast, N_2 fixation by Korean 468 accounted for 47 and 52% of current inputs of N. Lines D22-8, A82-3, A46-4, and E72-3 were outstanding in terms of N_2 fixation, with Pfix values of around 50%. The Pfix value for commercial cultivar Reynolds was around 41%, suggesting that it already had the desired symbiotic characteristics, i.e. tolerance of the suppressive effects of soil nitrate on nodulation and N_2 fixation.

Grain yields of the F_3-derived lines in the F_6 and F_7 generations, although substantially larger than yields of Korean 468, could not match those of the highest-yielding commercial cultivar, Forrest. Some lines gave yields comparable to older commercial cultivars such as Bossier. In particular, lines A46-4, K78-1 and D22-8 had average yields across the six trials of >2.0 t/ha.

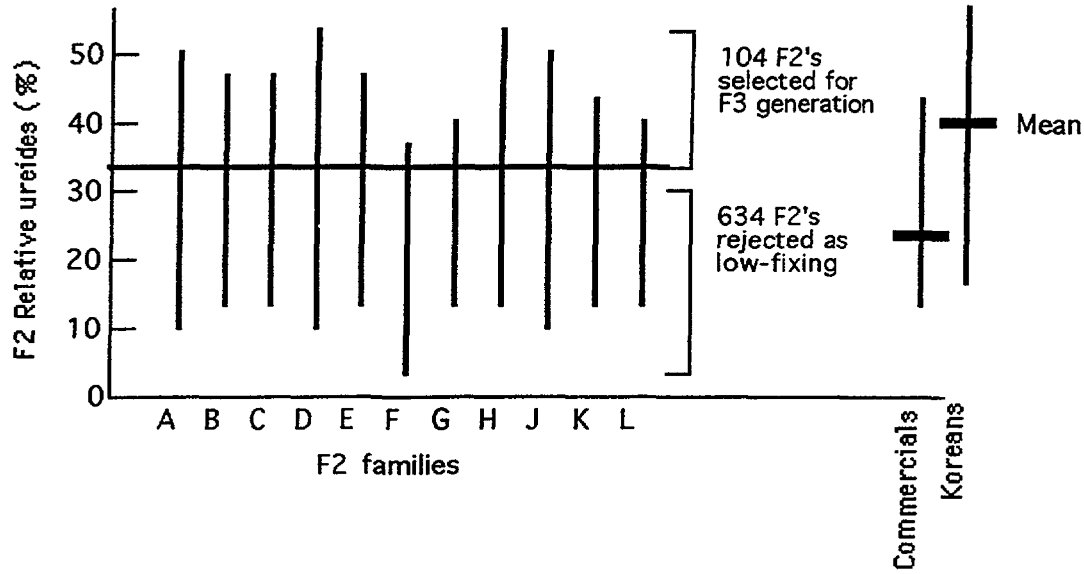

FIG. 3. Ranges of N_2 fixation (relative ureide-N of xylem sap) for the eleven F_2 families and for the commercial and Korean parents. The horizontal line through the families indicates the cut-off point for selection for F_3 generation.

We concluded that the field site chosen for assessing N_2 fixation was vital for discriminating the high-fixing, nitrate-tolerant lines. The enhanced capacity for N_2 fixation of these lines and of the Korean parents was expressed only when N_2 fixation of the commercial cultivars, e.g. Forrest, was suppressed by high-nitrate soil. Data from the F_6 and F_7 trials support this by showing that correlations across sites and/or seasons were improved when the soils were high in nitrate.

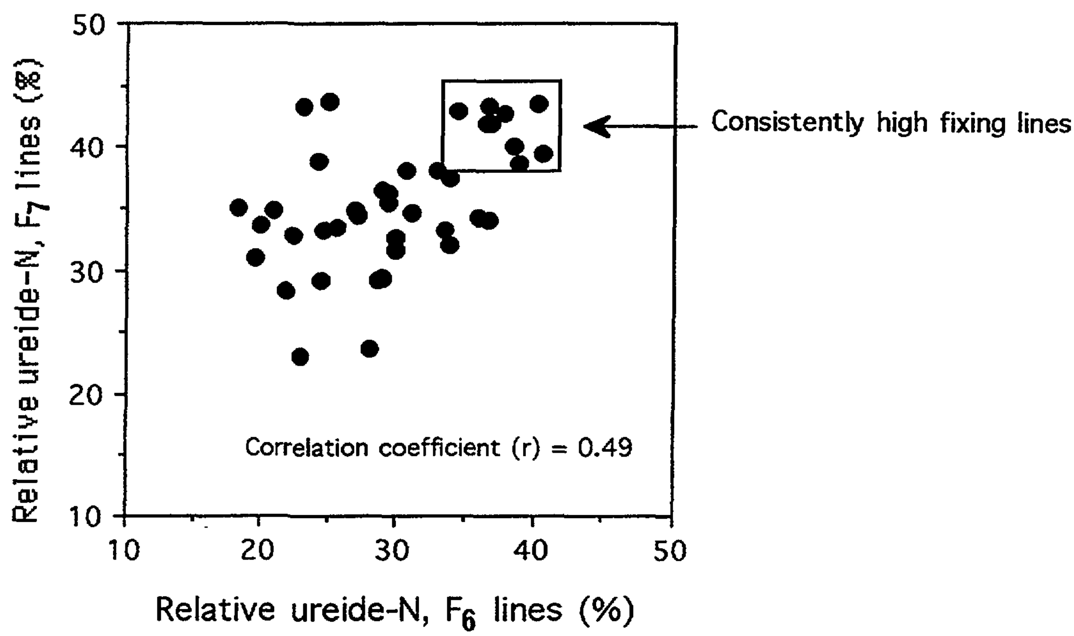

FIG. 4. Relative abundance of ureide-N for F_3-derived F_6 and F_7 lines of soybean, grown in successive years on high nitrate soils at Breeza and Narrabri, Australia.

Yields of the high-fixing, nitrate-tolerant material need to be improved by about 20% before commercial release. A second cycle of selection was commenced in 1991 with crossing of lines D22-8, A82-3, A46-4 and K78-1 with high-yielding genotypes. Single-seed descent lines were formed as F_4 single plant progeny and more than 1400 lines from six populations were field-tested in F_5 and F_6 trials for phenology, growth habit, lodging, disease resistance, yield, shattering and seed oil and protein. The best lines from those assessments were then evaluated for N_2 fixation in the F_7 generation during the 1994-95 summer season.

TABLE VIII. DAYS TO FLOWERING, SEED YIELD AND N_2 FIXATION AT F_6 AND F_7 FOR LINES SELECTED FOR BACKCROSSING AND FOR HIGH (KOREAN 468) AND LOW N_2-FIXING (FORREST) PARENTS

Line	Original cross	Flowering	Seed yield mean 3 sites		Pfix high nitrate sites	
			F_6	F_7	F_6	F_7
		(days)	(t/ha)		(%)	
Forrest		50	2.67	2.66	27	33
D22-8	Valder x Korean 468	46	2.08	2.29	47	55
A82-3	Korean 464 x Valder	52	2.00	1.70	49	49
K78-1	Korean 468 x Bossier	57	1.91	2.09	46	54
A46-4	Korean 464 x Valder	58	2.24	2.20	51	56
Korean 468		43	0.95	0.58	47	52

3.2. Pigeon pea

Pigeon pea has potential as a summer-crop alternative to winter legumes in Australia's northern wheat belt. There are problems, however. Research during the 1980s showed that pigeon pea nodulates poorly, fixes little N_2 and produces low yields when grown on the alkaline, black-earth soils that are common to the area. Table IX shows a typical set of results from Duri, near Tamworth - no effect of inoculation with rhizobia, virtually no N_2 fixation activity and a doubling of grain yield with applied fertilizer N. Such poor performance of unfertilized pigeon pea on these soils represents a barrier to adoption by farmers, even though the selling price of the grain is high ($350 - $400 per tonne) compared with other crops.

We believed that genotypes of pigeon pea better adapted to nodulating and growing on alkaline soils might be present in the Australian pigeon-pea germplasm collection at the University of Queensland. In 1988, we assembled some 500 genotypes from the collection and commenced screening for N_2 fixation, grain yield and maturity. Our aim was to identify genetic variation in nodulation and yield capacity on the alkaline, black earths, and to select superior genotype(s) for commercial release or for use as parent(s) in a breeding program.

In the first season (1988-89), the genotypes were grown on a black earth at Tamworth. In the following year (1989-90), 105 of the most promising lines were further evaluated at two nearby sites. The check cultivars, Quest, Quantum and Campea yielded consistently (range 1.42 - 1.49 t/ha), but showed no evidence of N_2 fixation in five of the six combinations of cultivar x site (Table X). Eleven of the 105 test genotypes showed potential for either N_2 fixation (Pfix range 10 to 19%, after ignoring a low value for E137 at Currabubula) or yield (range 1.47 to 1.65 t/ha). The relationship between Pfix and yield was not significant ($P = 0.05$) and none of the 11 genotypes gave high values for both characters. Nitrogen fixation activity could not be detected for 86 of the 100 genotypes at the TARC site, nor for 51 of the 62 genotypes at Currabubula.

At that time, it was found that low iron (Fe) availability depressed yields of pigeon pea on an alkaline black earth on the Liverpool Plains (Breeza) of northern New South Wales [20]. Seed yields were increased by more than 400% with additions of 20 kg Fe/ha to the soil as the iron chelate, FeEDDHA. Plant nodulation and N_2 fixation were not assessed in that study. It has also be shown that Fe deficiency specifically limits nodule initiation and development in peanut [21] and lupin [22], and that strains of rhizobia producing siderophores could form functioning nodules under conditions of low Fe availability. Similarly, plant genotypes with improved resistance to iron chlorosis had been identified for a range of agricultural species, including legumes [23]. Together, these results suggested that variation in tolerance to alkalinity/low Fe may exist for genotypes of pigeon pea [23] and of rhizobial strains [21], and that symbiotic combinations should be sought to replace the currently-recommended cultivar (cvv. Quest, Quantum)-*Bradyrhizobium* strain CB756 combinations. Although previous results [24] indicated that depressed symbiotic activity and yield of pigeon pea were associated with the host plant and not with CB756, there was no evidence that the latter was a siderophore-producer or was adapted to alkaline soils.

TABLE IX. EFFECTS OF INOCULATION AND FERTILIZER N (100 kg N/ha AS UREA) ON YIELD AND NITROGEN FIXATION OF PIGEON PEA GROWN ON AN ALKALINE, BLACK EARTH SOIL AT DURI (1987)

Cultivar/Treatment	Grain yield (t/ha)		Pfix (%)	
	-Fertilizer N	+Fertilizer N	-Fertilizer N	+Fertilizer N
Quantum				
Uninoculated	0.72	1.32	0	0
Inoculated	0.74	1.37	0	3

In the following season (1990-91), we evaluated effects of Fe, fertilizer N and strain of rhizobia on nodulation, N_2 fixation and yield. Results were encouraging (Table XI). We increased grain yields from a low of 0.5 t/ha (old strain of rhizobia, no fertilizer) to 2.4 t/ha with a new strain of rhizobia and Fe and N fertilizers. Curiously, the highest yields were obtained only when fertilizer Fe and N were applied together. In the absence of fertilizer N, when the pigeon pea was more dependent on N_2 fixation, yields

TABLE X. YIELDS AND N_2 FIXATION OF SELECTED GENOTYPES OF PIGEON PEA IN 1989-90. A TOTAL OF 108 GENOTYPES, INCLUDING THREE CHECKS, WERE SOWN INTO ALKALINE BLACK EARTHS AT TWO SITES NEAR TAMWORTH. EACH VALUE IS THE MEAN OF EITHER TWO (TARC) OR THREE (CURRABUBULA) REPLICATES

Genotype TARC [a]	UQ [b]	Days to flowering	Seed yield TARC (t/ha)	Pfix (%) TARC	Currabubula
Lines with N_2-fixation potential					
E25	4	71	1.30	10	11
E32	994	72	1.31	19	-[c]
E42	622	70	-	-	12
E93	1039	70	1.07	14	-
E137	1017	69	0.97	14	0
M63	356	71	1.08	11	-
Lines with yield potential					
E51	990	69	1.55	0	-
E111	116	74	1.54	0	-
M61	885	74	1.47	0	1
M77	748	72	1.65	0	-
M144	T30	71	1.50	0	6
Check lines					
Quest		72	1.44	0	7
Quantum		72	1.42	0	0
Campea		79	1.49	0	0

[a]Tamworth Agricultural Research Centre. [b]University of Queensland. [c]Not determined.

flattened out at 1.5 t/ha. With N applied but no Fe, yields were only marginally better at 1.7 t/ha. These results confirmed the importance of Fe to nodulation, N_2 fixation, and grain yield, but challenged us to bring the yields up to 2.4 t/ha without relying on fertilizer N.

To answer that challenge, we continued to screen for genetic variation in capacity of pigeon pea to nodulate, fix N_2 and yield on these high-pH soils. Thus, between 1991 and 1994, we evaluated 22 of the most promising genotypes from the original screenings at a number of sites in northern NSW.

In the summer of 1990-91, 14 genotypes were evaluated at Breeza in the presence and absence of Fe. The soil is a deep, black earth, pH 8.2, low in plant-available N following 15 years of cereals. In the unamended soil (-Fe), we found significant differences among genotypes in nodulation, N_2 fixation and yields of dry matter and N of shoots and grain. During during vegetative growth, Fe increased nodulation, N_2 fixation and dry matter of shoots but reduced shoot %N. Differences in the responsiveness of the genotypes to applied Fe were significant also. Four genotypes (E76, M48, M144 and Quest) showed a low level of response to Fe, whereas others (E33, M96, L133 and Campea) were highly responsive (Table XII). In the unfertilized plots, nodulation, N_2 fixation and shoot dry matter were generally higher for the non-responsive genotypes than for the responsive group. By late pod-fill, Fe increased shoot dry matter by an average of 28% for the non-responsive group and by 50% for the responsive group. Iron increased grain yields by an average of 0.32 t/ha (25%) (non-responsive group) and 0.70 t/ha (108%) (responsive

TABLE XI. EFFECTS OF STRAIN OF RHIZOBIA AND FERTILIZER FE AND N ON SYMBIOTIC ACTIVITY (NODULATION AND N_2 FIXATION) AND GRAIN YIELD OF PIGEON PEA, GROWN ON THE ALKALINE, BLACK EARTH SOILS OF NORTHERN NSW

Treatment	Nodulation (mg/plant)	Pfix (%)	Grain yield (t/ha)
Rhizobia			
Old strain (CB756)	12	22	0.47
New strain (CB1024)	81 (+575)[a]	50 (+127)	1.47 (+212)
Fertilizer			
None	81	50	1.47
+Fe	107 (+32)	46 (-8)	1.46 (0)
+N	13 (-84)	17 (-66)	1.67 (+14)
+Fe +N	40 (-50)	14 (-72)	2.43 (+65)

group). Genotype E76 was particularly promising, out-yielding the commercial check, Quest, by 0.20 t/ha (17%) in the absence of Fe and by 0.39 t/ha (26%) when Fe was applied. Campea nodulated poorly and produced the lowest yield of all 14 genotypes (0.51 t/ha). It was also the most responsive genotype to Fe (170% increase). Effects of Fe and genotype on %N of shoot dry matter and grain were less than during early growth (data not shown).

In the following season, 18 genotypes were grown under irrigation at Breeza. The experiment was sampled three times for nodulation, N_2 fixation (xylem-sap ureides) and yield parameters: at flowering/early pod-fill, late pod-fill and maturity.

Fertilizer Fe and N increased yields of shoot dry matter and grain (Table XIII). Although responses were negligible at flowering, by late pod-fill the fertilized plants were, on average, 23% larger than the unfertilized plants. At maturity, the fertilized plants contained 35% more dry matter and produced 27% more grain. We recorded an opposite effect of the fertilizers on symbiotic characters. The applied Fe and N depressed nodule weight and number by 85-90% and the N_2 fixation index (xylem sap relative ureide-N) by about 30%. It is probable that both Fe and N contributed to the growth and yield increases and that the N alone depressed nodulation and N_2 fixation.

A number of the genotypes performed reasonably well, although none was outstanding. The data in Table XIV are from the unfertilized (-Fe -N) plots; 11 of the 18 genotypes are included. Results were generally consistent with the two genotype-evaluation trials of the previous (1990-91) season.

In 1991-92 the highest-yielding group of genotypes, Quest, E76, M48 and M144 were all high yielders in the previous season, and showed low responsiveness to fertilizer Fe (Table XII). Genotypes E31 and E42 were intermediate in yield and responsiveness in that trial. The low-yielding genotypes, Campea and E33 were similarly identified in 1990-91 in the absence of fertilizer Fe and highly responsive to Fe.

TABLE XII. EFFECTS OF FE ON NODULATION, N_2 FIXATION AND GRAIN YIELD OF GENOTYPES OF PIGEON PEA, GROWN AT BREEZA, NSW, ON AN ALKALINE, BLACK EARTH (1990-91)

Genotype[a]	Nodulation		Pfix Flowering		Pfix Late pod-fill		Grain yield	
	-Fe	+Fe[b]	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe
	(mg/plant)		(%)				(t/ha)	
Low response to Fe								
E76	14.2	30.2	25	24	55	48	1.40	1.89
E88	4.2	22.6	17	19	42	38	1.25	1.29
M48	16.3	28.6	25	30	55	40	1.27	1.50
M144	10.6	30.8	24	21	58	68	1.18	1.42
Quest	19.0	31.0	17	20	63	50	1.20	1.50
Highly Responsive to Fe								
E33	3.2	17.3	12	16	28	36	0.90	1.41
M96	1.7	27.2	13	18	35	50	0.68	1.18
M132	3.4	39.8	16	19	58	60	1.02	1.68
L133	1.8	28.3	13	22	38	44	0.70	1.61
Campea	1.2	43.3	16	21	49	70	0.51	1.38

[a]Fourteen genotypes were in this experiment; E31, E42, E51 and E61 showed intermediate responsiveness to Fe.
[b]Fe applied at 16 kg/ha at sowing as FeEDDHA.

Only one genotype performed inconsistently: M132 was in the highest-yielding group in 1991-92, but was classified as low yielding and responsive to applied Fe in 1990-91. Nodulation and relative ureide-N values were inconsistent within the two groups of genotypes (Table XIV). The largest effects were due to fertilizer (N) (data not shown).

A summary of the yield data from the trials to evaluate potentially adapted genotypes is presented as Table XV. In 1990-91, 22 genotypes were sown at Narrabri, a number of which yielded as well as the standard cultivar Quest. Campea again yielded poorly. Grain %N values were low (range 2.77 - 3.45, equivalent to about 20% protein) and there was a tendency for the low yielders to have higher grain %N values.

In 1991-92, eighteen genotypes were included in the Premer and Moree trials. Yields were higher at Premer (range 1.37-2.38 t/ha) than at Moree (0.28-0.73 t/ha) (Table XV). All of the genotypes flowered within a few days of Quest. Genotypes E42 and E51 had the earliest anthesis of 72 days (mean of the two sites) compared with 74 days for Quest and 78 days for Quantum. Genotype E76 was the highest-yielding line overall.

TABLE XIII. EFFECTS OF FERTILIZER FE (4 kg/ha) AND N (200 kg/ha) AS UREA) APPLIED AT SOWING ON YIELD AND SYMBIOTIC COMPONENTS OF PIGEON PEA, GROWN ON AN ALKALINE VERTISOL AT BREEZA, NSW (1991-92)

Component	-Fe -N	+Fe+N	Response to +Fe+N
Yields (t/ha)			
Shoot DM - flowering	2.11[a]	2.13	+1%
- pod-fill	3.11	3.82	+23%
- final harvest	2.42	3.28	+35%
Grain yield	0.77	0.98	+27%
Symbiosis			
Nodule DM (mg/plant)	88	9	-90%
Nodule no./plant	19	3	-85%
Rel. ureides - flower (%)	46	34	-26%
- pod-fill (%)	35	24	-32%

[a]Values presented are the means of the 18 genotypes.

TABLE XIV. YIELD AND SYMBIOTIC TRAITS OF SELECTED GENOTYPES, GROWN WITHOUT FERTILIZER IN AN ALKALINE, BLACK EARTH AT BREEZA, NSW (1991-92)

Genotype[a]	Plant pop. (no/m^2)	Shoot dry wt. late pod-fill (t/ha)	Grain yield (t/ha)	Nodulation No.	Nodulation Wt. (mg)	Rel. ureides S1[b] (%)	Rel. ureides S2[c] (%)
Highest yielding							
Quest	16	3.22	0.93	22	137	45	41
E31	14	3.50	0.98	34	135	48	34
E42	23	3.23	0.85	16	60	47	38
E76	10	3.17	0.86	14	82	46	32
M48	19	3.38	0.87	39	132	54	38
M132	13	2.98	0.84	14	63	42	32
M144	20	3.38	0.94	15	90	50	33
Lowest yielding							
Campea	18	3.70	0.69	36	184	55	33
E28	16	2.75	0.70	18	79	27	34
E33	11	2.74	0.71	16	63	41	31
E61	16	3.20	0.55	17	80	57	36

[a]Data are not presented for genotypes Quantum, E6, E51, M58, M117, M136 and L133.
[b]Flowering/early pod-fill. [c]Late pod-fill.

TABLE XV. GRAIN YIELDS OF SELECTED PIGEON-PEA GENOTYPES, GROWN WITHOUT FERTILIZER ON ALKALINE BLACK EARTHS AT SITES IN NORTHERN NSW IN THE 1990-91 TO 1993-94 SEASONS

	1990-91	1991-92		1992-93	1993-94
Genotype[a]	Narrabri	Premer	Moree	Breeza	Spring Ridge
			(t/ha)		
High-yielding					
Quest	1.55	2.30	0.69	0.22	1.84
Quantum	1.56	*[b]	*	*	1.90
E31	1.61	2.26	0.73	0.21	2.03
E76	1.59	2.38	0.68	0.33	2.19
M48	1.50	2.16	0.61	*	*
M132	1.55	2.20	0.64	0.37	2.28
M144	1.69	*	*	*	2.20
ICPL85014	*	*	*	*	2.18
Low-yielding					
Campea	1.10	1.37	0.28	*	*

[a]Genotypes E28, E33, E51, E54, E61, M58, M114 and L133 were intermediate between the two listed groups.
[b]Designates that the genotype was either not sown or was not high-yielding in that trial.

In 1993-93 seventeen genotypes were grown without irrigation at Breeza. The trial was sown with little sub-soil moisture. Total rainfall was below average for the season, consequently yields were low, ranging from 0.19 to 0.37 t/ha. Genotype E76 was again in the high-yielding group. Quest produced only 0.2 t/ha grain, about 50% below that of the high-yielding genotypes. Relative ureide-N values, indicative of N_2 fixation, were low for all genotypes.

During the 1993-94 season, nineteen genotypes, including two early-maturing lines from ICRISAT, were grown without irrigation at Spring Ridge Yields ranged between 1.7 and 2.3 t/ha, generally higher than in previous years (Table XV). Genotypes M132 and M144 were the highest yielders, superior to the standard cv. Quest by about 0.4 t/ha. Genotype E42 was again the earliest to flower, at 4 days before Quest, 6 days before Quantum and simultaneously with an early-maturing accession from ICRISAT, India, ICPL85014. This genotype was acquired at TARC as two selections with the same maturity, but slightly different seed colour and a yield difference of 0.5 t/ha.

Our original objective in this project was to identify genetic variation in pigeon pea in capacity to nodulate and yield on the alkaline, black earth soils, and to select superior genotype(s) for commercial release or for use as parent(s) in a breeding program. Our strategy was to assemble a large collection of genotypes and to evaluate them on the black soils for maturity, grain yield, and nodulation and N_2 fixation

(symbiotic activity). During the course of this project, we were able to increase yields and, to some degree, symbiotic activity, through applications of fertilizer Fe and N, through the use of a new inoculant strain of rhizobia, and through plant-genotype selection.

Results from the four seasons of experiments with pigeon pea indicate that the problem of poor nodulation, little N_2 fixation and low grain yields on the alkaline, black earths is complex. In one experiment, we succeeded in raising yields from 0.5 to 2.4 t/ha through: rhizobial strain, fertilizer Fe and fertilizer N. In other trials, genotypes such as E76 outyielded Quest, the current commercial cultivar, by as much as 20% in the absence of fertilizer Fe and N applications and by an average of 6% over all trials. It was not clear, however, that these increases were large enough to be used in isolation as the solution to the problems of growing pigeon pea on the alkaline, black earths. In the experiments in which fertilizer Fe and N were applied as treatments, yields of Quest were increased by 65% when both Fe and N were applied. Thus, even higher yields than those recorded for E76 may be necessary before pigeon pea can be considered an economic alternative to soybean and mung bean on the alkaline, black earths.

Yields were, at best, adequate on the lighter and better-drained (Spring Ridge and Premer) soils and generally low on the heavier, poorly-drained, Breeza-type soils, suggesting that pigeon pea is better adapted to the lighter black-earth soils of the region rather than to the heavier soils of the plains. It may also have a place on the more acid, red-earth soils of the slopes, on which Fe would not be deficient and its ability to withstand dry conditions would hold it in good stead. Work at ICRISAT revealed low yields of pigeon pea on heavy, black earth (vertisol) soil types [25]; yields on the black earth were about half of those on a red earth (alfisol), and excess soil moisture was believed to contribute to the depressed yields. It is likely that waterlogging played a role also in our experiments.

In conclusion, although we have made progress in better understanding the critical role of Fe and in unravelling other problems linked to low N_2 fixation and poor yields on our alkaline soils, we believe that a major breakthrough in pigeon-pea genotype improvement (i.e. 50% increases in N_2 fixation and yield) may be difficult and will certainly require a larger research programme.

3.3. Common Bean

The many cultivated types of common bean (dry bean, kidney bean, navy bean, culinary bean, etc.) are inefficient N_2 fixers. This has been attributed to insufficient nodulation and to the plant's inherent inability to fix N_2, and, as a result, farmers must grow them either in highly fertile soils or apply fertilizer N to obtain economic yields. Therefore, improvement in N_2 fixation would offset or wholly replace the need for fertilizer N, thereby reducing costs of production. In Australia, the culinary and navy bean industries are currently worth about $8-10 million annually, involving 400-500 growers producing 12,000-15,000 tonnes from about 15,000 ha . Eliminating the need to apply fertilizer N to crops of navy and culinary bean would save growers $1-1.5 million annually.

The first stage of the Queensland Department of Primary Industries programme to introduce high N_2 fixing cultivars to the industry involved screening 1462 genotypes/cultivars of common bean for capacity to nodulate under a range of field conditions, using indigenous and introduced rhizobia [26]. From the initial screening, 92 genotypes were selected for further evaluation at a single site (Inglewood) in 1985, from which nineteen were selected from further assessment, together with four check cultivars, at four sites in 1985 (Rocklea) and 1987 (Hermitage, Applethorpe and Kingaroy). The major findings of those assessments are presented in Table XVI. The best performing genotype, over all management practices, was ICA21573, and others nodulated well under two of the three management treatments, e.g. Campbell

20, Small White 38. The low-nodulating check, Gallaroy, performed poorly. Surprisingly, Puebla 152, the high N_2-fixing parent from the University of Wisconsin breeding programme performed only moderately. No assessments were made of N_2 fixation.

In 1988, an experiment was conducted at Inglewood as a first step in developing and evaluating the ureide method for measuring N_2 fixation by common bean in the field, and to test whether genotypes that are selected for increased nodulation also display improved N_2 fixation [28]. Plant nodulation and relative ureide-N in xylem sap were significantly correlated at each of the two samplings, suggesting that the ureide method had promise as a field assay of N_2 fixation. There were no cultivar effects on relative ureide-N values.

We considered the results of our own programme (1983-88) and of overseas research indicating that nodule function, i.e. N_2 fixation, rather than nodulation *per se* was the most likely cause of low productivity in bean. Thus, a greater emphasis needed to be given to assessing N_2 fixation, rather than nodulation, of bean genotypes. The ureide method had shown potential as an assay of N_2 fixation in preliminary experiments. Further research was required to calibrate the method using ^{15}N, so that it could be used quantitatively, rather than as a qualitative assay for treatment comparisons. The poor performance in Canada and South America of rhizobial strain CC511, then used in Australian commercial inoculant for common bean, raised doubts about the wisdom of its continued use. These issues needed to be addressed.

In 1989, we conducted a field experiment in which the main-plot treatments were (i) fertilizer N (80 kg N/ha) plus rhizobial inoculum, (ii) inoculum only and (iii) untreated control. Subplots were 10 genotypes of bean, chosen from the 1985 and 1987 trials for high nodulation (BAT419, Campbell 20, Selection 46, Small White 38, BAT1198, ICA21573, Epicure and Amarillo 155) and low nodulation (Gallaroy and Kerman). Nitrogen fixation was assessed using the ureide method. Nodulation could not be assessed with confidence because of problems of recovery from the heavy clay soils.

Application of commercial inoculant (strain CC511) resulted in reduced shoot weights in two of the three samplings (Table XVII). Shoot weight for the third sampling and grain yield were reduced also by inoculation, although differences were not significant at $P=0.05$. Fertilizer N increased plant growth and grain yields, although effects were not statistically significant in all cases; time to maturity was extended by 5%. The 10 genotypes varied significantly in the yield and agronomic characteristics examined. The largest plants did not necessarily produce the highest grain yields, although two of the three smallest genotypes, Epicure and Small White, did produce the lowest yields. The majority of the genotypes were bush types (vining score <2.0).

These results confirmed previous observations that bean yields can be increased with fertilizer N, and provided additional evidence that their N requirements are not met entirely from N_2 fixation. They indicated also that the commercial inoculant strain, CC511, may not be highly effective in N_2 fixation.

The largest effects on N_2 fixation, assessed as ureide-N in xylem sap, were from fertilizer N (Table XVII). In the first sampling, inoculation increased relative ureide-N levels above those of the uninoculated controls for nine of the ten genotypes (data not shown), although when main-plot effects were considered the differences were significant only at the 10% level. At the two remaining samplings, values for the plus and minus inoculation treatments were identical. The best performers were BAT419, Selection 46 and BAT1198.

There were no differences between the high- and low-nodulating genotypes in N_2 fixation. Kerman and Gallaroy, the two low nodulators, had average relative ureide-N values (Table XVII). On the other hand, ureide values for the high-nodulating group were both high (BAT419 and Selection 46) and low (Amarillo 155 and Epicure). In research overseas, high levels of N_2 fixation have been associated

TABLE XVI. EFFECTS OF INOCULATION AND FERTILIZER N (100 kg/ha AS UREA) ON NODULATION OF COMMON BEAN CULTIVARS IN SCREENING TRIALS AT KINGAROY, HERMITAGE, APPLETHORPE AND ROCKLEA IN 1985 AND 1987 [27]

Genotype	Nodule mass[a]		
	Uninoculated	+Inoc. +N	+Inoc.
ICA21573	2.06	3.00	3.17
Campbell 20	2.19	*[b]	4.17
Amarillo 155	2.00	2.75	*
BAT1198	1.75	2.60	*
Small White 38	*	2.50	4.33
Selection 46	*	1.50	4.00
Epicure	1.63	3.00	*
Puebla 152	2.06	1.37	*
Gallaroy	1.00	1.00	2.08

[a]Nodule abundance (rating of 1, 2 or 3) x size (rating of 1, 2 or 3). [b]Nodul'n not sig. better than Gallaroy.

with late maturity and a climbing habit [29], implying a direct relationship between leaf-area duration and N_2 fixation. Our results did not support these findings. The most consistent in N_2 fixation, BAT419, was a bush type of intermediate maturity. The two late, vining genotypes, Epicure and Amarillo 155, had the lowest levels for N_2 fixation. All three had been identified previously as high nodulators [27].

The yield reductions with rhizobial inoculation suggested that the native soil rhizobia were more effective at fixing N_2 than was CC511, the strain used in the inoculant. Mr J. Brockwell, CSIRO Plant Industry, subsequently joined the programme to evaluate the effectiveness of CC511 by comparing it with a number of highly effective strains from overseas (Table XVIII) and with strains isolated from field soils in southern Queensland.

To gain a better understanding of the effectiveness of the native soil rhizobia in navy-bean areas, 15 isolates from three field soils were tested for N_2 fixation in a controlled-environment study. There were 80 host cultivar x rhizobial strain combinations, i.e. 5 cultivars of bean x the 15 strains plus an uninoculated control.

Results indicated that the 15 rhizobial isolates differed in symbiotic capacity with the five bean cultivars (Table XIX). The best (6, 3, 7, 13, 8) produced around 30% more growth than did the poorest (14, 9). There was a pattern in their origins: the five best were isolated from Rocklea or Inglewood soils, four or which were from BAT419 or Campbell 20. Three of the four poorest performers were isolated

TABLE XVII. EFFECTS OF MANAGEMENT PRACTICE AND GENOTYPE OF BEAN ON SHOOT WEIGHTS AND RELATIVE UREIDE-N VALUES AT THREE SAMPLINGS, AND AGRONOMIC CHARACTERISTICS (AT HERMITAGE, 1989)

Practice/Genotype	S1[a]	S2	S3	U1[b]	U2	U3	Y[c]	V[d]	M[e]
	(g/plant)			(%)			(t/ha)		(days)
Management practice									
Control	41	92	99	27	28	24	1.65	1.9	91
+Inoc.	36	82	85	33	27	25	1.53	1.8	92
+Inoc. +N	42	98	111	12	15	15	1.99	1.9	97
Genotype									
Gallaroy	43	99	92	22	24	25	1.68	1.0	80
BAT419	50	119	108	26	29	28	1.72	1.3	89
Campbell 20	41	100	98	26	22	18	1.60	2.3	90
Selection 46	40	95	103	29	25	25	2.04	1.0	85
Small White	29	79	78	25	22	21	1.22	2.4	87
Kerman	40	88	92	25	22	21	1.55	1.0	89
BAT1198	42	98	120	22	25	26	2.02	1.0	91
ICA21573	42	92	115	21	23	21	1.69	1.0	102
Epicure	34	57	86	22	17	12	1.39	4.0	106
Amarillo155	37	81	92	21	24	16	2.02	3.4	115

[a]Shoot dry weight. [b]Relative ureide-N from the 46- (1), 60- (2) and 76- (3) day samplings. [c]Grain yield. [d]Vining scale 1 (= 0) to 4 (= 100% viney). [e]Time to 80% maturity.

from Hermitage soil. Overall, genotype ICA21573 was again the highest yielder (mean over the 15 strains: 1.44 g/plant), followed by Selection 46 (0.84 g/plant). The others, in order, were BAT419 (0.74 g/plant), Campbell 20 (0.66) and BAT76 (0.62).

Nine rhizobial strains (designated in Table XVIII), plus CC511 and Isolate 6 from Table XIX (CC507) were used to inoculate the same five cultivars - ICA21573, Campbell 20, Selection 46, BAT419, and BAT76. Plants were grown in 25-cm pots as before, and were harvested at 40 days.

Results (Table XX) indicate three broad groups of rhizobial strains, of low, moderate and high effectiveness. The current Australian inoculant strain (CC511) and the field isolate from Rocklea (Isolate 6) were highly effective on all five cultivars - an unexpected result in light of the previous year's field experiment at Hermitage, needing verification under field conditions. The low effectiveness of strains 127K89, CIAT652 and CIAT7001 was unexpected also, since, presumably, they had been selected for high effectiveness in inoculation trials in the United States and Colombia (Table XVIII).

TABLE XVIII. INFORMATION ON STRAINS OF BEAN RHIZOBIA RECEIVED FROM THREE OVERSEAS LABORATORIES FOR EFFECTIVENESS TESTING AGAINST THE AUSTRALIAN INOCULANT STRAIN, CC511

Source	Country	Contact	Strain	Synonym
NifTAL	USA	Dr HH Keyser	TAL182[a]	
			TAL943[a]	KIM-5, 127K102a
			TAL1382	C-O5
			TAL1383	CIAT632, 21
			TAL1797[a]	CIAT899, M188, 127K119
Nitragin Co.	USA	-	127K119	CIAT899
			127K102a	KIM-5, TAL943
			127K89[a]	
			127K105[a]	CIAT161, Z164
CIAT	Colombia	Dr J Kipe-Nolte	CIAT632[a]	TAL1383, 21
			CIAT652[a]	
			CIAT7001[a]	
			CIAT2513[a]	

[a]Strains used to produce the data in Table XX.

A low N-fertility site was prepared at Applethorpe, southern Queensland, to field-test rhizobial strains that had been identified as highly effective on five bean cultivars under glasshouse conditions. However, the experiment was not sown as planned because of drought in early 1991. Another site at Applethorpe was prepared for sowing two experiments in January 1992. Treatments in the first were rhizobial strains CC511, CIAT 161, CIAT 2513, TAL 182 and commercial inoculant (strain CC511), either singly or in various combinations with and without fertilizer N, with cultivars BAT419, Selection 46, Kerman, ICA21573 and Epicure. The second experiment consisted of 80 genotypes of bean, inoculated with a mixture of effective rhizobial strains. Both experiments were destroyed by flooding.

Following the abandoned field experiments of 1991 and 1992, a pot experiment was done in a temperature-controlled glasshouse at Hermitage using essentially the same cultivar x rhizobia combinations. The potting medium was an Applethorpe soil. Rhizobial inoculation treatments were as for the abortive experiments, with cultivars BAT419, Selection 46, Kerman, ICA21573 and BAT1198 (instead of Epicure).

No strain of rhizobia produced greater nodulation or plant growth than did CC511 (pure strain) or the commercial inoculant containing CC511 (Table XXI). Surprisingly, the uninoculated plants had the best nodulation, although it was not reflected in enhanced plant growth. Predictably, nodulation was depressed in the +N treatment. We concluded from these results that CC511 was as good in nodulating and fixing N_2 over a range of cultivars as the most effective rhizobial strains assembled from overseas,

TABLE XIX. ORIGIN AND EFFECTIVENESS WITH COMMON BEAN OF 15 RHIZOBIAL ISOLATES FROM THREE BEAN-FIELD SOILS

	Origin		
Isolate	Soil	Trap host	Shoot dry wt.[a] (g/plant)
1	Inglewood	ICA21573	0.92
2	Inglewood	Selection 46	0.88
3	Inglewood	BAT419	0.99
4	Rocklea	Selection 46	0.88
5	Inglewood	BAT76	0.88
6	Rocklea	BAT419	1.04
7	Inglewood	Campbell 20	0.97
8	Rocklea	BAT76	0.95
9	Hermitage	Selection 46	0.74
10	Hermitage	BAT76	0.90
11	Hermitage	BAT419	0.79
12	Hermitage	Campbell 20	0.82
13	Rocklea	Campbell 20	0.95
14	Rocklea	ICA21573	0.76
15	Hermitage	ICA21573	0.80
Uninoculated control			0.46

[a]Mean growth of cvv. ICA21573, Campbell 20, Selection 46, BAT419, BAT76.

but that inoculation using any of the strains resulted in decreases in nodulation but not in plant growth. These findings must be treated with caution because of the restrictions of pot culture, and need to be confirmed under the more demanding conditions that exist in the field.

Cultivar effects on both nodulation and growth were significant (Table XXI). Cultivars BAT419, ICA21573, Selection 46 and BAT1198 were similar in nodulation and were superior to Kerman. Shoot dry matter and N reflected nodulation, with Kerman again inferior to the other four.

Field comparisons of the most promising strains of rhizobia against the current commercial navy bean inoculant, strain CC511, were made in 1993. The trial was sown at Applethorpe, on a coarse-textured soil and was sampled for nodulation, xylem sap, shoot dry matter and grain. Treatments were eight inoculant strains or strain mixes x five cultivars, arranged as split plot with cultivar as main plots. Results are summarized in Table XXII.

TABLE XX. EFFECTS OF STRAIN OF RHIZOBIA ON SHOOT DRY MATTER AND N ACCUMULATION BY COMMON BEAN

Strain	Dry wt.[a] (g/plant)	Shoot %N[a]	Total N[a] (mg/plant)
Highly effective			
127K105 (CIAT161)	0.88	3.52	30.7
TAL182	0.88	3.46	30.3
Isolate 6 (CC507)	0.84	3.53	29.4
CC511	0.84	3.73	30.7
TAL943 (127K102a)	0.86	3.35	28.2
CIAT2513	0.92	3.45	31.5
Moderately effective			
CIAT632 (TAL1383)	0.79	3.31	25.3
TAL1797 (127K119)	0.84	3.08	25.8
Low effective			
127K89	0.66	2.64	17.3
CIAT652	0.65	2.61	16.2
CIAT7001	0.77	2.83	21.5
Uninoculated	0.58	2.79	15.7

[a]Interactions of genotype x strain were essentially non-significant; therefore, shown are the mean values for the five lines, ICA21573, BAT419, Selection 46, Campbell 20, BAT76.

At flowering, we recorded responses to inoculation in nodulation and N_2 fixation (xylem ureides), but not in shoot dry matter. There were similarities, rather than differences, amongst the strains, with CIAT 2513 having consistently high values for all parameters measured. Fertilizer N suppressed nodulation and N_2 fixation, but had no real effect on shoot dry matter. By pod-fill, the effects of inoculation had disappeared; fertilizer N continued to depress N_2 fixation. At maturity, highest grain yields were recorded for the fertilizer-N treatment followed by strains CIAT 2513 and TAL 182. The uninoculated plants had the lowest yields. Cultivar effects were less significant than strain effects.

Overall, Selection 46 and BAT1198 had the highest values for symbiotic and/or yield traits. Kerman and ICA211485 were the poorest nodulators (Table XXII). The commercial inoculant strain, CC511, was about average for all parameters measured and all three samplings. Within a few months, however, the strain used in the commercial inoculants was changed to RCR3644 (G. Gemmell, personal communication), because of the development of colony variants in CC511, rather than because of questionable effectiveness. In summary, our results and those unpublished from the Australian Inoculants Research and Control Service (AIRCS) indicate that a number of strains, including CC511 and the other three in Table XXII, are effective on navy and culinary bean and would be suitable for use in commercial inoculants.

TABLE XXI. EFFECTS OF RHIZOBIAL STRAIN ON GROWTH AND NODULATION OF *P. VULGARIS*, IN A TEMPERATURE CONTROLLED GLASSHOUSE AT HERMITAGE IN 1992

Rhizobia/cultivar	Nodulation[a]		Shoot[a]		
	No. (per pot)	Wt. (g/pot)	Wt. (g/pot)	%N	N (mg/pot)
Rhizobia					
CIAT 161	204	0.24	21.1	1.85	388
CIAT 2513	162	0.18	20.1	1.85	374
TAL 182	159	0.16	21.3	1.88	398
CC511	157	0.18	20.4	1.79	372
Commercial inoculant	173	0.20	22.2	1.87	412
Comm'l inoc. + N[b]	141	0.16	19.4	1.68	335
Uninoculated	267	0.40	20.5	1.82	372
Cultivar					
BAT419	173	0.22	22.2	1.68	374
ICA21573	219	0.15	23.8	2.00	470
Selection 46	235	0.31	23.2	1.85	430
BAT1198	185	0.34	19.3	1.72	334
Kerman	87	0.07	15.0	1.89	286

[a]Values shown are for the strains/inoculation treatments averaged over the five cultivars, and the cultivar means averaged over the six strains.
[b]100 kg N/ha.

At the Applethorpe site, three additional trials were sown simultaneously to evaluate early- and late-maturing genotypes of navy and culinary bean for nodulation and N_2 fixation (xylem ureides). The genotypes were selected on the basis of good growth characteristics in previous screenings. A total of 116 genotypes were sown in the three trials - 30 for each maturity group, replicated twice; 56 in the 'Other' group, unreplicated. Plants were sampled twice for nodulation, xylem sap, shoot weight and grain.

Data for 27 of the best performing genotypes from the three trials and for the five check lines (Selection 46, Gallaroy, Spearfelt, Sirius and Rainbird) are presented in Table XXIII. Some showed particular promise, because of high nodulation (699X, 1128, 223, 1059, 220, 843 and 830), high relative ureide-N value (1128, 216, 1301, RIZ36, 830 and 1094), high plant yield (832, RIZ32, ICA153446 and Spearfelt) or high grain yield (96, 1325, RIZ32 and RIZ36). These genotypes will be further evaluated for symbiotic and yield traits in the field.

TABLE XXII. RHIZOBIAL AND CULTIVAR EFFECTS ON SYMBIOTIC AND GROWTH TRAITS OF COMMON BEAN, GROWN IN THE FIELD (AT APPLETHORPE, 1993.

	Flowering (51 DAP)[a]				Pod-fill (72 DAP)[a]		Maturity
Rhizobia/cultivar	Nodulation No.	Nodulation Mass (mg)	RUN[b] (%)	Shoot DM (t/ha)	RUN[b] (%)	Shoot DM (t/ha)	Grain yield (t/ha)
Rhizobia							
-Inoc.	16	22	15	1.45	29	3.43	1.31
+Inoc. +N[c]	11	10	12	1.39	24	3.76	1.70
CIAT161	31	45	18	1.34	31	3.44	1.36
CIAT2513	58	57	21	1.40	30	3.72	1.56
TAL182	34	40	17	1.42	31	3.72	1.56
CIAT161+2513	38	72	20	1.46	31	3.43	1.41
CC511	51	53	20	1.36	29	3.50	1.43
All-strain mix	45	49	19	1.42	28	3.53	1.51
Cultivar							
BAT419	39	48	19	1.22	28	3.36	1.52
ICA211485	33	23	13	1.55	29	3.61	1.35
Selection 46	45	48	17	1.51	31	3.91	1.64
BAT1198	41	64	21	1.41	28	3.45	1.40
Kerman	21	34	18	1.33	29	3.49	1.49

[a]Days after planting. [b]Relative abundance of ureide-N in xylem sap. [c]100 kg N/ha as urea.

3.3.1. 1991-92 calibration of the xylem ureide method for common bean using ^{15}N

Many crop legumes, e.g. soybean, cowpea, pigeonpea and mung bean, transport the bulk of fixed N from root nodules to shoots as ureide compounds (allantoin and allantoic acid). They also take up mineral N from the soil and transport it as nitrate and amino compounds. Therefore, since the nitrogenous compounds in the transpiration stream represent the current products of N uptake (including N_2 fixation), it has proved possible to distinguish the sources of incoming N as from soil or atmosphere, by analyzing the xylem sap.

The ureide method can be used without calibration as an index of N_2 fixation [28]. After calibration it can be used to quantify N_2 fixation, i.e. to determine Pfix and in combination with N-accumulation data, to determine the amount of fixed N in kg/ha. Preliminary experiments had indicated that the method could be applied in studies of common-bean N_2 fixation [30] and we considered that the method should be properly calibrated for use in this and subsequent programs. Calibration involved growing plants with ^{15}N-labelled nitrate applied in the glasshouse with sampling throughout growth for ^{15}N enrichment of dry matter and for ureides, amino-N and nitrate in xylem sap. At each sampling time, the relative abundance of ureide-N in the xylem sap could be related to Pfix, determined using the isotope-dilution method.

TABLE XXIII. EVALUATION OF EARLY- AND LATE-MATURING GENOTYPES OF COMMON BEAN FOR SYMBIOTIC AND YIELD TRAITS (AT APPLETHORPE, 1993)

Genotype	Nodulation		Rel. ureide-N	Shoot DM	Grain
	No. (per plt)	Mass (mg)	(%)	(t/ha)	(t/ha)
Early-maturing					
699X	37	130	30	3.22	1.04
1128	33	124	37	3.17	1.73
96	18	97	35	3.32	1.84
1261	31	85	30	3.12	1.18
1115	37	83	31	3.29	1.15
832	22	58	32	3.60	1.60
RIZ53W	30	50	35	3.20	1.41
1325	21	41	35	3.51	1.62
216	18	17	39	2.62	0.79
Selection 46	42	59	25	3.05	1.36
Gallaroy	21	41	30	3.54	1.48
Late-maturing					
223	89	332	33	3.33	0.80
1059	61	185	19	3.99	1.69
220	42	160	35	3.12	1.27
1341	55	147	31	2.85	1.06
Purple Pod	37	147	28	3.52	1.39
1301	53	129	36	3.39	0.59
RIZ32	29	110	31	4.07	1.83
ICA153446	27	99	35	4.51	1.50
RIZ36	43	93	40	3.85	1.80
Spearfelt	34	101	30	4.05	1.38
Sirius	31	73	31	3.31	1.41
Rainbird	29	71	20	4.00	1.66
Other					
843	39	184	26	-[a]	-
830	50	161	41	-	-
RIZ103	69	148	25	-	-
1294	35	146	24	-	-
RIZ104	74	126	27	-	-
1094	35	79	38	-	-
907	18	75	44	-	-
391	37	63	36	-	-
Mutiki 2	18	30	34	-	-

[a]Not determined

Navy bean cv. Gallaroy plants were grown in a mixture of sand and vermiculite in large pots in a temperature-controlled glasshouse. Once seedlings had emerged, pots were watered on alternate days during the first six weeks and daily thereafter with 2 to 3 L of a nutrient solution supplemented $K^{15}NO_3+Ca(NO_3)_2$ to give concentrations of 0, 1, 2, 5, or 10 m*M*. Enrichments (% atom excess) in ^{15}N were 0.52 (10 m*M* nitrate), 1.03 (5 m*M*) and 2.05 (1 and 2 m*M*). Plants were harvested on four occasions: during vegetative growth, and at the flowering, pod-fill and seed-filling stages, for dry matter, %N, ^{15}N, and xylem sap as root-bleeding sap (RBS) and vacuum-extracted sap (VES). Experimental details were similar to those previously reported for soybean [6].

Nitrate levels had a consistent effect on Pfix and relative ureide-N in RBS and VES (Fig. 5). The Pfix values ranged from 100% for plants supplied with the N-free nutrient solution to zero for uninoculated plants supplied with 10 m*M* nitrate. Values for Pfix for intermediate treatments were 88-91% (1 m*M* nitrate), 75-80% (2 m*M*), 40-53% (5m*M*) and 12-31% (10 m*M*) (Fig. 5A). Values for relative ureide-N were in the range 5 to 82% for RBS and 5 to 70% for VES (Figs. 5B, C). The higher values for RBS are consistent with those obtained with soybean [6].

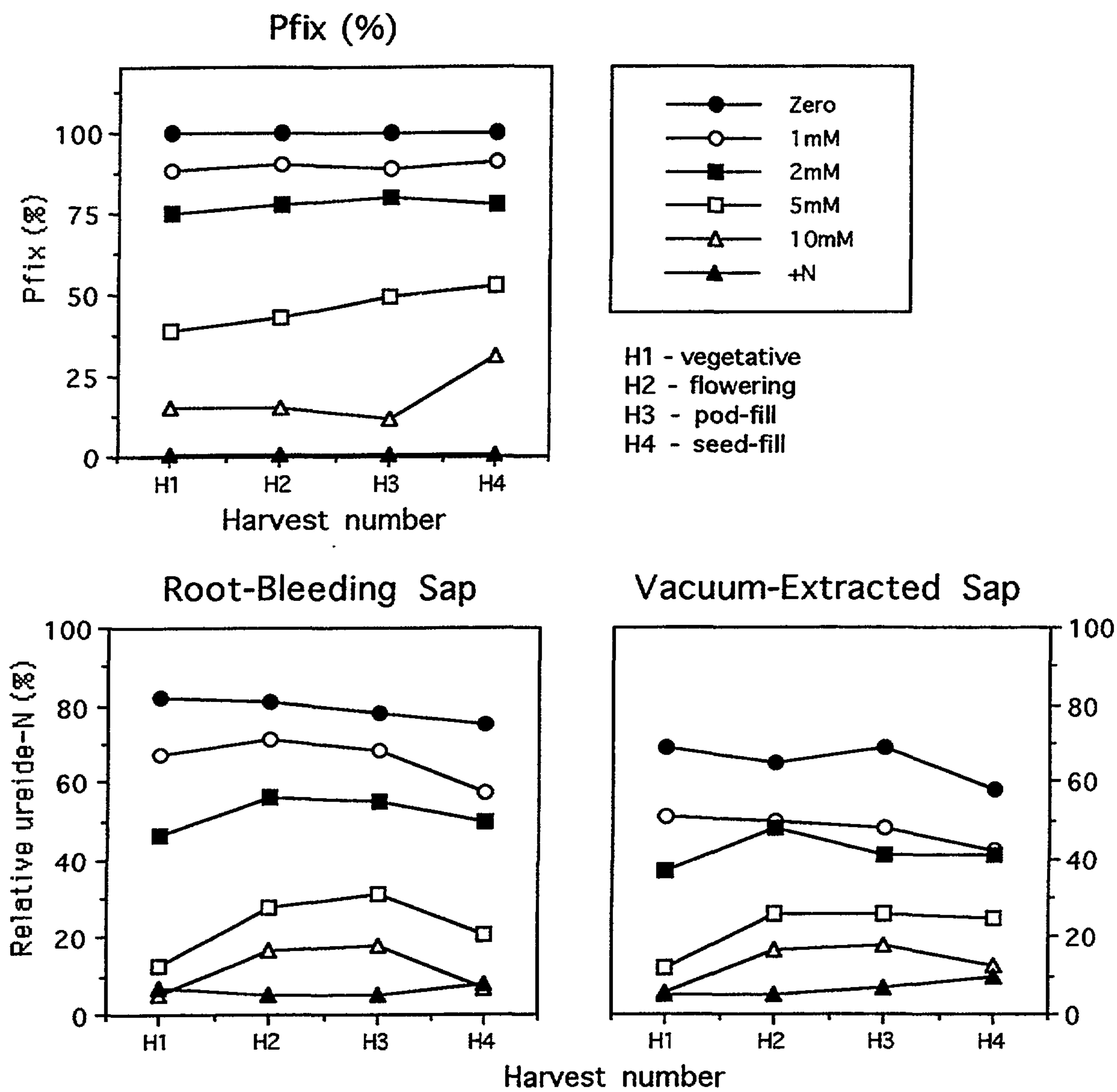

FIG. 5. Effects of nitrate supply on Pfix, relative ureide-N (%) of root-bleeding sap, and relative ureide-N (%) of vacuum-extracted sap of common bean over four harvest times: H1 - vegetative; H2 - flowering; H3 - pod-fill; H4 - seed filling.

The relative abundance values for ureide-N in RBS and VES were plotted against Pfix and subjected to regression analysis. Relationships were very strong with 97 (RBS) and 95% (VES) of the variation in relative ureides explained by variations in Pfix (Fig. 6). These regressions are very similar to those for pigeon pea and soybean [6, 31], and provide a means for calculating Pfix from solute analysis of xylem sap of common bean.

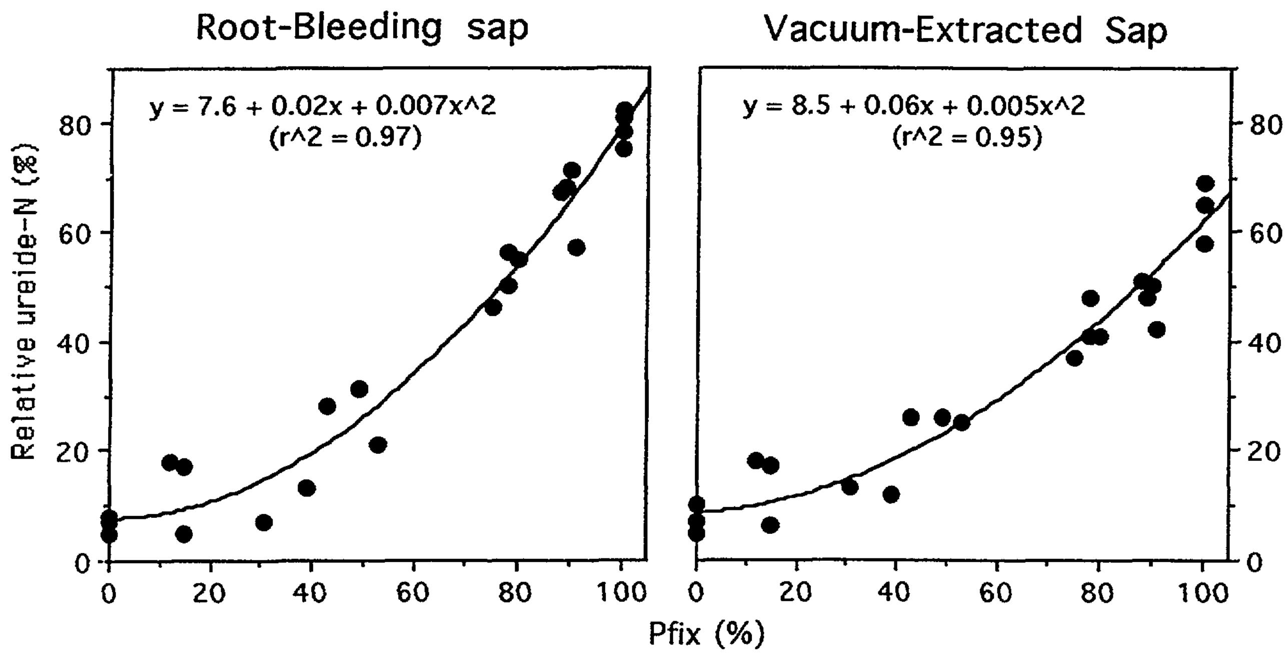

FIG. 6. Relationships between Pfix and relative abundance of ureide-N in root-bleeding sap and vacuum-extracted sap of common bean.

In conclusion, release of cultivars of navy and culinary bean with enhanced capacity for N_2 fixation could save Australian growers $1-1.5 million annually in fertilizer costs. Since 1988, we have made substantial progress towards this goal, having shown quantitative differences in nodulation and N_2 fixation capacity of bean genotypes and rhizobial-strain effects on symbiotic and yield traits of bean. With calibration, the xylem ureide method with ^{15}N, can be used as a field assay of N_2 fixation. Future research will continue these assessments until genotype(s) are identified with superior symbiotic and yield traits.

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FIELD EVALUATIONS OF N_2 FIXATION BY GRAIN LEGUMES IN PAKISTAN

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Abstract

FIELD EVALUATIONS OF N_2 FIXATION BY GRAIN LEGUMES IN PAKISTAN

Studies were undertaken with four legume species that are economically important in Pakistan, to gain an understanding of how host-genotype, rhizobial-strain, and environmental factors affect the root-nodule N_2-fixing symbiosis of field-grown plants. Strong responses to inoculation were obtained with lentil (*Lens culinaris*) that showed significant host-genotype×rhizobial strain interaction. In contrast, only one of eight mung-bean (*Vigna radiata*) genotypes and none of five black-gram (*V. mungo*) genotypes responded positively to inoculation; however, negative effects of inoculation were cautionary that host-genotype×rhizobial strain interactions must nevertheless be considered. Trials with chickpea (*Cicer arietinum*) indicated that biomass, grain yield and total N may be used as indicators of the amount of N fixed for large screening trials in which employment of the ^{15}N-dilution technique would be prohibitively expensive.

1. INTRODUCTION

In view of the persistent relatively high cost of fertilizer N, the exploitation of biological N_2 fixation is an attractive strategy for developing sustainable agricultural production. The role of N_2 fixation in legume N nutrition is well documented. However, it has been reported that various varieties/cultivars of grain legume, e.g. pigeon pea (*Cajanus cajan*), faba bean (*Vicia faba*), and cowpea (*Vigna unguiculata*), show significant differences regarding their ability to fix N_2 [1]. The influence of host-plant genotype on symbiotic effectiveness, nodulation and N_2-fixation is well documented [e.g. 2, 3, 4]. Strong cultivar×strain interactions have been reported for soybean (*Glycine max*) [5], common bean (*Phaseolus vulgaris*) [6] and peanut (*Arachis hypogaea*) [7] demonstrating that both symbionts, the host legume and the infecting rhizobium, have equal importance in influencing how much N is fixed; their co-selection for a given set of soil and environmental conditions may significantly enhance the amount of N fixed.

An essential component of legume studies is the accurate determination of amounts of N fixed, which requires separate measurements of N taken up from the soil and absorbed from the atmosphere. The ^{15}N-dilution technique is considered to be the most reliable for estimating N_2 fixation by nodulated legumes in the field. The method depends upon differences in the isotopic composition of the sources of N available to the plant, i.e. soil, fertilizer, and atmosphere [8]. It has the special advantage of providing an integrated determination over time of N derived from the atmosphere [9].

Lentil (*Lens culinaris*) cultivation in Pakistan covers 82,000 ha (26% of the area under pulses) with an annual grain production of approximately 30,000 t [10]. It is cultivated on marginal and less fertile lands with low indigenous rhizobial populations, therefore inoculation with rhizobia offers the potential to increase yields by stimulating N_2 fixation. There is little information available on the quantity of N_2 fixation by lentil as influenced by rhizobial strain and host cultivar, especially in soils with sparse native populations of its microsymbiont *Rhizobium leguminosarum* bv. *viceae*. Therefore, the integrated effects of inoculation and varietal diversity were examined in terms of growth, N_2 fixation determined by the ^{15}N-dilution technique, and yield of field-grown lentil.

Mung bean (*Vigna radiata*) and black gram (*V. mungo*) are also economically important legumes in Pakistan. Here we report studies of their responses to rhizobial inoculation, and patterns of nodulation, growth, grain yield and N_2 fixation over two seasons and at two locations.

The major limitation of the isotope-dilution technique is cost, of the instrumentation necessary to assay ^{15}N and of the isotopically labelled fertilizer [11]. Therefore, we set an ancillary objective: to determine how well nodulation, grain-yield and total-N data correlate with N_2-fixation data generated by the expensive and more laborious ^{15}N methodology, and to devise simpler methods for assessing fixation that would be applicable to field evaluation of large collections of germplasm, particularly for developing countries. We chose chickpea (*Cicer arietinum*) for this work, and evaluated 29 advanced mutant lines and cultivars in the field for nodulation, acetylene reduction activity, biomass, total-N accumulation, and ^{15}N dilution.

2. MATERIALS AND METHODS

2.1. Lentil

The experiment was done at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, located in semi-arid central Punjab, Pakistan, during 1992-93. The soil, a loam of the Hafizabad series, was non-saline, low in N and available P [electrical conductivity (ECe) 0.91 dS m^{-1}, total N 0.062%, available NH_4-N 0.90 ppm, NO_3-N 1.32 ppm, Olsen P 9 ppm, organic carbon, 0.54%, cation exchange capacity (CEC) 18.4 cmol kg^{-1}, extractable K 210 ppm, pH 7.8] with an indigenous population of *R. leguminosarum* bv. *viceae* of 36 cells g^{-1} soil, estimated by the most-probable-number (MPN) plant-infection technique [12]. Each plot was divided into two equal sub-plots, to one of which was applied ^{15}N-labelled fertilizer and to the other unlabelled fertilizer N; all other conditions were the same. The experiment was laid out in a randomized complete-block design with four replicates. Each replicate had five main plots.

Four *Rhizobium*-strain treatments were applied (Lc 6, Lc 26 and Lc 33 isolated from local soils, and exotic strain TAL 1397 obtained from NifTAL, HI, USA) with an uninoculated control as main plots. Peat-based single-strain inoculants were prepared from these strains. Each main plot was further divided into seven randomized sub-plots of six lentil varieties (Precoz, Precoz/F6-20-1 × M-85, Precoz/F6-23-1 × M-85, PL-406/M7-10-62, M-85, and PL-408) and wheat (*Triticum aestivum*) as the non-fixing reference crop. The lentil seeds were obtained from NIAB's Mutation Breeding Division.

The seeds were pelleted with the single-strain inoculants using gum arabic as adhesive. Two seeds were sown per hill spaced at 15 cm, with a row distance of 30 cm; seedlings were thinned to one per hill after a week. When the seedlings were 2 weeks old, liquid broth cultures of the same single strains (2 mL per seedling) were applied directly to the soil near the seedling roots in the respective inoculation plots, to maximize the possibility of inoculation-response.

At the 50% flowering stage, ten plants from each treatment-combination were excavated and nodule number and nodule dry weight recorded. At maturity, the plants were harvested and data for shoot and grain weight recorded. Total N determinations were made by the Kjeldahl method [13]. The $^{15}N/^{14}N$ analyses of leaves and grain were done at the IAEA's Seibersdorf Laboratory, Vienna, Austria with a mass spectrometer [14]. Treatment means were compared statistically using Duncan's Multiple Range Test [15]. To calculate Pfix (proportion of N from fixation) and Ndfa (amount of N derived from the atmosphere, i.e. from fixation) the following formulae were used [16]:

$$Pfix\ (\%) = \left(1 - \frac{\%^{15}N\ atom\ excess_{(legume)}}{\%^{15}N\ atom\ excess_{(reference)}}\right) \times 100$$

$$Ndfa\ (kg\ ha^{-1}) = \frac{Pfix}{100} \times Total\ N\ yield$$

2.2. Mung bean and black gram

The first experiment was sown in spring 1994, with eight mung-bean and five black-gram genotypes, whereas the second experiment (summer, 1994) examined three mung-bean and three black-gram genotypes. The seeds were obtained from the Mutation Breeding Division of NIAB.

2.2.1. Experiment 1

The experiment was conducted on a non-saline loamy soil of the Hafizabad series, low in N and available P (ECe 0.79 dS m^{-1}, total N 0.065%, available NH_4-N 1.10 ppm, NO_3-N 1.47 ppm, Olsen P 11 ppm, organic carbon 0.52%, pH 7.8) with an indigenous population of cowpea-miscellany rhizobia of $2x10^4$ cells g^{-1} soil, estimated by the MPN plant-infection technique [12].. Chickpea, lentil and maize (*Zea mays*) had been grown during the previous 10 years. The field was divided into two equal main plots and the soil of one was labelled by incorporating ^{15}N-enriched plant material. A basal dose of 60 kg ha^{-1} single super phosphate (SSP) was applied. The experiment was laid out in a randomized complete block design with three replications. Each replicate was divided into two sub-plots, inoculated and uninoculated. In both sub-plots each genotype was planted in two 3-m rows with a 15-cm spacing between plants and 30 cm between rows. After planting, a liquid mixture of three local strains of *(Brady)rhizobium* sp. (*Vigna*) (Vr16, Ma8 and K92) was applied to the soil as inoculum for all genotypes.

Harvesting protocols were the same for both experiments. A ten-plant sample of each genotype was collected from the unlabelled area at 50% flowering and maturity, to determine nodule number, and dry weights of nodules, shoot, and grain. At physiological maturity, plants from 0.45 m^2 of the labelled area of each sub-plot were collected for %N and ^{15}N-enrichment analyses. Calculations for %Pfix and Ndfa were made as described in **2.1.**

2.2.2. Experiment 2

The soil was a loam (ECe 0.98 dS m^{-1}, total N 0.057%, available NH_4-N 0.98 ppm, NO_3-N 1.54 ppm, Olsen P 8 ppm, organic carbon 0.7%, pH 7.6) with a native cowpea-miscellany rhizobial population was $5.2x10^2$ cells g^{-1} soil [12]. No leguminous crop had been grown during the previous 5 years. The experiment had a randomized complete-block design with five replications. In each

replicate there were five rows for each mung-bean or black-gram genotype and two non-N_2-fixing reference crops, maize and sorghum (*Sorghum bicolor*). An ^{15}N-labelled mini-plot of 1.5×14.4 m was marked in the center of each replicate with a 0.5-m spacing on both sides. ^{15}N was applied at the rate of 30 kg N ha^{-1} as $(NH_4)_2SO_4$ enriched with ^{15}N at 5% atom excess. The same amount of unlabelled $(NH_4)_2SO_4$ was added to each outer area. A basal dose of 60 kg ha^{-1} SSP was applied to each sub-plot. Seeds of three mung and three gram genotypes were sown with inter-plant and inter-row distances of 15 and 30 cm, respectively. After planting, a liquid mixture of the same above-mentioned strains of (brady)rhizobia was applied to the soil as inoculum for all genotypes. Average ^{15}N-enrichment values for the two reference crops were used to calculate Pfix.

2.3. Chickpea

Two experiments were conducted at NIAB.

2.3.1. Experiment 1

The soil was a sandy loam (pH 7.6) with initial available NH_4-N and NO_3-N concentrations of 0.98 and 1.54 ppm respectively. The plot was divided into two 20.5×11 m sub-plots. Twenty advanced chickpea mutant lines, cultivars, and hybrids (Table I) were selected for screening for N_2 fixation with wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) as reference crops, and sown in November 1990. The seeds were obtained from NIAB's Mutation Breeding Division. Genotypes C44, CM1918 and CM72 were commercial varieties and the others were either mutants or hybrids.

Ammonium sulphate, labelled with ^{15}N at 5.35% atom excess, was applied in one sub-plot at 30 kg ha^{-1} and the same amount of unlabelled $(NH_4)_2SO_4$ was added to the other. The label was applied in solution (80 mL m^{-2}) at sowing. A basal dose of 75 kg ha^{-1} P_2O_5 as SSP was applied to each sub-plot. The experiment had a randomized complete-block design with three replications. In each replicate there were three rows, 3 m in length, of each chickpea genotype, wheat and barley. Seeds were planted with inter-row and inter-plant spacing of 30 cm. Plants were not inoculated because of the known presence of effective indigenous chickpea rhizobia [17] enumerated at 200 cells g^{-1} [12].

2.3.2. Experiment 2

The second experiment was sown in the succeeding chickpea-growing season (November 1991) in the same field and with the same design and layout as the first. The number of chickpea genotypes tested again was twenty; nine that had been affected by gram-blight disease in the first experiment were replaced with disease-resistant genotypes (Table I). Thirty kg N ha^{-1} as $(NH_4)_2SO_4$ enriched with ^{15}N at 4.69% atom excess, was again applied in solution (80 mL m^{-2}) at sowing. The indigenous chickpea rhizobial population was estimated at 220 cells g^{-1} soil [12]; good nodulation had been confirmed in the first experiment, therefore inoculant was not applied. The same reference crops were used as in *2.3.1.*

A sample of ten unlabelled plants of each genotype was collected at 50% flowering to determine nodule number and dry weight, and nitrogenase activity, shoot and grain dry matter. At physiological maturity, 3-m lengths of middle rows (equivalent to 0.9 m^2) were collected from the labelled areas for each genotype, to determine %N and ^{15}N enrichment as described above, and at maturity ten unlabelled plants of each genotype were harvested to determine shoot dry matter and grain yield.

TABLE I. CHICKPEA ADVANCED MUTANTS/CULTIVARS TESTED

No.	Genotype	Parentage
1	C-44 (approved variety)	Local cultivated variety
2	CM-1	Mutant of local parent 6153
3	CM-72	Cultivated mutant of local parent 6153
4	CH-5[a]	Hybrid of two local parents Thel white×344
5	CM-1571-12A[a]	Mutant of exotic ICARDA genotype ILC-195
6	P12-45F	Hybrid of C44×ILC-195
7	CM-2[a]	Mutant of local parent 6153
8	CM-663	Mutant of C727 (hybrid cultivated variety)
9	CH-9	Hybrid of two local parents Thel white×344
10	P8-A	Hybrid of C44 and ILC-195
11	CM-1918[a] (approved variety)	Mutant of local parent 6153
12	CM-1571-22	Mutant of exotic genotype ILC-195
13	C-727 (approved variety)	Hybrid of F8×Punjab 7
14	CM-1913[a]	Mutant of local parent 6153
15	CM-687	Mutant of C727
16	CM-2197[a]	Mutant of CM72
17	P7-H[a]	Hybrid of CM72×ILC-195
18	CM-88[a]	Mutant of C727
19	CM-1571-12B[a]	Mutant of exotic genotype ILC-195
20	P5-B	Single plant selection
21	MB-75	Hybrid of C44×ILC195
22	50	Single plant selection of C727×C141
23	35	Single plant selection of C727×C141
24	Paidar 91 (approved variety)	Hybrid of C-235×ILC-191
25	Punjab 91 (approved variety)	Hybrid of RC-32×NEC 138-2
26	37	Single plant selection of C727×C141
27	19	Single plant selection of C727×C141
28	MB40	Hybrid of C44×ILC195
29	Noor 91 (approved variety).	Selected from Flip81-293C (hybrid of ILC-191×ILC-495)

[a]Infected in Experiment 1 with gram blight disease (*Ascochyta rabei*) and replaced by 21-29 in Experiment 2.

3. RESULTS AND DISCUSSION

3.1. LENTIL

Inoculation had significant beneficial effects on nodulation, biomass, grain yield, N yield, Pfix and Ndfa (Table II). Moreover, there were statistically significant differences amongst the strains in nodule number, nodule dry weight, plant biomass, grain yield, N yield, Pfix and Ndfa, as has been

shown before [18]. The locally isolated rhizobial strain Lc 26 out-classed the exotic TAL 1397, producing the highest number of nodules and the largest nodule dry weight, biomass, grain yield, total N and Ndfa, at 150, 200, 78, 57, 76 and 242%, respectively, higher than the uninoculated control. Lc 26 was statistically similar to TAL 1397 in Ndfa. The highest Pfix value (33%) was obtained with Lc 33.

Poor growth and yield by uninoculated lentil have been shown elsewhere [19], and other workers have similarly reported that inoculants improve legume growth and yield when the indigenous population of infecting rhizobia is sparse [20, 21, 22].

There were significant differences amongst the six lentil genotypes in response to inoculation (Table II). Although all were statistically similar in number of nodules, nodule dry weights differed significantly, with M-85 producing the highest and Precoz/F6-20-1×M-8 the lowest value, which not significantly different from those of Precoz and Precoz/F6-23-1×M-85. The highest values for biomass, grain yield and N yield were obtained with M-85, which was statistically similar to those of PL-406 and PL-406/M7-10-62. Maximum Pfix and Ndfa values were given by PL-406, statistically similar to thos of M-85 and PL-406/M7-10-62.

The six genotypes can be delineated into two groups: Precoz + Precoz/F6-20-1×M-85 + Precoz/F6-23-1×M-85, and M-85 + PL-406 + PL-406/M7-10-62. Precoz and its hybrids were statistically lower in nodule dry weight, biomass, grain yield, N yield, Pfix and Ndfa (Table II). Precoz is a bold-seeded variety from Argentina, apparently not well adapted to Pakistan, and, even in hybrid combination with M-85, nodulation, biomass and N_2-fixation were inferior. This strong host-genotype influence on N_2 fixation is consistent with previous research [e.g. 3, 18].

The effects of the lentil-host×*Rhizobium*-strain interaction on nodule number, nodule dry weight, biomass, grain yield, N yield, Pfix and Ndfa are shown in Fig. 1. Positive responses to inoculation were statistically significant for all host/strain combinations.

Nodule dry weight positively correlated with biomass (r = 0.90), grain yield (r = 0.78) and N yield (r = 0.74). There were highly significant correlations between biomass and N yield (r = 0.96) and biomass and Ndfa (r = 0.92). Generally, varieties or treatments with higher dry matter yield support more fixation and varieties with high Ndfa have high Pfix and *vice versa* [23].

It is clear from these results that the rhizobial strains and various lentil genotypes differed in their inherent potential to fix N_2. The range of Pfix values was broad: 3-52%, comparable with previous observations of 0-76% [24]. This demonstrates that care is needed in genotype selection to ensure a maximum input of fixed N.

In a previous study in the same experimental field, genotype M-85 produced 124 kg N ha^{-1} in response to the application of 150 kg N ha^{-1} (unpublished data), whereas, in this work, M-85 fixed a maximum of 48 kg N ha^{-1} from the atmosphere in accumulating total N values of <100 kg N ha^{-1} (Fig. 1 E and G). Such a gap between potential total-N yield and actual quantity of N fixed indicates the possibility of increasing N_2 fixation through careful selection of both *Rhizobium* strain and host genotype to ensure optimum compatibility between the symbionts.

3.2. Mung bean and black gram

There was significant variability among the genotypes of mung bean and black gram in both seasons, for nodulation, N_2 fixation, biomass accumulation, total N, and grain yield (Tables III, IV).

TABLE II. RESPONSE OF SIX LENTIL GENOTYPES TO INOCULATION WITH *R. LEGUMINOSARUM* BV. *VICEAE* STRAINS

Strain/ Genotype	Nod. no.	Nodule dry wt.	Biomass yield	Grain yield	Total N yield	Pfix	Ndfa
	(plt^{-1})	(mg plt^{-1})		(kg ha^{-1})		(%)	(kg ha^{-1})
Rhizobial strain							
Lc 6	5b[a]	7.3b	2389c	432bc	52c	25.9ab	15b
Lc 26	6a	8.7a	2849a	483a	67a	31.2a	24a
Lc 33	4c	6.6bc	2502bc	416c	57bc	33.0a	22a
TAL 1397	4c	6.1c	2533b	442b	63ab	30.2a	21a
Uninoc.	2d	2.9d	1603d	307d	38d	18.7b	7c
Lentil genotype							
1[b]	4b[c]	5.9c	1831c	358b	40c	8.9c	3.7b
2	4b	5.5c	1837c	326b	47c	8.5c	3.6b
3	4b	6.0c	1915c	328b	46c	22.9b	9.7b
4	5b	8.0b	3119b	521a	69b	45.5a	31.5a
5	6a	9.7a	3347a	546a	79a	46.2a	37.2a
6	5ab	8.0a	3258ab	537a	78a	48.4a	37.7a
$LSD_{0.05}$ for across-genotype/across-strain comparisons							
	1.3	1.3	324	41.2	12.9	18.3	5.9

[a]Averages of 18 observations, across genotypes. Numbers within strains and within genotypes followed by the same letter in a column are not significantly different ($P<0.05$). [b]Genotypes: 1 Precoz, 2 Precoz/F6-20-1×M-85, 3 Precoz/F6-23-1×M-85, 4 Pl-406/M7-10-62, 5 M-85, 6 Pl-406. [c]Averages of twelve observations, across strains.

3.2.1. *Experiment 1*

Of the eight mung-bean genotypes examined, only NM 92 showed a clear positive response to inoculation, with increases of 35-185% for biomass accumulation, total N, Ndfa, and grain yield (Table III). Genotype NM 121-25 appeared to be adversely affected by inoculation, with decreases in biomass, total N, Ndfa, and grain yield of 16-30%.

None of the five black-gram genotypes was positively affected by inoculant application (Table IV). On the contrary, inoculated Mash 33-40 and Mash 3-182 showed decreases in biomass, total N, Ndfa, and grain yield of 31-85% in comparison with uninoculated plants.

It has been reported that tropical legumes like *Vigna* spp. are not likely to show inoculation responses because compatible cowpea-miscellany rhizobia are widespread in tropical soils [11]. However, the positive responses of NM 92 to inoculation show that this is not a hard and fast rule. Furthermore, our negative effects of inoculant use, i.e. with rhizobia that were less compatible than indigenous strains with the genotypes in question, confirm that host-genotype×rhizobial strain interactions do occur, therefore before recommending a *Vigna* genotype for a specific location, its compatibility with the indigenous population should be checked.

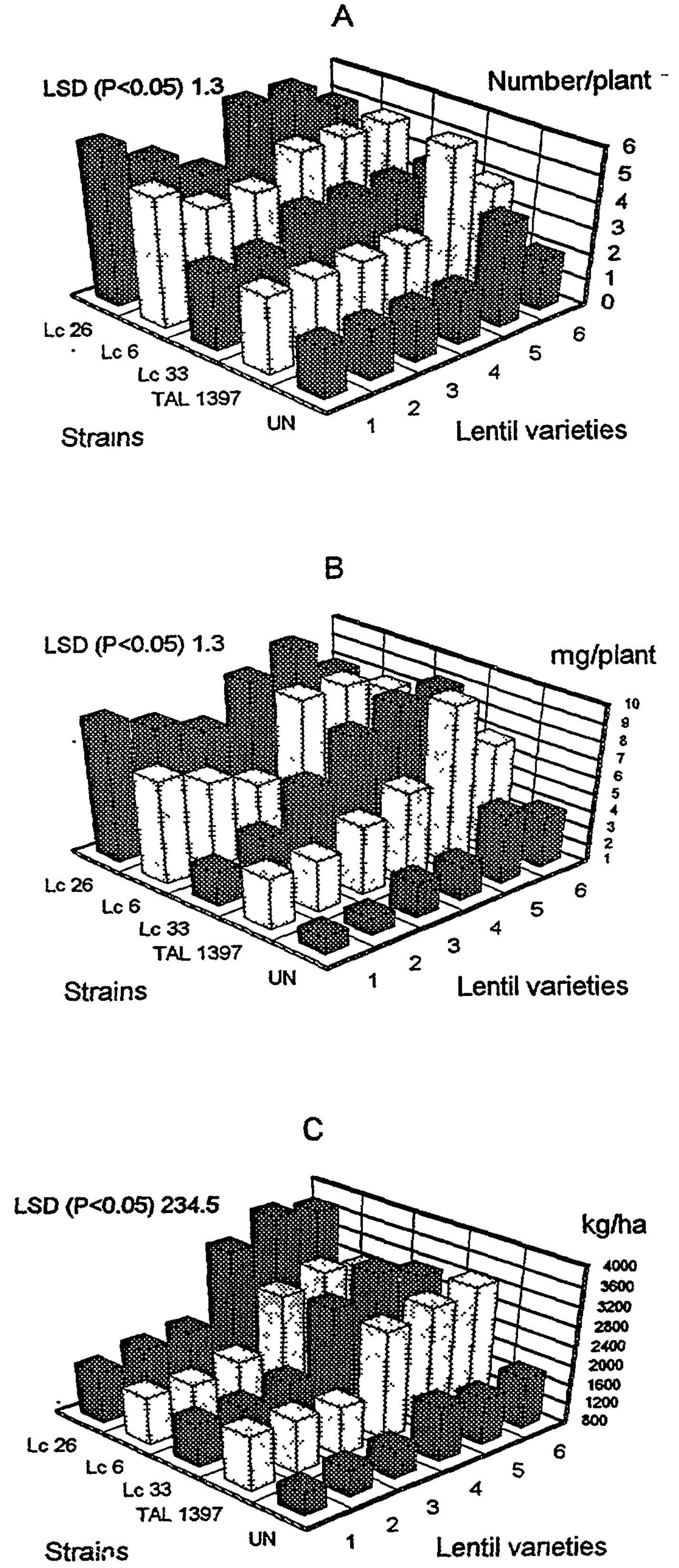
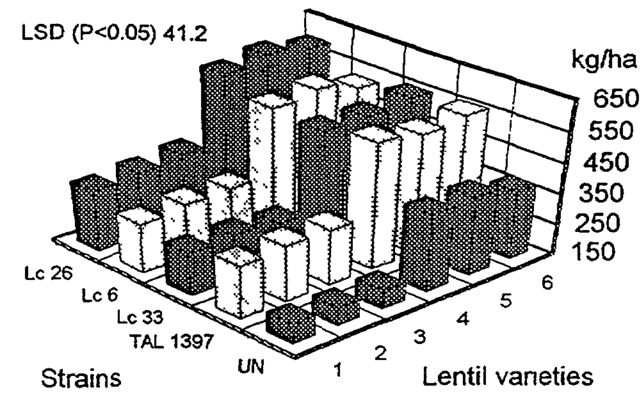
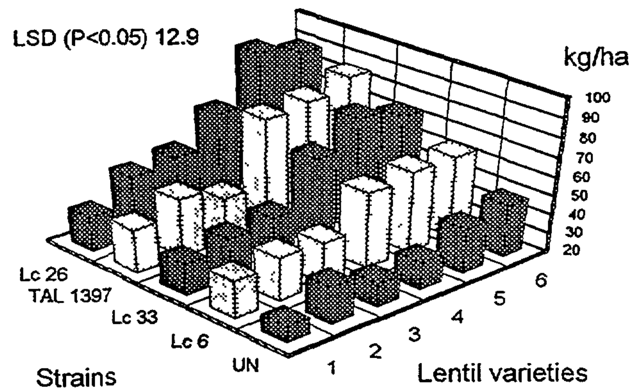


FIG. 1. Genotype × rhizobium-strain interaction. A nodule number, B nodule dry weight, C biomass yield, D grain yield, E total N yield, F Pfix, G Ndfa.

D



E



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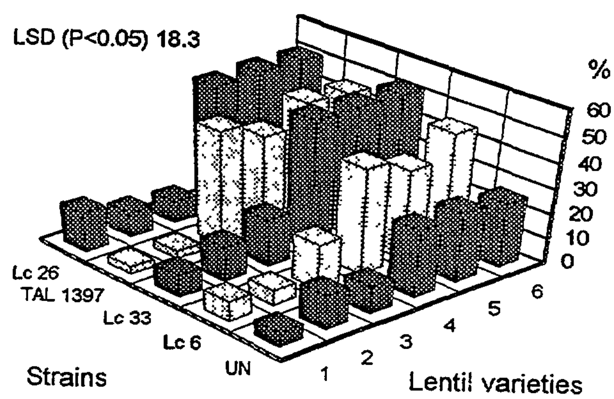

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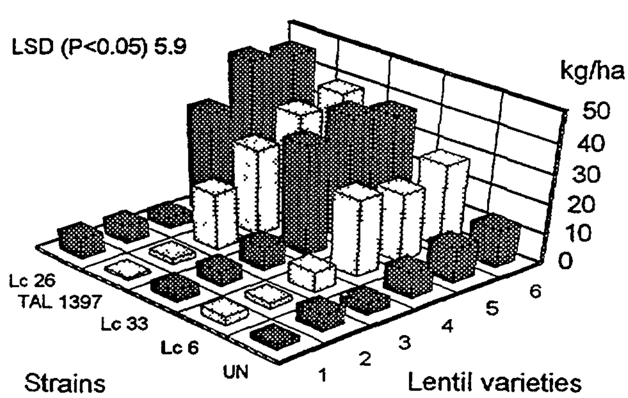

FIG.1 (Cont.)

TABLE III. MUNG-BEAN NODULATION, BIOMASS, GRAIN YIELD, TOTAL N YIELD AND N_2 FIXATION

Experiment Treatment Genotype	Nod. no.	Nod. dry wt.	Biomass yield	Grain yield	Nitrogen yield	Pfix	Ndfa
	(plt^{-1})	($mg\ plt^{-1}$)		($kg\ ha^{-1}$)		(%)	($kg\ ha^{-1}$)
Experiment 1, spring							
Inoculated							
NM 121-25	16	38.8	5207	1726	126	42	53
NM 19-19	14	31.9	4309	1624	103	45	46
NM 54	17	43.0	4511	1551	99	36	36
NM 51	14	35.6	4578	1127	99	38	38
NM 93	16	34.6	4085	1204	92	34	31
NM 96	16	37.2	4566	1227	95	37	35
NM 10-43	16	29.2	4358	1600	109	37	41
NM 92	15	15.1	3511	1234	89	39	34
$LSD_{0.05}$	3	9.9	1194	448	24	7	12
Uninoculated							
NM 121-25	13	21.4	7478	2171	181	35	63
NM 19-19	10	19.9	4472	1582	95	34	33
NM 54	19	41.3	4121	1618	94	47	43
NM 51	9	20.3	5056	1849	80	46	37
NM 93	11	24.3	4434	949	96	33	31
NM 96	19	33.8	4833	1379	109	34	37
NM 10-43	12	20.9	6045	1903	138	34	47
NM 92	14	17.3	2593	433	56	37	20
$LSD_{0.05}$	3	4.2	1301	380	26	7	7
Experiment 2, summer							
Inoculated							
NM 10-43	1.4	0.8	4062	935	93	31	29
NM 54	0.9	0.4	3845	1135	95	31	29
NM 92	0.8	0.3	3151	1111	76	26	20
$LSD_{0.05}$	0.2	0.1	765	186	16	5	6

TABLE IV. BLACK-GRAM NODULATION, BIOMASS, GRAIN YIELD, TOTAL N YIELD AND N_2 FIXATION

Experiment Treatment Genotype	Nod. no. (plt^{-1})	Nod. dry wt. (mg plt^{-1})	Biomass yield	Grain yield ($kg\ ha^{-1}$)	Nitrogen yield	Pfix (%)	Ndfa ($kg\ ha^{-1}$)
Experiment 1, spring							
Inoculated							
Mash 5-60	9	14.6	4255	747	108	43	46
Mash 88	12	18.7	3125	445	82	44	36
Mash 3	9	16.9	3104	986	74	40	30
Mash 33-40	11	11.6	2266	575	66	53	35
Mash 3-182	8	14.3	2396	109	52	43	22
$LSD_{0.05}$	2.3	4.3	632	166	23	6	9
Uninoculated							
Mash 5-60	8	14.8	3986	792	110	54	59
Mash 88	7	8.6	3913	514	99	48	47
Mash 3	7	12.3	3197	966	73	36	25
Mash 33-40	9	14.8	4413	1110	113	46	51
Mash 3-182	11	12.6	3852	712	86	50	43
$LSD_{0.05}$	3.3	4.7	1090	253	25	7	10
Experiment 2, summer							
Inoculated							
Mash 3	2.9	2.0	3601	1027	75	44	33
Mash 88	4.1	2.0	3510	481	84	48	40
Mash 33-40	4.0	2.1	3109	789	73	51	37
$LSD_{0.05}$	0.6	0.5	663	225	18	3.6	6.7

3.2.2. Experiment 2

Differences in growth, grain yield, total N and Ndfa between the two experiments possibly resulted from higher temperatures and drier conditions in the summer than in the spring season. For example, the three summer-grown mung-bean genotypes fixed less N, accumulated less N and less biomass, and yielded 10-41% less grain than in the spring (Table III). In contrast, however, the three black-gram genotypes grown in the summer showed patterns of N_2fixation, growth and yield that were similar to, or superior than, those of the inoculated spring-grown plants (Table IV). It may be inferred that black gram is more tolerant of heat and drought than mung.

3.3. Chickpea

Nodulation, N_2 fixation and yield data for the two successive years are presented in Tables V and VI. Table VII presents correlation values between various measured components in both experiments.

3.3.1. *Experiment 1*

There was significant genotypic diversity for biomass, grain and N yield, which ranged from 1333 to 5554, 373 to 1890 and 26 to 115 kg ha^{-1}, respectively, and for Pfix and Ndfa, which ranged from 23-68% and 4-68 kg ha^{-1} (Table V).

3.3.2. *Experiment 2*

In the second-year's experiment, nine varieties attacked by gram blight were replaced with resistant varieties (Table I). The disease-resistant genotypes and those repeated from the previous year's experiment accumulated more biomass and total N and, in general, fixed more N than in the previous year (Table VI). This may be attributable to the cultivation of chickpea in the previous season resulting in more rhizobia in the soil ($2.2x10^2$ cells g^{-1} soil), in view of the fact that rhizobia proliferate best in the rhizosphere of their host species [25, 26, 27]. On the other hand, nodule number averages were 16.7 for Experiment 1 and 19.1 for Experiment 2 (an increase of 14%), whereas nodule dry weight averages were 127 and 334 (+163%), respectively, indicating that a lower soil N level for Experiment 2 resulted in better nodule development and an increase in the overall average Ndfa value from 22 kg N ha^{-1} in Experiment 1 to 38 kg N ha^{-1} in Experiment 2.

Any factor that affects plant growth, such as disease, may indirectly influence N_2 fixation. The disease-resistant variety MB75 gave the highest values for biomass yield, N yield, Pfix and Ndfa. It was statistically similar to C44, the best performer in Experiment 1.

3.3.3. *Correlations*

Nodule number was not a useful correlate in either experiment; even the correlation with AR activity was not significant (Table VII). Nodule dry weight showed a highly significant correlation with AR activity in Experiment 1, which was not unexpected. AR activity, an indirect assay for nitrogenase activity, did not correlate signficantly with the other potential indicators of N_2 fixation: total N, Pdfa, or Ndfa. Such poor correlations between AR activity and N yield may be due to the fact that the indirect assay is a sampling at a single point in time [24], and it has been suggested that the AR assay is of limited utility even for pot-grown legumes, and not recommend without careful calibration for field studies [28]. Acetylene reduction assays were not conducted during the second year because of its largely non-significant contribution in the first screening (Table VII).

Nodule dry weight showed significant, although not strong, correlations (r^2 = 0.24-0.34) with biomass, grain and N yields in Experiment 2, but not in Experiment 1, probably because there was much better nodule development in Experiment 2.

In both experiments, Pfix correlated significantly with biomass yield, grain yield, total N, and Ndfa, but only with Ndfa was the correlation strong (r^2 = 0.69 and 0.71). In contrast, Ndfa showed highly significant and strong correlations in both experiments with biomass, grain yield and total N (r^2 = 0.56-0.83)

Isotope-dilution methodology is laborious and expensive, hence it cannot be employed for large field areas or many treatments [29]. The highly significant and strong correlations with N_2

TABLE V. CHICKPEA NODULATION, AR ACTIVITY, BIOMASS, GRAIN AND TOTAL-N YIELDS AND N_2 FIXATION (EXPERIMENT 1)

Genotype	Nodule no. (plt^{-1})	Nodule dry wt. (mg plt^{-1})	AR activity (μmol $plt^{-1}h^{-1}$)	Biomass	Grain	Nitrogen	Pfix (%)	Ndfa (kg ha^{-1})
				(kg ha^{-1})				
C-44	19	173	16.7	5554	1890	115	68	61
CM-1	10	63	5.7	3944	1397	82	56	36
CM-72	13	77	6.1	2706	1062	55	49	20
CH-5	14	197	19.1	4105	1140	78	45	25
CM-1571-12A	14	120	20.4	4056	1286	81	44	29
P12-45F	14	130	24.7	3829	925	79	40	21
CM-2	15	197	29.7	3018	674	60	36	17
CM-663	20	140	22.3	3665	1389	76	34	17
CH-9	20	153	8.7	3695	1411	74	36	20
P8-A	18	127	20.5	4041	1470	89	38	24
CM-1918	10	60	8.9	2137	745	40	39	12
CM-1571-22	14	170	27.9	4192	1531	86	32	21
C-727	29	150	17.6	4047	1401	84	32	24
CM-1913	19	87	10.4	4174	1167	83	36	19
CM-687	21	73	6.8	3768	1212	71	32	17
CM-2197	15	140	17.3	2831	985	56	29	18
P7-H	16	87	3.4	1333	373	26	23	4
CM-88	18	103	13.3	2598	1061	50	27	12
CM-1571-12B	21	197	56.1	2770	1337	62	23	20
P5-B	14	107	6.1	3627	998	71	24	15
$LSD_{0.05}$	5.0	26	5.7	2145	910	24	7.5	6

TABLE VI. CHICKPEA NODULATION, BIOMASS, GRAIN AND TOTAL-N YIELDS AND N_2 FIXATION (EXPERIMENT 2)

Genotype	Nod. no. (plt^{-1})	Nod. dry wt. (mg plt^{-1})	Biomass	Grain	Nitrogen	Pfix (%)	Ndfa (kg ha^{-1})
			(kg ha^{-1})				
MB-75	14	440	5693	2400	98	62	60
CM-687	29	150	4036	1867	77	57	45
P8-A	15	390	5567	2578	92	56	53
P12-45F	11	400	5340	2222	94	53	50
C-44	19	490	5275	2644	92	52	49
50	29	470	4610	2256	92	52	47
35	19	270	3463	1589	54	52	29
P5-B	14	220	4393	2044	74	51	38
Paidar 91	14	300	3797	1656	66	51	34
C-727	17	430	4786	2344	87	50	45
CM-1571-22	15	340	4562	2133	77	50	39
CM-72	13	220	3391	1567	66	50	31
CM-1	15	270	4747	2133	86	49	43
Punjab 91	38	340	4430	2256	68	47	32
37	22	280	3771	2011	78	47	36
19	25	420	3904	1844	65	43	26
MB40	16	440	4170	1533	75	43	32
M-663	30	200	4202	2044	72	41	30
CH-9	17	310	4448	1933	77	41	31
Noor 91	11	310	3952	1644	64	37	24
($LSD_{0.05}$	15	183	1446	667	27.6	17	18.6)

TABLE VII. CORRELATIONS OF MEASURED COMPONENTS IN EXPERIMENTS 1 AND 2

		Nod. dry wt.	Nod. no.	Biomass yield	Grain yield	N yield	Pfix	Ndfa
Exp. 1	Nodule no.	0.31^{NS}						
	Biomass yld.	0.33^{NS}	0.23^{NS}					
	Grain yld.	0.31^{NS}	0.37^{NS}	0.82^{***}				
	Nitrogen yld.	0.36^{NS}	0.26^{NS}	0.99^{***}	0.86^{***}			
	Pfix	0.07^{NS}	0.07^{NS}	0.58^{**}	0.47^{*}	0.57^{**}		
	Ndfa	0.27^{NS}	0.14^{NS}	0.79^{***}	0.75^{***}	0.81^{***}	0.84^{***}	
	AR activity	0.73^{***}	0.23^{NS}	0.17^{NS}	0.22^{NS}	0.17^{NS}	-0.20^{NS}	0.09^{NS}
Exp. 2	Nodule no.	-0.10^{NS}						
	Biomass yld.	0.58^{**}	-0.18^{NS}					
	Grain yld.	0.49^{*}	0.14^{NS}	0.87^{***}				
	Nitrogen yld.	0.53^{*}	-0.16^{NS}	0.89^{***}	0.80^{***}			
	Pfix	0.13^{NS}	-0.09^{NS}	0.49^{*}	0.47^{*}	0.53^{*}		
	Ndfa	0.40^{NS}	-0.16^{NS}	0.84^{***}	0.77^{***}	0.91^{***}	0.83^{***}	

^{NS}Not significant, or significant at $P<0.05^{*}$, $<0.01^{**}$ and $<0.001^{***}$.

fixation (Ndfa) in Table VII indicate that simpler indicators - total N, biomass, and grain yield - can be effectively used for screening genotypes for superior N_2 fixation. These findings are consistent with previous work that showed that the ranking of cultivars for N_2-fixing efficiency was the same whether derived from grain yield, N yield or using the ^{15}N-dilution technique [28, 30].

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BREEDING FOR HIGH N_2 FIXATION IN GROUNDNUT AND SOYBEAN IN VIET NAM

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Abstract

BREEDING FOR HIGH N_2 FIXATION IN GROUNDNUT AND SOYBEAN IN VIET NAM

Groundnut (*Arachis hypogaea* L.) and soybean [Glycine max (L.) Merr.] are grown mainly on two types of soil in Viet Nam: coastal-sandy and upland-degraded soils. These soils are deficient in N, and considering that fertilizer N is not only costly to farmers but also a threat to the environment, it is important to maximize productivity by exploiting the ability of these legumes to fix N_2 symbiotically in their root nodules. We initiated programmes of breeding and selection to combine high N_2 fixation and high grain-yielding capacity.

In the spring of 1992, breeding lines of groundnut and soybean were tested under greenhouse conditions for varietal differences in the capacity to fix N_2 using the acetylene reduction assay and the ^{15}N-dilution technique, with upland rice as reference plants. Varietal differences were found in nitrogenase activity, and percent N derived from fixation (%Ndfa) ranged from 11 to 63% for groundnut and from 9 to 79% for soybean. Field experiments in the autumn-winter season of 1992 again revealed significant varietal differences; %Ndfa ranged from 36 to 56% for groundnut and from 28 to 58% for soybean.

Gamma-irradiated seeds of groundnut and soybean were propagated in bulk from M_1 to M_4. Five high-yielding mutant lines of both species were selected from the M_5 populations, and N_2 fixation was estimated using the ^{15}N-dilution technique. The average values for %Ndfa of the mutants were 55 and 57%, significant improvements over the parent-cultivar values of 25 and 29% for soybean and groundnut, respectively.

1. INTRODUCTION

In Viet Nam, groundnut (*Arachis hypogaea* L.) and soybean [*Glycine max* (L.) Merr.] are important crops, although secondary to rice. Legume cultivation occurs mainly in mountain and seacoast provinces, on degraded and coastal-sandy soils that are low in N and poorly productive. Degraded soils occupy some 200,000 ha and coastal sandy soils 600,000 ha, and sustain many densely populated communities. Therefore, they are important socio-economically [1].

The sandy soils are prevalent in coastal plains with a sandstone bedrock. The degraded soils are prevalent in mountain provinces from the northern subtropics to the southern tropics, existing in part as alluvium terraces and in part as colluvium and degraded ferralitic soils derived from acid marma and sandstone, mostly with gently sloping topography; commonly they are highly leached and eroded.

Due to high cost, chemical N fertizers are not commonly used by farmers. Groundnut and soybean are legumes, capable of fixing N_2 and of yielding well without fertilizer N application.

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Legumes with high N_2-fixation capacity and high-yielding ability may benefit such impoverished soils by contributing N for subsequent cereal cultivation. Although synthetic chemical fertilizers promote plant growth and increase crop yields, their indiscriminate use exacts a penalty on the environment and on human health.

A great deal of research has focused on groundnut and soybean and their ability to fix N_2 [2-5]. In Viet Nam, prior to the initiation of recent research [6-8], very little had been done on legume nodulation and N_2 fixation. The results of efforts to select and breed for high N_2 fixation in groundnut and soybean have been summarized and are reported here.

2. MATERIALS AND METHODS

Three experiments were conducted to investigate genetic diversity among selected lines of groundnut and soybean, for nitrogenase activity by the acetylene reduction (AR) assay, and for accumulation of N and the contribution thereto of N_2 fixation using the ^{15}N-dilution technique.

2.1. Greenhouse trials

A sandy loam, pH 6.5, was collected at the village of Khuong Thuong (Dong Da district, Hanoi), air dried, and passed through a 2-mm mesh sieve. Thirteen-kg aliquots of soil were added to earthenware vessels (45.5 cm diameter). A solution of ^{15}N-enriched $(NH_4)SO_4$ (46.6% atom excess) was added at 60 mg N/kg soil. Five plants each of five genotypes were grown per vessel in a greenhouse with a temperature range of 18-35°C, 1 March to 9 June for groundnut and 25 February to 27 May 1992 for soybean. Upland rice cvs. CH1-NG90 and CH1-NG92 were used as non-fixing reference plants for groundnut and soybean, respectively [8].

For the AR assay, nodulated roots were collected at flowering and placed individually in 500-mL stoppered bottles, and incubated for 3 h in 10% acetylene; 0.5 mL samples were collected at 1, 2, and 3 h, and ethylene production was determined by gas chromatography (Series 204, Pye Unicam Ltd.) [9] .

For total-N determinations, plant samples were dried at 60°C for 3 days, then milled. A semi-micro Kjeldahl method was employed [10]. Nitrogen-15 analyses were done by emission spectrometry (JASCO N-150). Percent N derived from fixation (i.e. from air, %NDfa) was calculated as follows [11, 12]:

$$\%Ndfa = (1 - \frac{\%^{15}N\ atom\ excess_{(legume)}}{\%^{15}N\ atom\ excess_{(reference)}}) \times 100$$

2.2. Field trials

Field trials were conducted in 1992 at the village of Phu Minh (Tu Liem district, Hanoi), 1 June to 8 September for groundnut and 1 August to 9 November for soybean, on a sandy loam of pH 6.0. Five genotypes of groundnut and five of soybean were examined, with rice as the non-fixing reference crops, as above. Plots were 2x3 m, and there was a complete randomized block design of

Nitrogen-15 labelled $(NH_4)_2SO_4$, at 5.036% atom excess was applied as a solution at 20 kg N/ha, and then incorporated into the soil [8]. Basal fertilizer was applied as 60 kg P_2O_5/ha and 20 kg K_2O/ha [6], and, on areas not treated with ^{15}N, unlabelled $(NH_4)_2SO_4$ was applied at 20 kg N/ha.

Acetylene reduction assays, and total N and ^{15}N analyses were done as described above.

2.3. Induced-mutation programmes

Seeds of the commercial groundnut cv. Sen and soybean cv. Thuong Tin were exposed to a ^{60}Co source of γ-radiation, with a dosage of 10-20 kRad (at the Cancer Research Centre, K-Hospital, Hanoi) [7]. At the village of Co Nhue, Gia Lam district, Hanoi, the plants were propagated in bulk from M_1 to M_4, during which any plants of abnormal appearance were removed and used for other purposes. Five high-yielding mutant lines of both legumes were examined as described in 2.2., in the spring season of 1994.

3. RESULTS

3.1. Greenhouse trials

There were significant differences among the cultivars of both species for all of the components examined (Table I). Acetylene reduction activities ranged from 522 to 1087 nmol/plant/h for groundnut and from 493 to 1,348 nmol/plant/h for soybean. Total N content, %Ndfa and amount of N fixed correlated with AR activity. Across the five cultivars, there was a nine-fold range in the amounts of N fixed by groundnut, and a 15-fold range for soybean.

3.2. Field trials

The AR data again revealed varietal differences for nitrogenase activity (Table II). Statistically significant differences between cultivars of both species were obtained for total N accumulated, %Ndfa, and amount of N fixed. The AR rates were higher than those obtained in the greenhouse trial and, consistent with this, the determinations of total N accumulated and amount of N fixed were higher for the field-grown plants.

3.3. Induced-mutation programmes

The five high-yielding lines of each species, chosen from the M_5 populations, had significantly higher values for %Ndfa and for total N fixed than did the parent lines, groundnut cv. Sen and soybean cv. Thuong Tin (Table III).

TABLE I. AR ACTIVITY, TOTAL N ACCUMULATED, PERCENT N FROM FIXATION, AND AMOUNT OF N FIXED BY GROUNDNUT AND SOYBEAN (GREENHOUSE: SPRING 1992)

Genotype	AR activity (nmol/plant/h)	N content (mg/plant)	Ndfa (%)	N fixed (kg/ha)
Groundnut				
GTH1.SP92(G)	1,087a[a]	90.3a	62.6a	56.6a
GTH2.SP92(G)	935b	76.4b	28.2b	21.5b
GTH3.SP92(G)	886c	70.3c	23.7c	16.7c
GTH4.SP92(G)	712d	65.6d	12.7d	8.33d
GTH5.SP92(G)	522e	62.0e	10.7e	6.66e
Soybean				
STH1.SP92(G)	1,384a	115a	78.7a	90.5a
STH2.SP92(G)	1,109b	94.0b	27.2b	25.5b
STH3.SP92(G)	993c	88.6c	17.7c	15.7c
STH4.SP92(G)	769d	86.2c	13.8d	11.9d
STH5.SP92(G)	493e	65.9d	9.37e	6.17e

[a]Average of ten determinations, numbers within species and columns followed by different letters are significantly different (P=0.05, Duncan's Multiple Range Test).

4. DISCUSSION

Nitrogen fixation is strongly influenced by environmental factors, and the isotope-dilution technique has the advantage of providing a time-integrated value, provided that certain conditions apply, among them as follows [11, 12]:

- The non-fixing reference species does not, in fact, fix N_2. We verified this for our rice genotypes, using the AR assay.
- Non-fixing reference plants have similar rooting depth and exploit a similar soil volume. Pot trials in the greenhouse have the advantage of ensuring these conditions.
- The fixing and reference plants have similar growing-season length and reach maturity at the same time. The rice checks were chosen accordingly.

Our groundnut and soybean plants nodulated and fixed N_2 in combination with the rhizobia that are indigenous to the chosen soils. Sometimes nodulation, N_2 fixation, growth and yield can be improved by application of inoculants containing rhizobia that are more compatible with the legume of interest than are the indigenous strains [3]. Therefore it is important to combine symbionts prudently for maximum fixation. Furthermore, the criteria for legume breeding and selection for high N_2 fixation should be appropriate for the conditions under which the crop will be grown and this is particularly important for conditions of environmental or nutritional stress [4, 5, 13].

TABLE II. AR ACTIVITY, TOTAL N, PERCENT N FROM FIXATION, AND AMOUNT OF N FIXED BY GROUNDNUT AND SOYBEAN (FIELD: SUMMER-AUTUMN, 1992)

Genotype	AR activity (nmol/plant/h)	N content (mg/plant)	Ndfa (%)	N fixed (kg/ha)
Groundnut				
GTH1-SA92(F)	5,519a[a]	657a	56.0a	121a
GTH2-SA92(F)	5,025b	631b	52.2b	109b
GTH3-SA92(F)	4,217c	625c	41.4c	85.3c
GTH4-SA92(F)	3,945d	606d	38.1d	76.4d
GTH5-SA92(F)	3,528e	553e	35.6e	65.1e
Soybean				
STH1-AU92(F)	6,215a	713a	58.3a	137a
STH2-AU92(F)	5,749b	647b	49.3b	105b
STH3-AU92(F)	4,674c	637c	47.9c	101c
STH4-AU92(F)	4,031d	587d	37.1d	72.0d
STH5-AU92(F)	3,564e	572e	28.0e	52.9e

[a]Average of twenty determinations, numbers within species and columns followed by different letters are significantly different (P=0.05, Duncan's Multiple Range Test).

In order to select a genotype with superior N_2-fixing capacity by plant breeding using, for example, the SSD technique [14, 15], greenhouse experiments are needed and we have proposed similar greenhouse selections as a preliminary screening step [8].

The greenhouse and field trials reported here demonstrate genetic diversity for characteristics associated with symbiotic N_2 fixation by groundnut and soybean. This diversity represents a resource for the improvement of these legumes by producing genotypes with higher N_2-fixing capability, through induced mutation combined with hybridization. Five M_5 lines of both legumes, selected on the criterion of high yield, had significantly higher values for %NDFA and for the contribution of fixation to total N accumulation.

TABLE III. NITROGEN-15 ENRICHMENT, PERCENT N DERIVED FROM FIXATION, AND AMOUNT OF N FIXED BY HIGH-YIELDING M_5 LINES OF GROUNDNUT AND SOYBEAN COMPARED WITH PARENT CVV. (MUTATION BREEDING: SPRING 1994)

Genotype	^{15}N enrichment (% atom excess)	Ndfa (%)	N fixed (kg/ha)
Groundnut			
GMTH21-SP94(F)	0.9432f[a]	56.7a	130a
GMTH22-SP94(F)	0.9321e	52.2b	119b
GMTH23-SP94(F)	1.0454d	46.3c	105c
GMTH24-SP94(F)	1.1268c	42.2d	94.7d
GMTH25-SP94(F)	1.2450b	36.1e	80.6e
SEN	1.3834a	29.00f	63.8f
(Rice reference	1.9485)		
Soybean			
SMTH31-SP94(F)	0.7816f	54.9a	141a
SMTH32-SP94(F)	0.8634e	50.1b	135b
SMTH33-SP94(F)	0.9451d	45.5c	122c
SMTH34-SP94(F)	1.0186c	41.2d	117d
SMTH35-SP94(F)	1.1347b	34.5e	83.1e
THUONG TIN	1.2986a	25.0f	64.1f
(Rice reference	1.7321)		

[a]Average of twenty determinations, numbers within species and columns followed by different letters are significantly different ($P=0.05$, Duncan's Multiple Range Test).

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FIELD EVALUATION OF N_2 FIXATION BY MUNG BEAN IN THE PHILIPPINES, AND RESIDUAL EFFECTS ON MAIZE

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Abstract

FIELD EVALUATION OF N_2 FIXATION BY MUNG BEAN, AND RESIDUAL EFFECTS ON MAIZE

Seventeen genotypes of mung bean (*Vigna radiata)* were screened for growth, yield, and symbiotic N_2 fixation during the late-dry (March-May) and early-dry (October-December) seasons of 1992 at the University of the Philippines at Los Baños (UPLB). The ^{15}N-dilution method was used to determine amounts of N fixed. Soil mineral N availability was higher (average 22 kg N/ha) in the late- than in the early-dry season (9.2 kg N/ha), and, possibly in consequence, vegetative growth was better in the late- than in the early-dry season; however, in contrast, seed yields were better in the latter. Cultivar Pagasa 5 had the highest value (52 kg N/ha) for fixed N in the late-dry season, whereas PAEC 3 had the highest value (70 kg N/ha) in the early-dry season; Accession 2041 had the lowest values in both seasons (33 and 26 kg N/ha, respectively). Genetic variability, albeit slight, was observed for total N fixed, but not for percent N derived from fixation (%Ndfa).

Further field work at UPLB and at the Philippine Nuclear Research Institute (PNRI), Quezon City, investigated five mung genotypes, including three from the previous trials, for yield, N_2 fixation and residual effects on subsequent maize (*Zea mays*). Estimates for %Ndfa and for amounts of N fixed ranged from 64 to 87% and 43 to 85 kg N/ha, respectively, at PNRI, and from 37 to 72% and 21 to 85 kg N/ha, respectively, at UPLB. The highest mung-bean seed yields obtained were 1.99 t/ha at PNRI and 0.86 t/ha at UPLB in the two locations. When maize was planted after mung, dry matter, seed yields and total N were consistently higher than when planted after maize or cotton, although most of the differences fell short of statistical significance.

The data are discussed in terms of genetic diversity for yield and N_2 fixation in these soils, and potential to exploit mung-fixed N to improve cereal yields.

1. INTRODUCTION

Mung bean (*Vigna radiata*) is widely grown in the Philippines as a source of food, feed, green manure, and industrial materials. As a legume it can obtain N from the atmosphere by fixation in its root nodules in symbiosis with soil-borne rhizobia, and thus has the potential to yield well in N-deficient soils. This characteristic is particularly important in developing countries, including the Philippines, due to the relatively high cost and/or restricted availability of fertilizer N. Furthermore, incorporation into the soil of vegetative residues from a N_2-fixing legume crop may provide organic N for the subsequent benefit of a cereal.

In Chiang-Mai, Thailand, mung bean was estimated to fix 90% of its need for N, which amounted to 65 kg N/ha [1]. In recognition of the fact that no published data were available on the amount of N_2 fixed by mung in the Philippines, we utilized the ^{15}N-dilution technique [2] to quantify N_2 fixation by seventeen diverse genotypes. Cotton (*Gossypium hirsutum*), wheat (*Triticum aestivum*) and non-nodulating soybean (*Glycine max*) were compared as non-fixing reference crops. The objectives were to quantify the atmospheric N_2 fixed, and thus identify high- and low-fixing genotypes for further research.

In a second experiment, five mung genotypes were grown at two sites and N_2 fixation was quantified and related to subsequent effects on maize grown in the same plots without fertilizer N.

2. MATERIALS AND METHODS

2.1. Screening experiment

Seventeen mung-bean genotypes (Table I) were screened in the field at the Institute of Plant Breeding of the University of the Philippines at Los Baños (UPLB) during the late-dry period (March-May) of the 1991/92 growing season and the early-dry (October-December 1992) season of 1992/93. Some of the cultivars had been used in previous work at UPLB [3]. PAEC 3 and PAEC 10 are mutant varieties developed at the Philippine Nuclear Research Institute (PNRI); Pagasa 3, 5 and Taiwan Green are approved by the Philippine Seed Board; Accession 2041, 379, 58 and 71 have been identifed as poor fixers and were included for comparison; also included was the local progenitor of the PAEC varieties. The reference crops used were wheat ('Trigo 2'), cotton ('CRD1-1') and non-nod soybean ('Clay').

Ammonium sulphate, enriched in ^{15}N at 10% atom excess, was applied at 4 g N/m^2, in a sucrose suspension to encourage immobilization, at one week prior to planting the late-dry-season experiment [4-6]. Phosphorus and K were applied at 4.5 g/m^2 and 6.0 g/m^2, respectively, prior to planting. Soil pH was recorded at 5.8. For the subsequent early-dry-season experiment, no N fertilizer was applied.

A randomized complete block design was used, replicated four times. Each replicate had 20 rows with a 50-cm row spacing. Nitrogen-15 was applied to 1.5 m x 10 m, and yield was taken from 4.5 m x 10 m. Four seeds were sown per hill, spaced at 10 centimeters. A water slurry of a charcoal/forest-soil based rhizobial inoculant was applied to the seeds at planting. At two weeks after emergence, seedlings were thinned to two per hill, giving a population equivalent of 400,000 plants/ha. Irrigation was applied weekly until flowering during the late-dry season only. Appropriate practices ensured weed, insect, and disease control.

2.1.1. Sampling

The ^{15}N microplots were sampled (12 plants per replicate) at 45 days after planting, early pod-formation, to determine vegetative growth, including roots and nodules. The samples were oven dried, weighed and ground; 5-g samples were sent to the IAEA Soils Unit Laboratory, Seibersdorf, Austria for ^{15}N determination. The reference crops were harvested at the same time as the mung plants by cutting them 5 cm above ground level with similar procedures for sample preparation.

Mature pods were harvested from the yield plots when 80% had turned black, with two or three collections for each entry depending on duration of the pod-development period. Pods were dried and threshed, and seeds cleaned. Seed yields were determined after sun-drying, at a moisture content of approximately 12%.

2.1.2. Calculations

The percent N derived from the atmosphere (i.e. from fixation, %Ndfa), the amount of N fixed, percent N derived from fertilizer (%NdfF), and percent N derived from soil (%NdfS) were estimated as follows [7, 8]:

$$\%Ndfa = \left(1 - \frac{\%^{15}N\ atom\ excess_{(legume)}}{\%^{15}N\ atom\ excess_{(reference)}}\right) \times 100$$

$$N\ fixed\ (kg/ha) = \frac{\%Ndfa}{100} \times Total\ N\ yield$$

$$\%NdfF = \frac{\%^{15}N\ atom\ excess_{(plant)}}{\%^{15}N\ atom\ excess_{(fertilizer)}} \times 100$$

$$\%NdfS = 100 - (\%Ndfa + \%NdfF)$$

Analyses of variance were performed and comparisons between means assessed by Duncan's Multiple Range Test.

2.2. Residual-effects experiment

This experiment was conducted in duplicate, at PNRI, Quezon City, and at UPLB. The characteristics of the soils are presented in Table II; N was lower at PNRI (0.12%) than at UPLB (0.20%). Five mung-bean genotypes were used, with maize (*Zea mays*) and cotton as reference crops. Three mung genotypes had been included in the screening, PAEC 3, Taiwan Green, and Acc. 638, and two were obtained from the National Institute for Agriculture and Biology, Faisalabad, Pakistan (NIAB 54 and 92).

The experiments had a randomized complete block design with treatments replicated four times. The unit size was 6.5 m (1.5 m ^{15}N sub-plot and 5 m yield plot) x 2.0 m with four rows per plot. Plant density was 20 plants per linear meter or 400,000 plants/ha. Ammonium sulphate, at 10% ^{15}N atom excess was applied at 20 kg N/ha in solution, as split applications of 6, 6, and 8 kg N/ha at 0, 10, and 20 days after planting, respectively. The same rate of unlabelled $(NH_4)_2SO_4$ was applied in the yield plot. At planting, P and K were applied at 40 and 30 kg/ha, respectively. Mung bean seeds were moistened and coated with a *Bradyrhizobium* sp. inoculant in a charcoal/forest-soil carrier prior to sowing. Carbofuran (Furadan) was applied in furrows just after planting for insect control.

TABLE I. MUNG-BEAN GENOTYPES

Variety no.	Genotype name	Characteristics
V1	PAEC 3 Mutant (check)	PNRI developed mutant variety
V2	PAEC 10	"
V3	Pagasa 3	Phil. Seed Board approved variety
V4	Pagasa 5	"
V5	Pagasa 7	"
V6	Taiwan Green	"
V7	Acc 658	Good N-fixer
V8	Acc 687	"
V9	Acc 638	"
V10	Acc 211	"
V11	Acc 174	"
V12	Acc 188	"
V13	Acc 2041	Poor N-fixer
V14	Acc 379	"
V15	Acc 58	"
V16	Acc 71	"
V17	PAEC progenitor	Local variety

Samplings were made at early pod-fill for dry matter yield, nodule weight, nodule number, total N and ^{15}N determinations; seed-yield determinations were made at maturity. The harvest areas were 1.0 m^2 and 4.0 m^2 for ^{15}N analysis and seed yield, respectively. Samples were taken from the 2nd and 3rd rows, leaving the 1st and 4th as borders. Plant samples were weighed, chopped and quartered, and oven-dried at 70°C for two days until constant weight was obtained. Ground samples were sent to Seibersdorf for total N and ^{15}N determinations as above.

In the following season, maize was grown on the same plots without additional N fertilization. Planting distance was 25 cm between hills, and 50 cm between rows, with one plant per hill. With standard cultural practices of irrigation, weed and pest control, plant samples (fifteen plants/plot) were collected from the ^{15}N plots at 45 days after planting. Sub-samples were obtained, weighed and dried for several days at 70°C until constant weight was obtained. After grinding, 5-g sub-samples were sent to Joint FAO/IAEA Soil Science Unit, Seibersdorf, Austria, for total N and determination of ^{15}N enrichment. Kernel yields were determined at maturity.

Statistical comparisons of means were made as described above.

3. RESULTS AND DISCUSSION

3.1. Screening experiment

Vegetative dry-matter yields ranged from 1.99 to 3.05 (Table III) and 0.92 to 2.35 t/ha (Table IV) for the late- and early-dry seasons, respectively. Vegetative-N yields also were generally higher

TABLE II. CHEMICAL CHARACTERISTICS OF SOILS AT PNRI AND UPLB

	O.M.	Total N	Avail. P	Exchangeable bases Ca	Mg	Na	K	Zn	Micronutrients Cu	Mn	Fe	pH
	(%)	(%)	(mg/kg)	(meq/100g)				(mg/kg)				
PNRI	2.4	0.12	6.1	18.7	10.0	0.26	0.51	7.4	9.8	149	67	6.7
UPLB	4.0	0.20	21	8.23	3.83	0.84	2.48	2.0	8.1	79	130	6.9

during the late-dry season, by an average of 16%. This pattern of better early growth and accumulation of N can be explained in terms of greater availability of mineral N, from soil and fertilizer, during the late-dry season. It is noteworthy that, in contrast with vegetative growth and N accumation by early pod-fill, seed yields were generally better in the early-dry season. The reason for this incongruity is unclear, but it is possible that conditions for N_2 fixation during grain fill were superior during the early-dry season. In conditions of abundant N, some species invest relatively more resources in vegetative than in reproductive growth, however, with only 37% of the total N originating from soil and fertilizer (Table III) this explanation seems unlikely.

The three reference crops did not vary significantly in % atom-excess values (data not shown), therefore %Ndfa values were estimated using mean reference values. These data indicate that, at least in some conditions, plants of different rooting pattern may be used as controls for isotope-dilution determinations of N fixed by legumes [9-12].

Genetic variability for N fixed was not well pronounced. In the late-dry season screening, 15 genotypes did not fix significantly less N than did Pagasa 5, which had the highest value of 52 kg N/ha, and 14 genotypes did not fix significantly more than did Accession 2041, the lowest at 33 kg N/ha. In the early-dry season, 14 genotypes did not fix significantly less than did PAEC 3 at 70 kg N/ha; although Accession 379 alone did not fix more N than did Accession 2041 at 26 kg N/ha, twelve genotypes did not fix significantly more than did 379. No single genotype emerged as superior in both seasons; fifteen of the seventeen genotypes fixed amounts of N that were not significantly different from PAEC 3 or from Accession 687 in one or other of the trials, including Accession 58, 71, and 379, which had been previously designated as poor-fixing checks (Table I, [3]). Similarly, the estimates for %Ndfa were remarkably uniform, with no statistically significant differences. It may be inferred that N was not the chief factor limiting vegetative growth, and consistent with this deduction is the fact that seed yields were low, averaging only 680 and 730 kg/ha (Tables III and IV). With better growth conditions and improved potential for expression of symbiotic N_2 fixation, then superior N_2-fixing genotypes may have emerged more clearly.

TABLE III. DRY WEIGHT, N ACCUMULATION AND N_2-FIXATION CHARACTERISTICS AT 45 DAYS AFTER PLANTING, AND SEED YIELD, OF 17 MUNG-BEAN GENOTYPES DURING THE LATE-DRY SEASON OF 1991-92

Cultivar	Dry matter yield (t/ha)	Seed yield (t/ha)	N yield (kg/ha)	NdfF[a] (%)	NdfS[b] (%)	Ndfa[c] (%)	Amount N fixed (kg/ha)
V1 PAEC 3	2.45bc[d]	0.61def	73.3abc	5.5	32.5	62.0	44.7ab
V2 PAEC 10	2.50abc	0.5 efg	66.2bc	4.8	28.5	66.7	43.7ab
V3 Pagasa 3	2.81ab	0.31gh	68.8abc	7.3	43.2	49.5	35.1ab
V4 Pagasa 5	2.97ab	0.59def	76.6ab	4.7	28.2	67.1	52.0a
V5 Pagasa 7	2.52abc	0.80cd	68.9abc	4.8	28.4	66.8	45.6ab
V6 Twn. Grn.	2.91ab	1.06ab	72.8abc	4.3	24.9	70.8	50.9ab
V7 Acc 658	2.88ab	0.52efg	74.6abc	4.6	27.5	67.9	50.2ab
V8 Acc 687	3.05a	0.85bc	85.5a	5.8	34.7	69.5	51.5a
V9 Acc 638	2.44bc	0.80cd	73.1abc	6.4	37.8	55.8	40.6ab
V10 Acc 211	2.61ab	0.61def	71.1abc	4.2	25.5	70.3	49.5ab
V11 Acc 174	2.74ab	1.13a	75.4ab	6.1	36.8	57.1	43.4ab
V12 Acc 188	2.67ab	1.15a	75.6ab	4.8	28.2	67.0	50.6ab
V13 Acc 2041	1.99c	0.24h	56.6c	5.8	34.6	59.6	33.0b
V14 Acc 379	2.44bc	0.58def	68.2abc	5.4	32.3	62.3	44.0ab
V15 Acc 58	2.67ab	0.56def	67.7abc	5.2	30.5	64.3	43.6ab
V16 Acc 71	2.66ab	0.45fgh	66.1bc	4.4	25.7	69.9	46.1ab
V17 PAEC pro.	2.43bc	0.73cde	71.5 abc	5.3	31.8	62.9	44.9ab
Grand Mean	2.63	0.68	71.3	5.3	31.2	63.5	45.3
C. V. (%)	13	22	15	-	-	18	24

[a]N derived from fertilizer. [b]N derived from soil. [c]N derived from fixation.

[d]Numbers in columns followed by the same letter are not significantly different ($P = 0.05$).

3.2. Residual-effects experiment

3.2.1. Mung bean

The patterns of vegetative growth and seed yield differed between the PNRI and UPLB sites. The highest dry-weight (Table V) and total-N (Table VI) values were obtained with NIAB 54 at PNRI and with Taiwan Green. Total N accumulation at early pod-fill did not correlate well with seed yield; for example, Taiwan Green at UPLB had 130 kg N/ha and yielded 860 kg/ha of grain, whereas Accession 638 at PNRI accumulated only 64 kg N/ha but yielded 1.58 t/ha of grain. The low %N value of Accession 638 (2.12%, Table VI) was consistent with its low nodule weight (Table V) indicating poor compatibility with the inoculant and with indigenous rhizobia.

TABLE IV. DRY WEIGHT, N ACCUMULATION AND N_2-FIXATION CHARACTERISTICS AT 45 DAYS AFTER PLANTING, AND SEED YIELD, OF 17 MUNG-BEAN GENOTYPES DURING THE EARLY-DRY SEASON, 1992

Cultivar	Dry matter yield (t/ha)	Seed yield (t/ha)	N yield (kg/ha)	NdfF[a] (%)	NdfS[b] (%)	Ndfa[c] (%)	Amount N fixed (kg/ha)
V1 PAEC 3	2.35a[d]	1.22a	79.3a	0.28	11.6	88.1	69.9a
V2 PAEC 10	1.92abc	0.62c-g	71.2a	0.28	12.2	87.5	62.3ab
V3 Pagasa 3	1.85abc	1.23a	62.4ab	0.34	15.4	84.3	52.6abc
V4 Pagasa 5	1.79abc	0.66b-g	65.4ab	0.41	17.8	81.8	53.5abc
V5 Pagasa 7	1.78abc	0.87a-d	64.6ab	0.32	14.6	85.1	55.0abc
V6 Twn. Grn.	1.72abc	0.74b-f	62.9 ab	0.32	13.4	86.3	54.3abc
V7 Acc 658	1.90abc	0.61c-g	64.4ab	0.31	14.0	85.7	55.3abc
V8 Acc 687	1.91abc	0.44fg	67.7a	0.34	14.5	85.2	57.7ab
V9 Acc 638	1.95ab	0.73b-g	65.3ab	0.44	19.7	79.9	52.2abc
V10 Acc 211	1.75abc	0.46d-g	59.8ab	0.30	13.1	86.6	51.8abc
V11 Acc 174	1.91abc	1.06ab	61.9ab	0.44	18.7	80.6	50.1abc
V12 Acc 188	1.97ab	0.90abc	64.5ab	0.34	15.5	84.2	54.3abc
V13 Acc 2041	0.92d	0.37fg	31.5c	0.38	17.1	82.5	26.3d
V 14 Acc 379	1.25cd	0.27g	43.3bc	0.37	14.8	84.8	36.7cd
V15 Acc 58	1.69abc	1.00abc	60.4ab	0.28	12.0	87.7	53.0abc
V16 Acc 71	1.52bcd	0.84a-e	59.0ab	0.39	15.5	84.1	49.6bc
V17 PAEC pro.	1.74abc	0.36g	60.6ab	0.38	16.3	83.3	50.5abc
Grand Mean	1.76	0.73	61.4	0.35	15.1	84.6	52.1
C. V. (%)	23	35	23	-	-	5.4	23

[a]N derived from fertilizer. [b]N derived from soil. [c]N derived from fixation.

[d]*Numbers in columns followed by the same letter are not significantly different* ($P = 0.05$).

The lowest values for vegetative dry weight, total N, amount of N fixed, %Ndfa, and seed yield were obtained with PAEC 3 at UPLB. In contrast, these plants had the highest average value for %N, showing that in this case the limitations in growth and seed yield were not caused by incompatibility with the available rhizobia - some other nutritional or environmental factor(s) caused PAEC 3 to fail to achieve its potential at UPLB.

In contrast with the screening experiment, there was clear genotypic variability for amount of N fixed and for %Ndfa. These data were generated using cotton alone as the non-fixing reference crop; when maize was used, differences among genotypes were insignificant owing to high experimental error (coefficient of variation 82%), therefore we disregarded maize as a reference crop. Values for %Ndfa were lower at UPLB than at PNRI, consistent with the higher soil %N at the former location (Table II).

On the assumption that the highest benefit to a subsequent cereal will accrue from a legume crop that fixes a large amount of N but has low grain yield and thus contributes fixed N to the soil in the form of vegetative residues [13], then the best candidates for residual benefit to a following maize crop would be expected at UPLB from Taiwan Green, Accession 638, and NIAB 54.

3.2.2. Maize

Positive effects of mung bean on subsequent maize dry-matter yield were clear, and particularly well pronounced at UPLB where growth was better (Table VII). There was a similar trend in terms of maize seed yield, although there was statistical signficance only in the difference between the seed yield after cotton (0.63 t/ha) and after Acc. 638 (1.05). Highest maize dry-matter yields were obtained after Acc. 638 at both locations despite the fact that it was not superior in N_2 fixation (Table VI).

Very little of the fertilizer N applied to the mung was available to the maize, as revealed by the low values for %NdfF (Table VIII). It would be interesting to have applied ^{15}N-labelled mung residues to adjacent plots to determine how much of the organic N therein was available to subsequent maize.

3.3. General considerations

These data justify further work on mung bean, on its capacity to fix N_2 and its ability to improve subsequent cereal yields. There clearly exists genotypic variability in mung bean for N_2 fixation (Tables III, IV, VI) and in ability to utilize that fixed N to make grain yield (Tables III, IV).

TABLE V. SHOOT DRY WEIGHT, NODULE WEIGHT AND NODULE NUMBER AT EARLY POD-FILL, AND SEED YIELD OF FIVE MUNG-BEAN GENOTYPES AT PNRI AND UPLB

Genotype	Shoot dry wt. (g/plant)		Seed yield (t/ha)		Nodule d. wt. (mg/plant)		Nodule no. (per plant)	
	PNRI	IPB	PNRI	IPB	PNRI	IPB	PNRI	IPB
PAEC 3	8.03b	4.84d	1.87ab	0.19c	70.0ab	39.5c	41.3a	21.0ab
Twn. Grn.	8.22b	14.0a	1.41c	0.86a	46.5bc	68.3b	39.6a	24.4a
Acc. 638	10.2ab	11.0ab	1.58bc	0.59b	22.8c	46.3bc	28.1b	24.9a
NIAB 54	11.0a	10.3ab	1.99a	0.57b	81.8a	98.8a	26.5b	18.6ab
NIAB 92	8.84ab	7.53cd	1.96ab	0.55b	29.8c	27.5c	4.2c	12.3b
C. V. (%)	18	22	13	26	39	29	24	35

Differences in compatibility with the chosen inoculant strain and/or the indigenous rhizobia were also apparent (Table V), and must be accommodated in the future; if the indigenous flora are not suitable for a superior genotype then it becomes critically important that the appropriate strain be applied as inoculant.

TABLE VI. N CONCENTRATION, TOTAL N, PERCENT N DERIVED FROM FIXATION AND AMOUNT OF N FIXED BY FIVE MUNG-BEAN GENOTYPES AT EARLY POD-FILL, AT PNRI AND UPLB

Genotype	N conc. PNRI	N conc. UPLB	Total N PNRI	Total N UPLB	Ndfa PNRI	Ndfa UPLB	N fixed PNRI	N fixed UPLB
	(%)		(kg/ha)		(%)		(kg/ha)	
PAEC 3	3.19a	3.79a	76.9b	55.0c	78.5ab	36.6c	59.8bc	21.3b
Twn. Grn.	3.10a	3.10bc	76.3b	130a	70.8bc	66.0ab	53.9bc	84.9a
Acc. 638	2.12b	3.37b	64.4b	111ab	64.4c	59.9ab	43.1c	66.4a
NIAB 54	3.20a	2.97cd	105a	91.5b	80.8ab	71.7a	85.2a	65.3a
NIAB 92	3.06a	2.66d	80.9b	60.0c	87.0a	52.9b	70.3ab	32.0b
C. V. (%)	2.9	3.2	17	23	9.9	18	20	23

TABLE VII. TOTAL DRY MATTER AND SEED YIELDS OF MAIZE GROWN AFTER MUNG BEAN AT PNRI AND UPLB

Previous treatment	Dry matter yield PNRI	Dry matter yield UPLB	Seed yield PNRI	Seed yield UPLB
	(t/ha)		(kg/ha)	
PAEC 3	0.905abc[a]	4.47ab	-[b]	831ab
Taiwan Green	0.628bcd	3.85bc	-	819ab
Accession 638	1.145a	5.07a	-	1,049a
NIAB 54	0.988ab	4.67ab	-	890ab
NIAB 92	0.837abcd	4.50ab	-	755ab
Maize	0.565cd	2.67d	-	679ab
Cotton	0.518d	3.32cd	-	629b

[a]Numbers in columns followed by the same letter are not significantly different ($P = 0.05$).
[b]No grain yield obtained.

TABLE VIII. TOTAL N YIELD, ^{15}N ENRICHMENT, AND PERCENT N DERIVED FROM FERTILIZER OF MAIZE AT PNRI AND UPLB

Previous treatment	Total N yield PNRI	Total N yield UPLB	Atom excess PNRI	Atom excess UPLB	NdfF PNR	NdfF UPLB
	(kg N/ha)		(%)		(%)	
PAEC 3	8.84a-c[a]	52.9a	0.2345a	0.0862ab	2.34a	0.862ab
Taiwan Green	6.20bc	34.9b	0.2100a	0.0695b	2.10a	0.695b
Accession638	11.9a	48.7ab	0.2090a	0.0818ab	2.09a	0.817ab
NIAB 54	10.3ab	47.6ab	0.2197a	0.0762b	2.20a	0.762b
NIAB 92	8.42a	44.2ab	0.2147a	0.0848ab	2.15a	0.847ab
Maize	5.08a	26.9c	0.2375a	0.0982a	2.37a	0.982a
Cotton	5.23a	32.8bc	0.2410a	0.0880ab	2.41a	0.880ab

[a]Numbers in columns followed by the same letter are not significantly different ($P = 0.05$).

Genotypic diversity clearly exists also in the residual effects of mung in improving a subsequent cereal crop (Table VII). To understand whether such improvement results partially or completely from fixed-N inputs requires further research. Residual beneficial effects may be unrelated to N. Even if all of the vegetative residues of an effectively nodulated grain-legume crop are returned to the soil after grain harvest, a net gain in N does not necessarily accrue to the system - it depends on the amount of N that was fixed (which is affected by the availability of mineral N in the soil) and on the amount of N removed in the grain. When more N is removed than was fixed, then a net loss of N prevails [14]. Calculation of net change in N are possible by determining amounts of N fixed, seed N content, and total N content at physiological maturity. When the net change is positive, benefit to a subsequent crop will occur only under conditions conducive to microbial breakdown of the residues and if the N so mineralized is available during crop growth.

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SCREENING WITH NUCLEAR TECHNIQUES FOR YIELD AND N_2 FIXATION IN MUNG BEAN IN THAILAND

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Abstract

SCREENING WITH NUCLEAR TECHNIQUES FOR YIELD AND N_2 FIXATION IN MUNG BEAN IN THAILAND

For a farmer to reap benefit from mung bean's (*Vigna radiata*) capacity to fix N_2, the crop's requirement for N must come mainly from the atmosphere through symbiotic fixation in the root nodules. The aim of this study was to evaluate recommended mung-bean cultivars and advanced breeding lines, and identify high fixers. Preliminary investigations with the ^{15}N natural-abundance method indicated its utility for measuring N_2 fixation, and the examination of five recommended cultivars and two advanced breeding lines of mung using the ^{15}N-dilution method showed diversity in N_2 fixation and yield.

More than 400 lines of mung bean were screened in soil in cement containers for growth, nodulation, N accumulation and N_2 fixation at 35 days after planting, with the natural-abundance method used to determine N_2 fixation. Genetic variability was observed for all characteristics. Estimates of fixed N ranged form 0-300 mg N/plant. Whereas some lines obtained N mainly from fixation, recommended cultivars apparently obtained their N mainly from soil. The data are discussed in terms of reliability of the ^{15}N natural-abundance method.

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1. INTRODUCTION

Mung bean (*Vigna radiata*) is an economically important crop, and, as a legume, it is capable of fixing atmospheric N_2 in symbiosis with *Bradyrhizobium*. Rhizobial strains vary in their N_2-fixing effectiveness [1, 2], and the genotype of the legume host also influences the symbiosis. In peanut (*Arachis hypogaea*) for example, certain host-genotype traits were observed to be indicative of N_2 fixation [3]. Genotypic variation in host-plant control of nodulation and N_2 fixation has also been demonstrated with soybean (*Glycine max*); in high-nitrate soil, lines of Korean origin produced more nodules and fixed more N than did commercial lines of US origin [4]. And "supernodulating" soybean genotypes produced by mutation-breeding formed up to five times more nodules than did the parent cultivar [5], indicating the opportunity to improve nodulation in other legume species In this study, we were interested in identifying mung-bean genotypes with superior N_2-fixation ability.

There are several methods for measuring N_2 fixation by legumes, and it is generally accepted that the ^{15}N-dilution technique is the most reliable and accurate [6]. However, isotope dilution requires expensive ^{15}N-labelled fertilizers, whereas the ^{15}N natural-abundance technique does not [7]. This paper describes preliminary mung-bean work with the natural-abundance and isotope-dilution methods to investigate genetic diversity for N_2 fixation.

2. MATERIALS AND METHODS

2.1. Natural-abundance experiment

The experiment was conducted in the field in 1991 at Chainat Field Crop Research Center, Thailand, on a low humic glay of sandy clay loam texture, with two mung-bean lines, VC 2768A and VC 4000-7, the seeds of which were treated with a peat-based inoculant containing rhizobial strains THA302, THA305, and THA100. The non-N_2-fixing reference crops were sorghum (*Sorghum bicolor* cv. Chainat 60), maize (*Zea mays* cv. Suwan-1), upland rice (*Oryza sativa* cv. DOA) and non-nodulating soybean (*Glycine max* cv. D68-0099). Three seeds were planted per hill, spaced at 10 cm with 50 cm between rows, in plots of 4 x 6 m; the seedlings were thinned to one per hill at 7-10 days. The experiment had a randomized complete-block design with four replications. Phosphorus and potassium were applied at 55 kg/ha P_2O_5 and 37 kg/ha K_2O, respectively. Four plants per replicate were harvested at 12, 20, 30, 40, 50, 61, 71, and 81 days after planting. Plants were dried at 70°C and ground for analysis of natural ^{15}N abundance (at the National Institute of Agro-Resources, Tsukuba Japan, with a Finigan MAT251 mass-spectrometer). Estimates of percent N derived from fixation (i.e. from the atmosphere, %Ndfa), were made from $\delta^{15}N$ values as follows [7]:

$$\delta^{15}N\,(\text{‰}) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

$$\%Ndfa = \frac{\delta^{15}N_{reference} - \delta^{15}N_{legume}}{\delta^{15}N_{reference} - \delta^{15}N_{a}} \times 100$$

where $\delta^{15}N_a$ is the natural-abundance value for plants deriving N solely from fixation, determined by culturing inoculated plants with N-free nutrition.

2.2. Isotope-dilution experiment

This experiment was carried out in an irrigated farmer's field in Pakthongchai District, Nakhon Ratchasima. This soil also had a sandy clay loam texture (0.07% N). Three recommended mung-bean cultivars, CN36, PSU1 and KPS2, and two advanced breeding lines, 9-5 (CNM-I-8709-5) and 9-8 (CNM-I-8709-8) were used. Maize and sorghum were again used as non-fixing reference crops. Seed inoculation, P and K applications, plot size, planting distances, thinning, and experimental design were as described in **2.1.**

Labelled fertilizer, $(^{15}NH_4)_2SO_4$ at 10% atom excess, was applied at 20 kg N/ha to an area of 1 m^2 within each plot, by syringe-injection of 50-mL aliquots of solution into the soil near each plant. Unlabelled $(NH_4)_2SO_4$ was applied around each microplot at 20 kg N/ha. For the reference crops, $(^{15}NH_4)_2SO_4$ at 3.3% atom excess was applied at 60 kg N/ha.

At 45 days, mung-bean plants were sampled to determine nodulation and plant dry weight. Seed yield was determined at maturity. In the microplots, all plants were harvested at maturity, and dried, weighed, and ground vegetative components were mixed with ground seed from the respective microplot. Fifty-g aliquots were sent to the FAO/IAEA Soil Science Unit at Seibersdorf, Austria, for ^{15}N analysis.

The proportions of N derived from fixation and the amounts on N fixed by the mung-bean plants were determined as follows:

$$\%Ndfa = \left(1 - \frac{\%^{15}N\ atom\ excess_{legume}}{\%^{15}N\ atom\ excess_{reference}}\right) \times 100$$

$$N\,fixed\ (kg/ha) = \frac{\%Ndfa}{100} \times Total\ N\ yield$$

2.3. Screening experiment

Diverse lines of mung bean, 423 in total, were obtained from the Asian Vegetable Research and Development Centre, Taiwan, the Chainat Field Crop Research Center, Thailand, and Kasetsart University, Thailand. The screening was done in a single batch using cement tanks of 75x75x50 cm containing a low humic glay soil (0.07% N), with 2% by weight of ground corn cobs added to immobilize mineral N. One mung-bean line, fourteen plants, was planted per tank, with spacings of 10 cm intra-row and 50 cm inter-row. Maize (*Zea mays* cv. Suwan-1) was similarly grown as the non-fixing reference crop.

The soil was inoculated with a mixture of broth cultures (10^8 cells/mL) of ten strains of rhizobia that had previously been determined, in a trial of thirty-two strains, to be effective on four test cultivars of mung bean. The strains were: TAL420, TAL1000, TAL441, THA201, THA302, THA305, THA308, CB756, NC92, NC146. Aliquots of approximately 500 mL of the inoculum mixture were added per tank and incorporated into the surface 5 cm of soil. The shoots of four plants per tank were harvested at 35 days after planting to determine total dry weight, nodule number and weight, %N, and amount of N fixed using the ^{15}N natural-abundance method (see **2.1.**)

3. RESULTS

3.1. Natural-abundance experiment

The natural-abundance values for ^{15}N were higher for the 12-day seedlings than for the planted seeds (Fig. 1a). The values peaked at the 12- or 20-day samplings, and thereafter steadily decreased, with rates of decline highest for the mung beans. Nodules were present on the non-nodulating soybean, therefore N_2 fixation possibly contributed to the low $\delta^{15}N$ of 2.5‰ at 80 days. Similar low levels of $\delta^{15}N$ were found in corn and rice at the end of the experiment, possibly due to associative or endophytic N_2-fixing bacteria. The value of $\delta^{15}N$ for sorghum changed less, from 7‰ at 11 days to 5.5‰ at 80 days after sowing (Fig. 1a), therefore it may judged to be the best of the four reference crops. On the other hand, the fact that the $\delta^{15}N$ values increased during the first few days of growth shows that factors other than N_2 fixation influence natural-abundance levels.

In view of the presence of nodules and relatively low $\delta^{15}N$ values at 11 and 20 days, it seemed prudent to exclude the non-nodulating soybean data from the calculations of %Ndfa. Therefore, average values for the sorghum, maize and rice (Fig. 1b) were used to establish the trends of increasing inputs of fixed N for the two mung genotypes (Fig. 1c). The %Ndfa values for pods were closely similar to those for shoots, lending confidence in the method - on the other hand, the trend of decline in %Ndfa for mung cv. VC 2768 A between 50 and 71 days is difficult to explain other than in terms of experimental error. At 81 days, 60-70% of the N in both mung cultivars was derived from fixation.

3.2. Isotope-dilution experiment

At 45 days after sowing, there were no statistically significant differences among the seven mung-bean genotypes in shoot dry weight, nor in root-nodule number and dry weight (Table I). In contrast, at maturity there were significant differences in total dry weight and in stover and seed dry weights (Table II). Plant %N values did not vary significantly nor did values for %Ndfa, therefore the broad ranges in total N (25 - 67 kg N/ha) and amount of N fixed (16 - 42 kg N/ha) occurred as a result of genetic variability in total dry matter.

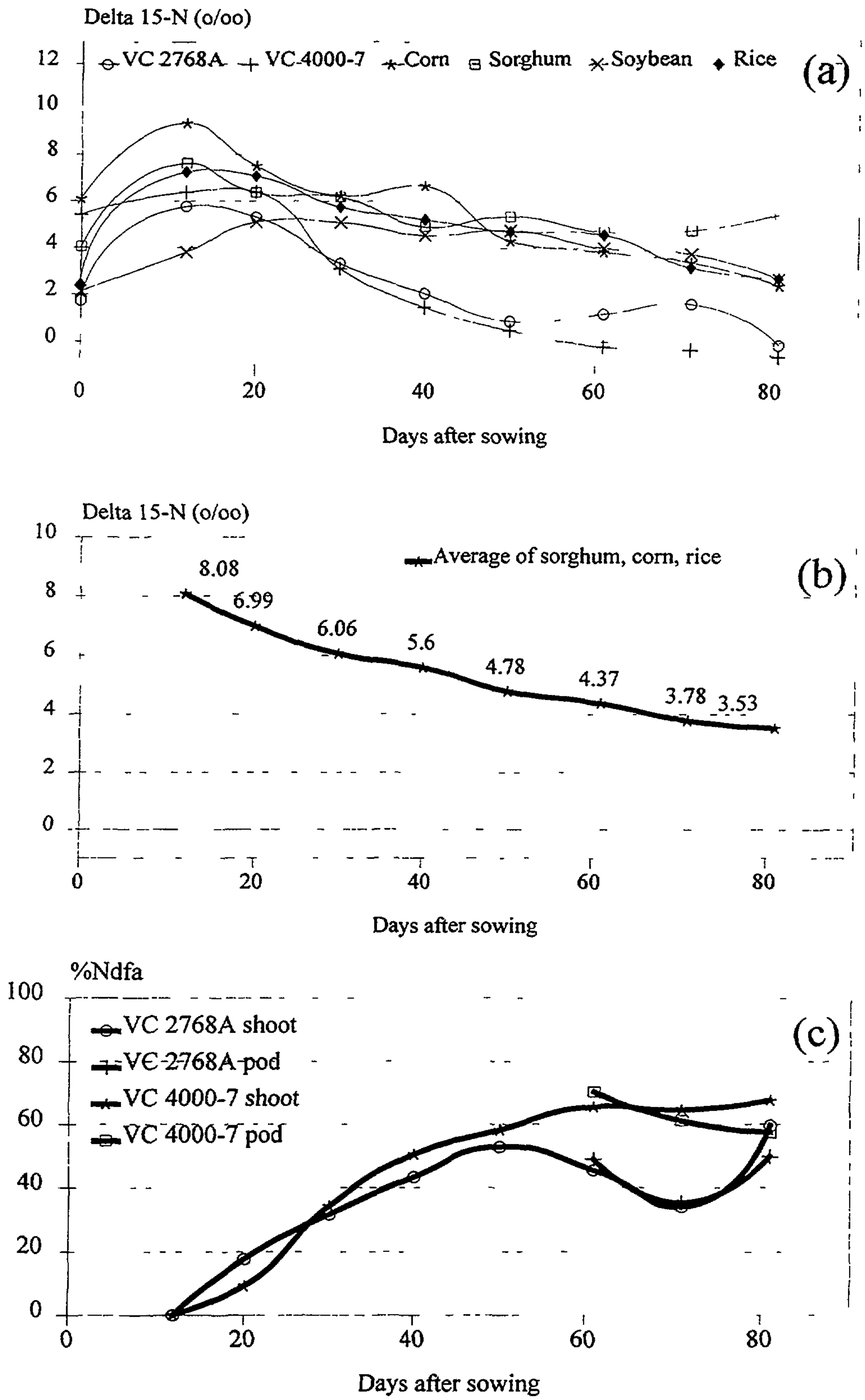

FIG. 1. Seasonal changes in $\delta^{15}N$ of two mung cultivars and four reference species (a), average $\delta^{15}N$ values of the three cereal reference crops (b), and %Ndfa values for the two mung-bean genotypes.

It is notewothy that cvv. KPS2 and 9-5, which fixed the highest amounts of N, are genetically similar: 9-5 (CNM-I-8709-5) was obtained from radiation of KPS2. This confirms the finding with cowpea (*Vigna unguiculata*) that N_2-fixation characteristics are transferable with yield potential [8].

3.3. Screening experiment

Among the 423 mung-bean lines examined, there was great diversity in terms of the total amount of N accumulated, and the degree to which that N was obtained from fixation. The amounts of N obtained from fixation and as mineral N from the soil are shown in Fig. 2 for 78 genotypes at 33 days after planting. The large majority of lines that accumulated less than 230 mg N/plant obtained the large proportion of that N from fixation, whereas the majority of lines that accumulated more than 230 mg N/plant obtained relatively more N from the soil. Three lines apparently did not utilize soil N, and had %Ndfa values of 100%.

Detailed data (Table III), for fifteen of the 423 gentoypes, show the following broad ranges at 33 days after planting: dry weight 4.2-10.1 g/plant, nodule number 17-168/plant, nodule dry weight 6-186 mg/plant, total N 152-467 mg/plant, and N from fixation 97-300 mg/plant. Also noteworthy are the determinations of amount of N from soil, giving the range 0-337 mg/plant.

These fifteen genotypes fell into two categories (Table III): five had %Ndfa values of <38 and ten had %Ndfa values of >89. The lines in the low %Ndfa category had average dry-weight and total-N values of 9.2 g/plant and 370 mg N/plant, respectively, whereas the high %Ndfa category had average values for dry weight and total N of only 6.4 and 235 mg N/plant. The group of five had a lower average nodule mass, 48 vs. 143 mg/plant, which inversely correlated with the amount of N derived from soil, averages of 251 vs. 17 mg N/plant. It is noteworthy that the hybrid 2768×1560, one of the group of ten, had plant dry weight and total N values similar to those of parent VC 2768 A, although its other characteristics were similar to those of parent VC 1560 D.

4. DISCUSSION

Preliminary work with the natural-abundance method for determining %Ndfa and amount of fixed N indicated that it provided meaningful data. Mung genotypes VC 2768 A and VC 4000-7 showed accumulation of fixed N with growth, with %Ndfa values of 60-70 at 80 days after sowing (Fig. 1c). On the other hand, there were three sources of concern: (i) increases in $\delta^{15}N$ values during early seedling growth (Fig. 1a), (ii) trends of decreasing $\delta^{15}N$ values in the non-fixing reference plants during growth (Fig. 1b), and (iii) a trend of increasing $\delta^{15}N$ values in VC 4000-7 from 50 to 71 days. Since items (i) and (ii) occurred across genotypes they appear to be real, and necessary subjects for further investigation; it is possible that item (iii) resulted from sampling an insufficient number of replicate plants.

The use of the istotope-dilution method revealed genotypic diversity for N_2 fixation among seven mung genotypes (Table III). This was a function of growth rather than of fixation per se; the genotypes that showed poorest growth, KPS 1 and CN 60, had %N values not signigicantly lower than those of the other genotypes, therefore their lack of vigour did not result from N deficiency, but from some other limiting factor.

TABLE I. MUNG-BEAN GROWTH AND NODULATION AT 45 DAYS

Genotype	Shoot dry wt. (g/plant)	Nodulation Dry wt. (mg/plant)	Nodulation Number (per plant)
CN 60	6.71	32	19
CN 36	5.95	34	20
PSU 1	7.05	24	17
KPS 1	8.42	37	36
KPS 2	6.28	32	25
9-5	7.42	42	34
9-8	8.56	45	43
CV (%)	27	49	60
F-test	ns[a]	ns	ns

[a]Not significant at $P = 0.05$.

TABLE II. YIELDS, N ACCUMULATION AND N_2 FIXATION AT MATURITY

Genotype	Dry weight Total (kg/ha)	Dry weight Stover (kg/ha)	Dry weight Seed (kg/ha)	N (%)	N yield (kg/ha)	^{15}N excess (%)	Ndfa (%)	N fixed (kg/ha)
CN 60	1126d[a]	758d	368c	2.22	25.0	0.271	65	16.2
CN 36	2858a	1425a	1034a	2.35	67.1	0.350	55	36.9
PSU 1	2239abc	1363bc	876ab	2.20	49.2	0.368	53	26.1
KPS 1	1702cd	1021cd	581bc	2.02	34.4	0.360	54	18.6
KPS 2	2747ab	1872ab	1174a	2.40	65.9	0.283	64	42.2
9-5	2487abc	1528abc	959a	2.25	55.9	0.254	68	38.0
9-8	1944bcd	1088cd	756ab	2.27	44.1	0.325	58	25.6
CV (%)	25	22	29	8.3	en[b]			en
F-test	5%	5%	5%	ns				

[a]Means within a column followed by the same letter are not significantly different at $P = 0.05$.

[b]Editor's note: These were not subjected to statistical analysis; the authors assume the same patterns of signficance shown by Total dry weight.

The screening of 423 strains revealed broad genotypic diversity for total N accumulated, and, using the natural-abundance technique, for the N-from-air/N-from-soil relationship (Fig. 2). The detailed data for growth, nodulation, N accumulation and N_2 fixation in Table III matched the N-accumulation/N source data in Fig. 2 in revealing that the genotypes fell largely into two broad groups: (i) those with high total N and low %Ndfa, and (ii) those with lower total N and high %Ndfa. The genotypes in category (i) utilized soil N efficiently, whereas those in category (ii) took up little N from the soil. A possible explanation for these two patterns is that high-yielding lines, such as PSU 1 and KPS (Table III) have been bred and selected for good performance with fertilizer N applied, as is the case in some breeding programmes. However, two of the genotypes listed in Table III and three in Fig. 2 were calculated to have assimilated no N from the soil, and other genotypes appeared to take up very little soil N. These genotypes did not show poor early growth that would be consistent with a lesion in ability to assimilate mineral N, indicating that the %Ndfa values were over-estimates.

The isotope-dilution experiment indicated that plant dry weight correlated positively with the amount of N fixed (Table II), whereas the detailed data from the screening experiment (Table III) revealed a negative correlation: the group of five had a dry weight average of 9.2 g/plant and an average amount fixed of 119 mg N/plant, whereas the group of ten had an average dry weight of 6.4 g/plant and an average amount fixed of 206 mg N/plant. This apparent discrepancy requires further investigation, but may be explainable also in terms of over-estimate of %Ndfa for the group of ten.

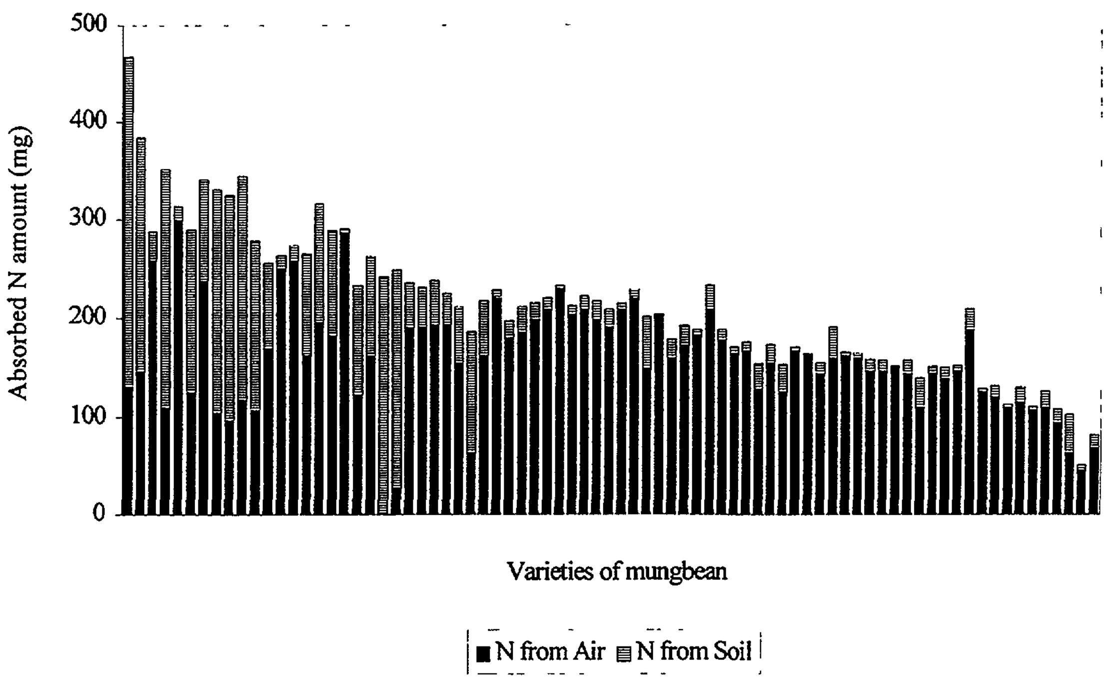

FIG. 2. Sources of N for selected mung-bean genotypes, at 33 days.

In conclusion, it is clear that genetic diversity exists in mung bean for N_2 fixation, as shown by the isotope-dilution experiment and the screening experiment. However, in neither trial was there evidence that inputs of fixed N were growth-limiting, as would be indicated by low %N values. The natural-abundance method holds promise as a convenient and relatively inexpensive method (provided that a four-decimal-place mass-spectrometer is available) for estimating %Ndfa in mung bean, but several questions must be addressed to provide assurance that the data are reliable.

TABLE III. PLANT DRY WEIGHT, NODULATION, AND SOURCES OF N FOR SELECTED MUNG-BEAN GENOTYPES AT 33 DAYS

Genotype	Dry wt.	Nodulation No.	Nodulation Dry wt.	N	Ndfa	Total N	Ndfa	Ndfs
	(g/plant)	(/plant)	(mg/plt)	(%)	(%)	(mg/plant)	(mg/plant)	(mg/plant)
VC2768(PSU1)	10.1	130	50	4.6	27.9	467	130	337
UT 8101	9.9	122	130	3.9	37.9	384	146	239
VC1973A(KPS)	8.5	37	25	4.0	34.2	345	118	227
VC 2917 A	8.8	68	24	3.7	31.8	331	105	225
VC 2991 A	8.5	17	6.0	3.8	29.9	325	97	228
VC 2754 A	8.2	168	186	3.2	95.1	264	250	13
VC 2764 B	8.0	83	173	3.4	94.3	274	258	16
VC 2755 A	7.5	110	126	3.8	98.3	291	286	5
VC 2750 A	6.3	96	132	3.6	96.3	226	220	8
UT 8102-13	5.9	167	181	3.9	98.0	233	228	5
UT 8104-5	5.3	166	150	3.9	99.6	204	203	1
VC 1560 D	4.9	32	56	4.7	89.5	233	208	24
UT 8103-8	4.5	100	120	3.6	100	164	164	0
CV 2802 A	4.2	58	173	3.6	100	152	152	0
2768×1560	9.0	95	130	3.5	95.6	313	300	13

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EVALUATION OF YIELD AND N_2 FIXATION OF MUTANT LINES OF GROUNDNUT IN MALAYSIA

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Abstract

EVALUATION OF YIELD AND N_2 FIXATION OF MUTANT LINES OF GROUNDNUT IN MALAYSIA

The ^{15}N-dilution technique was used to evaluate N_2 fixation in groundnut (*Arachis hypogaea* L.) in three field trials of cultivars Matjan and V-13 (parents), their selected mutant lines, and a other local and foreign genotypes. Matjan mutant MJ/40/42 consistently produced the highest pod yields, at above 4 t ha^{-1}, 14-22% higher yields than the parent. In contrast, none of the V-13 mutants had consistently better yields than the parent. The mutant lines did not show consistent agronomic performance from year to year. Total dry matter yield did not correlate with pod yield, and pod yield did not correlate with amount of N fixed.

1. INTRODUCTION

Groundnut or peanut (*Arachis hypogaea* L.) is the most important legume crop in Malaysia. It is cultivated mostly by farmers in the north-eastern states of peninsular Malaysia. Inoculation of seeds with rhizobia is not normally practised by groundnut farmers, in contrast with the leguminous cover crops that are sown in rubber and oil-palm plantations. However, most farmers know that groundnut growth is better in soils previously cultivated with the crop than in soils without such a history. The application of 150 kg ha^{-1} of fertilizer N was needed to produce yields similar to those obtained on soil previously cultivated with groundnut with only 20 kg N ha^{-1} added [1].

Since the early 1980s, the yields of fresh groundnut in Malaysia have averaged 3,400 kg ha^{-1}, with maximum values rarely exceeding 3,500 kg ha^{-1} [2]. There have been few attempts to quantify the inputs of fixed N by these crops.

The present study attempted to measure N_2 by several genotypes including mutants of Matjan and V-13, using the ^{15}N-dilution technique [3, 4]. The trials were part of a programme initiated in 1984 at the Malaysian Institute for Nuclear Technology Research, with the objective of producing mutant lines resistant to *Cercospora* leaf-spot disease with high-yielding capability and other desirable agronomic traits.

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2. MATERIALS AND METHODS

The seeds of locally adapted cultivars of groundnut, Matjan and V-13, were exposed to γ-irradiation of 16 kGy h^{-1} at the Malaysian Institute for Nuclear Technology Research (MINT), with doses ranging from 0 to 100 Gy. A preliminary test was conducted in the greenhouse to gather information on radio-sensitivity, and six doses were chosen for subsequent field examination: 20, 25, 30, 35, 40 and 45 Gy. For the M_1 field population, 2000 seeds were irradiated per dose per cultivar, and planted at the MINT Agricultural Experimental Plot in Bangi. Selections were carried into the M_2 populations, whereby ten pods (twenty seeds) were collected from each plant. From M_4 to M_5, evaluations were made to confirm mutant stability. After further evaluation and screening up to the M_6 population, twenty-three mutants were identified as potentially high yielding. From 148 lines tested for *Cercospora* leaf-spot resistance, five were resistant, eighteen were tolerant and forty-eight moderately tolerant. Seeds of the M_8 populations were used for field trials.

2.1. Experiment 1

An experiment to quantify N_2 fixation in fifteen mutant lines, their parents, and thirteen other cultivars of groundnut (Table I) was conducted at MINT in 1990. The trial was conducted on soil of the Bungor series (Typic paleudult) with the following characteristics: sandy clay loam, pH(H_2O) 4.85, organic matter 1.17%, total N 0.12%, available P 57 ppm, exchangeable K 0.20 meq per 100 g, exchangeable Ca 2.80 meq per 100 g.

Groundnut seeds were sown with 50 cm between rows and 25 cm between plants on 1 x 2 m plots. The seeds were coated with inoculant containing *Bradyrhizobium* strains NC 83 and 32H1 in a sterilized-peat and coconut-fibre medium, obtained from the Rubber Research Institute of Malaysia. The non-N_2-fixing reference crops were upland rice (*Oryza sativa*) cv. Seri Pelanduk, okra (*Hibiscus esculentus*), and two genotypes of chilli (*Capsicum annuum* and *Capsicum* sp.), each planted at 50 cm x 25 cm on separate plots. There was a randomized complete-block design with three replications.

Ammonium sulphate, enriched in ^{15}N at 5% atom excess, was applied at 20 kg N ha^{-1} as a solution by spraying evenly on the plots. Fertilizer P and K were both applied at 60 kg ha^{-1}.

The crops were harvested at 100 days after planting. Fresh-pod yield and total plant dry matter yields (including pods and roots) were measured. Total N in plant samples was determined [5], and ^{15}N was determined by mass-spectrometery at the IAEA Soil Science Unit, Seibersdorf, Austria [6]. The amount of N fixed and the relative contributions from fixation (i.e. percent from the atmosphere %Ndfa), from the soil (%NdfS) and from fertilizer (%NdfF) were determined as follows, using overall average enrichment values for the non-fixing reference checks:

$$\%Ndfa = (1 - \frac{\%^{15}N\ atom\ excess_{(legume)}}{\%^{15}N\ atom\ excess_{(reference)}}) \times 100$$

$$N\,fixed\,(kg/ha) = \frac{\%Ndfa}{100} \times Total\,N\,yield$$

$$\%NdfF = \frac{\%^{15}N\,atom\,excess_{(plant)}}{\%^{15}N\,atom\,excess_{(fertilizer)}} \times 100$$

$$\%NdfS = 100 - (\%Ndfa + \%NdfF)$$

2.2. Experiment 2

In 1991 at the MINT experimental plot in Bangi, nine mutant lines from Experiment 1, the parent cultivars and two other genotypes (Table II) were assessed for fresh pod yield, total plant dry-matter yield, nodule dry weight and plant %N. The sources of N, from fixation, soil and fertilizer were determined as in **2.1.** The selections were made on the basis of high yield and/or high N_2 fixation. Nodules were sampled at 30, 60 and 90 days after planting for dry-weight determination.

2.3. Experiment 3

A further experiment to assess yield and N_2-fixation capability using the ^{15}N-technique was conducted in 1992 with ten of the previously tested genotypes and two new cultivars, MKT1 and NC-7. Seeds were planted on 2 x 2 m plots, with okra as the reference species. Each genotype was replicated three times. The objective was to determine the degree of constancy of previously identified characteristics with comparisons made between the selected mutant and the local cultivar, MKT1, and the foreign cultivar NC-7, known for its high pod yield.

3. RESULTS AND DISCUSSION

The main objective of the induced-mutation breeding programme was to select mutant lines with high pod yield and superior capacity for fixing N_2 when nodulated with effective *Bradyrhizobium*. The mutants had the added advantage of originating from genotypes relatively resistant or tolerant of *Cercospora* leaf-spot disease.

In Experiment 1, most of the mutant lines produced pod yields that were within the range observed with the thirteen diverse genotypes (2.7-3.9 t ha^{-1}) (Table I). Mutant line MJ/20/5 (i.e. line 5 from Matjan after a 20-Gy γ-radiation dosage) looked particularly promising, with a pod yield of 4.2 t ha^{-1}. It is noteworthy that none of the MJ-mutant lines had total crop dry matter yields more than 50% of that produced by the parent-cultivar Matjan. In contrast, three of the six V-mutant lines had crop dry-matter yields greater than that of the V-13 parent.

TABLE I. COMPARISON OF 30 GENOTYPES OF GROUNDNUT FOR POD YIELD, TOTAL DRY-MATTER YIELD, SHOOT %N, N YIELD, %N DERIVED FROM FERTILIZER, FROM SOIL AND FROM FIXATION, AND AMOUNT OF N FIXED (EXPERIMENT 1)

Genotype	Fresh pod (kg ha^{-1})	Dry matter (kg ha^{-1})	%N	Total N yield (kg ha^{-1})	NdfF (%)	NdfS (%)	Ndfa (%)	Amount N fixed (kgha^{-1})
Matjan (parent)	3437	3941	2.94	116	3.7	47	49	57
MJ/20/1	3737	1516	2.28	34.6	4.4	55	40	14
MJ/20/5	4212	1667	2.41	40.2	3.7	47	49	20
MJ/20/88	3125	1864	3.37	62.8	3.6	45	52	32
MJ/20/96	2916	1410	1.73	24.4	6.0	75	19	4.7
MJ/20/105	3812	1410	2.09	29.4	3.7	46	50	15
MJ/20/165-5	3187	1761	2.44	43.0	2.8	35	62	27
MJ/20/165-10	2800	1718	2.42	41.6	3.0	38	59	25
MJ/40/42	4050	1570	3.00	47.2	5.0	63	32	15
MJ/40/81	3000	1385	2.71	37.5	4.4	56	40	15
V-13 (parent)	3812	2269	2.64	59.9	6.4	80	13	8.0
V13/35/16	3837	3220	2.34	75.3	6.4	81	12	9.4
V13/35/20	3575	2124	2.05	43.5	3.6	45	52	22
V13/35/23	3075	3348	2.67	89.4	4.1	52	44	39
V13/35/64	3325	1993	2.37	47.2	3.8	48	48	23
V13/35/89	3312	2407	2.46	59.3	5.6	71	23	14
V13/35/189	3237	1802	2.54	45.8	2.9	36	61	28
Alabama	2962	2509	2.34	58.8	5.2	65	29	17
C-421	3675	3059	2.13	65.3	6.4	80	13	8.5
Canton 1	3752	2484	3.40	84.4	5.5	70	24	21
Ces 2.75	3085	2570	2.19	56.3	5.1	64	31	18
Golden 1	3022	2126	2.36	50.2	5.0	32	63	16
Kuala Berang	3069	2444	2.75	67.3	5.5	69	26	17
M-10	3510	2610	2.45	63.9	4.9	62	33	21
NC-7	3676	1958	1.91	37.5	4.0	45	51	17
Red variety	3029	2224	3.20	71.2	7.6	96	-3.7	-2.6
Tainan # 9	2721	2978	3.68	110	4.3	54	41	45
Tifspan	3551	2553	2.36	60.3	4.6	57	38	23
UPL-PN-2	3882	2136	2.33	49.8	4.9	62	33	17
79-2	3850	5380	2.92	157	4.5	57	39	61

TABLE II. COMPARISON OF THIRTEEN GENOTYPES OF GROUNDNUT FOR POD YIELD, CROP DRY-MATTER YIELD, % N, N YIELD, PERCENT N DERIVED FROM FIXATION AND AMOUNT OF FIXED N (EXPERIMENT 2)

Genotype	Fresh pod (kg ha^{-1})	Dry matter (kg ha^{-1})	%N	Total N yield (kg ha^{-1})	Ndfa (%)	Amount N fixed (kg ha^{-1})
Matjan (parent)	3850	4159	1.70	76.8	57	44
MJ/20/1	3256	4023	1.92	77.3	53	41
MJ/20/5	3009	3997	1.78	71.1	59	42
MJ/20/105	3079	3941	1.90	74.9	51	38
MJ/20/165-5	1492	1804	1.80	32.5	69	22
MJ/40/42	4396	4340	1.73	75.1	61	46
V-13 (parent)	1598	2100	1.95	40.9	59	24
V13/35/16	3580	1260	1.77	22.3	65	14
V13/35/20	3517	1326	1.87	24.8	55	14
V13/35/164	1962	1060	1.88	19.9	65	13
V13/35/189	3009	3309	1.89	62.5	59	37
UPL-PN-2	1562	2357	2.00	47.2	59	28
79-2	1003	2158	2.34	50.5	61	31

In Experiment 2, MJ/40/42 again yielded and fixed N well, as did V13/35/189 (Table III). The two selected foreign genotypes yielded poorly by comparison with their previous year's performance. In contrast with Experiment 1, total crop dry-matter yields for four of the five MJ mutant lines were comparable to that of the parent Matjan, and although all four of the the V-13 mutant lines produced pod yields that were superior to that of the parent, three had crop-dry matter yields much less than that of the parent, similar to the poor vegatative growth of the MJ mutant lines in Experiment 1.

In Experiment 2, nodule dry weights were uniformly high, with a range 385-553 mg plant^{-1} at 60 days, and did not correlate well with amount of N fixed. In all three experiments, seeds were inoculated at sowing to encourage nodulation. In Experiment 1 mutant lines MJ/20/96 and MJ/20/105 had low values for dry-matter accumulation, for N yield, and for amount of N derived from fixation. Most significantly, MJ/20/96 and MJ/20/105 had low values for %N, 1.73 and 2.09, respectively, indicating that N was the chief factor limiting their growth and that their N_2-fixing capability had been compromised by radiation. On the other hand, MJ12/20/105 did not have a low %N value in Experiment 2 (Table II), indicating that ineffective inoculation rather than an adverse effect of radiation explains the Experiment 1 data.

TABLE III. DRY WEIGHT OF NODULES OF THIRTEEN SELECTED GENOTYPES OF GROUNDNUT AT 30, 60 AND 90 DAYS AFTER PLANTING (EXPERIMENT 2)

Genotype	Nodule dry weight		
	30 days	60 days	90 days
	(mg plant^{-1})		
Matjan (parent)	182	447	487
MJ/20/1	360	548	487
MJ/20/5	175	542	585
MJ/20/105	338	385	597
MJ/20/165-5	574	678	583
MJ/40/42	323	513	437
V-13 (parent)	405	550	526
V13/35/16	326	461	481
V13/35/20	339	421	435
V13/35/164	260	427	407
V13/35/189	320	379	411
UPL-PN-2	515	553	535
79-2	450	464	446

Of the reference crops used in the measurement of %Ndfa, okra emerged as the most suitable as compared to chilli and dry rice, in terms of its physiological attributes, as well as in its ^{15}N-uptake pattern (data not shown).

In Experiment 3, MJ/40/42 and V-13 (parent) were the best performers (Table IV). The newly introduced MKT1 showed promise as a high-yielding local cultivar.

The effects of γ-radiation were not clearly manifested as consistent trends, in terms of depressing or improving growth, pod yield and N_2 fixation in comparison with the parent cultivars. Furthermore, total dry matter yield did not correlated with pod yield. and pod yield did not correlate with %Ndfa or amount of N fixed.

One mutant line of Matjan showed particular promise in terms of yield: MJ/40/42. And in terms of high values for %Ndfa another mutant line emerged as consistently superior: MJ/20/165-5. It is hoped that further work on the improvement of groundnut for Malaysia will include crossing of these genotypes.

TABLE IV. COMPARISON OF TEN GENOTYPES OF GROUNDNUT FOR CROP YIELD, POD YIELD AND PERCENT N DERIVED FROM FIXATION (EXPERIMENT 3)

Genotype	Fresh crop ($kg\ ha^{-1}$)	Fresh pod ($kg\ ha^{-1}$)	Ndfa (%)
Matjan (parent)	14,146	3230	55
MJ/20/105	9,555	3119	51
MJ/20/165-5	11,784	3422	68
MJ/40/42	10,064	3950	63
V-13 (parent)	19,287	3339	21
V13/35/16	5,700	1432	14
V13/35/164	10,607	3288	45
V13/35/189	7,573	2167	33
MKT1	10,374	3879	42
NC-7	16,111	3655	30

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EVALUATION OF N_2 FIXATION BY NODULATION-VARIANTS OF CHICKPEA IN INDIA

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Abstract

EVALUATION OF N_2 FIXATION BY NODULATION-VARIANTS OF CHICKPEA IN INDIA

Five nodulation-variants of chickpea (*Cicer arietinum* L.) cv. ICC 5003, delineated on the basis of visual ratings ('S1' for minimum nodulation to 'S5' for maximum), were used to investigate the optimum levels of nodulation and N_2 fixation for growth and yield. Two field experiments were conducted, with fertilizer N (enriched in ^{15}N) applied at 10 ('N1') or 100 kg ha^{-1} ('N2') on contrasting soils in different years; plants were evaluated for nodulation, growth, N_2 fixation and yield. Experiment 2 included high-nodulating (HN) and low-nodulating (LN) selections of cvv. ICC 4948, ICC 14196 and Kourinsky. Non-nodulating selections of chickpea were included as references to quantify N_2 fixation. In both experiments the trends in amounts of N fixed by the five selections at N1 were similar when assessed by ^{15}N-enriched and by N-difference methods. The percent N derived from N_2 fixation (estimated from ^{15}N data) correlated with yield and amount of N fixed in Experiment 2, but not Experiment 1. The relative nodulation differences were consistent across locations; the S4 and S5 (and HN) lines were generally superior to S1 and S2 (and LN) for nodulation, N_2-fixation, total dry matter and grain yield. Nodule number and mass correlated signficantly ($P<0.05$) with total dry matter, grain yield, total N and quantity of N_2-fixed in both experiments.

1. INTRODUCTION

Chickpea (*Cicer arietinum*) is an important cool-season legume of the semi-arid tropics considered to sustain cropping-system productivity. Among other factors, this is due to its ability to fix atmospheric N_2 in its root nodules and to beneficially affect subsequent crops. It is nodulated by *Bradyrhizobium* sp. (*Cicer*) bacteria [1, 2]. Although the extent of nodulation and N_2 fixation varies among cultivars [3, 4], high-nodulating and high-N_2-fixing genotypes are not necessarily high yielding, as has been reported for common bean (*Phaseolus vulgaris* L.) cv. Dunadja [5] and some Korean lines of soybean [*Glycine max* (L.) Merr.] [6]. Similar examples may have led some breeders to think that maintaining nodules and their N_2-fixing functions diverts significant resources of energy to roots, resulting in low legume yields compared to cereals [7]. On the other hand, groundnut (*Arachis hypogaea* L.), pigeon pea [*Cajanus cajan* (L.) Millsp.] and chickpea plants devoid of nodules (i.e. genetically non-nodulating) when supplied with abundant fertilizer N produce yields similar to those of nodulated plants [8, 9, 10].

What is optimum nodulation and N_2 fixation for a legume? It is an important question, but difficult to answer. The extent of nodulation and N_2 fixation of a given cultivar may vary with moisture, nutrient availability, and temperature of the root and shoot. Large plant-to-plant differences in nodulation capacity have been observed within bred cultivars and land races [11], with the natural occurrence of non-nodulating plants [9]. Chickpea cv. ICC 5003 (= K850 = 850-3/27) was found to have plants with a range of nodulation capacities; when rated visually on a '1' (minimum nodulation) to '5' (maximum nodulation) scale [12], about 70% of 4,965 plants observed had a rating of '4' and only 1% had ratings of '1' and '5'. Selected plants of rating '1' (designated S1) to '5' (S5) were developed into separate accessions using pure-line selection procedures. The reasons for wide-ranging nodulation capacity within a cultivar are not clear, and need to be studied.

These nodulation-variants offer the opportunity to address the question of optimum nodulation and N_2-fixation capacity in chickpea. In an experiment with pots containing a vertisol, the different nodulation ratings were found to be generally stable [13, 14]. This paper reports performance of these selections in field experiments at two locations set up to address the following questions.

- Does the nodulation response of the five selections hold across locations?
- Does nodulation capacity correlate with amount of N fixed?
- Do the high-nodulating selections yield more?

2. MATERIALS AND METHODS

2.1. Nodulation selections and experimental sites

The five nodulation-variants (selections S1 to S5) of chickpea cv. ICC 5003 were initially developed on an entisol at the research farm of the College of Agriculture, Gwalior (26°N) India, 1987-90 [11]. In the post-rainy season of 1991-92, one representative of each selection was studied at the Agricultural Research Station Durgapura (near Jaipur), Rajasthan (27°N), India (Experiment 1). This provided a different agroclimate and soil type, and perhaps a different rhizobial population, from those at Gwalior. A non-nodulating (Nod^-) selection of ICC 5003 (ICC 5003 NN) was included as a non-fixing reference. The soil at the site was sandy, pH 8.2, EC 0.72 dS m^{-1}, mineral N 7.0 mg kg^{-1} soil and total N 359 mg kg^{-1} soil, with 5.2 x 10^3 chickpea rhizobia g^{-1} soil in the top 15 cm at the time of sowing. Minimum temperatures at Durgapura range between 3 and 13°C and maximum temperatures between 22 and 34°C during the growing season.

In the post-rainy season of 1992-93, the five selections were evaluated on a vertisol at the ICRISAT Asia Center (IAC, 18°N), India, along with the parent cultivar (Experiment 2). The vertisol site of this experiment had pH 8.2, EC 0.25 dS m^{-1}, mineral N 9.8 mg kg^{-1} soil and total N 549 mg kg^{-1} soil, with 8.9 x 10^3 chickpea rhizobia g^{-1} soil in the top 15 cm at the time of sowing. Included were high-nodulating (HN, visual rating '3' to '5') and low-nodulating (LN, visual rating '1' to '2') selections (one each) of cvv. ICC 4948, ICC 14196 and Kourinsky, along with their parents, and one non-nodulating (NN) selection each of ICCV 2, ICC 4918 (= Annigeri), ICC 4993, and their parents. The Nod^- chickpea lines were included as non-fixing references. Winter at IAC is mild and short, and the average temperatures for October to February range from 28 to 30°C during the day and from 15 to 20°C at night.

2.2. Experiment 1

Two soil N levels, achieved by application of ammonium sulphate at 10 kg N ha^{-1} (designated 'N1') and 100 kg N ha^{-1} ('N2'), were the main plots, and five nodulation-variants and the parent cv. ICC 5003 were the sub-plot treatments of a split-plot design with four replications. The N2 plots had 39 mg mineral N kg^{-1} soil in the top 60-cm soil profile, when measured eight days after N application at the time of sowing. The sub-plot size was 4 x 2.4 m (9.6 m^2).

Furrows were opened using a bullock-drawn plough, and single super phosphate (200 kg ha^{-1}) was applied in the 30-cm rows. Each sub-plot had a microplot of 0.8 m x 1.2 m (0.8-m long, 4 rows) in the center, for ^{15}N application. The ammonium sulphate, applied as single doses, was enriched with ^{15}N at approximately 10% atom excess for N1 and 1% atom excess for N2. The required quantities were dissolved in water and 500-mL aliquots were applied to each 0.8-m-long furrow. The remainder of the each sub-plot received similar applications of unenriched ammonium sulphate. After N application, furrows were manually covered with a thin layer of soil to minimize surface movement of fertilizer during manual sowing. Peat-based inoculant (strain IC 59) containing 2.1 x 10^9 rhizobia g^{-1} was applied at 500 mg m^{-2} after mixing with local soil (1 g inoculant kg^{-1} soil); the field was then irrigated. Bunds were constructed around each plot to prevent fertilizer transfer. After eight days, on 23 October 1991, seeds were dibbled at a 20-cm spacing, on fertilizer rows spaced at 30 cm. The seeds had been coated with inoculant to provide about 2.3 x 10^4 cells per seed. Two irrigations were provided, at 32 days after sowing (DAS) and at 78 DAS. There were no serious pest problems and preventative action against *Helicoverpa* pod-borer was taken at early signs of damage, by spraying 2 L ha^{-1} of Endosulphan (35 EC) at 102 DAS. Physiological maturity was reached at about 132 DAS and final harvesting was at 152 DAS.

Plants from 0.36 m^2 were sampled at 42 DAS, and the shoots dried (70°C). Roots were excavated and acetylene reduction (AR) activities [15], nodule numbers and nodule dry weights determined. The presence of nodules on some Nod^- plants caused us to sample them again, at 92 DAS.

At final harvest, plants from 4.8 m^2 were dried in the sun and processed for total dry matter including fallen leaves, and grain yield. Of the four 0.8-m-long rows of the ^{15}N-micro plots, 0.6-m lengths of the two middle rows were harvested. Stover and seeds from the micro plots were weighed and milled separately to < 1 mm.

2.3. Experiment 2

The field used had been prepared as a long-term ^{15}N plot for screening germplasm accessions for high N_2-fixation. Soil profiles with two different N levels were created by applying ^{15}N-enriched ammonium sulphate at 10 and 100 kg N ha^{-1} (N1, N2), at approximately 10% and 1% atom excess, respectively. The ^{15}N was applied to the preceding crop of millet (*Pennisetum glaucum* (L.) R.Br. cv. ICMS 7703) sown on 8 July 1992, i.e. during the rainy season. The ammonium sulphate ^{15}N-enrichment values were . The millet crop was ratooned at 34 DAS and finally harvested at 65 DAS. It was chopped and incorporated into the plots from which it had been cut. The crop harvested at 34 DAS was retained after chopping and oven drying, and later incorporated. The field was prepared as 60-cm ridges after another 45 days when the incorporated millet had apparently decomposed.

There were no micro plots; ^{15}N had been applied to the whole area. The N-treatments formed main plots of the split-plot design, with four replications. The twenty-one chickpea entries (see **2.1.**), including the five nodulating variants (S1 to S5) of ICC 5003 used in Experiment 1, were the sub-plot treatments. The sub-plot size was 2 x 1.2 m.

The experiment was sown on 31 October 1992, on 60-cm ridges, with two rows per ridge spaced at 30 cm, and 10 cm between plants. Single super phosphate (250 kg ha^{-1}) was manually applied before sowing. The experiment was sprinkler-irrigated (38 mm) on the day after sowing, and a flood irrigation was made at 54 DAS. Weeding was done at 25 and 68 DAS and Endosulphan (35 EC, 2 L ha^{-1}) was sprayed against insects at 34 and 81 DAS. Some areas were affected by wilt caused by *Fusarium oxisporum* f. sp. *ciceri*, and data from such were excluded from statistical analysis. Net plot size for the final harvest was 1.8 m^2. Most entries matured at about 95 DAS and were harvested between 101 and 104 DAS.

Plants of cv. ICC 5003 and its five nodulation-variant selections (S1 to S5) were excavated from a 0.36 m^2 area at 42 DAS, for determinations of nodule number and nodule mass. For the other lines, plants from only two replications were similarly sampled from 0.36 m^2. The final harvest was from a 1.8-m^2 area of each sub-plot between 101 and 104 DAS, before excessive leaf-shedding had occurred. The plants were placed in cloth bags and dried (70°C), followed by threshing for assessment of seed yield and stover mass. Sub-samples of stover and seed from each sub-plot were milled separately to < 1 mm.

2.4. Calculations for assessment of N_2 fixation

The ^{15}N-enriched ammonium sulphate solutions were prepared separately to produce enrichments of approximately 1% and 10% atom excess [16]. Analysis using a Jasco 150 emission spectrometer gave values (in solution) of 0.924 and 8.723% atom excess for Experiment 1, and 0.989 and 9.225% atom excess for Experiment 2. Ground samples of stover and seed from the micro-plots were analyzed for ^{15}N at the Joint FAO/IAEA Soil Science Unit, Seibersdorf, Austria, using a VG 602 mass spectrometer.

In Experiment 1, at least 25% of nominally non-nodulating reference plants in N1 had nodules when observed at 92 DAS, whereas those at N2 were practically devoid of nodules. Also, yields (total dry matter 5.97 t ha^{-1} and grain yield 1.8 t ha^{-1}) were close to those of the best-growing chickpea at N1, therefore we ignored the ^{15}N-enrichment data generated with the nodulation-variants at N2, using only the non-nodulating plants at N2 as reference with the A_N-value method to calculate fixed N at N1 [17]:

$$N\ fixed\ (kg\ ha^{-1}) = (A_{N\ (legume)} - A_{N\ (reference)}) \times \%NFU_{(legume)}$$

$$where\ A_N\ (kg\ ha^{-1}) = \frac{(100 - \%NdfF)}{\%NdfF} \times Rate\ of\ N\ applied$$

$$\textit{where } \%NFU = \%\ N\text{-fertilizer utilization} = \frac{\%NdfF \times total\ N}{Rate\ N\ applied}$$

$$\textit{where } \%NdfF = \frac{\%^{15}N\ atom\ excess_{(crop)}}{\%^{15}N\ atom\ excess_{(fertilizer)}} \times 100$$

In Experiment 2, the Nod$^-$ selections from cultivars ICCV 2, ICC 4918, and ICC 4993 were free of nodules and were used as reference with the ^{15}N-dilution method to calculating %Ndfa at N1 and N2:

$$\%Ndfa = (1 - \frac{\%^{15}N\ atom\ excess_{(legume)}}{\%^{15}N\ atom\ excess_{(reference)}}) \times 100$$

$$N\ fixed\ (kg\ ha^{-1}) = \frac{\%Ndfa}{100} \times Total\ N\ yield$$

Means of the N-yield data (N uptake by all above-ground plant parts) of the non-nodulating line(s) were deducted from the N yield of a given entry to arrive at the quantity of N fixed by the N-difference method. The net plot size for final harvest was only 1.8 m^2 in Experiment 2, therefore those data would be most appropriately expressed as kg m^{-2}, but, in order to compare the two experiments, they are presented as t ha^{-1}.

3. RESULTS

3.1. Experiment 1

Plants of selections S4 and S5 were best nodulated and difficult to differentiate visually. Means (of the two N-levels) for nodule number and mass of S3, S4, and S5 plants were significantly ($P<0.05$) different from each other (Tables I, II). Also, the N $\times$ selection interactions were significant ($P<0.05$). Mean (of all the five selections) nodulation (both nodule numbers and mass) at N2 was less than half of that at N1. Differences in the five selections were also apparent for acetylene reduction (AR) activity at 42 DAS: barely detectable in S1 and S2 plants, and increasing from S3 to S5 (Table III).

TABLE I. NODULE NUMBER OF NODULATION-VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003 AT TWO N-LEVELS, AT 42 DAS (EXPERIMENT 1)

N level	Nodule number S1	S2	S3	S4	S5	Mean
	(per plant)					
N1	1	0	20	31	37	18
N2	0	0	9	11	16	7
SE			±3.0 (3.2)[a]			±2.3
Mean	1	0	14	21	26	
SE			±1.1			

[a]To compare means for same level of N.

TABLE II. NODULE DRY WEIGHT OF NODULATION-VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003 AT TWO N-LEVELS, AT 42 DAS (EXPERIMENT 1)

N level	Nodule dry weight S1	S2	S3	S4	S5	Mean
	(mg $plant^{-1}$)					
N1	2	1	43	74	78	40
N2	0	0	19	21	32	14
SE			±6.5 (6.6)[a]			±5.5
Mean	1	0	31	47	55	
SE			±2.8			

[a]To compare means for same level of N.

Later (92-100 DAS), all plants of all ratings had nodules at N1, and where nodules were few (< 10 per plant) some were large, approximately 2 cm in diameter. At N2, some plants did not nodulate (data not shown). About 25% plants of the non-nodulating line of ICC 5003 had nodules at N1, but at N2 fewer than 5% plants were nodulated.

Germination and plant establishment were good. However, growth was poor in patches. At 42 DAS, when plants were first sampled for nodulation, poor plant growth was apparently associated with root-knot nematodes (*Meloidogyne incognita* and *M. javanica*).

Total dry-matter (all above-ground parts) yields of the five selections at N2 were significantly ($P < 0.05$) superior to those at N1 (Table IV). Total dry-matter yield generally increased with better nodulation (from S1 to S5) both at N1 and N2, but the differences were non-significant. Mean grain yield (2.19 t ha^{-1}) of the five selections at N2 was also significantly ($P < 0.05$) greater than that at N1 (1.73 t ha^{-1}) (Table V). Also, grain yield increased with nodulation capacity (from S1 to S5) both at N1 and N2. Mean (of the two N levels) yield of the high-nodulating (S4 and S5) selections was significantly superior to those of low-nodulating (S1 and S2) selections. Interactions between N levels and nodulation ratings were non-significant.

The average N yield (of the two N levels) of the five selections increased with nodulation (Table VI). The N yields of the high-nodulating selections (139, 141 kg ha^{-1}) were significantly ($P < 0.05$) superior to those of low-nodulating selections (101, 106 kg ha^{-1}). With the exception of S4, the nodulation-variants contained more N at N2 than at N1; the interaction with applied N was non-significant.

All five selections had similar values for %Ndfa both in stover and grain (Table VII). The %Ndfa values of selections S4 and S5 were only marginally (2 to 3%) higher than those of S1 and S2, although substantially more N was fixed by the high-nodulating selections; S4 and S5 had 32% more fixed N in stover and 67% in grain than did S1 and S2.

When assessed using the N-difference method, the high-nodulating selections had 77% more fixed N in stover, and 117% in grain, than did the low-nodulating selections (Table VII). On a total-N basis, the high-nodulating selections fixed 53% more N when measured by the A-value method and 100% more when measured by the N-difference method, compared to the low-nodulating selections (Fig. 1). The S4 plants fixed marginally more N than did the S5 plants.

3.2. Experiment 2

The S1 plants of cv. ICC 5003 formed the fewest nodules and S5 the most at 42 DAS (Table VIII). Fewer nodules (44% averaged over all entries) were formed at N2 than at N1. The parent cv. ICC 5003 formed 29 nodules at N1, similar to S3.

The HN selections of cvv. ICC 4948, ICC 14196 and Kourinsky formed (average of the two N levels) 1.8- to 4-fold more nodules than did the LN selections of their respective cultivars (Table VIII). The number of nodules formed at N2 for all of the nodulating lines were substantially lower than those at N1, except for ICC 4948 HN. All of the non-nodulating selections were devoid of nodules at both N1 and N2. Plants of ICC 5003 S5 formed the highest number of nodules per plant at both N1 and N2.

TABLE III. ACETYLENE REDUCTION ACTIVITY OF NODULATION-VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003 AT TWO N-LEVELS, AT 42 DAS (EXPERIMENT 1)

N level	AR activity (μmoles C_2H_4 plant^{-1} h^{-1}) S1	S2	S3	S4	S5	Mean
N1	0.1	0.0	4.1	5.0	7.1	3.3
N2	0.1	0.0	0.7	0.8	1.3	0.6
SE			±1.19 (0.94)[a]			±0.66
Mean	0.1	0.0	2.4	2.9	4.2	
SE			±0.85			

[a]To compare means for same level of N.

Nodule mass (mg plant^{-1}) of selection S1 from ICC 5003 was least, and increased with nodulation rating to S5 (Table VIII). At N1, the parent line ICC 5003 formed a nodule mass of 92 mg plant^{-1} close to that of S3 (88 mg). The HN selections of the three cultivars ICC 4948, ICC 14196, and Kourinsky had substantially greater nodule mass than those of the respective LN selections. The HN selections from these three varieties were better nodulated than were their parents at both N levels, with the exception of ICC 4948 at N1. The mean nodule mass at N2 (all entries) was 31 mg plant^{-1}, 47% less than that at N1. Nodule mass of all entries was less at N2 than that at N1 (range 9-95%) except for ICC 4948 HN, with which nodule mass was 33 mg plant^{-1} at N1 and 87 mg plant^{-1} at N2.

The highest total dry-matter yields were obtained with ICC 5003 S5 at N1 and N2, and were significantly ($P<0.05$) superior to those of selections S1 and S2 (Table IX). At N1, the yield was similar to that of its parent, whereas at N2 it was substantially (34%) superior to that of the parent.

Among the other entries, the total dry matter yields of the Nod$^-$ selections were generally low, and that of the locally adapted cv. ICC 4918 highest, both at N1 (with the exception of ICC4993 NN) and at N2. The HN selection of ICC 14196 (a wilt-resistant line) yielded more than did the LN selection from the same cultivar (Table IX), whereas the HN selections of ICC 4948 and Kourinsky, some of which died due to fusarium wilt, did not yield well.

Grain yields of selection S5 of ICC 5003 were highest both at N1 and N2 and significantly ($P<0.05$) superior to those of S1 or S2, although only marginally better than those of the parent (Table IX). Yield of selection S1 was lowest of the five nodulation-variants of ICC 5003 at N1, whereas at N2 its yield was similar to that of ICC 5003 S4. The non-nodulating selections gave the lowest yields (0.40 to 0.68 t ha^{-1} at N1, 0.97 to 1.05 t ha^{-1} at N2). Grain yields of ICC 14196 HN were greater (by 31 to 32%) than those of ICC 14196 LN at both N1 and N2 (Table IX). Compared to its parent, it yielded 61% more at N1 and 4% less at N2.

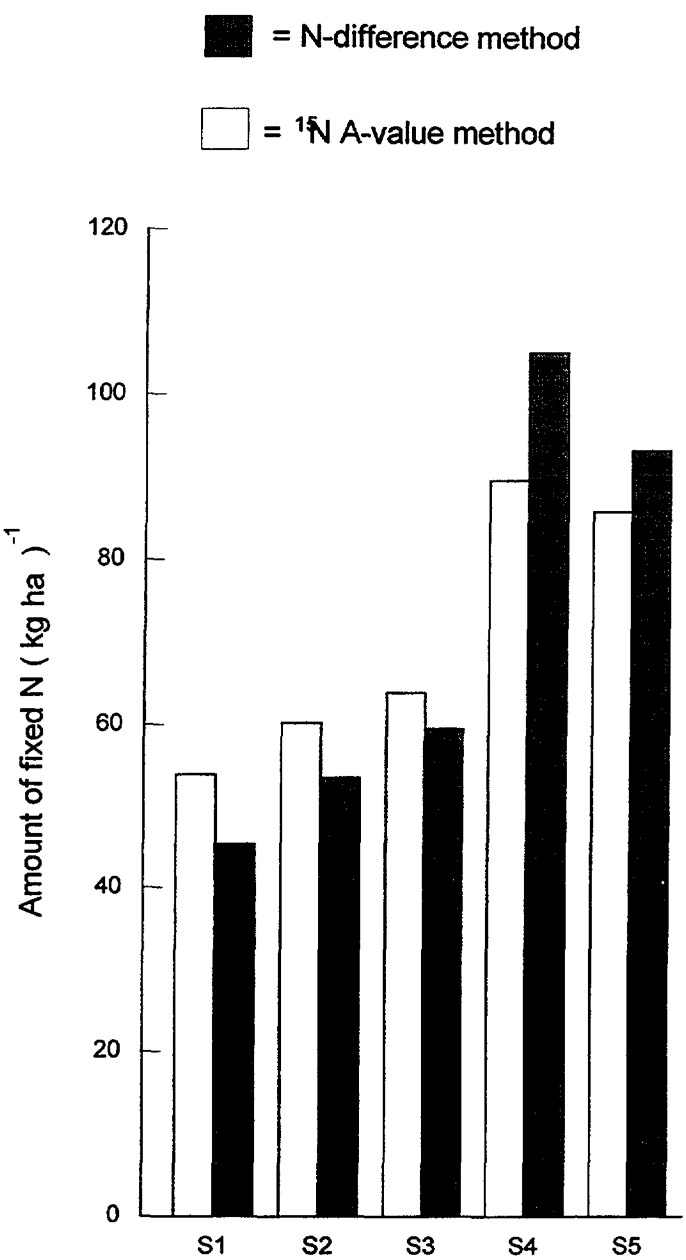

FIG. 1. Amounts of N fixed by nodulation-variants S1-S5 of chickpea cv. ICC 5003, by the A-value and N-difference methods, at N1 (Experiment 1).

TABLE IV. TOTAL DRY WEIGHT AT FINAL HARVEST OF NODULATION-VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003, AT TWO N-LEVELS (EXPERIMENT 1)

N level	S1	S2	Total mass S3	S4	S5	Mean
			($t\ ha^{-1}$)			
N1	4.70	5.01	5.25	6.22	6.19	5.48
N2	6.20	5.99	6.30	6.65	6.99	6.42
SE			±0.591 (0.607)[a]			±0.233
Mean	5.45	5.50	5.77	6.43	6.60	
SE			±0.429			

[a]To compare means at same level of N.

TABLE V. GRAIN YIELD AT FINAL HARVEST OF NODULATION-VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003, AT TWO N-LEVELS (EXPERIMENT 1)

N level	S1	S2	Grain yield S3	S4	S5	Mean
			($t\ ha^{-1}$)			
N1	1.22	1.57	1.67	2.09	2.11	1.73
N2	2.02	2.05	2.18	2.24	2.45	2.19
SE			±0.196 (0.175)[a]			±0.118
Mean	1.62	1.81	1.93	2.167	2.28	
SE			±0.124			

[a]To compare means at same level of N.

The total-N yields followed the trends for total dry matter and grain. The non-nodulating lines generally accumulated relatively little N, but more at N2 than at N1 (Table IX). The nodulating lines, however, did not show such a trend; only five of eighteen nodulated lines accumulated more N at N2 than at N1. The locally adapted line ICC 4918 had the highest dry matter, grain, and N yields, at N1.

Fifteen of the eighteen nodulated entries had lower %Ndfa values at N2 than at N1, both in stover and in grain (Table X), with ranges in grain of 65-83% at N1 and 61-82% at N2, and in stover of 55-78% and 45-77%, respectively. The low-nodulating selections S1 and S2 of ICC 5003 had the lowest %Ndfa values for stover both at N1 and at N2, but not for %Ndfa in grain.

When measured by the ^{15}N-dilution method, fixed N for S5 was numerically the highest of the ICC 5003 selections and substantially greater (59% at N1 and 85% at N2) than the means for S1 and S2 (Table XI). The parent line ICC 5003 fixed more (11%) than S5 at N1, but 15% less at N2. At N1, the HN selection of ICC 14196 fixed 42% more than did the LN selection, and 70% more than its parent, whereas at N2 the parent and the HN selection fixed similar quantities (62-63 kg N ha^{-1}). The locally adapted average-nodulating line ICC 4918 fixed the most N (107 kg N ha^{-1}) at N1, 48% more than did the highest-nodulating line of ICC 5003; however, they fixed similar quantities at N2. The N_2-fixation values obtained by the N-difference method were similar ($r = 0.9$) to those obtained by the ^{15}N-dilution method.

4. DISCUSSION

4.1. Non-nodulating selections as reference

In Experiment 1, 25% of plants of the non-nodulating selection of ICC 5003 had nodules at N1, whereas at N2 nodules were lacking or were very small. This genotype had been reported as Nod^- based on greenhouse tests using rhizobial strain IC 59 and on field evaluation at Gwalior using the same rhizobial strain [9]. Clearly, at Durgapura, the site of Experiment 1, some native rhizobia were infective. Furthermore, on a vertisol at IAC, about 50% of this putative Nod^- selection had nodules (O.P. Rupela, unpublished), therefore it was decided not to use the ^{15}N-dilution method to assess N_2-fixation by the five selections (S1 to S5) of ICC 5003. Instead the ^{15}N A-value method was adopted, using data generated with Nod^- ICC 5003 at N2, because the few nodules formed thereon were virtually non-functional.

In Experiment 2, the non-nodulating selections from cvv. ICCV 2, ICC 4918 and ICC 4993 formed no nodules, therefore the ^{15}N-dilution method was used for quantification of N_2 fixed [17].

4.2. Nodulation, N_2 fixation and soil N

Chickpea nodules have an indeterminate growth habit [18] and may develop to about 2 cm in diameter [19]. It is likely that the formation of large nodules is associated with rhizobial genotype, and certainly nodule size is influenced by soil moisture and temperature. In Experiment 1, large nodules were observed on plants of Nod^- ICC at N1 and on S1 and S2 selections both at N1 and at N2. As a result, some plants of low-nodulating selections (S1 or S2) with few nodules had nodule dry weights close to those of their S4 and S5 counterparts (based on spot checks on vigorous versus poorly-growing plants of S1 and S2 at 74 DAS). Thus, it appears that under irrigated conditions (as

in both experiments) and in light soil (Experiment 1), the expected differences in nodule mass and amount of N fixed by plants of different nodulation-capacity may be blurred. Nevertheless, the use of high-nodulating selections may have utility in environments in which the formation of only a few or no nodules would minimize the growth potential of low-nodulating selections.

In Experiment 2, HN selections of cvv. ICC 4948, ICC 14196 and Kourinsky formed more nodules with greater mass at both N1 and N2 (Table VIII) than did their LN counterparts, and were generally better nodulated than were their parents. However, the level of their superiority was influenced by N level. For example, ICC 14196 HN had 1.8-fold more nodule mass than did its parent at N1, whereas they were of similar nodule mass at N2: a 63% reduction in the case of 14196 HN and only a 29% decrease for the parent. In the case of ICC 4948 HN, instead of suppression in nodule mass, a 1.6-fold increase was recorded (from 33 at N1 to 87 mg plant^{-1} at N2), while its parent registered an 80% reduction at N2. Unfortunately, this selection is more susceptible to fusarium wilt than is its parent and ICC 4948 LN, and, as a result, its N_2-fixation was not high (Table XI). In a field experiment in the post-rainy season of 1993-94 where N2 plots had 2.5-fold more mineral N than did N1, ICC 4948 HN recorded a 71% suppression in nodule mass [20]. Therefore improvement or suppression in nodulation is dependent on the concentration of mineral-N, perhaps particularly during early plant growth, and on the host cultivar. It was apparent that, at a given soil-N level, some selections fixed more N than did their parents (Table XI). Nitrogen is known to be a dynamic nutrient, therefore strategies are required to effectively manage soil mineral-N to maximize harnessing N_2-fixation by legumes for high cropping-system productivity. Also, there is a need to better understand the interactions between soil mineral-N and nodulation/N_2-fixation to effectively identify high-fixing selections for particular soil-N levels.

TABLE VI. N YIELD AT FINAL HARVEST OF VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003, AT TWO N-LEVELS (EXPERIMENT 1)

N level	S1	S2	Total N S3	S4	S5	Mean
			(kg ha^{-1})			
N1	89.5	97.6	104	149	137	115
N2	114	115	133	133	141	127
SE			±12.1 (13.1)[a]			±3.19
Mean	101	106	118	141	139	
SE			±9.24			

[a]To compare means at same level of N.

TABLE VII. PERCENT N DERIVED FROM FIXATION AND AMOUNT OF FIXED N, ESTIMATED BY THE A-VALUE AND N-DIFFERENCE METHODS, IN STOVER AND GRAIN OF NODULATION-VARIANTS OF CHICKPEA CV. ICC 5003 AT N1 (EXPERIMENT 1)

Nodulation-variant	Ndfa (%)		Amount of fixed N: A-value ($kg\ ha^{-1}$)		Amount of fixed N: N-difference ($kg\ ha^{-1}$)	
	Stover	Grain	Stover	Grain	Stover	Grain
S1	56	64	24.6	29.3	23.2	22.2
S2	56	66	21.4	38.7	19.1	34.4
S3	55	66	22.3	41.7	20.4	39.1
S4	53	68	30.3	59.2	41.0	64.1
S5	56	66	30.2	54.4	34.0	59.1
SE	±6.8	±5.7	±6.53	±5.26	±10.4	±6.27

Soil mineral-N, particularly nitrate-N, is known to suppress N_2-fixation by legumes [21]. In the two experiments reported here, mineral N was assayed in air-dried soil samples collected at sowing, with part of the mineralizable N accounted for [S.P. Wani, pers. comm.]. In Experiment 2, the mineral N levels in the top 15 cm soil profile at N1 and N2 were not disparate (9.9 vs. 12.1 mg kg^{-1} soil, respectively) at the time of sowing. Between 15 and 60 cm depths, both profiles had similar N levels. However, all of the nodulated chickpea accessions observed at 42 DAS showed suppressed nodule mass at N2 (range 9-96 %) compared to that at N1 (Table VIII). These data indicate that the plant's nodulation response is more sensitive than the measurement of mineral N in soil. Further studies are needed on response-relationships between soil-mineral N level (and form) and nodule mass/N_2-fixation by the nodulation-variants in controlled-environment conditions. Also, it will be important to determine if measuring mineral-N in fresh soil samples at sowing would be a better indicator of suppression of nodulation/N_2-fixation in chickpea.

4.3. Total dry matter and grain yield

The sites of the two experiments have contrasting environments (see **2.1.**). Generally, chickpea growth and yield are better at site 1, which is cooler with a longer growing season. The temperatures at site 1 (<30°C) also favor N_2-fixation by chickpea [19].

TABLE VIII. NODULE NUMBER AND DRY WEIGHT OF NODULATION-VARIANTS OF CHICKPEA AND THEIR PARENT CULTIVARS, AT TWO N-LEVELS, AT 42 DAS (EXPERIMENT 2)

	Nodule number			Nodule mass		
Genotype	N1	N2	Mean	N1	N2	Mean
	(per plant)			(mg $plant^{-1}$)		
ICC 5003 S1	4	3	3	11	7	9
ICC 5003 S2	22	1	11	46	2	24
ICC 5003 S3	30	19	25	88	74	81
ICC 5003 S4	38	23	30	102	47	74
ICC 5003 S5	41	31	36	129	102	119
ICC 5003	9	8	19	92	19	55
ICC 4948 HN	22	29	25	33	87	60
ICC 4948 LN	6	7	6	23	21	22
ICC 4948	16	4	10	46	9	28
ICC 14196 HN	30	10	20	103	38	70
ICC 14196 LN	14	5	10	73	15	14
ICC 14196	11	10	11	58	41	49
KourinskyHN	33	13	23	127	38	82
Kourinsky LN	17	8	13	53	24	38
Kourinsky	30	7	19	105	22	63
ICCV 2	22	15	18	117	29	73
ICCV2 NN	0	0	0	0	0	0
ICC 4993	23	12	17	62	47	55
ICC 4993 NN	0	0	0	0	0	0
ICC 4918	17	18	17	57	83	70
ICC 4918 NN	0	0	0	0	0	0
SE	±6.5 (6.6)[a]		±4.7	±19.0 (19.2)[a]		±13.6
Mean	18	10		59	31	
SE	±0.9			±3.0		

[a]To compare means at same level of N.

Better plant growth and greater yield may be expected at N2 than at N1, which was true for all entries at site 1 (Tables IV, V), but not at site 2 where at least 10 of the 18 nodulated lines produced less foliage and grain at N2 than at N1 (Table IX). The yield of non-nodulating lines was consistently higher at N2 than at N1. The overall (average of all entries) yield at N2 was marginally lower than at N1. It seems that temperature stress at site 2 interacted differentially with the various entries. This, along with the interactions between soil N with N_2 fixation, suggests the need for investigation of how the two sources of N (symbiosis and soil) are differentially affected by soil temperature.

Long-duration varieties of chickpea are known to yield poorly at site 2 [22] because pod development coincides with unfavorably high ambient temperatures. It is expected that plentiful N (from fixation or soil) would delay maturity, and therefore yield at site 2 would be adversely affected. Lower grain yields at N2 than at N1 have been recorded in other studies [20, O.P. Rupela unpub.].

4.4. High nodulation versus yield

When evaluated in pots [13] nodule numbers of selections S1 to S5 of ICC 5003 were well delineated at low soil-N, providing a broad range of nodule mass (mg plant^{-1}, < 10 by S1, 12 by S2, 56 by S3, 69 by S4 and 79 by S5). Therefore, it was hoped that we were closer to answering the question, "What is optimum nodulation and N_2 fixation?" particularly by using ^{15}N-based methods. However, it seems that more factors influence this trait in field conditions than in the glasshouse. Also, the data reported here were affected by nematodes in Experiment 1 and small plot size in Experiment 2. As a result, only large differences in nodulation capacity, e.g. S4/S5 vs. S1/S2 of ICC 5003 or ICC 14196 HN vs. ICC 14196 LN, were significantly different, reflecting the trends in grain yield and N_2-fixation.

Reported "super-nodulating" mutants of soybean [23, 24] and of common bean [25] produce yields lower than their normal-nodulating parents. In contrast, our high-nodulating selections generally produced yields at least marginally higher than did their parents. Of the five variants of ICC 5003, even S5, with the highest nodulation so far recorded for chickpea, produced yields not lower than those of its parent, which should remove any apprehension from the minds of skeptics that selection (not mutation) for high nodulation will lead to yield reduction.

It has been observed repeatedly, including in these experiments (data not shown), that N_2-fixation efficiency (as measured by AR activity per nodule weight) of low-nodulating selections is greater than that of high-nodulating selections. As a result, 100% nodule-mass differences in low- and high-nodulating selections may result in fixed-N differences of only 30%. Furthermore, differences in yield (a result of several factors including N nutrition) may be only 5-10% in favor of the high-nodulating selections. Nevertheless, promotion of high-nodulating variants is worthwhile as a buffer in conditions of stress in which low-nodulating lines would lack nodules. In addition, high-nodulating selections may contribute 20 kg ha^{-1} (assessment based on nodule mass at 42 DAS in Experiment 1) or more biomass (as nodules) of high N-content (at least 4% N), beneficial to soil health.

TABLE IX. DRY MATTER, GRAIN AND N YIELDS OF NODULATION-VARIANTS AND PARENT CULTIVARS, TWO N-LEVELS (EXPERIMENT 2)

	Total dry matter			Grain yield			Total N		
Genotype	N1	N2	Mean	N1	N2	Mean	N1	N2	Mean
	($t\ ha^{-1}$)			($t\ ha^{-1}$)			($kg\ ha^{-1}$)		
ICC 5003 S1	2.50	3.00	2.75	0.92	1.33	1.12	44	77	60
ICC 5003 S2	2.36	1.64	2.00	1.08	0.59	0.84	45	26	36
ICC 5003 S3	3.51	2.70	3.11	1.49	1.18	1.33	69	63	66
ICC 5003 S4	4.10	3.18	3.64	1.88	1.40	1.64	80	57	69
ICC 5003 S5	4.30	4.18	4.24	1.94	1.63	1.79	82	81	81
ICC 5003	4.30	3.12	3.71	1.71	1.37	1.54	89	70	80
ICC 4948 HN	2.35	4.14	3.25	0.82	1.94	1.38	41	78	60
ICC 4948 LN	2.67	3.72	3.19	0.97	1.46	1.21	49	69	59
ICC 4948	4.06	3.64	3.85	1.85	1.56	1.71	84	64	74
ICC14196 HN	4.88	4.38	4.63	1.97	1.73	1.85	92	81	86
ICC 14196 LN	3.91	3.50	3.70	1.49	1.32	1.41	69	60	65
ICC 14196	3.83	4.25	3.04	1.22	1.80	1.51	62	81	71
Kourinsky HN	3.62	2.82	3.22	1.30	1.10	1.20	64	50	57
Kourinsky LN	4.37	4.21	4.29	1.49	1.68	1.59	86	79	82
Kourinsky	5.06	2.82	3.94	2.07	1.09	1.58	94	48	71
ICCV 2	4.37	4.22	4.29	1.88	2.01	1.95	84	84	84
ICCV 2 NN	1.09	2.58	1.84	0.40	0.97	0.69	13	45	29
ICC 4993	4.14	4.12	4.13	1.43	1.50	1.46	75	76	75
ICC 4993 NN	2.38	2.71	2.55	0.68	1.05	0.87	40	49	45
ICC 4918	5.03	4.80	4.92	2.23	1.91	2.07	138	90	114
ICC 4918 NN	1.71	2.73	2.22	0.61	1.04	0.83	29	50	39
SE	±0.676(0.662)[a]		±0.468	±0.378(0.373)[a]		±0.264	±15.2(15.1)[a]		±10.7
Mean	3.55	3.45		1.39	1.37		66	64	
SE	±0.206			±0.097			±3.5		

[a]To compare means at same level of N.

TABLE X. PERCENT N DERIVED FROM FIXATION IN STOVER AND GRAIN OF NODULATION-VARIANTS OF CHICKPEA AND PARENT CVV., AT TWO N-LEVELS (EXPERIMENT 2)

	Ndfa					
	Stover			Grain		
Genotype	N1	N2	Mean	N1	N2	Mean
	(%)					
ICC 5003 S1	55	56	56	75	68	72
ICC 5003 S2	55	45	50	77	72	75
ICC 5003 S3	75	57	66	80	61	70
ICC 5003 S4	77	62	70	79	74	77
ICC 5003 S5	79	77	78	82	82	82
ICC 5003	71	69	70	78	79	78
ICC 4948 HN	67	63	65	72	68	70
ICC 4948 LN	63	67	65	65	76	70
ICC 4948	78	73	76	79	76	78
ICC 14196 HN	76	73	74	81	78	80
ICC 14196 LN	75	67	71	78	71	74
ICC 14196	57	66	61	71	80	75
Kourinsky HN	71	60	66	79	72	76
Kourinsky LN	77	65	71	83	76	80
Kourinsky	74	68	71	78	77	77
ICCV 2	73	60	67	79	70	74
ICC 4993	79	72	75	81	79	80
ICC 4918	72	67	70	79	77	78
SE	±8.1(7.4)[a]		±5.2	±5.5(4.9)[a]		±3.5
Mean	71	65		77	74	
SE	±3.8			±2.7		

[a]To compare means at same level of N.

TABLE XI. ESTIMATES OF FIXED N, USING ^{15}N AND DIFFERENCE METHODS, BY NODULATION-VARIENTS OF CHICKPEA AND PARENT CULTIVARS, AT TWO N-LEVELS (EXPERIMENT 2)

	Total amount of fixed N					
	^{15}N-dilution			N-difference		
Genotype	N1	N2	Mean	N1	N2	Mean
	(kg ha^{-1})					
ICC 5003 S1	38	49	43	15	42	28
ICC 5003 S2	44	21	33	17	-[a]	4
ICC 5003 S3	54	59	57	40	28	34
ICC 5003 S4	64	41	52	52	22	37
ICC 5003 S5	65	65	65	53	46	49
ICC 5003	72	55	63	60	35	48
ICC 4948 HN	33	51	42	12	43	28
ICC 4948 LN	34	50	43	20	34	27
ICC 4948	66	77	71	55	29	42
ICC 14196 HN	75	62	68	63	45	54
ICC 14196 LN	53	53	53	40	25	33
ICC 14196	44	63	53	33	46	39
Kourinsky HN	50	40	45	35	15	25
Kourinsky LN	71	57	64	57	44	50
Kourinsky	71	43	57	64	13	39
ICCV 2	65	58	62	55	49	52
ICC 4993	59	72	66	46	41	43
ICC 4918	107	67	87	109	54	82
SE	±11.7(11.5)[b]		±8.11	±16.9(14.3)[b]		±10.1
Mean	45	42		46	34	
SE	±3.64			±9.7		

[a]Negative value recorded.
[b]To compare means at same level of N.

4.5. Selecting for high N_2 fixation based on nodulation versus ^{15}N-enrichment methods

In Experiment 2 at N2, ICC 5003 S1 yielded (and therefore fixed N_2) similarly with ICC 5003 S4, which is difficult to explain (Table IX). ICC 5003 S1 may be more tolerant of soil nitrate than ICC 5003 S4. As a result ICC 5003 S1 not only fixed more, it also utilized soil-N more effectively.

Measurement of $^{15}N/^{14}N$ ratios by mass-spectrometry is a sensitive procedure. The values of % ^{15}N atom excess so obtained are used with yield data to calculate the amount of N fixed. Therefore, any factor affecting yield also affects the determination of fixed N. Also, the ^{15}N methods are based on assumptions [26] including what constitutes an appropriate non-fixing reference crop. It is difficult to assess if all such assumptions are satisfied in a given experiment. Using the ^{15}N-dilution method in Experiment 2, the top three entries at N1 were ICC 4918, ICC 14196 HN and ICC 5003, and at N2 are ICC 4948, ICC 4993 and ICC 4918 (Table XI). On the basis of nodule mass per plant, the top-three entries at N1 were ICC 5003 S5, Kourinsky HN, and ICCV 2, and at N2 were ICC 5003 S5, ICC 4948 HN and ICC 4918 (Table VIII). Therefore, only ICC 4918 would be selected by both

TABLE XII. CORRELATION COEFFICIENTS BETWEEN N_2-FIXATION AND YIELD TRAITS

N_2-fixation trait	Experiment 1 (n = 20) Total dry wt.	Grain yield	N yield	Experiment 2 (n = 60) Total dry wt.	Grain yield	N yield
		(r)			(r)	
Nodule no.	0.55*[a]	0.64*	0.66*	0.32*	0.34*	0.38*
Nodule wt.	0.56*	0.70*	0.67*	0.39*	0.35*	0.36*
AR activity	0.37*	0.64*	0.39*	-[b]	-	-
%Ndfa, stover	-0.26	0.01	-0.22	0.53*	0.53*	0.47*
%Ndfa, grain	-0.11	0.25	-0.13	0.45*	0.46*	0.37*
Amount N fixed (^{15}N)	0.28	0.27	0.49*	0.90*	0.95*	0.88*
Amount N fixed (N-diff.)	0.44*	0.34*	0.66*	0.90*	0.95*	0.89*

[a]Statistically significant ($P < 0.05$).
[b]Not determined.

methods. Nodule number and mass were significantly correlated with yield traits (total dry matter, grain, and N-yield) in both experiments (Table XII), whereas the ^{15}N-based estimates of %N derived from fixation in stover and grain were significantly correlated with the yield traits in Experiment 2 but not in Experiment 1. The ^{15}N-dilution method requires the use of expensive ^{15}N-labelled fertilizer and special equipment (mass spectrometer or emission spectrometer) generally unavailable in developing countries. Selecting varieties (or plants) greatly superior in nodulation capacity with even marginally superior yields may obviate the need for ^{15}N in selection programs for high N_2-fixation. Confirmation studies, however, should be conducted using ^{15}N-based methods.

5. CONCLUSIONS

These two experiments with nodulation-variants did not answer the difficult question, "What is optimum nodulation and N_2 fixation for chickpea?" However, they confirmed that the relative nodulation differences in low-nodulating (S1 and S2) and high-nodulating (S4 and S5) variants were consistent over locations. Also, the high-nodulation selections generally fixed more N_2 than did the low-nodulating counterparts and gave yields at least marginally superior to those of their parents. The various selections responded differently to soil-N level. For most, nodulation was suppressed at the high soil-N levels, whereas a few lines showed enhanced nodulation at high soil-N, confirming the possibility of selecting for high-soil-N tolerant symbiosis in chickpea, as previously proposed [27].

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STUDIES IN SRI LANKA ON COWPEA: N_2 FIXATION, GROWTH, YIELD, AND EFFECTS ON CEREALS

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Abstract

STUDIES IN SRI LANKA ON COWPEA: N_2 FIXATION, GROWTH, YIELD, AND EFFECTS OF CEREALS.

The impact of seed inoculation and N-fertilization on nodulation, plant dry-matter production, and seed yield was studied through a series of field experiments with cultivars of cowpea. In some instances there were positive growth responses to applied N, indicating the potential to improve N_2 fixation and yields by combining compatible genotypes and bradyrhizobial strains.

Beneficial residual effects on growth of subsequent maize could not be related to N_2 fixation by the preceding cowpea. Although there was no evidence of direct transfer of N from cowpea to intercropped maize, there was greater efficiency of use of N for total crop production during intercropping.

1. INTRODUCTION

More than half of the world's population is dependent on rice for sustenance, and it has been estimated that, within the next 30 years, a 65% increase in production will be necessary to meet the requirements of the growing population. This need forces the development of higher-yielding varieties that will require increased fertilizer inputs. In the face of prices that are currently prohibitive for growers in much of the developing world, as well as undesirable environmental effects, alternatives to chemical N-fertilizers are needed urgently. Hence, new attention must be paid to exploiting biological sources of N to meet the requirements of rice and the other important cereal crops.

Grain legumes - cowpea, mung bean, black gram, groundnut, etc. - are planted increasingly in rice-based cropping systems as insurance crops in place of leguminous green manures, because, in addition to the contributions of symbiotically fixed N to the soil, they produce an economically valuable seed yield.

Sri Lanka's population of approximately 18 million obtains about 75% of its dietary protein from plants. Hence, legumes are of significant nutritional as well as economical importance. However, pulse production in Sri Lanka is insufficient to meet national needs (Table I). Over Rs2 billion (ca.US$35 million) are spent annually on importation, mainly of lentil and chickpea, a policy that cannot be sustained due to escalating costs. Cowpea, mung bean, black gram, and groundnut are the major pulses grown, on about 80,000 ha. Average productivity is only 700-800 kg/ha as a result of low levels of nutrient input, drought stress, insect and disease infestations, and lack of improved, locally adapted, cultivars.

If Sri Lanka is to achieve and maintain self-sufficiency in rice, emphasis must be placed also on grain legumes. Cowpea, the major legume crop and a good substitute for imported lentil, is grown on 25,000 ha in the dry zone. In symbiosis with compatible rhizobia, it can derive 80-90 kg N/ha from the atmosphere. Therefore, yields of cowpea in Sri Lanka may be increased by the

enhancement of N_2 fixation, and, at the same time, the residual N in the stover may contribute to the requirements of cereal crops.

Although it has been often observed that the yield of a non-leguminous crop is greater when following a legume [e.g. 1], such benefits cannot always be explained in terms of N. Nitrogen contribution from a grain-legume crop depends on the amount of N fixed and on the amount of N removed in the harvested grain, assuming that all of the stover is returned to the soil [2]. If more N is removed in harvested grain than was fixed, a net depletion of N occurs [3]. Therefore, studies on the impact of available N in the soil and other factors that affect the root-nodule symbiosis would contribute to the efficient use of legumes in multiple-cropping systems in Sri Lanka.

Although cowpea has been grown in Sri Lanka since time immemorial, no systematic effort has been made to exploit its N_2-fixing ability towards improving productivity. Hence, a series of studies was undertaken with the following objectives.

- To ascertain if N is a limiting factor for increasing productivity.
- To determine the nature and magnitude of variation in cowpea traits associated with yield and with N_2 fixation, and how they are affected by applied N.
- To evaluate possible beneficial effects from cowpea on intercropped and rotationally cropped cereals.

2. MATERIALS AND METHODS

2.1. Effects of inoculation and N application on growth and yield of cowpea

2.1.1. Experiment 1

The effects of inoculation and N application on growth and yield of cowpea cvv. MI 35, Bombay and Arlington were studied in a field experiment at the regional research station of the Department of Agriculture at Bata-atha, Hungama, (6°15'N, 80°54'E) in the dry zone of Sri Lanka. Two treatments were compared to controls: inoculation with a peat-based indigenous bradyrhizobial multi-strain inoculant, and fertilizer-N application. A randomized complete block design (RCBD) was used, with five replications.

The above-ground parts of the plants in ^{15}N-labelled micro-plots were harvested at physiological maturity, dried (80°C), milled, and %N [4] and ^{15}N % atom excess [5] values determined. Estimates of the amounts of N fixed by Bombay and Arlington were made using the ^{15}N-dilution method with thanhal (*Setaria italica)* as the reference crop. The percent N derived from fixation (i.e. from the atmosphere, %Ndfa), amount of N fixed, and values for percent N derived from fertilizer and from soil, respectively, were calculated as follows [6].

$$\%Ndfa = \left(1 - \frac{\%\,^{15}N \text{ atom excess}_{(legume)}}{\%\,^{15}N \text{ atom excess}_{(reference)}}\right) \times 100$$

$$N \text{ fixed (kg/ha)} = \frac{\%Ndfa}{100} \times \text{Total N yield}$$

$$\%NDfF = \frac{\%^{15}N \text{ atom excess}_{(plant)}}{\%^{15}N \text{ atom excess}_{(fertilizer)}} \times 100$$

$$\%NdfS = 100 - (\%Ndfa + \%NdfF)$$

2.1.2. Experiment 2

This field trial was conducted May - July, 1992, in the field at Bata-atha, on a Reddish Brown Earth. Cultivars Bombay and MI 35 were grown with thanhal as the non-fixing reference crop. The following treatments were applied.

N_{10} uninoc.	10 kg N/ha applied at 10 days after planting (DAP)
N_{10}	Inoculated and 10 kg N/ha at 10 DAP
N_{40}	Inoculated and 40 kg N/ha: 20 at 10 DAP and 20 at 25 DAP
N_{80}	Inoculated and 80 kg N/ha: 20 at 10 DAP and 60 at 25 DAP
N_{120}	Inoculated and 120 kg N/ha: 20 at 10 Dap, 60 at 25 DAP and 40 at 35 DAP
N_{160}	Inoculated and 160 kg N/ha: 20 at 10 DAP, 60 at 25 DAP, 60 at 35 DAP and 20 at 55 DAP
N_{200}	Inoculated and 200 kg N/ha: 20 at 10 DAP, 60 at 25 DAP, 60 at 35 DAP and 60 at 55 DAP.

The inoculant, containing bradyrhizobial strains THA 291 and THA 30, was applied at 10 days after planting. The fertilizer N was enriched with ^{15}N.

The treatments were laid out in an RCBD with five replications. The following determinations were made to evaluate the response to N: nodule number and fresh weight and shoot dry weight at 45 DAP, and pod yield, shoot dry weight and total N at physiological maturity. Values for %Ndfa were calculated as described above.

2.2. Effects of cowpea on intercropped and rotationally cropped maize

2.2.1. Experiment 3

This investigation was carried out in two stages at Mapalana in the Matara District of the low-country wet zone of Sri Lanka, on a Red Yellow podsol.

In Stage 1, N_2 fixation by previously screened cvv. IT 81 D-994, IT 86 D-1004, IT 85 D-3428, IT 82 D-504, MI 35 and Bombay was measured using ^{15}N with maize and sorghum as reference crops. In Stage 2, the effects of incorporation of cowpea stover on the growth, yield and N content of a succeeding maize crop were compared with the effects of a previous crop of maize.

The treatments for Stage 1 were replicated four times and had an RCBD. One week after seed germination, ammonium sulphate was applied at 10 kg N/ha, enriched in ^{15}N at 10% atom excess for application to micro-plots, and unenriched for the remainder of each plot. All plots also received 50 kg P/ha and 75 kg K/ha as superphosphate and muriate of potash, respectively. The plot size used was 3x1 m for the legumes and 3x3 m for the cereals. Plots and blocks were separated by 40-cm-wide drainage channels.

Spacings were 30 x 15 cm for cowpea and 60 x 30 cm for maize and sorghum, and rows had an east-west orientation to minimize mutual shading. Two seeds were planted per hill, and seedlings were thinned to one per hill.

At 45 DAP and at physiological maturity, plants were sampled and determinations of %N, ^{15}N enrichment and %Ndfa made as described above. At maturity, the above-ground components in the non-labelled areas were harvested, dried and weighed.

In Stage 2, the stovers were chopped and incorporated into the soil at 3 weeks prior to planting maize (cv. Badra) in the same experimental plots. No fertilizers were used. At maturity, the above-ground parts of the maize plants were harvested, and yields of grain and stover recorded. Correlation coefficients between N_2 fixed by the legume and dry-matter production and N yield of the succeeding maize were determined.

2.2.2. Experiment 4

This field experiment was also carried out at Bata-atha on a Reddish Brown Earth, during May-August 1993. Maize cv. Badra was grown as a monocrop and as an intercrop with cowpea cvv. Bombay and MI 35. The experiment had an RCBD with four replicates. Plots were 3.6 x 2.1 m in size, and were separated by 40-cm drainage ditches. The treatments were as follows.

1. Maize/Bombay intercrop
2. Maize/MI 35 intercrop
3 Bombay monocrop
4. MI 35 monocrop
5. Maize monocrop

The maize seeds were planted two per hill with a 60 x 30-cm spacing, and thinned to one per hill 2 weeks later. In the intercropping plots, each alternative row of maize was replaced with three rows of cowpea with a 30 x 15-cm spacing. The rows had an east-west orientation to minimize shading of the cowpea by the maize.

Before crop establishment, 1 x 1 m micro-plots were demarcated and applied with 20 kg N/ha as ammonium sulphate 5% enriched in ^{15}N, and to the remainder of each plot unenriched ammonium sulphate was applied at the same rate. In addition, 50 kg P/ha as superphosphate and 75 kg K/ha as a muriate of potash were applied to all plots and incorporated.

Irrigation, weeding and spraying against pests and diseases were done when necessary. At physiological maturity, the above-ground components were harvested, separated into pods and stover, dried (80°C) and weighed. Samples were ground and used for determinations of %N and ^{15}N and %Ndfa as described above. Evidence of N transfer from cowpea was examined in terms of dilution of ^{15}N in intercropped maize as compared with monocropped maize.

3. RESULTS AND DISCUSSION

3.1. Effects of inoculation and N application on growth and yield of cowpea

3.1.1. Experiment 1

Inoculation had no significant effect on total dry weight or seed yield (Table II). The application of N fertilizer improved the seed yields of MI 35 and Arlington, but not of Bombay. These data indicate that Bombay, which originated in Asia, formed effective symbioses with the native strains of bradyrhizobia, whereas Arlington and MI 35, both of foreign origin, were

TABLE I. ACTUAL PRODUCTION, DESIRED PRODUCTION AND DEFICIT OF SOME GRAIN LEGUMES IN SRI LANKA

Crop	Production Actual	Desired	Deficit
	(MTx10^3)		(%)
Cowpea	32.5	70	67
Green gram	12.5	35	64
Black gram	5.5	24	77
Groundnut	14	27	49
Soya bean	1.5	8	84
(Rice	3000	3250	8)

TABLE II. EFFECT OF SEED INOCULATION AND N FERTILIZATION ON GROWTH AND YIELD OF COWPEA (EXPERIMENT 1)

Cultivar	Treatment	Seed yield	Total d.wt.
		(kg/ha)	
MI 35	Control	1876c[a]	6078abc
	Inoculated	1942c	5260c
	Fert. N	2836ab (51)[b]	6500abc
Arlington	Control	2209bc	6993ab
	Inoculated	2276bc	5536bc
	Fert. N	3098a (40)	7922a
Bombay	Control	2462abc	5818abc
	Inoculated	2311bc	5638abc
	Fert. N	2662ab (8)	7587a

[a]Values within columns followed by the same letter are not significantly different ($P < 0.05$).

[b]% increase over the control.

incompatible with the indigenous strains and with the inoculant strains, and therefore responded positively to fertilizer N.

Genotype Bombay fixed 54 kg N/ha (48% Ndfa) whereas Arlington fixed only 18 kg N/ha (18% Ndfa) (Table III), consistent with the possibility that the former genotype was compatible with the indigenous rhizobia and the latter was not.

TABLE III. NITROGEN FIXED BY BOMBAY AND ARLINGTON (EXPERIMENT 1)

Cultivar	Ndfa (%)	N_2 fixed (kg N/ha)
Bombay	48	54
Arlington	18	18

TABLE IV. NODULE FRESH WEIGHT, NODULE NUMBER AND SHOOT WEIGHT AT 45 DAP (EXPERIMENT 2)

Treatment	Nodule fresh wt. (g/plant)	Nodule number (per plant)	Shoot d. wt. (g/plant)	Total N (g/plant)
MN_{10}	0.46cd[a]	21.8cd	4.13	0.108
MIN_{10}[a]	0.74abcd	25.8bcd	3.80	0.097
MIN_{40}	0.43cd	19.8cd	3.58	0.095
MIN_{80}	0.22d	16.6d	3.29	0.105
MIN_{120}	0.12d	10.1d	2.87	0.084
MIN_{140}	0.15d	17.3cd	3.79	0.119
BN_{10}	1.22ab	75.6a	4.49	0.115
BIN_{10}	1.39a	68.6a	5.35	0.142
BIN_{40}	0.99abc	51.3abc	4.37	0.120
BIN_{80}	0.61bcd	61.8ab	5.02	0.143
BIN_{120}	0.38cd	41.5abcd	4.25	0.127
BIN_{140}	0.64abcd	38.2abcd	4.45	0.125
	(**)	(**)	(NS)	(NS)
TN_{10}			3.76	0.049
TN_{40}			4.16	0.069
TN_{80}			6.01	0.117
TN_{120}			4.91	0.121
TN_{140}			5.96	0.153

[a]Values within columns followed by the same letter are not significantly different ($P<0.05$).
[b]MI 35, inoculated, 10 kg N/ha applied, etc.

TABLE V. POD YIELD, SHOOT WEIGHT AND TOTAL PHYTOMASS PRODUCTION AT PHYSIOLOGICAL MATURITY (EXPERIMENT 2)

Treatment	Pod yield	Shoot d. wt.	Total d. wt.	Total N
	(g/plant)			
MN_{10}	5.21	3.40d	8.61ef	0.172
MIN_{10}[a]	4.40	2.95d	7.34f	0.151
MIN_{40}	5.57	4.40d	9.97bcdef	0.198
MIN_{80}	5.09	4.67d	9.76cdef	0.202
MIN_{120}	4.72	3.27d	7.99ef	0.188
MIN_{160}	6.64	5.30bc	11.8abcde	0.272
MIN_{200}	5.08	4.15d	9.23def	-
BN_{10}	3.98	5.09cd	9.06def	0.191
BIN_{10}	5.07	9.25a	14.3ab	0.339
BIN_{40}	5.23	7.95abc	13.3abcd	0.289
BIN_{80}	5.96	8.20ab	14.2abc	0.299
BIN_{120}	6.71	8.46a	15.2a	0.301
BIN_{160}	6.73	8.38a	15.1a	0.338
BIN_{200}	6.60	8.04abc	14.6a	-
	(NS)	(**)	(**)	(NS)
TN_{10}			7.86	0.036
TIN_{10}			11.0	0.051
TIN_{40}			11.0	0.060
TIN_{80}			13.5	0.060
TIN_{120}			14.00	0.085
TIN_{160}			17.1	0.170

[a]Values within columns followed by the same letter are not significantly different ($P < 0.05$).
[b]MI 35, inoculated, 10 kg N/ha applied, etc.

3.1.2. *Experiment 2*

Inoculation did not significantly increase nodule number or fresh weight in cv. MI 35 or Bombay at 45 DAP (Table IV). In both cultivars, application of fertilizer N resulted in trends of decrease in nodule number and nodule fresh weight. Shoot dry weights showed no significant response to inoculation or to added N, indicating compatibility with the indigenous rhizobia. On the other hand, the shoot weights were only 2.9 to 5.0 g/plant, therefore it is possible that a factor other than N was growth-limiting.

At physiological maturity, pod yields showed no significant response to inoculation or to N (Table V). Shoot dry weight of Bombay responded significantly to inoculation, and that of MI 35 to 160 kg N/ha. Bombay had consistently higher shoot weights than did MI 35. Total-production data showed trends similar to those for shoot dry weight. In neither cultivar was total N accumulation significantly affected by inoculation or applied N indicating compatibility with the indigenous rhizobia; again, however, the low total dry weight values suggest that N was not the chief growth-limiting factor, in which case neither inoculation nor applied N would improve growth.

Treatment effects on %Ndfa and fixed N were not significant at 45 DAP (Table VI). In contrast, significant effects were observed at physiological maturity; the values %Ndfa and for the amounts of N fixed were significantly higher for Bombay compared to MI 35. The highest value for %Ndfa with 10 kg N/ha, only 48%, again suggests that N was not the chief growth-limiting factor for the cowpeas. As was to be expected, increased application of fertilizer N significantly reduced the %Ndfa values for both genotypes. The non-fixing reference crop, thanhal, responded positively to fertilizer N.

3.2. Effects of cowpea on intercropped and rotationally cropped maize

3.2.1. Experiment 3

The %Ndfa values were highest for cvv. IT 81D-994 and MI 35 at 60 DAP (i.e. at physiological maturity) and all genotypes derived 49 - 76% of their N requirement through symbiotic N fixation (Fig. 1). Thus, dependency on mineral N was significant.

At Stage 2, maize grew better after cowpea than after maize, by 11 - 49% (Fig 2). The increases in the N yield of the succeeding maize crops in comparison to maize after maize ranged from 15-50% (data not shown). There was no correlation between the amount of N fixed by the preceding cowpea genotype and the magnitude of the increases in dry-matter production of the succeeding maize showing that factors other than N were contributory. The highest residual effects were observed after IT 1054 and IT 994 (Fig. 2), which ranked second and fifth, respectively, in terms of %Ndfa (Fig. 1).

3.2.2. Experiment 4

The cowpeas had significantly lower values for ^{15}N atom excess compared to monocropped maize showing that they derived considerable proportions of their N requirement from fixation (Table VII). When intercropped, cv. Bombay fixed the highest amount of N (137 kg/ha), although not significantly more than the amount fixed by MI 35. Both genotypes fixed significantly more N when intercropped than when monocropped, presumably the result of depletion of mineral N by the accompanying maize.

Maize intercropped with either genotype of cowpea was not significantly different from monocropped maize in terms of ^{15}N enrichment (Table VIII), suggesting that the legume did not transfer fixed N of to the cereal.

The highest total dry-matter production was obtained with monocropped maize, followed by the maize/Bombay intercrop and maize/MI 35 (Table VIII). In contrast, the highest N yield was observed with maize/Bombay, which accumulated 84% more N than did monocropped maize and 108% more N than monocropped Bombay.

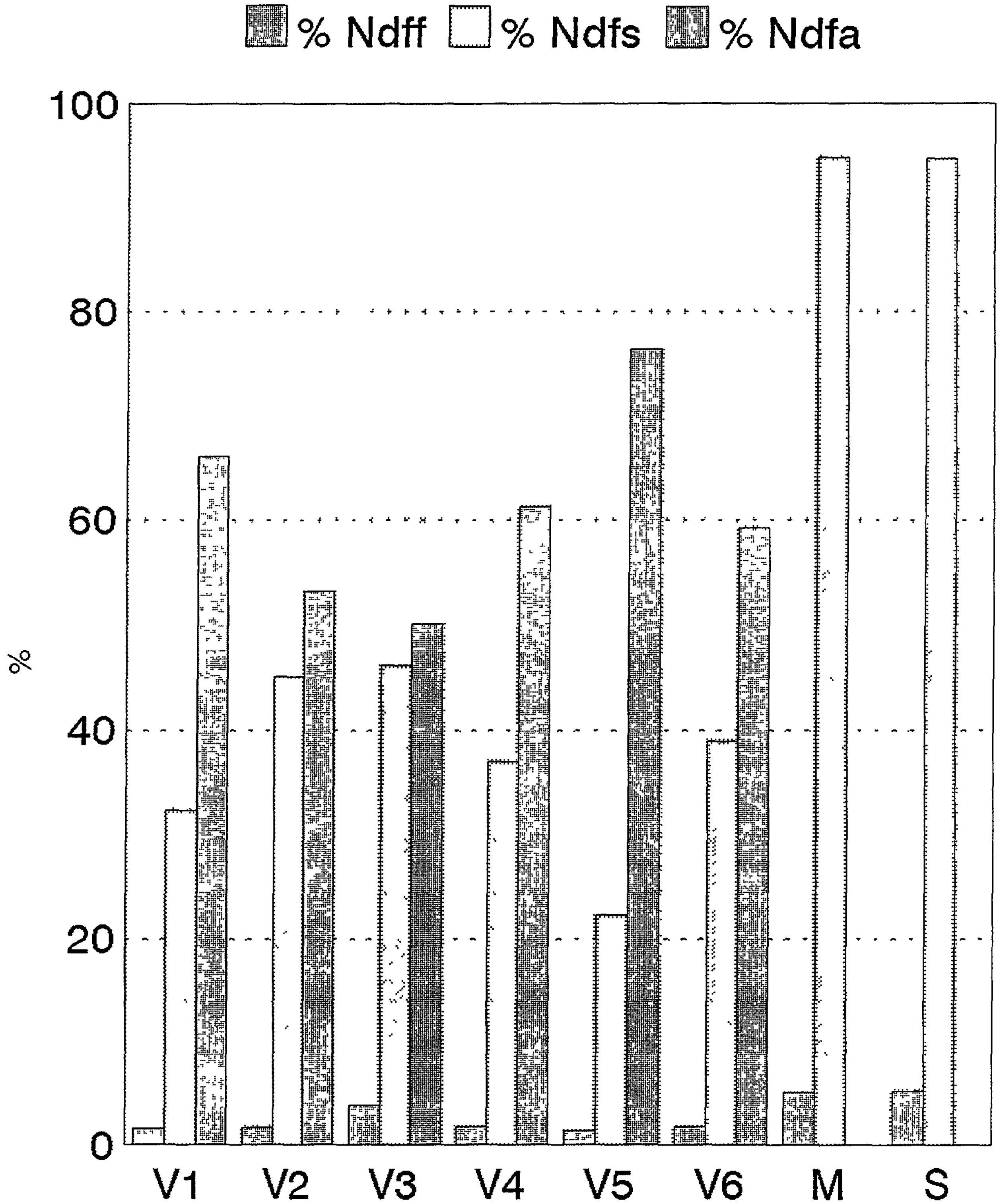

FIG. 1. Percent N derived from fertilizer, soil and N_2 fixation by six cultivars of cowpea, and non-fixing reference crops. V1 = IT 81 D-994, V2 = IT 86 D1004, V3 = IT 85 D-3428, V4 = IT 82-D-504, V5 = MI 35, V6 = Bombay, M = maize, S = sorghum.

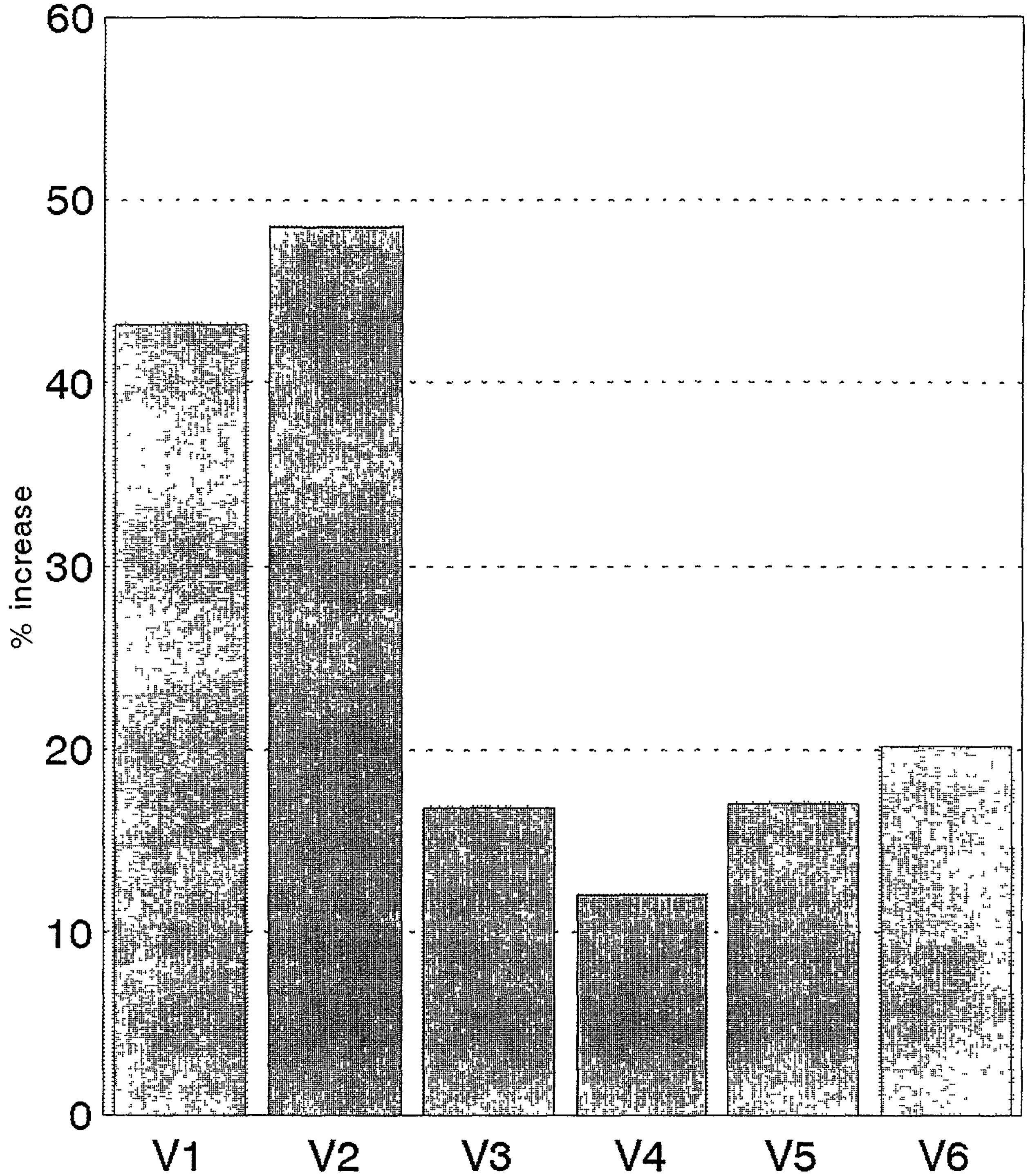

FIG. 2. Percent increase in dry weight of maize planted after one of six cultivars of cowpea, compared to maize planted after maize. V1 = IT 81 D-994, V2 = IT 86 D1004, V3 = IT 85 D-3428, V4 = IT 82-D-504, V5 = MI 35, V6 = Bombay.

TABLE VI. %Ndfa AND AMOUNT OF N FIXED OF COWPEA AT 45 DAP AND AT PHYSIOLOGICAL MATURITY (EXPERIMENT 2)

Treatment	At 45 DAP		At PM	
	Ndfa (%)	Fixed N (g/plant)	Ndfa (%)	Fixed N (g/plant)
MN_{10}	22	0.068	37	0.064
MIN_{10}[a]	21	0.017	27	0.041
MIN_{40}	35	0.042	27	0.064
MIN_{80}	20	0.020	16	0.033
MIN_{120}	40	0.033	13	0.023
MIN_{140}	29	0.400	12	0.035
BN_{10}	33	0.084	35	0.081
BIN_{10}	43	0.045	48	0.167
BIN_{40}	43	0.048	39	0.112
BIN_{80}	28	0.039	34	0.101
BIN_{120}	33	0.044	34	0.101
BIN_{140}	34	0.042	29	0.103
	(NS)	(NS)	(*)[b]	(*)[c]

[a]MI 35, inoculated, 10 kg N/ha applied, etc.
[b]Significant for cv. and N level.
[c]Significant for cv. only.

TABLE VII. ^{15}N ATOM EXCESS AND DETERMINED VALUES FOR N DERIVED FROM FIXATION (%Ndfa) AND AMOUNT OF N FIXED FOR COWPEA AND MAIZE (EXPERIMENT 4)

Crop	^{15}N a.e. (%)	Ndfa (%)	N fixed (kg/ha)
Bombay (intercrop)	0.275b[a]	55	137a
MI 35 (intercrop)	0.268b	56	117a
Bombay (monocrop)	0.332b	46	55.6b
MI 35 (monocrop)	0.336b	45	44.1b
Maize (monocrop)	0.614a		
Maize (intercropped with Bombay)	0.707a		
Maize (intercropped with MI	0.661a		
		(NS)	

[a]Values within columns followed by the same letter are not significantly different ($P<0.05$).

TABLE VIII. DRY MATTER AND N YIELDS OF MAIZE/COWPEA INTERCROPS AND THE COMPONENT MONOCROPS (EXPERIMENT 4)

Crop	Total d. wt.	Total N yield
	(kg/ha)	
Maize/Bombay	13720a[a]	248a
Maize/MI 35	11090ab	210ab
Bombay	6560bc	119c
MI 35	4980c	102c
Maize	14560a	135bc

[a]Values within columns with the same letter are not significantly different ($P < 0.05$).

4. CONCLUSIONS

1. Variability was observed in amount of N fixed, total dry matter production and yield among cowpea genotypes. Choice of cultivar for maximum yield and maximum fixation is important for Sri Lanka.

2. Nitrogen fertilizer inhibited nodulation and N_2 fixation. The maximum exploitation of the symbiotic potential of cowpea is possible only in soils of low N availability.

4. Where total dry-matter production, total N and the pod yield responded positively to N fertilization it indicated less than optimal expression of symbiotic potential; therefore, in certain soils, which must be appraised on a case-by-case basis, cowpea growth and yield parameters may be enhanced by inoculation of seed with appropriate bradyrhizobial strains. Non-response to inoculation and to applied N must be interpreted with caution, and in terms of the crop's growth potential. If, for example, drought is growth-limiting, then there is little scope for significant effects from inoculant or N application.

5. The observed positive effects of cowpea on subsequent maize could not be explained only in terms of amounts of N fixed. Further investigation of the source of such positive residual effects is needed. In addition, better expression of symbiotic potential with higher amounts of N fixed may result in improved effects on succeeding and associated non-leguminous crops.

6. Intercropping maize with cowpea resulted in greater efficiency of crop production although ^{15}N-enrichment data did not suggest direct N transfer.

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EVALUATION OF CHICKPEA AND GROUNDNUT FOR N_2 FIXATION AND YIELD IN BANGLADESH

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Abstract

EVALUATION OF CHICKPEA AND GROUNDNUT FOR N_2 FIXATION AND YIELD IN BANGLADESH

Field experiments on chickpea and groundnut were variously carried out at four locations in Bangladesh. Generally consistent trends were obtained in terms of positive effects of inoculation with rhizobia, and genotypic diversity for components of N_2 fixation and yield.

Inoculation of groundnut increased average nodule number by 77% at Rajshahi, 99% at Mymensingh and 148% at Jamalpur. The increases in nodule dry weight, plant dry weight, pod and stover yields due to inoculation ranged from 93 to 146%, 55 to 77%, 43 to 50% and 29 to 80%, respectively. At all three locations, significant differences were found amongst the genotypes for nodulation, dry matter production and yield. Mutant genotype 62-30 was superior for most components, and statistically better than the parent variety Dacca-1 for all characteristics investigated.

Inoculant application to chickpea resulted in at least a doubling of nodule number at Ishurdi and Mymensingh; on average, there was a three-fold increase in nodule mass as a result of inoculation. Seed-yield increases due to inoculation ranged from 24 to 50%. Inoculated cv. G-97 recorded a seed yield of about 1.5 t/ha at Ishurdi, 47% higher than that produced by Nabin, a variety widely cultivated in Bangladesh. Total-N yield and the amount of N fixed by G-97 with inoculant were also higher than for Hyprosola, which is known for high yield and protein content [23].

In a screening trial at Mymensingh the commercial chickpea Nabin and Hyprosola were consistently inferior to advanced lines produced by mutation breeding. Of 12 mutant groundnut genotypes tested, D1-15KR/62-30 maintained superiority for almost all components. Most of the mutants performed better than the commercial variety Dacca-1.

The data show the potential for increasing chickpea and groundnut yields in Bangladesh by improving N_2 fixation via selection of superior genotypes in conjunction with compatible rhizobia.

1. INTRODUCTION

In Bangladesh, groundnut (or peanut, *Arachis hypogaea*), a rich source of protein, is third in importance as an oil crop after rape and mustard. Being multipurpose, it can help to reduce Bangladesh's shortages of edible oil, food and fodder [1]. Despite this potential importance, groundnut-improvement programmes have been limited in the past. Recent attempts to increase yields by improving N_2 fixation have met with little success.

Among the grain legumes grown in Bangladesh, chickpea (*Cicer arietinum*) ranks third in acreage and production, contributing about 20% of all pulses [2]. Its low average yield (765 kg/ha) is attributed to various factors, including lack of availability of genotypes with higher yield potential, and incompatible host x rhizobia associations.

The total area for pulse production in Bangladesh has been declining since the mid-1970s due to increased demands for cereals. As a consequence, pulse cultivation has been increasingly relegated to marginal soils with concomitant loss of per-hectare productivity [3, 4]. A possible means of

augmenting yields would be to develop varieties with higher potential for symbiotic N_2 fixation, for use possibly in conjunction with inoculants containing compatible rhizobia. Genetic diversity has been found to exist for N_2 fixation and yield of grain [5-8] and forage [9-13] legumes. Considerable progress has been achieved in breeding soybean alfalfa for increased N_2 fixation [9, 10, 13], but not with chickpea [14] for which it has been suggested that genetic variability for fixation should be studied using ^{15}N-based techniques [15].

The application of rhizobial inoculants as a means of increasing N_2 fixation by legume crops is widely recognized. The success of this strategy depends on the host cultivar, the rhizobial strain, and the environmental conditions. Many legumes are nodulated by diverse rhizobia present in soil, but the introduction of superior strains may be necessary to maximize fixation for a particular environment. Differences in N_2 fixation among host cultivars and rhizobial strains are well documented, as are cultivar x strain interactions [16-19]. Furthermore, rhizobial strains vary in the ability to survive and nodulate under adverse soil conditions [20, 21]. This heterogeneity may be exploited by screening and selecting host x rhizobia combinations that are compatible for particular environmental situations.

This paper describes field work with chickpea and groundnut, including mutant lines developed through γ-radiation, in three agro-ecological regions of Bangladesh. Yield responses to inoculation with rhizobia were examined, with N_2-fixation quantified using the ^{15}N-dilution technique.

2. MATERIALS AND METHODS

2.1. Inoculation experiments

2.1.1. Groundnut

The experiment was conducted at three locations, Rajshahi, Mymensingh and Jamalpur, during the period January to June, 1994. The soil at Rajshahi was a High Ganges River Floodplain (Sara series) with pH 7.8 (H_2O), organic C 0.49%, total N 0.05% and Olsen's P 7 ppm. At Mymensingh the soil was an Old Brahmaputra Floodplain (Sonatala series) with pH (H_2O) 6.8, organic C 0.68%, total N 0.05% and Olsen's P 10.5 ppm. And at Jamalpur it was a non-calcareous Dark Grey Floodplain (Kendua series) with pH 6.3, organic C 0.67%, total N 0.06% and Olsen's P 7.8 ppm.

In each experiment, five genotypes of groundnut (43-17, 62-30, 86-54, 64-82 and 16-90), developed at the Bangladesh Institute of Nuclear Agriculture (BINA) through γ-radiation, were investigated along with the commercial parent variety Dacca-1. The genotypes were selected on the basis of extensive field trials over several years, from a screened lot of 25 advanced lines. Seeds were treated with a peat-based inoculant (BINA-GN-2, 30 g/kg seed) that contained locally isolated *Bradyrhizobium* strains G-24 and G-26. Sugarcane molasses (35 g/kg seed) was used as an adhesive [22].

The ^{15}N-dilution technique was used in the Mymensingh experiment to estimate N fixed by the groundnut genotypes, using maize (*Zea mays*) as the non-fixing reference crop. Ammonium sulphate, enriched in ^{15}N at approximately 5% and 1% atom excess, was applied at 20 and 100 kg N/ha to the groundnut and maize plots, respectively. Nitrogen-15-treated plots of 1 x 3 m were established adjacent to the yield plots of 3 x 4 m which were fertilized with unlabelled urea at the same rates of N

as above. Phosphorus (27 kg/ha as triple superphosphate) and K (35 kg/ha as muriate of potash) were applied.

A split-plot design was adopted, with genotype as the main plot. The treatments were replicated three times.

Data for number and dry weight of nodules and dry weight of whole plants were recorded at 120 days. Yields (air-dried pod and stover) were recorded at maturity (180 days). The plants were dried (65°C) and %N values determined by Kjeldahl digestion. Nitrogen-isotope ratio analyses of sub-samples were made by mass spectrometry at the FAO/IAEA Soil Science Unit, Seibersdorf, Austria. The percent N derived from fixation (i.e. from the atmosphere, %Ndfa) and amount of N fixed were calculated as follows.

$$\%Ndfa = \left(1 - \frac{\%\,^{15}N \text{ atom excess}_{(legume)}}{\%\,^{15}N \text{ atom excess}_{(reference)}}\right) \times 100$$

$$N \text{ fixed (kg/ha)} = \frac{\%Ndfa}{100} \times \text{Total N yield}$$

Values for percent N derived from fertilizer and from soil, respectively, were calculated as follows.

$$\%NDfF = \frac{\%\,^{15}N \text{ atom excess}_{(plant)}}{\%\,^{15}N \text{ atom excess}_{(fertilizer)}} \times 100$$

$$\%NdfS = 100 - (\%Ndfa + \%NdfF)$$

Analysis of variance was performed and significance of differences between means classified according to Duncan's Multiple Range Test ($P<0.05$).

2.1.2. Chickpea

Field experiments were conducted, at Ishurdi and Mymensingh during November 1993 to March, 1994. The soil at Ishurdi was a clay loam (Calcareous Dark Grey Floodplain, Sara series) with C 0.82%, total N 0.07%, Olsen's P 12 ppm, pH 7.8 (H_2O). At Mymensingh it was a sandy loam (Old Brahmaputra Floodplain, Sonatala series) with organic C 0.07% total N 0.05%, P 10.5 ppm, pH 6.8. Phosphorus (25 kg/ha) and K (33 kg/ha) were applied at the time of land-preparation, one day before sowing.

Four genotypes of chickpea were used in each experiment: Nabin released by the Bangladesh Agricultural Research Institute (BARI); Hyprosola, a high-yielding high-protein variety of BINA; G-97, a bold-seeded variety of BINA, and a mutant line, G-299. Seeds of chickpea were treated with peat-based inoculant BINA-CP-2 (30 g/kg seed) containing local *Bradryrhizobium* strains CP Dhaka and CP 2200. Sugarcane molasses (40 g/kg seed) was used as a sticking agent.

In the Mymensingh experiment, the ^{15}N-dilution technique was used to estimate N_2 fixed, using wheat (*Triticum aestivum*) as the non-fixing reference crop. Nitrogen-15-labelled ammonium sulphate was applied to the chickpea and wheat plots at 20 and 100 kg N/ha at approximately 5% and 1% atom excess, respectively. Isotope-treated plots of 1 x 3 m were adjacent to the 3 x 4 m yield plots.

The experimental design used split plots, with varieties in the main block. The treatments were replicated three times. Data on number and dry weight of nodules and dry weight of whole plants were recorded at 90 days. Grain and stover yields were recorded at maturity, 130 days. Total-N

and isotope-ratio determinations and statistical analyses were made as described above. Percent N derived from fixation and amount of N fixed were calculated as shown above.

2.2. Screening experiment

Field experiments on chickpea and groundnut were conducted at BINA farm, Mymensingh in 1993. The soil was a sandy loam (Sonatala series, Old Brahmaputra floodplain) with pH 6.8, organic C 0.63%, total N 0.08% and available P 0.03%. Nine chickpea genotypes (ICCT-DS-56, ICCT-DS-79, ICCT-DS-80, ICCV-90039, ICCV-90040, ICCV-90041, ICCV-90043, ICCV-90044 and ICCV-90051) from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, India), obtained from BARI, were evaluated against Nabin and Hyprosola. Eleven groundnut genotypes, developed through g-radiation [15-35 krad (KR)] at BINA, (D1-15KR/24-29, D1-15KR/28-38, D1-15KR/36-2, D1-15KR/62-30, D1-25KR/43-17, D1-35KR/26-54, M6-15 KR/16-90, M6-15KR/60-37, M6-15KR/79-71, M6-25KR/64-82 and M6-25KR/90-39), were compared with Dacca-1. Wheat and maize, each of four maturity groups, were used as reference crops for chickpea and groundnut, respectively.

The experiments were set out using a strip-plot technique with three replications. Three rows (2 m long) of each genotype had row distances of 30, 20, 45 and 45 cm for chickpea, wheat, groundnut and maize, respectively. Inter-plant spacings were 10 cm for chickpea, 5 cm for wheat and 15 cm for groundnut and maize.

The seeds were coated with peat-based inoculant mixtures; for chickpea, BINA-CP-11 + BINA-CP-12 + BINA-CP-19, and for groundnut, BINA-GN-7 + BINA-GN-24 + BINA-GN-26), using sugarcane molasses as a sticking agent. Triple super phosphate (25 kg P/ha) and muriate of potash (33 kg K/ha) were applied as a basal dose during land preparation.

The ^{15}N-dilution technique was used to quantify fixed N. Ammonium sulphate, enriched with ^{15}N at approximately 5% atom excess for the legumes and 1% atom excess for the reference crops, was applied at 15 kg N/ha and 100 kg N/ha, respectively, to the middle 1-m portion of each plot as a solution spray. The remaining portion of each plot was fertilized with the required rate of unlabelled urea.

For nodulation and dry matter weight determinations, five plants of each genotype were collected from the unlabelled area of the plots at mid-flowering, 90 days for chickpea and 120 days for groundnut. For grain and stover yields, plants were harvested at full maturity. Total-N and isotope-ratio determinations and statistical analyses were made as described above. Percent N derived from fixation and amount of N fixed were calculated as shown above.

3. RESULTS

3.1. Inoculation experiments

3.1.1. Groundnut

Significant effects of inoculation and genotype were recorded at all three sites for nodule number, nodule dry matter, pod and stover yield, and N_2 fixation (Table I). A significant genotype x inoculation interaction was obtained only for stover yield.

TABLE I. STATISTICAL SIGNIFICANCE OF TREATMENT EFFECTS ON COMPONENTS OF GROUNDNUT

Site	Source of variation	Nodule number	Nodule d. wt.	Plant d. wt.	Pod yield	Stovar yield	Total N	%Ndfa	%Ndfs	Ndfa
Rajshahi	Genotype	4063*	7490*	67.1**	1.74*	284				
	Inoculation	56249**	246843**	503**	6.93**	1013*				
	G x I	330	2674	30.6	0.26	38.1*				
Mymensingh	Genotype	2799*[a]	21821*	132	0.49*	7.59*	3171*	6195*	721*	0.39**
	Inoculation	222034**	733878**	1590**	10.6**	66.2**	85370**	48561**	521**	0.55**
	G x I	1339	10747	5.26	0.11	1.68	478	512	51.9	0.03
Jamalpur	Genotype	1472**	8984**	175**	1.0*	26.9**				
	Inoculation	36290**	142318**	2003**	4.9**	57.9**				
	G x I	127	901	32.6	0.11	1.0				

[a]Significant at $P <$ *0.05, **0.01.

TABLE II. NODULATION AND PLANT DRY WEIGHT OF GROUNDNUT AT 120 DAP AS INFLUENCED BY INOCULATION, ATRAJSHAHI, MYMENSINGH AND JAMALPUR

Genotype	Nodules (per plant)			Nodule d. wt. (mg/plant)			Plant d. wt. (g/plant)		
	Raj.	Mymen.	Jam.	Raj.	Mymen.	Jam.	Raj.	Mymen.	Jam.
43-17 Uninoc.	99.0fg[a]	110fg	36.3ef	129d	182f	159def	12.7de	26.9def	22.1cd
Inoc.	196ab	255ab	102b	327ab	602a	252bc	17.3bcd	38.6ab	41.5a
62-30 Uninoc.	116efg	123fg	50.7e	170cd	228ef	174de	15.1cde	29.1cde	21.9cd
Inoc.	200ab	280a	128a	382a	536ab	325a	31.5a	41.1a	41.8a
86-54 Uninoc.	95.3fg	114fg	44.3e	191cd	247ef	176de	15.2cde	22.4efg	21.1cd
Inoc.	154cde	229bcd	97.3bc	306ab	537ab	278ab	2.8b	39.2ab	37.1ab
64-82 Uninoc.	134frg	113fg	56.0de	175cd	186f	128efg	13.3cde	22.2efg	25.3d
Inoc.	225a	190de	124a	341a	422cd	246bc	18.9bc	35.5abc	31.0bc
16-90 Uninoc.	98.3fg	147ef	47.7e	126d	164f	107fg	13.3cde	20.1fg	19.0d
Inoc.	177bc	243abc	110ab	241bc	458bc	256bc	17.8bcd	32.4bcd	33.0ab
Dac-1 Uninoc.	72.3g	94.7g	21.0f	107d	163f	68.3g	11.6e	15.2g	12.8d
Inoc.	137def	205cd	75.3cd	294ab	328de	209cd	17.8bcd	28.9cde	21.6cd
CV (%)	7	14	17	18	18	18	19	16	22

[a]Values in the same column followed by the same letter are not significantly different ($P<0.05$).

Inoculation increased average nodule number by 77% at Rajshahi, 99% at Mymensingh and 148% at Jamalpur (Table II). The increases in nodule dry weight, plant dry matter, pod and stover yields due to inoculation ranged from 93 to 146%, 55 to 77%, 43 to 50% and 29 to 80%, respectively (Tables II and III). The magnitude of response varied with component and location. At Jamalpur, the responses were highest for nodule number (148%) and plant dry matter (77%) at 120 DAP, and for pod yield (50%). At Mymensingh, nodule dry weight was increased by 146%, whereas at Rajshadi stover yield showed an 80% increase. At all three locations, significant differences were found amongst the genotypes for nodulation, plant dry matter production and yield. Mutant genotype 62-30 was superior to the others for most components, and statistically better than the parent variety Dacca-1 for all characteristics investigated; it produced the highest pod yields (4 t/ha at Mymensingh, 3.6 at Rajshahi and 2.8 at Jamalpur).

The total N determinations and the %Ndfa values also showed genotypic diversity (Table IV). The highest total N accumulation (281 kg/ha) occurred with genotype 43-17 with inoculation. The %Ndfa values ranged from 38 (86-54, uninoculated) to 75% (Dacca-1 inoculated) and amount of fixed

TABLE III. POD AND STOVER YIELDS OF GROUNDNUT AT 120 DAP AS INFLUENCED BY INOCULATION, AT RAJSHAHI, MYMENSINGH AND JAMALPUR

Genotype	Pod yield Raj. (t/ha)	Pod yield Mymen. (t/ha)	Pod yield Jam. (t/ha)	Stover yield Raj. (t/ha)	Stover yield Mymen. (t/ha)	Stover yield Jam. (t/ha)
43-17 Uninoc.	2.6bcd[a]	2.8cd	1.3d	17.0d	6.7c-f	8.9d
Inoc.	3.2ab	3.3b	2.2bc	27.3b	10.2ab	10.7bc
62-30 Uninoc.	2.0def	3.0bc	2.2bc	18.8cd	7.8cd	11.7b
Inoc.	3.6a	4.0a	2.8a	37.9a	9.8ab	15.4a
86-54 Uninoc.	2.8bc	2.4de	1.5d	17.4d	6.4def	9.3cd
Inoc.	3.5a	3.5ab	2.6ab	26.0b	10.9a	11.5b
64-82 Uninoc.	1.8ef	2.3de	1.5d	10.1ef	5.1f	6.7e
Inoc.	3.0ab	3.5ab	2.3abc	23.7bc	7.3cde	8.8d
16-90 Uninoc.	1.6ef	2.2e	1.4d	7.6f	6.5c-f	9.1d
Inoc.	2.2cde	3.5ab	2.1c	14.5de	8.4bc	11.0b
Dacca-1 Uninoc.	1.4f	2.0e	1.1d	8.3ef	5.5ef	6.1e
Inoc.	2.1de	3.3bc	1.4d	13.6def	7.7cd	9.0cd
CV (%)	13	10	15	19	14	8

[a]Values in the same column followed by the same letter are not significantly different ($P < 0.05$).

TABLE IV. GROUNDNUT TOTAL N, N DERIVED FROM FERTILIZER (NdfF), SOIL (NdfS) AND FIXATION (Ndfa) AS INFLUENCED BY INOCULATION, AT MYMENSINGH

Genotype	Total N	NdfS	NdfF	Ndfa
		[kg/ha (%)]		
43-17 Uninoc.	170def[a]	53.2cde(31st)	1.95b-e(1.1qr)	115cd(68pq)
Inoc.	281a	87.4b(30st)	2.18bcd(0.8s)	192a(69pq)
62-30 Uninoc.	197cde	100b(51pq)	2.47b(1.3pq)	93.8de(48st)
Inoc.	276a	90.2b(33st)	2.22bc(0.8rs)	183a(66pq)
86-54 Uninoc.	153ef	93.3b(61p)	2.29bc(1.5p)	57.9e(38t)
Inoc.	257ab	143ab(56p)	3.52a(1.4pq)	111cd(43t)
64-82 Uninoc.	140f	48.3de(34st)	1.18de(0.8rs)	90.4de(65pq)
Inoc.	261ab	79.3bc(30st)	1.92b-e(0.7s)	180ab(70pq)
16-90 Uninoc.	159ef	72.0bcd(45qr)	1.77b-e(1.1qr)	85.3de(54rs)
Inoc.	236abc	89.3b(37rs)	2.17bcd(0.9rs)	144bc(62qr)
Dacca-1 Uninoc.	125f	41.0e(33st)	1.00e(0.8rs)	82.8de(66pq)
Inoc.	216bcd	53.1cde(25t)	1.31cde(0.6s)	162ab(75p)
CV (%)	13	21	28	17

[a]Values in the same column followed by the same letter are not significantly different ($P < 0.05$).

N ranged from 53 (64-82, uninoculated) to 210 kg N/ha (43-17, inoculated). The %NdfS values ranged from 31 to 61% (uninoculated) and 25 to 56% (inoculated), and the %NdfF values were also lower with inoculation. However, as with Ndfa, the amounts of NdfF and NdfS were generally higher with inoculation.

Correlation analysis (Table V) showed that Ndfa, but not %Ndfa, correlated with nodule number and mass and plant dry matter at 120 DAP, and with pod, stover and total N yields at maturity. The negative correlation between Ndfa and %NdfS might be taken to suggest that higher-fixing genotypes assimilated less soil N. However, improved growth with inoculation generally resulted in greater total uptake of soil N (Table IV).

3.1.2. *Chickpea*

Significant effects of genotype and inoculation with rhizobia, and their interactions, were observed for almost all of the investigated components, viz. nodulation (number and mass), grain yield, %Ndfa, %NdfS and %NDdF (Table VI).

TABLE V. CORRELATION COEFFICIENTS FOR GROUNDNUT, ATMYMENSINGH

Component %NdfF	Nodule d. wt.	Dry matter	Pod yield	Stover yield	Total N	Ndfa		%Ndfa
Nodule no.	0.854	0.689	0.764	0.760	0.733	0.707	0.2630	0.383
Nodule d. wt.		0.701	0.762	0.767	0.752	0.598	-0.060	-0.159
Dry matter			0.772	0.745	0.714	0.554	-0.057	-0.231
Seed yield				0.713	0.742	0.578	-0.055	-0.195
Stover yield					0.699	0.403	0.042	-0.066
Total N						0.796	-0.080	-0.124
Ndfa							-0.651	-0.616
%Ndfa								-0.863

Inoculation resulted in at least a doubling of nodule number for all four genotypes at both locations (Table VII). On average, there was a three-fold increase in nodule mass as a result of inoculation. Although dry matter yield increases at 90 DAP were similar to those for nodule number, the increases in grain and stover were less-well marked. Seed-yield increases due to inoculation ranged from 24 to 50% at Ishurdi and from 28 to 46% at Mymensingh, with corresponding increases in stover of 14 to 60% and 59 to 96%, respectively, (Table VIII).

Inoculated plants were highest for the proportion and total amount of N obtained from fixation (Table IX). The %Ndfa values ranged from 70 to 81% and amounts of fixed N ranged from 80 to 87 kg N/ha. The %NdfS and %NdfF values were significantly lower as a result of inoculation, although the total amounts of N derived from soil and fertilizer were generally higher with inoculation.

The results clearly show genotypic diversity for nodulation (number and mass) and plant dry weight at 90 DAP, and for grain, stover and total-N yields at maturity. Variety G-97 maintained superiority for almost all components except that it was identical with G-299 in grain yield at Ishurdi and in dry matter and total N yield at Mymensingh, with Nabin in stover yield at Ishurdi, and with Hyprosola in total-N yield at Mymensingh. With inoculation, G-97 recorded a seed yield of about 1.5 t/ha (Table VIII) at Ishurdi, 47% higher than that produced by Nabin, a variety that is widely cultivated in Bangladesh. Total-N yield and the amount of N fixed by G-97 with inoculant were also higher than for Hyprosola, a variety known for high yield and protein content [23](Shaikh *et al.* 1982).

TABLE VI. STATISTICAL SIGNIFICANCE OF GENOTYPE AND INOCULATION EFFECTS ON COMPONENTS OFCHICKPEA

Site	Nodule no.	Nodule mass	Dry matter	Grain yield	Stover yield	Total N	Ndfa	%NdfS	%NdfF
Mymen.									
Genotype	96.0**[a]	1757**	56.0*	14723**	0.44*	437*	125	181**	5.4**
Inoc.	610**	28563**	189**	293046**	7.84**	10930**	10443**	651**	19.6**
GxI	28.8*	892**	2.0	4540*	0.04	57.7	24.3*	24.7*	0.87*
Ishurdi									
Geno.	64**	63829**	57.2**	114585**	6.5**				
Inoc.	620**	327601**	1106**	822214**	11.9**				
GxI	28.3**	32981**	9.8	32408*	1.0*				

[a]Significant at $P <$ *0.05, **0.01.

TABLE VII. NODULATION NUMBER AND DRY WEIGHT OF CHICKPEA GENOTYPES AT 90 DAP AS INFLUNCED BY INOCULATION, AT ISHURDI AND MYMENSINGH

Genotype	Nodule no. (per plant) Ishurdi	Nodule no. (per plant) Mymen.	Nodule d. wt. (mg/plant) Ishurdi	Nodule d. wt. (mg/plant) Mymen.	Plant d. wt. (g/plant) Ishurdi	Plant d. wt. (g/plant) Mymen.
G-97 Uninoc.	14.3c*	10.0cd	165cd	30.4de	16.6c	12.oc
Inoc.	30.7a	26.3a	611a	135a	35.5a	18.0a
G-299 Uninoc.	8.0d	7.3d	91.7e	20.2de	13.5c	8.9de
Inoc.	18.0b	17.0b	197c	69.7c	27.8ab	14.9b
Nabin Uninoc.	7.7d	6.0d	123de	17.7e	12.2c	7.3ef
Inoc.	14.3c	12.3c	295b	79.7bc	23.5b	11.2cd
Hyprosola Uninoc.	8.7d	6.3d	140cde	35.5d	13.4c	4.9f
Inoc.	16.3bc	14.3bc	351b	95.3b	25.2b	11.4c
CV (%)	19	11	14	15	16	12

[a]Values in the same column followed by the same letter are not significantly different ($P<0.05$).

Correlation studies (Table X) showed that nodule number and dry weight and plant dry weight at 90 DAP, and seed, stover, total N and amount of N fixed at final harvest were all well correlated. As with groundnut (see *3.1.1.*), significant negative correlations were obtained between Ndfa and %NdfS.

3.2. Screening experiment

Genotypic diversity was clear for both species for all components except total-N content and %NdfF in groundnut (Tables XI and XII). For chickpea, ICCT-DS-79 recorded maximum nodules, grain weight, total dry matter, total N and amount of N fixed, whereas ICCV-90044 had higher nodule dry weight, ICCT-SDS-56 higher stover weight, and ICCV-90041 higher %Ndfa (although not statistically superior to ICCT-DS-79. The commercial variety Nabin and the high-protein BINA genotype Hyprosola were consistently inferior to these advanced lines. Of the 12 groundnut genotypes, D1-15KR/62-30 maintained superiority for almost all components; M6-25KR/64-82 and M6-15KR/16-90 recorded statistically similar values for %Ndfa and total N, respectively. Most of the mutants performed better than the commercial variety Dacca-1.

Correlation studies (Table XIII) showed that amount of N fixed had significant positive relationships with most of the components under investigation. In the case of chickpea, nodule number was positively correlated with total N content, fixed N and grain yield, with significance at the 1% level of probability.

TABLE VIII. GRAIN AND STOVER YIELDS OF CHICKPEA GENOTYPES AS INFLUENCED BY INOCULATION, AT ISHURDI AND MYMENSINGH

Genotype	Grain yield (kg/ha)		Stover yield (t/ha)	
	Ishurdi	Mymensingh	Ishurdi	Mymensingh
G-97 Uninoc.	907c	651de	3.63de	1.93c
Inoc.	1454a	952a	5.82a	3.12a
G-299 Uninoc.	870c	593e	2.95cd	1.43d
Inoc.	1304b	775c	3.38bc	2.61b
Nabin Uninoc.	717d	631de	3.75b	1.33d
Inoc.	988c	843b	5.75a	2.62b
Hyprosola Uninoc.	937c	672d	2.27d	1.52d
Inoc.	1167b	860b	3.27bc	2.42b
CV (%)	8	5	11	9

[a]Values in the same column followed by the same letter are not significantly different ($P < 0.05$).

TABLE IX. TOTAL N, N DERIVED FROM FERTILIZER (NdfF), SOIL (NdfS) AND FIXATION (Ndfa) OF CHICKPEA GENOTYPES AS INFLUENCED BY INOCULATION, AT MYMENSINGH

Genotype	Total N	NdfS	Ndff	Ndfa
	[kg/ha (%)]			
G-97 Uninoc.	74.9c[a]	26.1b(34p)	4.34b(5.9p)	44.3bc(59s)
Inoc.	126a	32.9a(26qr)	5.55a(4.4qr)	87.34a(70qr)
G-299 Uninoc.	69.2cd	24.3bc(39p)	4.27b(6.7p)	34.6c(54s)
Inoc.	109b	23.8bc(23r)	4.25b(3.9rs)	79.8a(73q)
Nabin Uninoc.	58.2d	16.9d(29q)	2.93c(5.0q)	38.4bc(66r)
Inoc.	102b	18.3cd(18st)	3.17c(3.1st)	81.0a(79p)
Hyprosola Uninoc.	68.2cd	15.0d(23rs)	2.51c(3.8rs)	48.5b(74q)
Inoc.	105b	17.3d(16t)	2.99c(2.8t)	84.4a(81p)
CV (%)	10	15	13	9

[a]Values in the same column followed by the same letter are not significantly different ($P < 0.05$).

TABLE X. CORRELATION COEFFICIENTS BETWEEN COMPONENTS OF CHICKPEA, AT MYMENSINGH

Component	Nodule d. wt.	Plant d. wt.	Seed yield	Stover yield	Total N	Ndfa	%NdfS	%NdfF
Nodule no.	0.855	0.792	0.835	0.835	0.829	0.686	- 0.102	-0.298
Nodule d. wt.		0.686	0.938	0.903	0.877	0.882	-0.515	-0.566
Plant d. wt.			0.655	0.805	0.800	0.642	0.019	-0.071
Seed yield				0.875	0.854	0.852	-0.476	-0.661
Stover yield					0.957	0.910	-0.426	-0.479
Total N						0.927	-0.419	-0.456
Ndfa							-0.704	-0.678
%NdfS								0.789

4. DISCUSSION

4.1. Inoculation experiments

4.1.1. Groundnut

Nodulation, dry-matter production, yield and N_2 fixation were significantly enhanced by inoculation with *Bradyrhizobium* in all three soils. Although similar benefits to groundnut due to inoculation have been reported before [e.g. 24](Hadad et al. 1986), it is noteworthy that the uninoculated plants were also profusely nodulated. Our strong responses to inoculation indicate that the indigenous bradyrhizobia were not optimally compatible with the tested genotypes.

Legume-genotype effects on nodulation, dry-matter production, yield and N_2 fixation also have been reported before [e.g. 7, 25, 26]. This study is unusual in that no genotype x inoculant interaction was observed; however, this can be explained in terms of the narrow genetic base of the six genotypes.

For varietal screening for high biological N_2 fixation potential and yield, the ^{15}N technique has the advantage of estimating N obtained from different sources, viz. soil, fertilizer and the atmosphere. Genotypes that use less soil N and fix more N from the atmosphere may be identified for use in crop-rotation programmes. Further such work is needed with groundnut under our conditions, using diverse genotypes.

TABLE XI. COMPONENTS OF INOCULATED CHICKPEA GENOTYPES, SCREENING EXPERIMENT AT MYMENSINGH

Genotype	Nodules (per plt)	Nodule d.wt. (mg/plt)	Seed yield (g/plant)	Stover yield (g/plant)	Plant d. wt. (g/plant)	Total N (mg/plt)	%NdfF	%Ndfa	Ndfa (mg/plt)
ICCT-DS-56	17.0de	60.0cd	1.93cde	4.17a	6.10abc	82.9bc	2.3d	88ab	73.1bc
ICCT-DS-79	43.3a	88.3ab	2.90a	3.93ab	6.83a	147a	2.8bc	86ab	126a
ICCT-DS-80	32.3c	85.0ab	2.33abc	3.00bc	5.33a-d	72.7bc	3.6a	81c	59.2c
ICCV-90039	38.7ab	26.7f	2.23a-d	4.10ab	6.33ab	143a	2.5bcd	87ab	125a
ICCV-90040	12.3e	36.0ef	1.40e	2.97bc	4.37cd	67.9c	2.35d	88ab	59.7c
ICCV-90041	20.7d	50.0de	1.50de	2.53c	4.03d	59.3c	2.3b	88a	52.50c
ICCV-90043	16.7de	91.7a	2.10d-e	2.63c	4.73bcd	81.2bc	2.8b	85b	69.3bc
ICCV-90044	42.7a	96.7a	2.30abc	3.40abc	5.70a-d	133a	2.39cd	88ab	117a
ICCV-90051	18.7de	71.7bc	1.83cde	4.03ab	5.87abc	76.6bc	3.9a	80c	61.7c
Hyprosola	28.0c	55.3cd	2.73ab	3.53abc	6.27ab	110ab	2.9b	86ab	94.3ab
Nabin	33.0bc	91.7a	2.43abc	4.07ab	6.50ab	122a	2.8bc	86ab	105a
CV(%)	16	14	19	17	16	20	8.6	1.5	19

4.1.2. *Chickpea*

Nodules on all uninoculated plants showed that both soils contained infective rhizobia. However, with inoculant application, the nodules were more numerous and larger, indicating that the native rhizobia were not only insufficient in number but also less than optimally effective with the tested genotypes. Similar enhancement of chickpea nodulation from inoculant application has been shown before [27]. Better nodulation correlated with higher yield and N_2 fixation (Table X).

Nodulation, dry matter and yield were superior at Ishurdi (Tables VII and VIII) due to unfavourable soil and climatic conditions at Mymensingh for chickpea. Such dependence of the legume-rhizobia symbiosis on environmental conditions has been reported before [e.g. 28, 29]. Genotype x inoculant interactions (Table VI) have been reported for other legumes [e.g. 30, 31]. Our data show the importance of identifying optimally compatible genotype/strain combinations, particularly for use in conditions of environmental stress.

4.2. Screening experiment

All of the genotypes were well adapted to our local environmental conditions. However, there was genotypic diversity in terms of the ability to form nodules, fix atmospheric N_2 and produce dry matter and grain yield, for both chickpea and groundnut. There were differences also between the species, with groundnut (Table XII) producing more nodules and dry matter and fixing more N_2 than did chickpea (Table XI). The data demonstrate the importance of choosing the most appropriate species and cultivar for prevailing conditions in accordance with the priorities of the grower, e.g. high grain yield of a particular legume, high level of fixed N in stover for animal feed, or high level of fixed N in stover to be incorporated into the soil for the benefit of a subsequent cereal crop.

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