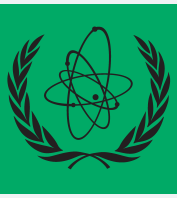
IAEA-TECDOC-1493

Mutational analysis of root characters in food plants

Proceedings of a final research coordination meeting organized by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture and held in Antalya, Turkey, 11–15 October 2004



IAEA
International Atomic Energy Agency

April 2006

IAEA-TECDOC-1493

Mutational analysis of root characters in food plants

Proceedings of a final research coordination meeting
organized by the
Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture
and held in Antalya, Turkey, 11–15 October 2004

IAEA
International Atomic Energy Agency

April 2006

The originating Section of this publication in the IAEA was:

Plant Breeding and Genetics Section
Joint FAO/IAEA Division
International Atomic Energy Agency
Wagramer Strasse 5
P.O. Box 100
A-1400 Vienna, Austria

MUTATIONAL ANALYSIS OF ROOT CHARACTERS IN FOOD PLANTS

IAEA, VIENNA, 2006
IAEA-TECDOC-1493
ISBN 92–0–103106–8
ISSN 1011–4289

Printed by the IAEA in Austria
April 2006

FOREWORD

Efficient exploration for water and nutrient uptake from the soil is achieved by adaptation of root architecture, plasticity of root construction, specialized root structures, root physiological responses and beneficial relationships with microorganisms. Roots may also serve as storage organs for carbohydrates and as perennial structures that last for many years. Historically, the genetic analysis of root traits has been neglected, largely because of the difficulty in accessing this below ground organ. Consequently, few characterised root mutants of crop plants are available. The scarcity of root mutants has resulted in the inability to evaluate specific root traits in breeding programmes.

This Coordinated Research Project (CRP) on Mutational Analysis of Root Characters in Annual Food Plants Related to Plant Performance was initiated in 1999, with the objective of assisting Member States in the application of mutation techniques and related biotechnologies to generate and utilise mutants for the identification of root properties and genes for improving productivity and sustainability of crop plants. Mutational analysis results obtained over the five year life span of the CRP have shown that changes in root architecture are correlated with crop plant response to stress. Some of the major outputs of the CRP have been the development and genetic analysis of new germplasm and populations in various crops and the development of new methods for phenotyping root architecture. Furthermore, mutants for root traits have been used in the production of new cultivars suitable for stressed environments and new specific root mutations provided a means to identify genes important for root traits. Moreover, new research collaborations have been formed and a consortium, "Crops Root Research", was established (http://www.crop-roots.org), which extends the collaborative scientific network created by this CRP.

Plant research is entering a new era that will permit the use of functional genomics and proteomics to address important agricultural issues, supporting successful agricultural production with minimal impact on the environment. Improvements in screening techniques and development of molecular markers are ways to alleviate the need for direct root assessment and speed up selection in plant breeding programmes. Root architecture models provide a tool to address the space-time dynamics of the soil/plant system. Genetic analysis of quantitative traits and methods in forward and reverse genetics will also contribute to breeding crops with root systems suitable for a range of agricultural environments. Recent evidence indicates that plant stresses are mediated by changes at the root system level.

In this context, mutants and mutational analysis of root characters are becoming significant elements in research that seeks to clarify the ways by which root structure and function respond to environmental cues and alleviate the impact of stress.

This publication summarizes the results presented at the third and final Research Coordination meeting (RCM) of the CRP, which was held in Antalya, Turkey, 11–15 October 2004. The IAEA officers responsible for this publication were M. Maluszynski, who initiated this CRP, and Q.Y. Shu of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. Special acknowledgement goes to Z. Dhlamini (Zimbabwe) for compiling and preparing this publication.

EDITORIAL NOTE

The papers in these proceedings are reproduced as submitted by the authors and have not undergone rigorous editorial review by the IAEA.

The views expressed do not necessarily reflect those of the IAEA, the governments of the nominating Member States or the nominating organizations.

The use of particular designations of countries or territories does not imply any judgement by the publisher, the IAEA, as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.

The mention of names of specific companies or products (whether or not indicated as registered) does not imply any intention to infringe proprietary rights, nor should it be construed as an endorsement or recommendation on the part of the IAEA.

The authors are responsible for having obtained the necessary permission for the IAEA to reproduce, translate or use material from sources already protected by copyrights.

CONTENTS

SUMMARY

A. INTRODUCTION

Merging the study of plant ecophysiology and genetics has great potential for improving adaptability of crops to stressful environments. Root systems play a key role in acquiring resources and avoiding stress, but they also represent a major cost to plants in terms of carbon expenditures. Additional allocation of assimilates to roots potentially affects crop growth and yield, either positively in the case of stress avoidance, or negatively in situations where the costs of root allocation outweigh the benefits in yield. Knowledge of these relationships is necessary for optimizing plant breeding for root system characteristics, and suggests that breeding goals for root systems may vary by species, genotype, resource availability and local agricultural conditions. Root systems are highly dynamic and respond to changes in environmental parameters, particularly to stresses such as drought, nutrient deficiencies, water logging, etc. Understanding of root form and function is inherently important for creating root ideotypes that are well-adapted to such specific environments.

Regulation of root architecture, lateral root formation and root symbiosis with host plants are examples of how rates of water and nutrient acquisition can be altered to enhance survival and productivity. Each of these is the result of intricate processes determined by genotype × environment interactions that are associated with specific adaptations. Mutational analysis provides a tool to understanding these processes and to discovering genes that might be exploited to improve root function. Genetic studies on the recognition of regulatory pathways will be aided by the use of various types of mutants. Specific genomic and proteomic tools, such as EST libraries and protein/metabolite profiles of mutants, provide a new dimension to understanding root function.

As with most aspects of plant growth and development, genetic variation of root characters is controlled by genes with both major and minor effects. Quantitative trait locus (QTL) mapping relates phenotypic variation to specific genetic regions. This method has the advantage of not being limited to known and cloned genes. Accurate assessment of phenotypic variation for root characters can be difficult as described above, and this can present problems for identifying highly heritable traits. Use of QTL mapping methods to identify genetic regions that explain a high percentage of the variation in a trait paves the way for further studies to identify the specific genes in a genomic region delimited by a QTL. In a later stage, identification of candidate genes related to QTL, their cloning and their phenotypic effects could clarify mechanisms controlling root traits.

Induced mutations have particular relevance to crop improvement for stressful environments where breeders are often constrained by maintaining a specific plant ideotype. Wide crossing or breeding with novel germplasm may be used to introgress traits, but the genetic background of the locally adapted lines will be disrupted and may require many years of breeding effort to correct. Induced mutations for desired traits, including root traits, offers a rapid means for crop improvement of elite adapted germplasm. Exploitation of mutants is supported by the development of structured mutant populations for forward and reverse genetics. These capitalize on the increasing abundance in sequence data to validate gene function. Mutation grids or 'TILLING' populations are established for model species such as *Arabidopsis*, *Medicago truncatula*, *Lotus japonicus* and rice, but are also being developed in crop plants such as barley and wheat. This list will be extended to other species as sequence data are amassed. The populations are evaluated for mutations in genes of interest using high throughput genotyping and changes in phenotype are determined (reverse genetics). In

addition, novel mutant genotypes are detected in field or specially devised screens to select a range of morphological variants (forward genetics). Both approaches are of interest to researchers and breeders as they provide novel germplasm and a scientific basis for future work.

Biotic stresses that occur as a result of pests and diseases inevitably impact root growth and function through depleted assimilate supply and impaired shoot/root interactions and by changes of root system architecture. All are mediated by translocated hormones and other signals. Root diseases have a direct impact on function and overall plant performance. Study of diseases in model species are revealing new insights into genes for resistance and are beginning to provide potential markers for use in species lacking sequence data. Induced mutant populations in crop plants will provide a further tool for genetic analysis of specific disease and pest susceptibilities. Research in the establishment of both beneficial and pathogenic root/microbial interactions has highlighted the importance of specific features of the root surface. The microbial signals involved are being described and synthesized. These provide new tools for screening mutant populations to reveal genes that regulate the progress and effectiveness of symbiosis and pathogenicity. The specific traits of roots that attract or deter specific organisms are unknown and require investigation.

Plant traits that are important for agricultural production include adaptation to soil-based environmental stresses such as drought, salinity, soil compaction, nutrient imbalances, acidity, heavy metal toxicity, and water logging. Each is mediated through the root system and all have drastic effects on plant development. Eventually they affect yield quantity and quality. Root screening procedures have been improved during this CRP and can be correlated with plant performance of field-grown plants. Soil-free systems (aeroponics, hydroponics, use of vermiculite, sand, gel or paper) and measurements of electrical capacitance in the field have provided detailed information on the potential of root growth. Specific information on the behaviour of seminal, shoot-borne, crown, nodal, lateral, cluster and nodulated roots, root hairs and storage roots is gradually accumulating. Some of these can be scaled up for practical plant breeding purposes, such as selection for tolerance to aluminium toxicity, extreme pH, low nutrient supply, salinity, drought and water logging.

B. RESULTS AND ACHIEVEMENTS

The results and achievements of the CRP are catalogued in the table below by crop and trait. Numbering relates to the researchers involved, for which details are provided below.

Crop / Trait	Wheat	Barley	Maize	Tomato	Soybean	Lettuce	Faba bean	Rice	Lupin	Cowpea	Chickpea	Bambara	Banana
Aluminium	3				5								
Salinity	1,7	14											
Drought	1,3,6,7	13,14			5	15			2	12		12	
Waterlogging		1											
Root growth	1,3,6,7	1,4,11,13,14	8	9,10		15	10	4	2,4	12	13	12	4
Root hairs		1,11											
Anchorage			8										
Root development		1,4,11	8	9	5	15	10	4	2	12		12	4
(Root) physiology	1,7	1,14		9,10		15	10		2	12		12	
Rhizospere dynamics						15	10		2		13		

1. Argentina – Selection for drought tolerance in mutagenized wheat

- A mass screening methodology consisting of germinating M_2 seeds in closed boxes with 2.5% NaCl solutions was useful for selecting mutants for seminal root and/or coleoptile characteristics.

- Some selected mutants have shown improvement to tolerating transient drought.

- Differential effects of NaCl or PEG treatments were found and it is possible that these osmolytes can be affecting different mechanisms during root development.

- Analysis of a barley root mutant showing no tropic response to submergence in water indicated that the typical growth with windings and turnings, usually observed in wild type barley seedlings (grown in hydroponics by the "blotting paper sandwich method") is controlled by a single semi-dominant nuclear gene. This wild type barley root character is associated with an overproduction of ethylene and is interpreted as an adaptative response to ameliorate the impact of waterlogging.

- Ethylene production is critical in the development of root length and there is an optimal concentration for root growth.

2. Australia – Mutational and transgenic analysis of root characters in lupins

- We have initiated a significant research programme to describe the proteome and metabolome of phloem in sufficient detail to discover the unique translocated signals that regulate meristem activity in shoot and root organs.

- A number of species of lupin (*Lupinus albus*, *L. angustifolius* and *L. mutabilis*) have been used because phloem can be sampled directly and reliably with negligible contamination from incisions made to their vasculature. Phloem stream directed towards the shoot apical meristem (SAM), developing embryos, to the root and sites of nodulation can be collected separately, allowing a comparison of signals moving upwards and downwards in the plant. A range of known plant growth regulators (ABA, cytokinins, GA, ACC, jasmonates and salycilic acid) are reliably analysed in phloem and xylem. However, the study is seeking to identify specific translocated signals that are involved in regulating stem cell activity and differentiation at root and shoot apices.

- The potential signals include low molecular weight peptides (1-15kD) and RNA. Among RNA species are mRNA, micro RNA (miRNA) and short interfering RNA (siRNA).

- Proteomic analyses indicate at least 200 polypeptides in phloem and a number of the larger of these have been identified. These include ubiquitin (8.5kD), SOD, membrane proteins and a range of enzymes associated with C metabolism. Among the smaller peptides a number have been detected and some sequenced using an LC-ms/ms technique. They will be assessed in relation to initiation of nodulation and in *L. albus* the development of lateral roots as clusters in relation to low P stress.

- The many more miRNA that appear to be present in lupin phloem have been cloned and their sequences will be determined. This information will permit the target genes

to be identified in *Arabidopsis*, rice and lupin. The next step in the project is to associate these miRNA with target genes in meristems.

3. Belgium – Root architecture in annual plants: genetics bases and plasticity in response to nutrient supply

- Model formulation of geometric interactions between root growth, vascular architecture and branching provide a powerful means to assess phenotype.
- Scaling up of aeroponic culture for high throughput phenotyping of root system architecture beyond the seedling stage.
- QTL analysis of root architecture traits of rice (static traits) and barley (dynamic traits) is ongoing.
- Elaboration of a system to induce lateral roots suitable for the analysis of gene expression.
- Implementation of a generic space-time model of root system architecture.
- Design and implementation of an image analysis software for the quantification of root architecture and localisation of root features from root image sequences.

4. Brazil – Primary root growth: genetics and differences among wheat mutant lines in nutrient solutions

- Wheat mutant lines produced and included in breeding programmes: Anahuac M1, Anahauc M3, IAC-17M, KAUZ "S" / IAC-24-M2, KAUZ "S" / IAC-24-M8, IAC-24/TUI "S"-M2 and TUI"S"7IAC-24-M1.
- Genetic variability was obtained among evaluated Al-tolerant genotypes (wheat cultivars and inbred lines) in relation to primary root growth in the first stages of development in nutrient solutions, that was independent of P concentration, temperature, pH, salt concentration and period of growth.
- Primary root growth is genetically controlled by a few genes with additive effects.
- Selection for Al-tolerance and high rates of primary root growth under acid soil conditions would be effective if selection could be realized in early segregating generations.

5. China – Identification, classification and functional characterization of spontaneous and induced root mutants of soybean

- Three hundred soybean accessions were assessed for tolerance to rhizospheric stresses, including drought, Al toxicity and low phosphorus. Fifteen lines were selected, two were tolerant to drought at both seedling and reproductive stages, and two were tolerant to both Al and low phosphorus stresses. All four have been incorporated into breeding programmes.
- Correlations were found between drought tolerance and the relative values for total root length, root volume and dry root weight.

- Correlations were also found between tolerance to Al and the stressed to unstressed ratio values of number of lateral roots, tap root length and total root length.

- Recombinant inbred lines (RILs) were used to investigate the inheritance of these traits and major and minor gene effects were revealed. Genetic mapping in the RILs showed that three major loci for relative values of dry weight, root length and root volume were found to be coincident, suggesting that the observed effects result from pleiotropy of a single gene.

- The results of segregation analysis and QTL mapping are consistent and can be used in genotypic verification.

6. China – Genetic improvement of wheat roots for high efficiency of water and nutrient absorption by radiation induced mutations

- Twelve wheat genotypes, including the partial amphidiploid of Zhong 4 Awnless with *Thinopyrum intermedium*, and derived chromosome addition lines (Z_1, Z_2, Z_4, Z_6 and L_1) were tested for root characteristics in the field. Seedling and immature embryos were also tested for resistance to PEG. The results demonstrated that the chromosome addition lines had stronger root systems than the parent wheat line and other wheat varieties.

- Six mutant lines with strong PEG resistance were developed following γ-rays irradiation of embryonic calli from the chromosome addition lines. Two of these were identified as translocation lines, four other lines were chromosome substitutions. Field tests of these lines indicated that they had enhanced drought tolerance, longer roots, and higher yield than the controls.

- Mutants with longer root systems were more tolerant to drought stress and one was found to be resistant to stripe rust.

- Four contemporary wheat cultivars were irradiated with γ-rays and four root mutants were identified. One of these mutants had a compact spike, and has been used in transformation studies as it has greater transformation efficiency than cv. Bobwhite.

7. Cuba – Morphological characterization of wheat mutants with improved drought tolerance

- Seven wheat mutants were evaluated and characterized for morphological, physiological and anatomical features of their roots, along with biochemical markers and yield.

- Roots exhibit large variations in structure and function and response to drought and salinity.

8. Germany – Genetic analysis of root formation in maize

- To overcome the lack of information on the genetic basis of root formation in cereals, a programme to isolate monogenic recessive maize mutants with deficiencies in important steps of post embryogenic shoot-borne and lateral roots was initiated. This approach led to the identification of the mutant *rtcs* with a complete lack of seminal lateral and crown roots. Furthermore, the two lateral root mutations, *lrt1* and *rum1,*

were isolated and displayed temporal absence of lateral root initiation. In the case of *rum1* seminal laterals roots were also absent.

- The phenotypic and genetic analysis of these mutants demonstrated the high specificity of the defects in respect of the root type and tissue affected and the strict developmental timing of the mutant action prior to primordial formation. The characterization of double mutants generated from the various mutants indicated the existence of different genetic mechanisms for post embryogenic root initiation.

- The forward genetic approach outlined above has been complemented by reverse genetic methods towards the identification of genes with root specific expression patterns. Subtractive proteomic studies and RNA profiling with micro array chips done with materials from the root initiating tissues were performed to obtain more information on the complex genetic network governing the build-up of the root stock.

9. India – Isolation and genetic characterization of mutants defective in root development in tomato

- Several root mutants of tomato have been isolated, of which 3 types were characterized in detail.

- Short root mutants: these mutants have diminutive stature, delayed onset of flowering and few flowers. The reduction in plant size was related to reduction in cell size of plants. The rescue of mutants with known plant hormones was not successful. The mutant leaves showed resistance to bacterial infections.

- Profusely branching root mutants: these mutants showed vigorous growth and produced more green foliage and bigger leaves. The plants had more flowers and bore more fruits. Attempts are now underway to transfer these mutations to other local cultivars.

- Polycotyledon mutant: this mutant showed short roots at the seedling stage. The short root phenotype could be rescued by application of a polar auxin transport inhibitor, TIBA. Results of physiological and biochemical experiments were consistent with enhancement in polar transport of auxin in the mutant. The gene for this mutation has been mapped on chromosome 9, and cloning of the gene is envisaged.

10. Israel – Mutational analysis of root characters in selected annual food plants

- Physiological and structural differences between root types of individual root systems were identified. Apparently, taproots differ from lateral roots in their ABA content in the number and activity of several iso-enzymes, in their lipid content and composition, and in the bacterial population of the root surface.

11. Poland – Mutational analysis of root system characters related to agronomic performance of barley

- Barley mutants developing short seminal roots in different stages of plant development were selected and identified using paper-rolls and PVC tube methods.

- Allelism tests indicated five specific loci for short seminal roots.

- Root shortening could be accounted for by specific changes in cell length and number along the root axis.

- DNA markers were used to locate the gene responsible for root length reduction in mutant 035AR in the short arm of chromosome 7H (33.5 cM distance to the locus *brh1*).

- Growth dynamics of seminal roots in these mutants were also described and compared to their parental lines.

12. South Africa – Development and evaluation of drought tolerant mutant germplasm of *Vigna unguiculata* and *Vigna subterranea*

- The irradiation of cowpea and bambara groundnut seed led to the creation of various mutants.

- Six cowpea mutant lines were similar to or better than the parent line. One exhibited high yield under watered conditions, and three under drought stress conditions.

- Six bambara mutants were similar to, or better than the parent line. Three mutants outyielded the parent, and one showed relatively high yield under drought stress.

- It was possible to examine mutant plants at the seedling stage in wooden boxes. Mature plants were screened in rain out shelters and physiological traits were distinguished among the tested lines for drought stress. Roots of mature plants were also assessed and variation could be correlated with tolerance.

- Chlorophyll fluorescence was found to be a good predictor of plant performance in drought conditions.

13. Turkey – Selection and molecular characterization of root-system induced mutants in barley and chickpea

- A γ-ray induced barley mutant collection was produced and established.

- Wide genetic variability was observed for root traits in this collection and improved lines (F_7 and F_8) were derived from a number of mutants.

- A rapid outdoor screen has been established using sand culture. This system is well suited to single spike progeny testing.

- One of the long root and early heading mutants, M-K-88, and a land race cultivar, *Tokak*, had the lowest $\delta\ ^{13}C$, consistent with better water use efficiency.

- Bulked segregant analysis is under way to assign genetic markers to the mutant trait.

14. United Kingdom – Effects of mutant dwarfing genes on root traits in barley

- A mutation grid comprising >20 000 M_3 families has been developed in barley cv Optic. Publicly available: URL: http://bioint.scri.sari.ac.uk/distilling/distilling.html

- The two commercially important semi-dwarf mutations in barley, *sdw1* and *ari.e-GP* have different pleiotropic effects on some root traits.

- An efficient two-dimensional screen has been developed to study seedling root traits.

- A complete genome scan has been carried out for using over 100 barley backcross mutant lines, and evidence for genomic region controlling seedling root traits has been obtained.

- QTL mapping of seedling root traits has been carried out in a double haploid population and show QTL clustering at the *sdw1* and *ari.e-GP*loci.

- Short root mutants have beneficial effects in surface irrigation system in saline soils.

15. United States of America – Ecology and genetics of root architecture and soil water extraction

- This research project focused on the deep acquisition of water by a vegetable crop from the soil profile, as a means to reduce water stress and irrigation inputs. The goal was to determine the type of root architectural system that was conducive to rapid shoot growth and deep soil water extraction in lettuce, a shallow-rooted crop with high water demand.

- QTLs for a deeper taproot and for more laterals at the tip of the taproot were compared in recombinant inbred lines from an initial cross between cultivated (*Lactuca sativa*) and wild (*Lactuca serriola*) lettuce. Lines with the QTL for more deep laterals at the tip of the taproot appear to benefit from greater water availability, i.e. they had higher shoot biomass, deeper soil water extraction, and greater discrimination for ^{13}C.

- A marker-assisted breeding programme was initiated to introgress this QTL region in *L. sativa*, with the potential for allowing irrigation less frequently and with less water without a negative impact on yield.

C. CONCLUSIONS

- Mutation induction has proven to be an ideal tool for generating material to be used in investigating root form and function, and in developing new cultivars.

- More than one screening method and environment should be used to assess root characters in mutant populations and germplasm.

- Where mutant selection is based on root architecture at the seedling stage, selected lines should be assessed under field-based conditions through the crop cycle. Selection at early generations in a breeding programme is recommended.

- Genetic analysis of mutants should proceed to identify and isolate genes responsible for specific components of response to stresses.

- Where possible, stress components (e.g. pH and Al concentration) should be assessed separately when sreening for root screens for mutants.

- Indirect physiological measurements for root traits should be explored further to derive new, high throughput, screening methods.

ISOLATION AND CHARACTERIZATION OF ROOT MUTANTS OF TOMATO (*Lycopersicon esculentum*)

S. NEGI, M.S. SHARADA DEVI, R. SHARMA
School of Life Sciences,
University of Hyderabad, Hyderabad, India

Abstract

Plant roots perform a myriad of functions right from the anchorage of plants to storage of reserve food material for the adverse weather. Roots forage around the soil for acquisition of ions and water and continually develop new branches. However, very little is known about the molecular mechanisms regulating root development and differentiation. We have endeavoured to decipher the genetic regulation of root formation in tomato by screening mutants defective in root development. We have isolated several mutants with altered morphology of roots. These mutants have been characterized for their phenotypes throughout the life cycle. In this report we show that for few of the mutants the vigour of plant is directly related to modification in the root structure. The potential utility of these mutants in understanding root development and also improving yield of tomato is discussed.

1. INTRODUCTION

Plant roots perform a multitude of functions such as water and nutrient uptake, anchorage to the soil and interactions with the other organisms such as bacteria and fungi in the rhizosphere. The performance of root to carry out these functions can be improved by selecting for traits that improve one or more of these functions. Induced mutation therefore provides an easy tool through which one can induce variability in the root system and thus change the architecture and also functional performance of the roots. At the same time the induced mutations also provide a tool that can be used to elucidate the role of different genes involved in root development. The knowledge about these genes can therefore provide the genetic basis of root development and differentiation, which can then be utilized as an index for plant breeding and cultivation.

The root development in tomato follows pattern typical to dicotyledons plants. The growth of tomato root begins after imbibition of tomato seed; in fact the emergence of radicle from seed coat is the first outward signal of initiation of germination. The first root or tap root elongates and also develops the lateral branches during subsequent growth. Since tomato plants, grown in field, are transplanted from the nursery bed, the tap root is usually broken during transplantation. The root system for transplanted tomato consists of mostly fibrous roots. The roots developed from tomato cuttings are also of fibrous nature. The final form of the root system of mature tomato plants largely depends on the rhizosphere environment. However, the endogenous factors such as plant hormones also strongly influence the root development. Among the plant hormones auxin is considered to be the principal hormone regulating root development and the positive gravitropic movement of root. Other hormones such as abscisic acid and gibberellic acid also significantly influences root growth and development.

At the cellular level root elongation is determined by two successive processes: root elongation and root expansion. To uncover the mechanisms controlling root development, genetic analysis of root mutants has proven to be a useful approach. In crop plants such as tomato [1], maize [2, 3], and rice [4–8], root mutants have been isolated and characterized. Most information about genetic regulation of root development has come from analysis of root mutants of *Arabidopsis thaliana.* Many of Arabidopsis mutants have abnormal

morphogenesis such as root meristemless mutants [9–10], short root mutants [11–13] and root expansion mutants [14, 15]. Two mutants, *scarecrow* (*scr*) and *short root* (*shr*) show the defect in radial patterning of the root [13, 16]. Studies of these two mutants led to the identification of a pair of transcription factors viz; SHR and SCR that are required to set up and maintain the radial organization of the ground tissue.

Currently, the genetic analysis of mutants coupled with technology of gene cloning has emerged as a powerful technique to identify the genes regulating plant development. Such information to some extent has been already obtained for *Arabidopsis* root development [17]. By contrast, only limited information is available about tomato [18–20]. Genetic analysis by crossing of two different roots-less tomato mutants revealed that genetically roots could be characterized into four distinct types. Most importantly, these studies [18–20] showed that the root characteristics could be genetically manipulated to introduce specific changes in root systems. Some of the mutants are available in public domain from Tomato Genetics Research Cooperative, California (http://www.tgrc.ucdavis.edu/Monogenic-stocks-2002.doc)

We have screened tomato seedlings for mutants defective in root development. Here we report physiological and morphological characters for these mutants. The genetic analysis and gene mapping of these mutants would be helpful in identifying the genes regulating the root development in tomato.

2. MATERIALS AND METHODS

2.1. Plant material

The plant used in the present studies was *Lycopersicon esculentum* cv Ailsa Craig, obtained from RE Kendrick, University of Wageningen, Netherlands. The mutants were obtained by screening the Ethyl Methane Sulfonate (EMS) and γ-irradiated M_2 populations. *Short root* (*shr*) and profuse *branching root* (*pbr)* mutants were obtained from a γ-irradiated population of cv Ailsa Craig. *Polycotyledon* mutant in tomato was obtained by screening the EMS mutagenized population of cv Ailsa Craig [21].

2.1.1. Plant growth conditions

Seed germination: The seeds were surface sterilized with 0.1% (v/v) sodium hypochlorite solution for 10 min at 25±1°C. Thereafter seeds were washed in distilled water and germinated in transparent plastic boxes (9.5 cm *l* × 9.5 cm *b* × 5 cm *h*) on filter papers moistened with distilled water for 24 hours in darkness at 25±1°C [22]. After the radicle emergence, the germinated seeds were transferred to wet paper rolls, these paper rolls opened after seven days and eleven days. Then the putative mutant seedlings were transferred to vermiculate (Vermiculate and peat mixture, Karnataka Explosive Limited Bangalore, India) in plastic glasses and grown at 25±1°C under continuous white light (100 μmol m^{-2} s^{-1}) for 10 to 12 days. Seedlings were then transferred to the pots and kept in green house. The phenotype of the mutants was recorded through out the life cycle of the plants using the descriptors of tomato according to those provided by International Plant Genetic Resources Institute (http://www.ipgri.cgiar.org/publications/pdf/286.pdf).

2.2. Rooting assay

To examine the formation of adventitious roots in the *poc* mutant, one-week-old de-etiolated wild type and *poc* mutant seedlings were used. The taproots of these seedlings were

excised above the base of hypocotyls. The rootless seedlings were immediately planted on agar (1.7% w/v) supplemented with 10 μM kinetin and appearance of the adventitious roots was observed after one week. Control seedlings were planted in agar without kinetin.

To observe the mature roots, plants were grown in pots for 3 months, thereafter they were carefully removed so as to avoid any damage to the root system. The roots were then extensively washed with water to remove excess soil debris.

2.3. Mutant Screening

We have used two different populations for screening of mutants defective in root development as per the characters given in Table I. The first population comprised of 50,000 EMS-mutagenized M_2 seeds, which were bulked from nearly 1000 M_1 plants for ease of screening. While bulking of seeds facilitates easy screening of mutants, it is also a least desirable method. The bulking of mutant material leads to repetitive occurrence of mutants that may have come from same M_1 plant. To eliminate these one has to undertake extensive allelism test for the mutants with similar phenotype. We noticed this problem when we isolated nearly 25 *polycotyledon* mutant lines of tomato, which turned out to be allelic to each other.

TABLE I. CHARACTERS USED FOR SCREENING TOMATO ROOT MUTANTS

Primary target for mutation	Characteristics examined	Remarks
Tap root	Absence of tap root	Lethal mutation
	Degeneration of tap root after slight elongation	Lethal mutation
	Plants with long tap root	-
	Plants with short tap root	-
Lateral roots	Profuse branching of lateral roots	-
	Increased number of lateral roots	-
	Long lateral roots	-
	Small lateral roots	-
	Absence of lateral roots	-
Adventitious roots	Presence of roots on the hypocotyl	-
Agravitropic roots	Loss of geotropism	Lethal mutation

The second population consisted of 10,000 γ-irradiated M_2 seeds. The M_0 seeds were irradiated with 250 Gy of gamma rays. In this population each M_1 plant was labelled and M_2 seeds were harvested from the individual M_1 plants. The majority of root mutants appeared to belong to the lethal group and plants died during vegetative growth.

2.4. Validation of root mutants

The growth and development of plant roots is greatly influenced by the environmental conditions. This demands that root mutants should be further verified under rigorously standardized conditions to eliminate false mutants in the M_2 generation. The following precautions are required during the screening of mutants.

Firstly, the mutagenized seeds of tomato loose vigour for the germination as compared to wild type seeds of the same age. In addition, tomato seeds on imbibition do not show uniform germination as seen for species such as wheat and barley. In view of random seed germination spread over two to six days from imbibition; extreme care has to be exercised to eliminate false positives. Therefore it is recommended to germinate a large number of seeds. The germinated seeds were selected on a given day and then screened as single lot for defects in the root development.

Secondly, the root growth and development is strongly influenced by the ambient conditions. Unlike the shoot, the phenotype of root is very deceptive. While the seedling shoot develops as a hypocotyl and cotyledons and thereafter leaves, roots show a wide range of variation in their growth patterns even in wild type population.

It has been observed in wild type plants if taproot is damaged during the transplantation of the seedlings the mature root shows fibrous type of root characteristics and the taproot with intact seedlings show the normal taproot (Fig. 1).

Therefore the screening for mutants should be carried out under the controlled conditions. It entails that the seedlings should be grown under conditions of uniform light intensity, photoperiod and temperature condition. In our case we have screened for mutants among the seedlings grown under continuous white fluorescent light.

Thirdly, it is essential that the substrate used for the screening of root mutants is free of residues that might influence the root development. We have typically screened for mutants from the seedlings that were grown in paper rolls made from germination paper. Each batch of the paper rolls were tested for the uniformity for germination and growth with wild type seedlings and the paper material where the seedlings show deviation from a set standard was not used. Since the mineral nutrients also influence the development of root, we have screened for root mutants using only distilled water.

Finally, the seedlings with altered root phenotypes were examined at two different time points for development as mentioned earlier. The phenotype of mutant was recorded through out the life cycle of plants using the descriptors of tomato. The mutant plants that do not set flowers are automatically rejected, as this would hinder further genetic analysis of these mutants. In extreme case it is also desirable to make mutant survive by vegetative propagation.

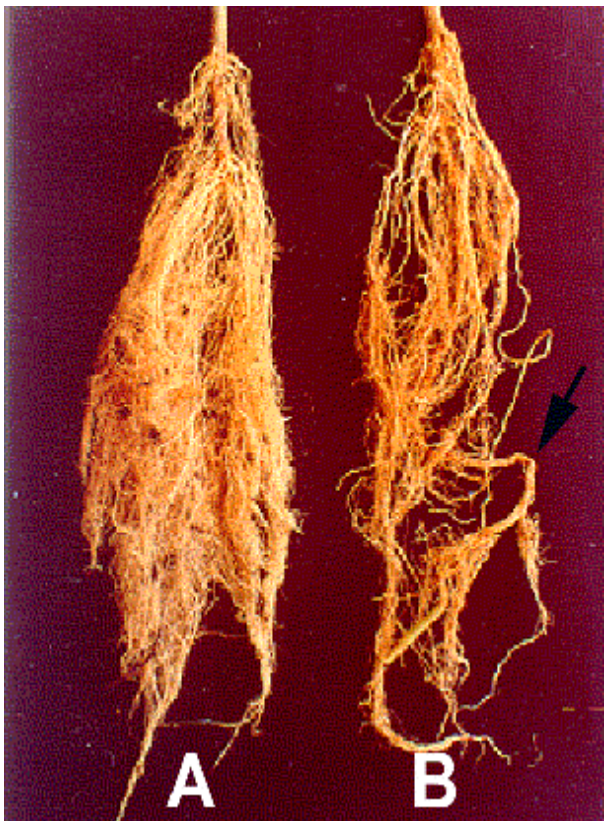


Fig. 1. Root morphology of three-months-old adult wild type tomato plants. A. Plant transplanted after seedling stage in a new pot, shows the fibrous roots characteristic of transplanted plants, where the tap root gets broken during transplantation. B. Plant not transplanted and has retained its original tap root (arrow). It shows less vigorous branching of roots.

2.5. Genetic analysis

These mutants were crossed to wild type. The F_1 and F_2 progenies were analyzed using the same screening parameters as mentioned above.

3. RESULTS AND DISCUSSION

3.1. Mutants from γ-irradiated lines

*3.1.1. **Sh**ort **r**oot mutant **(shr)***

The table II shows the phenotype of short root mutant lines that were re-screened in M_3 and M_4 generation. In our lab we confirm a mutant only if it retains phenotype in M_4 generation. It is evident from the results that several mutant lines in M_3 generation did not retain the observed root phenotype or showed segregation of the phenotype even in seeds harvested from the same fruit. Some of the extreme phenotypes turned out to be lethal and plants did not set flowers and these lines were lost. Some of the mutants that retained the phenotype in M_3 generation did not survive in M_4 generation. In fact only two mutant lines from 20 putative mutant lines have maintained short root character in M_4 generation. This supports the logic for re-screening phenotypes of all the putative M_2 mutants in M_3 and M_4 generation.

TABLE II. THE STABILITY OF SHORT ROOT PHENOTYPE FOR MUTANTS IN M_3 AND M_4 GENERATION

Mutant No. in M_2 generation	Phenotype in M_3 generation	Phenotype in M_4 generation
53-1	Short root	No fruits
53-2	Short root	Short root
53-3	No fruiting	-
53-5	Short root	Only one fruit with five seeds. Seedlings died after two weeks
53-6	No fruiting	-
53-7	No fruiting	-
53-8	No fruiting	-
53-12	No short root	-
53-13	No short roots	
13-4	Short root	Short root
F2-3	Strong segregation of phenotype, out of 30 seeds only three showed short roots	-
45-7	No short roots	-
45-9	No short roots	-
64-1	No short roots	-
26-A	No short roots	-
26-C	No short roots	-
29-9	No short roots	-
32-2	No short roots	-
N6-5	No short roots	-
76-1	No short roots	-
45-6	No short roots	-

For subsequent studies we used above two *short roots* (*shr*) mutants, which showed same phenotype consistently. The short root mutant plants grew sluggishly and took considerably longer time to complete their life cycle. In addition the *shr-1* mutant shows very low fertility level and sets very few fruits, whereas the *shr-2* mutant showed self-incompatibility and was manually pollinated. These mutants were crossed to wild type and F_1 progeny were analyzed. F_1 from both lines had a wild type phenotype indicating that the mutations are recessive in nature. The F_2 plants segregated in 3:1. This indicates *shr-1* and *shr-2* phenotype is caused by mutation in a single gene.

*3.1.2. **P**rofuse **br**anching **r**oot mutant **(pbr)***

The mutant plants with profusely branching roots show more robust growth than the wild type. The mature plants of the profuse branching (*pbr*) mutant also has more extensive root than the wild type. The *pbr* root mutant has five distinct mutant lines that can be distinguished based on phenotypes (Table III). The mutant lines named as *pbr-1* has green fruits. The line *pbr-2* and *pbr-3* bear light green fruits and *pbr-3* also shows delayed fruit ripening. Similar variations were also seen in the number of flowers in an inflorescence or number of inflorescence per plants.

The *pbr-4* mutant has more inflorescence than the wild type. Moreover this mutant is also more fertile and sets more fruits. It also has dark red fruits after ripening (Fig. 2). The *pbr-5* line has aerial roots on the hypocotyl and stem.

TABLE III. COMPARISON OF PHENOTYPES OF DIFFERENT PROFUSE BRANCHING ROOT (*PBR*) MUTANTS WITH WILD TYPE PLANTS

Plant stage	Character	Mutant				
		pbr-1	*pbr-2*	*pbr-3*	*pbr-4*	*pbr-5*
Seedling	Root length	Long tap root with more lateral roots.	Long tap root with more root.	Long tap root with more lateral roots.	Very long tap root with more lateral roots.	Long tap root with more lateral roots
	Cotyledon size	Normal	Broad	Broad	Broad	Broad
	Hypocotyl length	Same as wild type	Longer	Longer and shows more anthocyanin	Longer	Longer
Mature plant	Plant Growth	Fast	Fast	Fast	Fast	Fast
	Leaf size	Bigger	Bigger	Bigger	Bigger	Bigger
	Root size	Bulkier	Bulkier	Bulkier	-	-
	Aerial roots	-	-	-	-	Present
Fruits	Number of fruits wild type (+)	++	++	++	++	++
	Ripening	-	-	Delayed ripening	-	-
	Fruit colour	Green	Light green	Light green	Light green Fruits. Ripened fruits are dark red	Dark green Fruits with 5 lobes. Ripened fruits dark red
Inflorescence	Number of flowers in a inflorescence wild type (+)	++	++	++	+	+
	Number of Inflorescences wild type (+)	+	+	+	++	++

A close examination of the phenotype of these *pbr* mutants in the field revealed differences among several characters of the mutants. We are now examining whether these five mutant lines represent mutation in different loci or are alleles of the same gene. The *pbr* mutant lines crossed with wild type showed wild type phenotype in F_1 indicating that it is likely to be a monogenic recessive gene. The F_2 of each mutant line segregated in a 3:1 ratio and indicated that in each case a single gene causes the mutation.

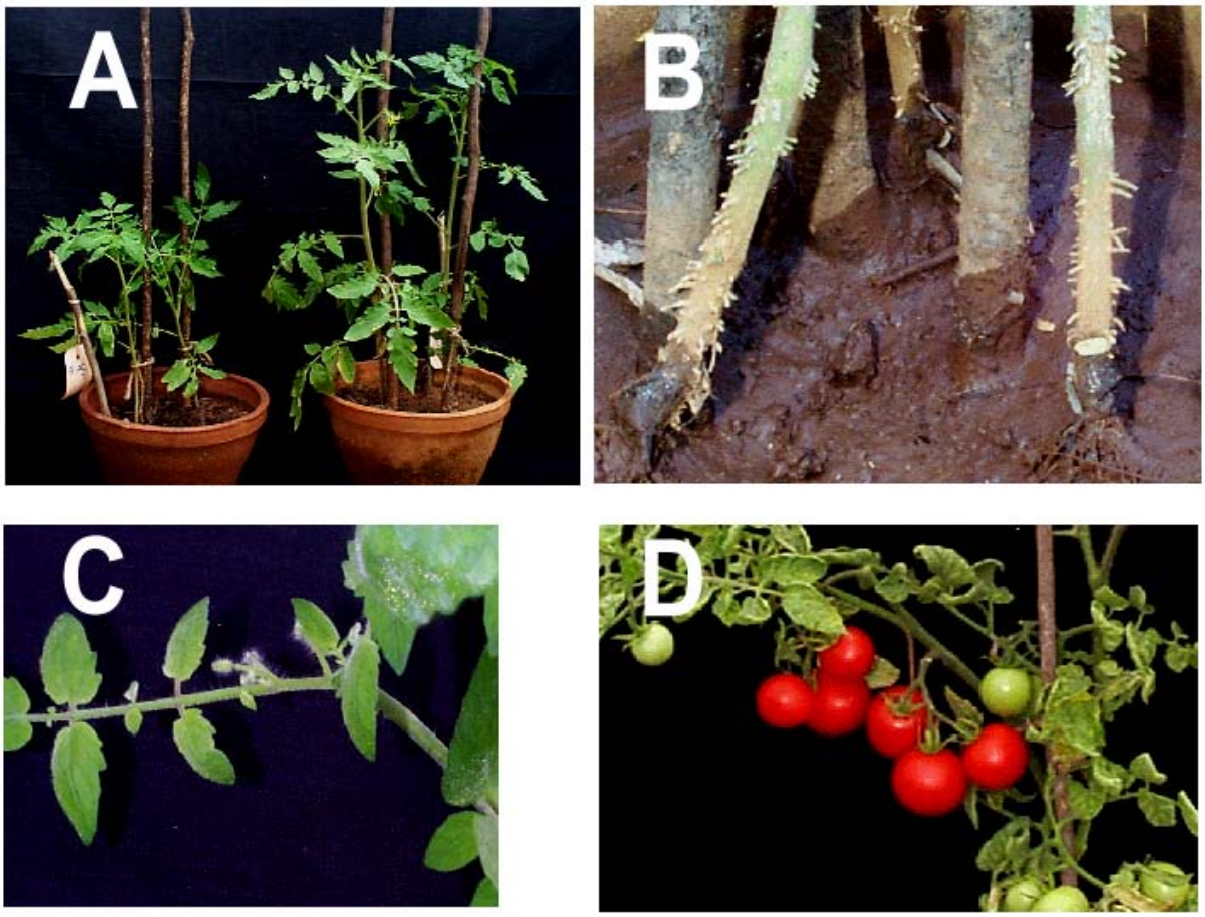


Fig. 2. The adult phenotype of some pbr mutants. A. 45 days old WT (left) and pbr-1 mutant plants (right). B. pbr-5 mutant showing aerial roots. C. pbr-4 mutant leaf showing ectopic inflorescence. D. Fruits of pbr-4 mutant.

3.2. Mutants from EMS-mutagenized lines

*3.2.1. Auxin transport and rooting in **po**ly**c**otyledon **(poc)** mutant*

The *polycotyledon* mutant of tomato shows shorter root in the seedling stage and more branched root in the adult plants. The short root phenotype of this mutant could be rescued by the application of an inhibitor of the polar transport of auxin-TIBA. The results obtained indicated that the above mutation enhances the polar transport of auxin in root [21].It is also supported by the experiment wherein we observed the stimulation of geotropic curvature of root in the *poc* mutant. In addition enhanced polar transport of auxin also reduced the lag period needed to manifest the root bending under gravitational influence.

Since auxin regulates the rooting of cuttings in plants, we examined whether enhanced auxin transport in *poc* mutant can lead to enhanced induction of adventitious roots. The role

of auxin transport on induction of rooting was studied by examining the capacity of excised hypocotyls to initiate roots. One-week-old de-etiolated seedlings were excised close to the base of the hypocotyl. While both wild type and the *poc* mutant hypocotyls showed adventitious root formation, the wild type hypocotyls failed to form adventitious roots in agar supplemented with 10 μM kinetin. In contrast *poc* hypocotyls showed adventitious root formation in agar supplemented with similar concentration of kinetin (Fig. 3). Since both wild type and *poc* mutant generated the adventitious roots on excised hypocotyl grown on agar, without any supplemental hormone, it indicates that the amount of endogenous auxin present in these two plants is sufficient to induce the rooting. However in case of *poc*, kinetin suppression is offset by the enhanced polar transport of auxin [21].Since auxin and kinetin act antagonistically to regulate the root induction, the presence of kinetin in media suppresses rooting of wild type cuttings. In view of faster transport of auxin more auxin reaches the cut end of hypocotyl and therefore the cytokinin is unable to antagonize action of endogenous auxin.

Fig. 3. One-week old seedlings of wild type (WT) and poc mutant (poc) grown on vermiculate were excised close to root base and were grown on agar for one week. Both wild type and poc mutant produce normal adventitious roots when grown in plain agar. On the other hand, wild type (WT+K) seedling produces no adventitious roots on agar supplemented with 10 μM of kinetin (K). The poc (poc+K) mutant produces adventitious roots on agar media supplemented with 10 μM of kinetin after one week albeit with reduced length and number.

3.2.2. Root mutants from EMS-mutagenized lines

From the EMS-mutagenized population we isolated several mutant lines defective in root formation and function. One of the mutants had agravitropic roots, in M_2 generation. Subsequent analysis showed that this agravitropic mutant had high mortality and inconsistency of phenotype. Only few of seedlings on germination produces root, which grows randomly for two days and later regains the normal geotropism. This mutant also shows delayed ripening trait in fruits.

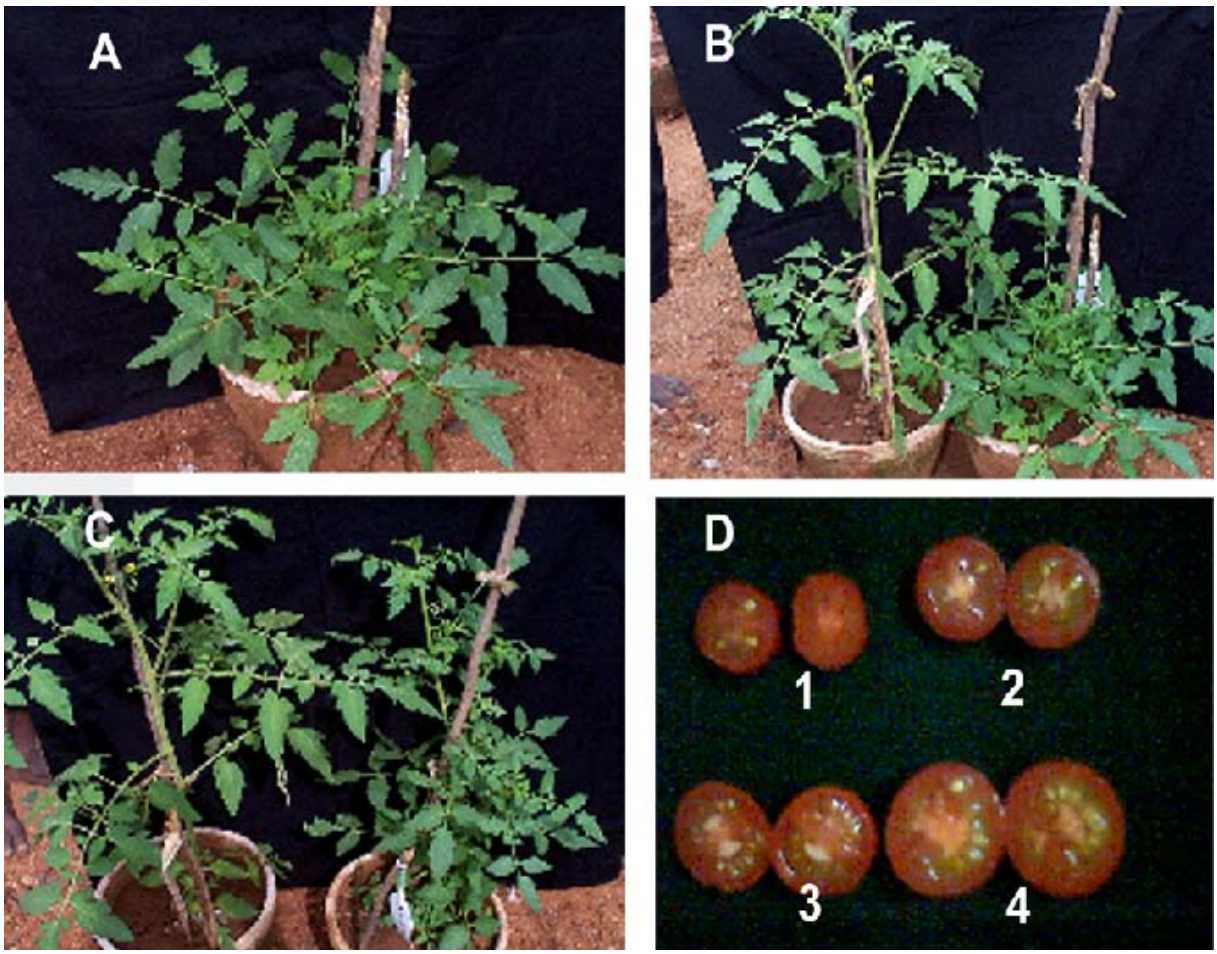


Fig. 4. The adult phenotype of mutant RT 34-2. A. green bushy plant. B. 34-2 bushy green plant in comparison with wild type. C. 34-2 bushy plant with many buds in comparison with wild type. D. Comparison of fruit size with the wild type. 1, 2, 3 fruits of mutant, 4 wild type fruit.

Several of these mutants also showed alteration of vegetative and reproductive phenotype. The mutant with more lateral roots (RT-31-4) showed bushy growth of stem and abortive inflorescence. The mutant RT 34-2 showed a degenerated taproot with long lateral roots at the seedling stage and a bushier phenotype the adult stage when compared with wild type (Fig. 4). The mutant plants had more buds and smaller fruits than the wild type. In contrast though RT-45 mutant had short taproot and also displays a dwarf phenotype, but at the same time produced large fruits with delayed ripening. The detailed feature of these mutants is presented in Table IV. These traits are retained until the M_4 generation.

3.3. Stability of mutations

In view of the vast influence of the environmental conditions on root phenotype, it is prudent to examine the stability of phenotypes in M_3 and M_4 generation. Many of putative mutants isolated may in-fact represents the aberrant root phenotype due to some environmental interactions and may not retain the phenotype in the next generation. Moreover, for many putative mutants it is likely that the observed phenotypes may in fact represents mutation in more than one gene and combination thereof. The mutant lines that do not show the originally scored phenotype in M_3 and M_4 generation are rejected. Essentially it

leads to rejection of more than the 90% of putative mutants, allowing only mutants with genetically stable phenotype to be selected. Moreover for the selected mutant lines the majority of the seedlings should reproduce the phenotype in M_3 and M_4 generation. It is also required that the re-screening of phenotype is carried out with more or less similar condition and protocols as used for original selection.

TABLE IV. PHENOTYPES OF DIFFERENT ROOT MUTANTS DERIVED FROM EMS-MUTAGENESIS IN M_4 GENERATION

Mutant	Root Phenotype	Adult phenotype
RT-31	Very long tap-root with small lateral roots	Mutant line segregated in two types. RT31-4-1 showed abortive inflorescence and deformed fruit. RT31-5-5 showed bushy kind of growth.
RT-45	Short tap root, lateral roots absent	This mutant showed the feature of delayed ripening of fruits.
RT-70	Short tap root with long lateral roots	The adult plants show high amount of anthocyanin. The mutant line RT70-2 also shows the unilocular fruit. Mutant plants show high amount of anthocyanin in roots.
RT-34	Degenerated tap root with long lateral roots.	The vegetative phenotype is split into two types (a) dark green bushy leaved plant (b) yellow virescent bushy plant. Both plants are highly sterile and difficult to self. The later plants plant lacks the pollen sac in the flower. The fruit size of these plants is very small.

4. CONCLUSION

The study of phenotypes of root mutants brought forward several interesting observations. Firstly there was a distinct correlation between plant growth and root size. The mutants with short roots (*shr-1* and *shr*-2) at the seedling stage grew sluggishly as compared to the wild type plants. In contrast, the profuse branching mutants (*pbr-1, pbr-2, pbr-3, pbr-4* and *pbr-5*) showed very vigorous growth with increase in the number of inflorescences, flowers and fruits as compared to their wild type counterpart. The correlation between the root size at the seedling stage and vigorous growth at the adult stage gives an important handle to screen for plants with robust growth phenotype. The tomato plants can be screened at seedlings stage for the profusely branched root and these plants are likely to grow faster. Most importantly these plants would likely bear more flowers and consequently more fruits. This is one character that can be easily exploited commercially.

Secondly it appears that profuse branching phenotype is caused by some changes in the level of the plant growth hormones. While several plant hormones are known, none has been specifically associated with profuse branching phenotype. It could be well that this phenotype may arise via a novel regulator that still remains to be identified. By same corollary the short root phenotype may represent either accumulation of a root growth inhibiting molecule or some deficiency in growth promoting hormones.

The molecular basis of these mutations would be known only after the mapping of the mutant loci on tomato chromosomes and subsequent isolation of the genes. However, a close examination of physiology and phenotype of the mutant, at times allow one to infer the possible cause of the mutant phenotype. Particularly mutant rescues are highly useful for making guess about the likely cause of the mutation. It can also be used to predict the nature of the gene responsible for that phenotype. For example, the root of the tomato *polycot* mutant seedling is much shorter than the wild type. However, application of TIBA an inhibitor of the polar auxin transport restores its length equal to wild type. We have concluded that this phenotype arises due to increase in rate of polar transport of auxin. Currently the experiments are under way to map the genes of these mutants. We are also doing detailed physiological and anatomical investigations of these mutants to decipher the likely cause of mutant phenotype. We hope that experiments on these lines would identify the physiological and biochemical basis of root phenotypes in tomato.

ACKNOWLEDGEMENTS

The financial support by International Atomic Energy Agency (IAEA; grant to Prof. R. Sharma) is gratefully acknowledged.

REFERENCES

[1] Muday, G.K., Lomax, T.L., Rayle, D.L. 1995. Characterization of the growth and auxin physiology of roots of the tomato mutant, *diageotropica.* Planta 195:548-553.

[2] Hetz, W., Hochloldinger, F., Schwall, M., Feix, G. 1996. Isolation and characterization of *rtcs*, a maize mutant deficient in the formation of nodal roots. Plant J. 10:845-857.

[3] Hochloldinger, F., Feix, G. 1998. Early post-embryonic root formation is specifically affected in the maize mutant *lrt 1*. Plant J. 16:247-255.

[4] Kitano, H., Fustuhara, Y. 1989. Inheritance and gene expression of a root-growth inhibiting mutant in rice (*Oryza sativa* L.), Proceedings of the Sixth International Congress, SABRAO, pp. 349-352.

[5] Liang, Z.W., Ichii, M. 1997. Genetic analysis of short-root mutant LM10 induced from rice (*Oryza sativa* L.) cultiver IR8. Breed Sci. 46:373-377.

[6] Ichii, M., Ishikawa, M. 1997. Genetic analysis of newly induced short-root mutants in rice (*Oryza sativa* L.). Breed Sci. 47:121-125.

[7] Ichii, M., Liang, Z.W. 1997. Morphological characteristics and its inheritance of a short-root mutant in rice. Breed Sci. 47:310 in Japanese.

[8] Liang, Z.W. 1997. Research on characteristics and genetic mechanism of short-root mutation in rice (*Oryza sativa* L.), Ph.D Thesis of United Agriculture Department of Ehime University. pp :61-98. in Japanese.

[9] Cheng, J.C., Seeley, K.A., Sung, Z.R. 1995. *RML1* and *RML2*, *Arabidopsis* genes required for cell proliferation at the root tip. Plant Physiol. 107:365-376.

10] Vernoux, T., Wilson, R.C., Seeley, K.A., Reichheld, J.P., Muroy, S., Brown, S., Maughan, S.C., Cobbett, C.S., Montagu, M.V., Lnze, D., May, M.J., Sung, Z.R. 2000. The root *meristemless 1/ cadmium sensitive 2* gene defines a gluthione – dependent pathway involved in initiation and maintenance of cell division during post embryonic root development. Plant cell 12:97-109.

[11] Baskin, T.I., Betzner, A.S., Hoggart, R., Cork, A., Williamsom, R.E. 1992. Root morphology mutants in *Arabidopsis thaliana*. Aust. J. Plant Physiol. 19:427-437.

[12] Baskin, T.I., Cork, A., Williamson, R.E., Gorst, J.R. 1995. *STUNTED PLANT 1*, a gene required for expansion in rapidly elongating but not in dividing cells and mediating root growth responses to applied cytokinin. Plant Physiol. 107:233-243.

[13] Benfey, P.N., Linstead, P.J., Robert, K., Schiefellbein, J.W., Hauser, M.T., Aeschbacher, R.A. 1993. Root development in Arabidopsis: four mutants with dramatically altered root morphogenesis. Development 119:57-70

[14] Cano-Delgado, A., Metzlaff, K., Bevan, M.W. 2000. The *eli1* mutation reveals a link between cell expansion and secondary cell wall formation in *Arabidopsis thaliana*. Development 127:3395-3405.

[15] Fagard, M., Desnos, T., Desprez, T., Goubet, F., Refregier, G., Mccann, M., Rayon, C., Vernhettes, S., Hofte, H. 2000. *PROCUSTE1* encodes a cellulose synthase required for normal cell elongation specifically in roots and dark-grown hypocotyls of *Arabidopsis*. Plant cell 12:2409-2424.

[16] Wysoka-Diller, J., Helariutta, Y., Fukaji, H., Malamy, J.E., Benfey, P.N. 2002. Molecular analysis of SCARECROW function reveals a radial patterning mechanism common to root and shoot. Development 127:595-603.

[17] McCully, M.E. 1999. Roots in soil: Unearthing the Complexities of Roots and Their Rhizospheres. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50: 695.

[18] Zobel, R.W. 1973. Some physiological characteristics of the ethylene requiring tomato mutant *diageotropica*. Plant Physiol. 52:385-389.

[19] Zobel, R.W. 1975. The genetics of root development. In: J.G. Torrey and D.F. Clarkson (eds.). The development and function of roots. Academic Press, London. 261.

[20] Zobel, R.W. 1991. Genetic control of root systems. In "Plant Roots: The Hidden Half", Y. Waisel, Eshel, A., Kafkafi, U. eds (New York: Marcel Dekker), pp:27-38.

[21] Al-Hammadi, A.S.A, Sreelakshmi, Y., Negi, S., Siddiqi, I., Sharma, R. 2003. The *polycotyledon* mutant of tomato shows enhanced polar auxin transport, Plant Physiol 133:113-125.

[22] Koornneef, M., Bosma, T.D.G., Hanhart, G.J., van der Veen, J.H., Zeevaart, J.A.D. 1990. The isolation and characterization of gibberellin deficient mutant of tomato. Theor. Appl. Genet. 80:852-857.

MUTATIONAL ANALYSIS OF ROOT SYSTEM CHARACTERS RELATED TO AGRONOMIC PERFORMANCE IN BARLEY

M. NAWROT, J. GUZY-WROBELSKA, A. BRASZEWSKA, I. ANDRZEJAK, I. SZAREJKO, M. MALUSZYNSKI
Department of Genetics,
University of Silesia, Katowice, Poland

Abstract

Analyses of seminal roots of 381 dwarf and semi-dwarf barley mutants showed great variability in root length at seedling stage. Two mutants (035AR from cv. 'Aramir' and 225DV from cv. 'Diva') expressing shorter roots also at spike emergence stage were chosen for further analysis. Both selected mutants developed shorter seminal roots and shoots in relation to their respective parents. In each mutant the recessive root and shoot phenotype was controlled by linked mutated genes. Anatomical studies revealed that root shortening resulted from smaller cell dimensions in all specific cell zones of the root. Linkage analysis was performed for the mutant 035AR, which had a semi-dwarf phenotype caused by the mutation at the *brh1* locus. The mutation mapped in a distal part of the short arm of chromosome 7H. The molecular studies focused on markers in this region from the 'Steptoe'/'Morex' genetic map, confirmed the linkage between locus *brh1* and gene responsible for the root length, 33.5 cM distal from *brh1*.

1. INTRODUCTION

Roots play a decisive role in plant growth and development. They anchor the plant to the soil, provide a system for water and nutrients uptake, and influence tolerance to various abiotic stresses. Although plant growth and reproduction rely on a properly developed root system, knowledge on genetic controls of root characters is still very limited. This is caused by the difficulty in observing and analysing root systems and the ability to screen for mutants in large plant populations. Several root system mutants have been developed in the model plant, *Arabidopsis thaliana*, which is more amenable to high throughput phenotyping. In agronomically important cereal plants, such as maize and rice only a few root mutants are known [1–3]. In barley, one agravitropic and one root hairless [4, 5] and curly roots [6] mutants were described. However, a preliminary study easily detected root mutants in our collection of semi-dwarf barley lines. The objective of this study was to select, characterise and genetically analyse barley mutants with changes in the development of seminal roots, and to localise genes controlling the mutated traits in chromosomes using DNA markers.

2. MATERIAL AND METHODS

2.1. Plant material

The studies were based on 381 dwarf and semi-dwarf (sd) spring barley mutants contained in a collection at the Department of Genetics, University of Silesia, Poland. The mutants were obtained after mutagenic treatment of 12 spring barley varieties with N-methyl-N-nitrosourea (MNH=MNU), sodium azide (NaN_3), gamma rays and fast neutrons. The sd mutants are in M_{15} or further generations after mutagenic treatment. Prior to this study, the semi-dwarf mutants had not been evaluated for any root character. After analysis of seminal root growth at seedling and spike emergence stages, two mutants; 035AR from cv. 'Aramir' and 225DV from cv. 'Diva' were chosen for further studies.

2.2. Seminal root growth analysis

2.2.1. The paper roll method

Seminal root growth analysis was performed with the use of paper rolls made of filter paper, wrapped tightly around Hagedorn tube (ϕ 25 mm). Five plastic-coated wires were enclosed between each layer of paper, and the surface of rolls was covered with black foil. Sterilized and pre-germinated seeds (with coleorhizae emergence of 1–2 mm) were placed embryo down, one beside each of two sides of a wire. Paper rolls were placed in containers with equal level of distilled water and kept in a growth chamber under controlled conditions (illumination 180 $\mu Em^{-2}s^{-1}$, 16/8h photoperiod and temperature 24/22°C day and night, respectively). The experiments were carried out in three replications with 10 seedlings per replication. After 8 days of growth, the length of the longest seminal root and the number of roots were measured. The measurements included also the length of the first leaf.

2.2.2. The PVC tube method

Plants were grown in the PVC tubes, 125 cm long and 7.5 cm in diameter, filled with sand. To facilitate the extraction of intact roots, plastic foil used to line the inside of each tube. Pre-germinated seeds were sown into the tubes, and covered with 2–3 cm layer of soil. The experiments were conducted in a glasshouse, under semi-controlled conditions (illumination 200 $\mu Em^{-2}s^{-1}$, 16/8h photoperiod and temperature 25/15°C day and night, respectively). Every two days, plants were nourished with the same volume (100 ml) of ½ MS mineral medium [7]. The excess solution flowed out freely from the tubes. Plants were gently sprinkled with tap water throughout to prevent desiccation. The experiments were performed in three replications with 3–5 plants per replication. Plants were harvested and washed after 6 weeks and the length of the longest seminal root was measured.

2.3. Genetic analysis of selected barley mutants

From the experiments described above two mutants were selected for further investigation. These mutants, 035AR and 225DV were selected because they had significantly shorter seminal roots and shoots at both analysed stages of development in comparison to parent varieties. The selected mutants were crossed to each other and with their respective parental line. The genetic analysis of F_1 and F_2 was carried out at the seedling and spike emergence stages. Genetic mapping was performed in populations derived from crosses of the mutants with ‘Steptoe’ and ‘Morex’ (S/M) parents of a well defined barley genetic mapping population.

2.4. Cytological and anatomical studies

Mitotic activity of the root meristem was assessed during the first 196 h of seedling growth using Feulgen staining [8]. The first measurement was taken after 48 h from the beginning of seed germination and thereafter at 24 h intervals.

Anatomical observations were based on paraffin slides preparations of longitudinal and cross sections of 5 day-old seminal roots. Longitudinal sections included meristematic, elongation and maturity zones. The roots were fixed in Nawashin fixative [8] and observations were performed under the light microscope.

2.5. Molecular analysis

2.5.1. DNA extraction

Total DNA was extracted according to the micro-CTAB method [9], from the young leaves (0.03 g), dried in plastic bags with Silica gel (Sigma) and finely ground in Eppendorf tubes (2.2 ml) using a ball mill. For each mutant line, its parent variety and parents of S/M mapping population, the DNA was extracted from bulk tissue of 10 individual plants. In linkage analysis, the DNA was extracted separately from each F_2 individual. The DNA concentration was determined using GeneQuant™ (Pharmacia Biotech, UK). The samples were diluted with sterile water and stored at 4°C.

2.5.2. AFLP marker analysis

AFLP assay was performed according to the method of [10] with a modification in the selective amplification step in detecting products using a *Li*-Cor automated sequencer (IRD800 fluorescence labelled *Eco*RI primers and increased concentration of magnesium ions were applied in PCR reactions).

2.5.3. SSR marker analysis

SSR marker analysis was conducted using primers designed after [11] and following their PCR protocol and amplification conditions for particular loci amplified. Visualisation of products was carried out in 3% agarose gels or sequencing polyacrylamide gels, followed by ^{33}P autoradiography.

2.5.4. Linkage analysis

Linkage analysis was performed using a computer programme MAPMAKER 3.0 [12], the order of markers was calculated with LOD of 3.0 and map distances were estimated with the use of the Kosambi mapping function [13].

3. RESULTS AND DISCUSSION

3.1. Selection of root growth mutants

The analysis of 381 dwarf and semi-dwarf mutants showed a great variability in the seminal root length, and the length of the first leaf. More than 40% (163) of mutants produced seedling roots significantly shorter than the parent, while 3% (12) of semi-dwarfs had root significantly longer than their respective parent (Fig. 1). The majority of mutants had the same number of seminal roots as their parent variety, and only two mutants with significantly higher or lower root number were found (Fig. 1). The first leaf of semi-dwarfs was shorter than in the parental variety in more than 50% (197) of mutants, while 30% (109) of them did not differ in this character in relation to the parent (Fig. 1). Among analysed mutants there were forms showing the lack of correlation between leaf and root length, although in the majority of mutant genotypes the correlation between root and stem length was strong ($r = 0.585$ for the whole collection).

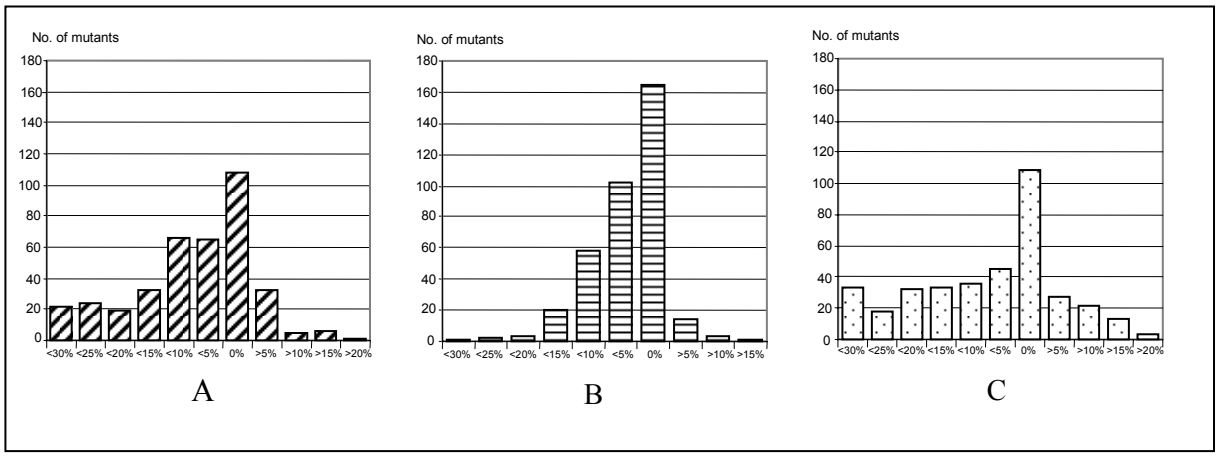


Fig. 1. Distribution of percentage changes in seedling root length (A), root number (B) and first leaf length (C) of barley mutants compared with the parent variety.

3.2. Genetic analysis of selected root mutants

For further analysis, two mutants were selected: 035AR from variety 'Aramir' and 225DV from variety 'Diva'. At the spike emergence stage (6 week-old plant), both mutants developed shorter roots than the respective parent variety (Fig. 2).

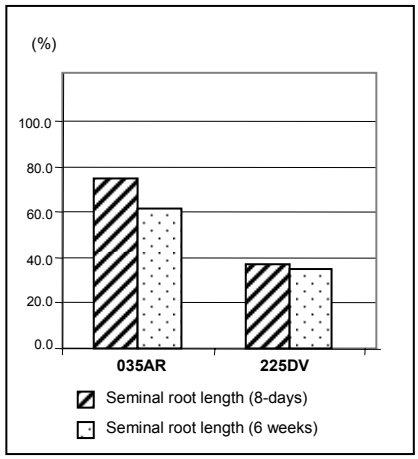


Fig. 2. The relative seminal root length of two selected barley mutants in relation to the parent variety.

3.2.1. Characteristics of the selected mutants

Mutant 035AR showed significant reductions in the seminal root and the first leaf length at the seedling stage. The length of the longest seminal root and the first leaf reached 70% and 65% of the parent variety, respectively. At 6-weeks, seminal roots were shorter than roots of the parent variety by 40%. The mutant's shoot length at maturity stage was also reduced by 40%. The results of previous studies indicated that the semi-dwarf shoot of mutant 035AR was controlled by the allele at the *brh1* locus (previously designated *br)* [14], on the short arm of chromosome 7H [15, 16].

Mutant 225DV showed three altered traits in comparison to its parent Diva. At both, seedling and spike emergence stages, the mutant developed seminal roots 65% shorter than the parent. The shoot length at seedling and maturity stages reached only 40% of the parent variety. Additionally, mutant 225DV developed very short root hairs [17].

3.2.2. Seminal root length at seedling stage

Genetic analysis of root length at the seedling stage indicated that observed changes in the root growth were recessive characters in both mutants. F_1 plants developed the same root phenotype as the respective parent variety. Analysis of seminal root length in F_2 generation indicated that a single gene is responsible for mutated character in each mutant. Values of chi^2 test calculated for 3:1 segregation were consistent with monogenic inheritance of mutated genotypes (Table I).

TABLE I. SEGREGATION FOR SEMINAL ROOT LENGTH IN THE F_2 PROGENY OF CROSSES BETWEEN ROOT LENGTH MUTANTS AND THEIR PARENT VARIETIES

Genotype	Number of seedlings	Seminal root length (cm) $\bar{x}\pm$ SD	Number of F_2 seedlings with the phenotype of		$\chi^2_{3:1}$
			parent variety	mutant	
Aramir	30	30.6±0.9[a*]			
035AR	30	20.0±1.7[b]			
F_1 Aramir x 035AR	4	30.1±1.1[a]			
F_2 Aramir x 035AR	145		109	36	0.002
Diva	30	28.3±0.3[a]			
225DV	29	7.7±0.7[b]			
F_1 Diva x 225DV	5	27.1±0.8[a]			
F_2 Diva x 225DV	187		134	53	1.11

* Values in the same column for the set of data (mutant, parent variety and F1) designated with the same letter do not differ significantly, according to the Duncan range test, P=0.05

3.2.3. Root and shoot length at seedling stage

The analysis of the first leaf and the longest seminal root length of the F_2 progeny of the cross Aramir x 035AR performed at the seedling stage demonstrated that two separate genes controlled these traits (Fig. 3). A small grouping of recombinants was present in the F_2 generation indicating linkage between the analysed loci. The result was confirmed by $chi^2_{Linkage}$ test (Table II). The distance between the locus controlling length of the first leaf and the locus responsible for seminal root length was 27.4 cm.

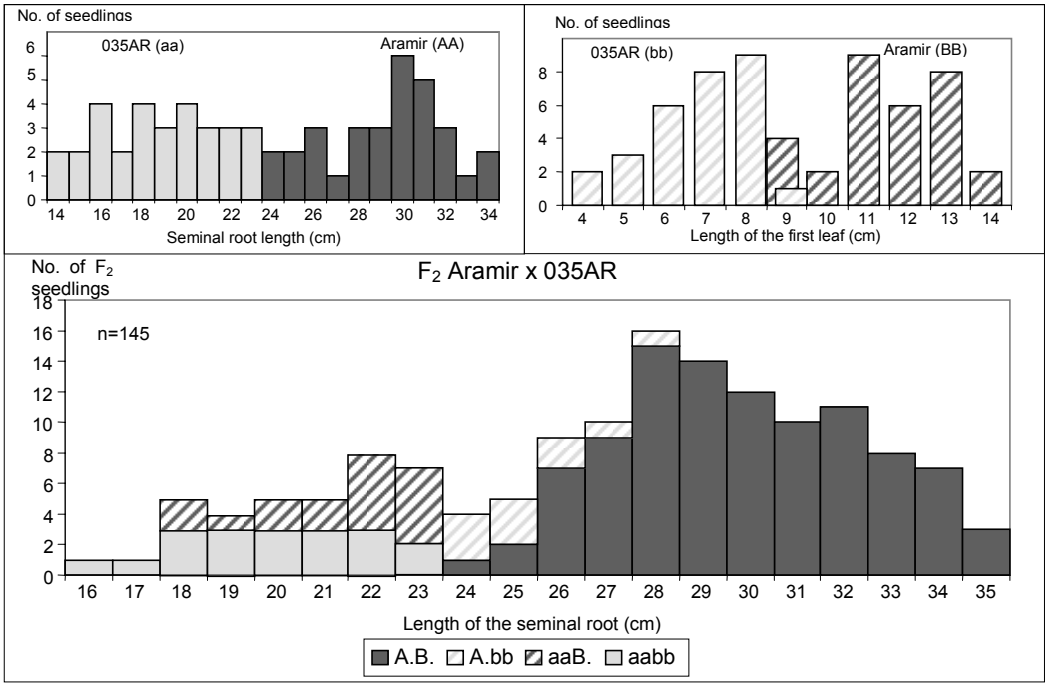


Fig. 3. Distribution of seminal root length and the length of the first leaf in F_2 generation of the cross 'Aramir' x 035AR in four phenotypic classes: A.B. – 'Aramir' phenotype, (A) long root (B) long first leaf, aabb – 035AR phenotype (a) short root (b) short first leaf, and recombinant phenotypes A.bb (long root, short first leaf) and aaB. (short root, long first leaf).

TABLE II. GENETIC ANALYSIS OF SEMINAL ROOT LENGTH AND THE FIRST LEAF LENGTH IN THE F_2 GENERATION OF THE CROSS 'ARAMIR' X MUTANT 035AR

Number of seedlings with a phenotype				$\chi^2_{3:1A,a}$	$\chi^2_{3:1B,b}$	χ^2_L	Recombination (%)
A.B.	A.bb	aaB.	aabb				
99	10	13	23	0.002	0.39	43.04*	27.4

*χ^2_L>3.84, P=0.05

A.B. – parental phenotype (long seminal root, long leaf), A.bb – recombinant phenotype (long seminal root, short leaf), aaB. – recombinant phenotype (short seminal root, long leaf), aabb – mutant phenotype (short seminal root, short leaf)

A similar analysis was performed in the F_2 of the cross 'Diva' and mutant 225DV. The length of the longest seminal root, first leaf and root hair morphology were evaluated in F_2 progeny. The results presented in Figs 4–6 indicated linkage between three genes responsible for these traits. The values of $chi^2_{Linkage}$ tests confirmed these observations (Table III-V).

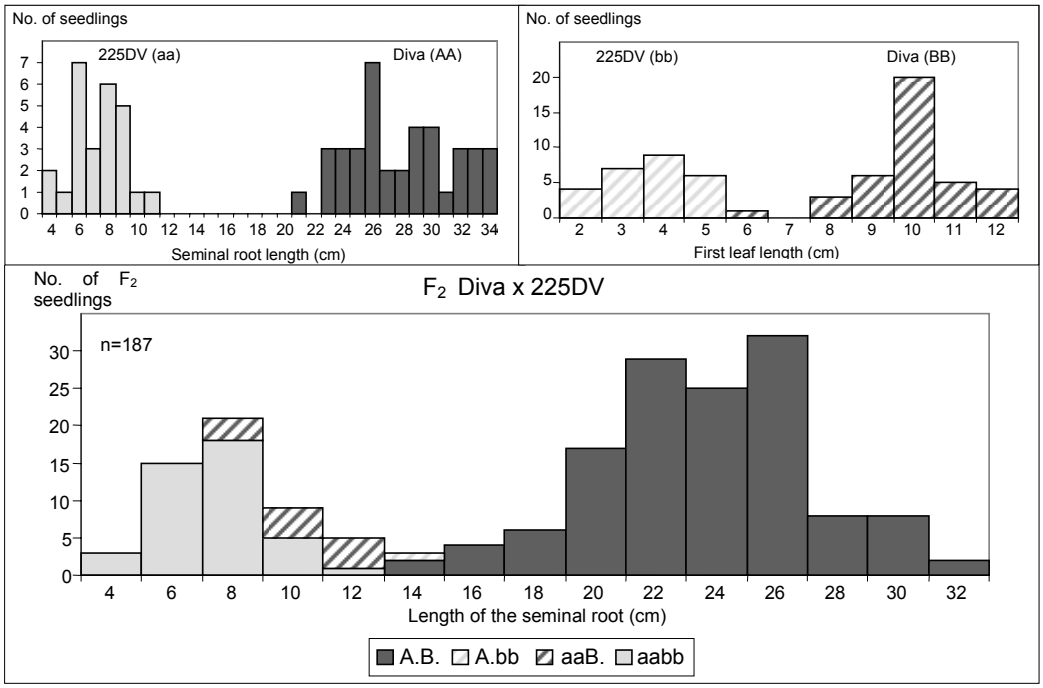


Fig. 4. Length distribution of seedlings with various seminal root length and the length of the first leaf in the F2 'Diva' x 225DV in four phenotypic classes: A.B. –'Diva' phenotype, (A) long root (B) long first leaf, aabb – 225DV phenotype (a) short root (b) short first leaf, and recombinant phenotypes A.bb (long root, short first leaf) and aaB. (short root, long first leaf).

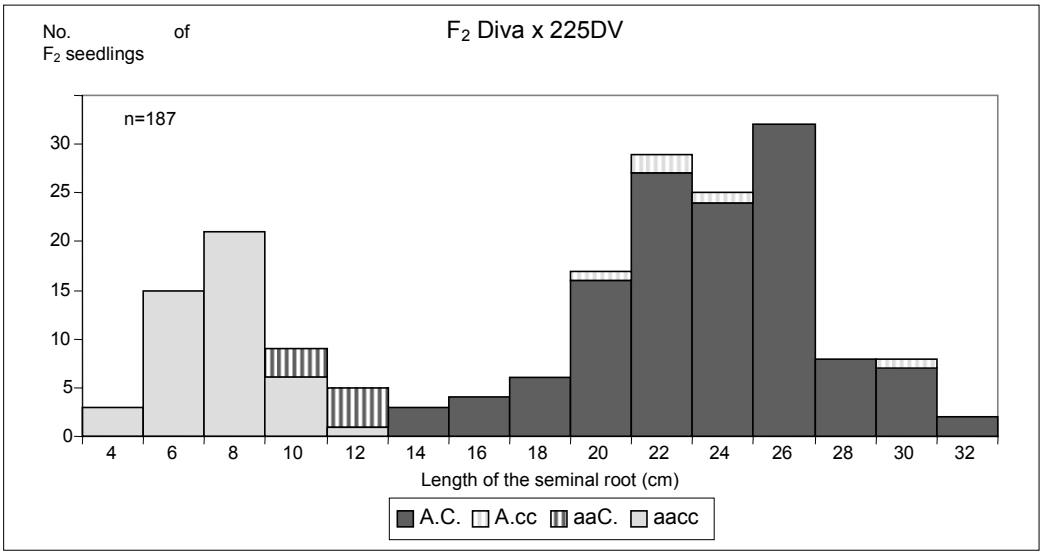


Fig. 5. Distribution of seminal root length and root hair morphology in the F_2 generation of the cross 'Diva' x 225DV in four phenotypic classes: A.C. – 'Diva' phenotype (A) long root (C) normal root hairs; aacc – 225DV phenotype (a) short root (c) short root hairs and recombinant phenotypes A.cc (long root, short root hairs) and aaC. (short root, normal root hairs).

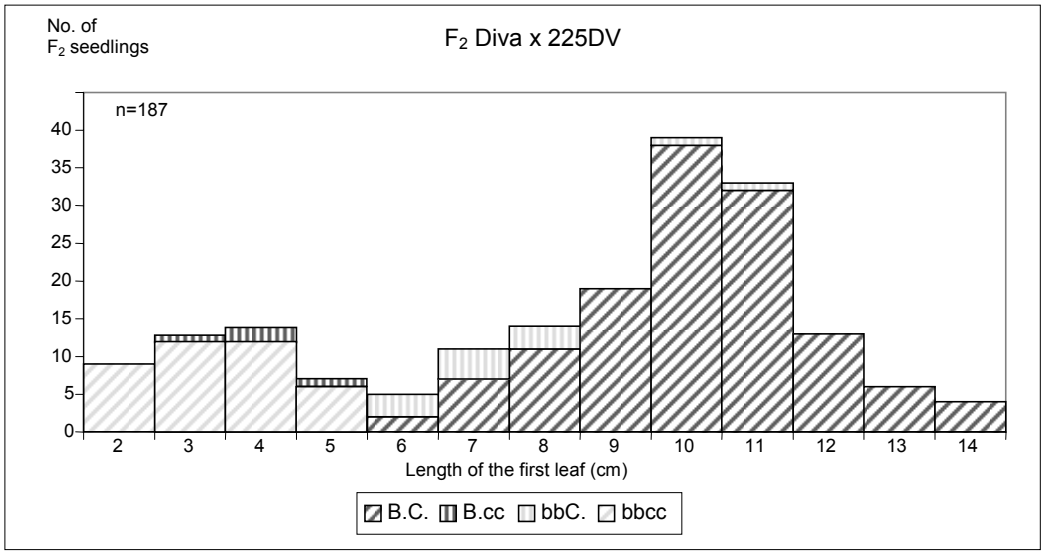


Fig. 6. Distribution of length of the first leaf and root hair morphology in the F_2 generation of cross 'Diva' x 225DV in four phenotypic classes: B.C. – Diva phenotype (B) long first leaf (C) normal root hairs; bbcc – 225DV phenotype (a) short first leaf (c) short root hairs; and recombinant phenotypes B.cc (long first leaf, short root hairs) and bbC. (short first leaf, normal root hairs).

TABLE III. GENETIC ANALYSIS OF SEMINAL ROOT LENGTH AND THE FIRST LEAF LENGTH IN THE F2 GENERATION OF THE CROSS 'DIVA' X MUTANT 225DV

Number of seedlings with a phenotype				$\chi^2_{3:1A,a}$	$\chi^2_{3:1B,b}$	χ^2_L	Recombination (%)
A.B.	A.bb	aaB.	aabb				
133	1	11	42	1.11	0.40	134.06*	7.0

*χ^2_L>3.84, P=0.05. A.B. – parental phenotype (long seminal root, long leaf), A.bb – recombinant phenotype (long seminal root, short leaf), aaB. – recombinant phenotype (short seminal root, long leaf), aabb – mutant phenotype (short seminal root, short leaf)

TABLE IV. GENETIC ANALYSIS OF SEMINAL ROOT LENGTH AND ROOT HAIR MORPHOLOGY IN THE F2 GENERATION OF THE CROSS 'DIVA'X MUTANT 225DV

Number of seedlings with a phenotype				$\chi^2_{3:1A,a}$	$\chi^2_{3:1C,c}$	χ^2_L	Recombination (%)
A.C.	A.cc	aaC.	aacc				
129	5	7	46	1.11	0.52	152.72*	6.8

*χ^2_L>3.84, P=0.05

A.C. – parental phenotype (long seminal root, normal root hair), A.cc – recombinant phenotype (long seminal root, short root hairs), aaC. – recombinant phenotype (short seminal root, long root hairs), aacc – mutant phenotype (short seminal root, short root hairs)

TABLE V. GENETIC ANALYSIS OF THE FIRST LEAF LENGTH AND ROOT HAIR MORPHOLOGY IN THE F2 GENERATION OF THE CROSS 'DIVA' × MUTANT 225DV

Number of seedlings with a phenotype				$\chi^2_{3:1B,b}$	$\chi^2_{3:1C,c}$	χ^2_L	Recombination (%)
B.C.	B.cc	bbC.	bbcc				
132	12	4	39	0.40	0.52	112.4*	13.8

*χ^2_L>3.84, P=0.05. B.C. – parental phenotype (long first leaf, normal root hair), A.cc – recombinant phenotype (long first leaf, short root hairs), aaC. – recombinant phenotype (short first leaf, long root hairs), aacc – mutant phenotype (short first leaf, short root hairs).

3.2.4. Root and shoot length of 6-week old and mature plants

One hundred and eight F_2 seedlings of the cross 'Aramir' x mutant 035AR were analysed in PVC tubes. For each plant the following measurements were taken: - first leaf length of 8-day seedlings, - length of the longest seminal root of 6-week plants, - length of the shoot at maturity. The results of measurements proved that the length reduction of the first leaf and the shoot at maturity was controlled by the same gene, i.e. locus *brh1* (Fig. 7).

The presence of F_2 recombinants with long shoot and short root (and otherwise) confirm the results obtained at seedling stage that two separate genes are responsible for root and shoot length reduction in 035AR. Root length reduction in 035AR was caused by the separate mutation in a locus linked to *brh1* gene on the short arm of chromosome 7H.

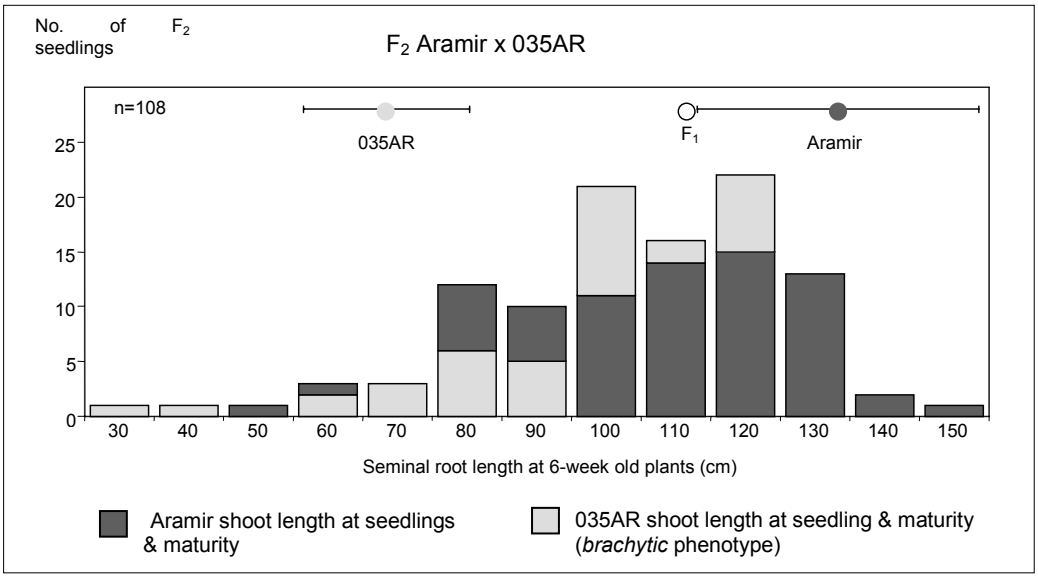


Fig. 7. Distribution of shoot length of 6-week old plants and the shoot length at maturity in an F_2 population of the cross 'Aramir' x 035AR.

3.2.5. Allelism test

Analysis of the F_2 generation of the cross 035AR x 225DV was performed at the seedling stage. F_1 plants developed roots as long as the parent lines. In the F_2 generation a segregation ratio 9:3:4 for root length was observed. The results indicated non-allelic relationships between loci controlling root length, with epistatic effect of the gene responsible for root shortening in mutant 225DV (Table VI, Fig. 8).

3.3. Cytological and anatomical studies

The aim of cytological and anatomical studies was to find out if mitotic activity or anatomical changes are responsible for the shortening of seminal roots in analysed mutants. The mitotic activity of mutant 035AR did not differ significantly from the parent variety (Fig. 9). However, the analysis of anatomical slides (cross and longitudinal sections) indicated smaller dimensions of mature cells in different root zones and tissues of mutant 035AR, compared to the parent 'Aramir' (Table VII).

TABLE VI. RESULTS OF ALLELISM TEST IN THE F_2 GENERATION OF CROSSES BETWEEN ROOT MUTANTS IN COMPARISON TO PARENT VARIETY

Genotype	Seminal root length (cm)	No. of plants with the phenotype			$\chi^2_{9:3:4}$
		Aramir or Diva	035AR	225DV	
Aramir	31.9±1.2[a*]				
Diva	28.2±0.4[b]				
035AR	18.1±1.1[c]				
225DV	8.4±0.4[d]				
F_2 035AR x 225DV	30.6±1.4[ab]	47	14	21	0.16

* Values in the same column for the set of data (mutant, parent variety and F_1) designated with the same letter do not differ significantly, according to the Duncan range test, P = 0.005

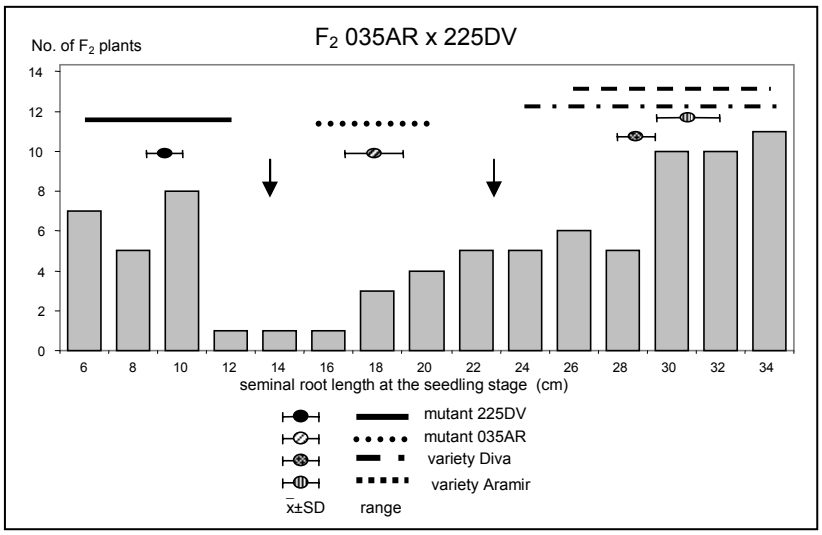
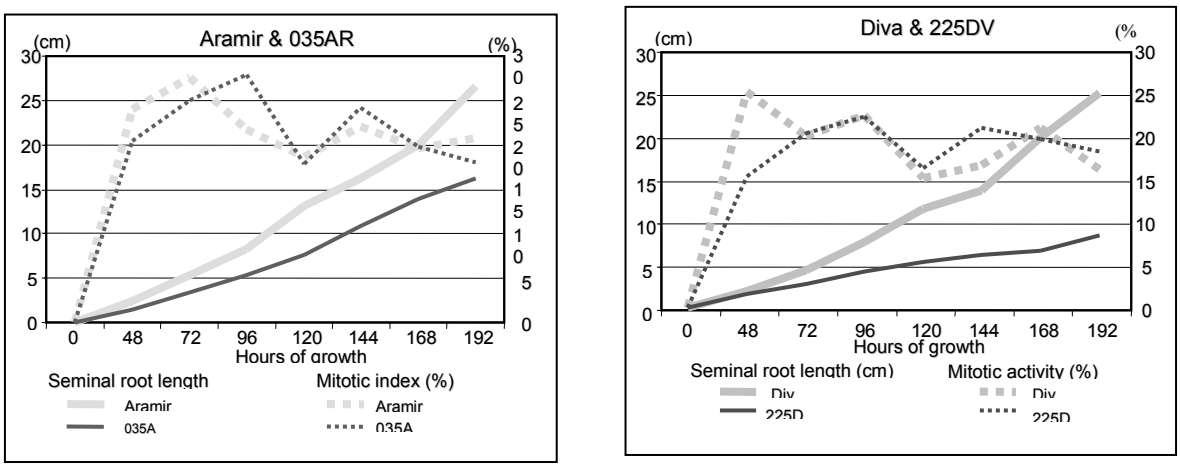


Fig. 8. Results of allelism test – seminal root length distribution in the F_2 of the cross 035AR x 225DV.



A B

Fig. 9. Comparison of mitotic index and dynamics of root growth in 'Aramir' and its mutant 035AR (A) and 'Diva' and its mutant 225DV (B) during the first 192 h.

TABLE VII. LENGTH DIFFERENCES IN ANATOMICAL PARAMETERS BETWEEN MUTANT 035AR AND IT'S PARENT 'ARAMIR'

Length (μ)	Aramir	035AR
	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Root diameter	412.3±17.8[a]	381.6±14.1[b]
Cortex cell diameter	23.2± 1.6[a]	22.3±1.4[a]
Meristematic zone	889.5±87.5[a]	662.5±42.8[b]
Elongation zone	1523.2±48.5[a]	1173.2±42.9[b]
Cells of differentiation zone	154.6±2.9[a]	126.8±1.8[b]

* Values in the same row for the set of data (mutant and parent variety) designated with the same letter do not differ significantly, according to the Duncan range test, P = 0.005

The mitotic index of mutant 225DV was lower than the mitotic index of 'Diva', but only in the first period analysed (48 h from the beginning of germination), there was no significant difference afterwards (Fig. 9). Root anatomical studies showed that mutants' short root length could be caused by the smaller cell dimensions than in the parent variety in all root zones and tissues (Table VIII).

TABLE VIII. LENGTH DIFFERENCES IN ANATOMICAL PARAMETERS BETWEEN MUTANT 225DV AND IT'S PARENTAL, 'DIVA'

Length (μ)	Diva	225DV
	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Root diameter	462.1±44.6[a]	307.7±3.0[b]
Cortex cell diameter	33.9± 5,5[a]	23.2±1.4[b]
Meristematic zone	928.6±3.4[a]	460.5±39.2[b]
Elongation zone	1501.8±30.3[a]	587.5±32.1[b]
Cells of differentiation zone	167.2±13.0[a]	124.1±10.2[b]

* Values in the same row for the set of data (mutant and parent variety) designated with the same letter do not differ significantly, according to the Duncan range test, P = 0.005

3.5. Molecular analysis

3.5.1. Analysis of AFLP polymorphism between root system mutants and their parents

'Aramir' and mutant 035AR were identical in AFLP profile. The comparison of 'Diva' and mutant 225DV resulted in detection of 0.59% changes between them (Table IX).

TABLE IX. LEVEL OF AFLP POLYMORPHISM BETWEEN PARENT LINES AND INVESTIGATED MUTANTS

Genotype	Primer combinations	Total bands	Polymorphic bands	Polymorphism (%)
Aramir/ 035AR	20	1330	0	0
Diva /225DV	60	4258	25	0.59

4.5.2. Linkage analysis

Linkage analysis followed by localization of mutated genes responsible for the reduction of root system and the length of the stem was performed for the mutant 035AR from variety 'Aramir'. Genetic analysis made in former years showed that the two genes were linked, and that the semi-dwarf phenotype of mutant was caused by the mutation in the locus *brh1*, mapped in a distal part of the short arm of chromosome 7H. Therefore, molecular analysis focused on markers that mapped in the region of *brh1* gene, and parents of genetic mapping populations were targeted in the search for polymorphic markers. Altogether 6 AFLP primers combinations from the map for 'Steptoe' x 'Morex' mapping population [18] and 5 SSR markers from the map for 'Lina' x *H. spontaneum* (L/*H.sp*) population [9] were screened for polymorphism between mutant line 035AR and 'Morex' or 'Steptoe' varieties. The highest number of polymorphic markers from the target region was identified between 035AR and 'Steptoe', consequently searching for linkage between genes of interest and markers was performed within a F_2 population (148 plants) specially developed from the cross 035AR x 'Steptoe'. The AFLP and SSR markers used in the linkage analysis are presented in Table X.

TABLE X. MARKERS ANALYSED IN THE F2 GENERATION OF THE CROSS 035AR × 'STEPTOE'

Marker assay	Primer combination	Marker from 7H chromosome
AFLP	E32M60	E32M60S167
		E32M60M166
	E41M47	E41M47S138
	E35M47	E35M47S85
SSR		EBmac 0603

In total, the linkage map of the chromosome 7H, created in this analysis, spanned 158,6 cM and contained both genes responsible for analysed traits, 3 AFLP markers (Fig. 10) and EBmac0603 SSR marker (Fig. 11), mapped previously in S/M and L/*H.sp* mapping populations, respectively [11]. Additionally, two new AFLP markers: E41/M47nm1 and E41/M47nm2 were assigned to this linkage group. The gene responsible for the short root

system, designated *srt1* (*short root* 1) was located; with a high LOD score (4.2), 33.5 cM distal of the *brh1* locus (Fig. 12). The distance between the pairs of other markers were also long, however the LOD scores for each pair of markers were high, and ranged from 4.07 to 8.33 Saturation of this region was attempted by seeking additional markers (known from the Lina and *H. spontaneum* genetic map). The data are also consistent with a genome scan using the Bowman backcross mutant series where the *brh1* line (accession 1820 was found to be associated with short roots, Forster, personal communication).

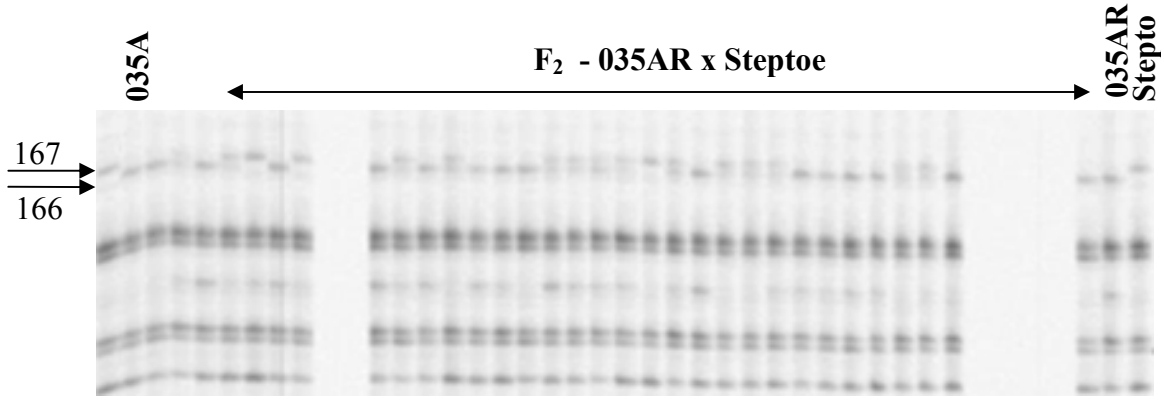


Fig. 10. Linkage analysis in the F_2 population of the cross 035AR x 'Steptoe' with AFLP markers E32M60S167 and E32M60S166.

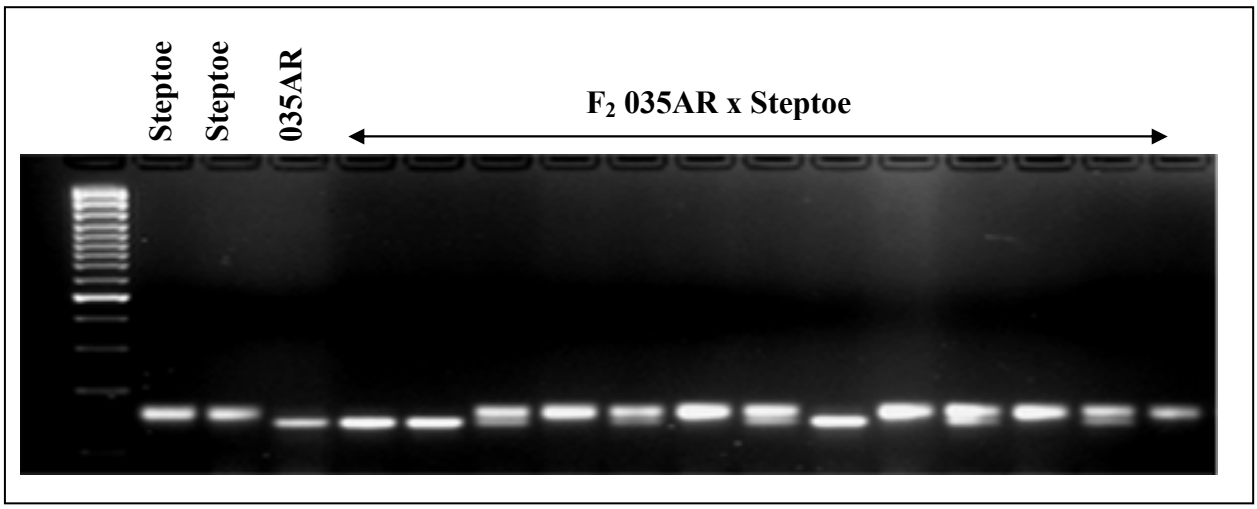


Fig. 11. Linkage analysis in the F_2 population of the cross 035AR x 'Steptoe' with SSR marker Bmac0603.

The identification of a gene responsible for short root in the mutant 035AR, revealed in this study, increased the number of known genes responsible for root development in barley. Up to now only a limited number of root mutants have been described. The most of them were reported in maize [19–21] and rice [22–24].

The majority of known genes responsible for root morphology and development have been described in *Arabidopsis*, the model plant species [25–29] It is anticipated that further mutational analysis in barley will reveal many genes controlling a wide range of root traits.

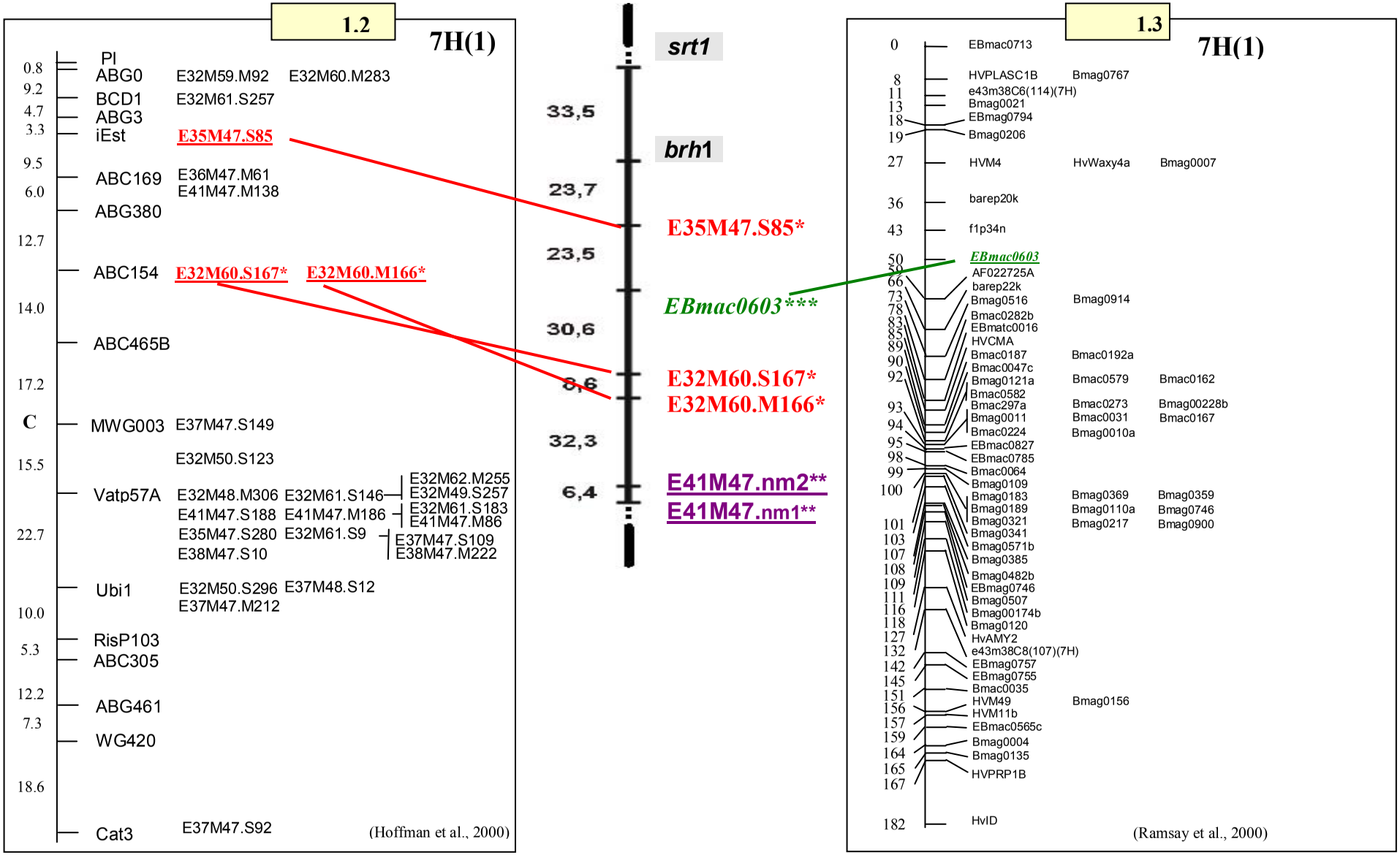


Fig. 12. The linkage group of the 7H chromosome with genes for the traits of interest and linked AFLP and SSR markers.
** – AFLP markers from S/M map; ** – non mapped AFLP markers assigned to the group; *** – SSR markers from the L/H.sp map*

REFERENCES

[1] Hetz, W., Hochholdinger, F., Schwall, M., Feix, G. 1996. Isolation and characterization of *rtcs* a maize mutant deficient in the formation of nodal roots. The Plant Journal 10:845-857.

[2] Hochholdinger, F., Feix, G. 1998. Early post-embryonic root formation is specifically affected in the maize mutant *ltr1*. The Plant Journal 16:247-255.

[3] Yao, S.G., Tageta, S., Ichii, M. 2002. A novel short-root gene affects specifically early root development in rice (*Oryza sativa* L.*)*. Plant Science 163:207-215.

[4] Tagliani, L., Nissen, S., Blake, T.K. 1986. Comparison of growth, exogenous auxin sensitivity, and endogenous indole-3-acetic acid content in roots of *Hordeum vulgare* L. and agravitropic mutant. Biochem. Genet. 24:839-848.

[5] Gahonia, T.S., Nielsen, N.E., Joshi, P.A., Jahoor, A. 2001. A root hairless barley mutant for elucidating genetic of root hairs and phosphorus uptake. Plant Soil 235:211-219.

[6] Tsuchiya, T. 1974. Root characters of curly mutants in barley. BGN 4:88-91.

[7] Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:437-497.

[8] Gerlach, D. 1972. Zarys mikrotechniki botanicznej. PWRiL, Warszawa pp. 37-78.

[9] Doyle, J.J., Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11-15.

[10] Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 21:4407-4414.

[11] Ramsay, L., Macaulay, M., Degli Ivanissevich, S., Maclean, K., Cardle, L., Fuller, J., Edwards, K.J., Tuvesson, S., Morgante, M., Massari, A., Maestri, E., Marmiroli, N., Sjakste, T., Ganal, M., Powell, W., Waugh, R. 2000. A simple sequence repeat-based linkage map of barley. Genetics 156:1997-2005.

[12] Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., Newburg, L. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

[13] Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. 12:172-175.

[14] Lundqvist, U., Franckowiak, J., Konishi, T. 1996. New and revised names for barley genes. BGN 26:22-44.

[15] Szarejko, I., Maluszynski, M. 1984. New *brachytic* mutant of spring barley variety Aramir. BGN 14:33-35.

[16] Franckowiak, D.J. 1991. Allelism tests among selected semi dwarf barleys. BGN 21:17-23.

[17] Szarejko, I, Kwasniewski, M., Janiak, A., Chmielewska, B., Karcz, J., Nawrot, M., Maluszynski, M. 2004. Mutational analysis to study root hair formation in barley. The International Conference on the Status of Plant and Animal Research. PAG XII. San Diego, CA. p. 65.

[18] Hoffman, D.L., Hang, A. Burton, C. 2000. Interval mapping of AFLP markers for barley using a subset of doubled-haploid lines. Journal of Agricultural Genomics http://www.cabi-publishing.org/jag/papers00/paper200/indexp200.html.

[19] Dolfini, S., Landoni, M., Consonni, G., Rascio, N., Vecchia, F.D., Gavazzi, G. 1999. The maize *liliputian* mutation is responsible for disrupted morphogenesis and minute stature. Plant J. 17:11-17.

[20] Hochholdinger, F., Park, W.J., Feix, G. 2001. Cooperative action of *SLR1* and *SLR2* is required for lateral root specific cell-elongation in maize. Plant Physiol. 125:1529-1539.
[21] Hochholdinger, F. 2002. ZMBP – Research Activities 2002. http://www.Frank Hochholdinger.html.
[22] Debi, B.R., Mushika, J., Taketa, S., Miyao, A., Hirochika, H., Ichii, M. 2003. Isolation and characterization of short lateral root mutant in rice (*Oryza sativa* L.). Plant Sci. 165**:**895-903.
[23] Hao, Z., Ichii, M. 1999. A mutant RM109 of rice (*Oryza sativa* L.) exhibiting altered lateral root initiation and gravitropism. Jpn. J. Crop. Sci. 68:245-252.
[24] Ichii, M., Ishikawa, M. 1997. Genetic analysis of newly induced short-root mutants in rice (*Oryza sativa* L.). Breed Sci. 47:121-125.
[25] Benfey, P.N., Linstead, P.J., Roberts, K., Schiefelbein, J.W., Hauser, M-T., Aeschbacher, R.A. 1993. Root development in *Arabidopsis*: four mutants with dramatically altered root morphogenesis. Development 119:57-70.
[26] Aeschbacher, R.A., Schiefelbein, J.W., Benfey, P.N. 1994. The genetic and molecular basis of development. Plant Physiol. Plant Mol. Biol. 45:25-45.
[27] Baskin, T.I., Cork, A., Williamson, R.E., Gorst, R. 1995. *STUNTED PLANT1*, a gene required for expansion in rapidly elongating but not in dividing cells and mediating root growth responses to applied cytokinin. Plant Physiol. 107:233-243.
[28] Hauser, M-T., Morikami, A., Benfey, P.N. 1995. Conditional root expansion mutants of *Arabidopsis*. Development 121:1237-1252.
[29] Di Laurenzio, L., Wysocka-Diller, J., Malamy, J.E., Pysh, L., Helariutta, Y., Freshour, G., Hahn, M.G., Feldmann, K.A., Benfey, P.N. 1996. The *SCARECROW* gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. Cell 86:423-433.

GENETIC ANALYSIS OF ROOT FORMATION IN MAIZE

G. FEIX[1], M. SAUER[2], K. WOLL[2], F. HOCHHOLDINGER[1+2]
[1] Institute of Biology III,
University of Freiburg, Freiburg, Germany
[2] Center for Plant Molecular Biology (ZMBP),
University of Tuebingen, Tuebingen, Germany

Abstract

Monogenic recessive mutants deficient in the initiation of post-embryonic shoot-born and lateral roots were isolated from segregating M_2 populations of mutated maize stocks and phenotypically and genetically analysed indicating the existence of root-type specific mechanisms of root formation. Complementary to the formal genetic approach, molecular genetic tools including proteomics and RNA profiling with microarray chips were applied to obtain more information on the complex genetic network governing the build-up of the root stock.

1. INTRODUCTION

The agricultural use of maize has greatly profited from the longstanding genetic analysis of the development and performance of the above ground part of the maize plant. A corresponding study of the root stock was, however, neglected in the past. Great efforts are now undertaken to overcome this lack of genetic analysis showing that the currently available genetic tools can be successfully applied for the study of root development. The root system of maize represents a highly variable structure and consists of different root types initiated late in embryogenesis (primary- and seminal lateral roots) or post embryogenically (crown-, brace- and lateral roots). The anatomical structure of the different root types is very similar, they are, however, initiated from different tissues. A more detailed description of the maize root system is given elsewhere [1, 2]. The contribution of our group in the genetic analysis of root formation was focussed on the dissection of the genetic basis for the initiation and development of shoot-born roots, namely the stem node-derived crown roots and the lateral roots initiated at all root types. These postembryonic roots start growing at the young seedling level and represent later the root stock of the mature plant.

2. GENETIC ANALYSIS OF ROOT INITIATION

Monogenic recessive mutants deficient in the initiation of root development were isolated from segregating M_2 population of mutated (by chemical mutagenesis or transposon mutagenesis) maize lines. The mutant screen either performed with young seedlings on a wet paper towel test or with more grown-up young plants in field experiments led to the isolation of several mutants with deficiencies in root initiation and development. Emphasis will be given here on the description of experiments performed in the time period of this CRP which deal with the initiation mutants *rtcs* (lacking crown roots) and *lrt1* and *rum1* (lacking lateral roots).

The loss of function mutant *rtcs* (deficient in the formation of crown roots emerging from nodes) acts at an early stage of root growth (no primordia formation) [3]. The only main root-type that remains in *rtcs* is the primary root. The effect of the mutation in *rtcs* seems to be highly specific since no pleiotropic effects on other parts of the plant have been observed. The deficiency of the mutant is confined to the root initiation part of the nodes with no influence on their tiller forming part as tested by introgressing the *rtcs* locus into the heavily

tillering gaspe flint line. The RTCS locus was mapped with the help of microsatellite markers and by a B-A translocation test to the small arm of chromosome 1.

The mutant *lrt1* lacks the initiation of lateral roots at the early seedlings stage (no primordia formation), i.e., no lateral roots emerge from the primary root or from the crown roots that grow out of the coleoptilar node [4]. Lateral root formation is, however, only affected in the embryonic primary- and seminal roots but not in the shoot-born root system as lateral root growth resumes later leading to normally developed root systems of the mature plant. The LRT1 locus was mapped to chromosome 2S by B-A translocation analysis. The chromosomal map positions of the mutant loci of *rtcs* and *lrt1* indicate that a larger root locus combining root relevant genes is not evident from the available data. The mutant *rum1* is not only deficient in the primordia formation for lateral roots on the primary root but also in the primordia formation for the embryonic lateral seminal roots showing an embryonic and post-embryonic phenotype [5]. Interestingly, the mutant *rtcs* shows a comparable phenotypic behaviour in that the lateral roots on the primary root are formed normally but all later postembryonic roots, including all shoot-born roots are missing.

Double mutants prepared from *lrt1* and *rtcs* show a strict additive behaviour indicating at least two independent mechanisms in the formation of root primordia. Gene functions with an influence on several root types had been seen by most of the genes impaired in the available mutants. The transient occurrence of the deficiency of most mutants indicates that various steps of root formation depend on sets of signals operating for defined periods of time during the development of the plant. A more extensive characterization of the genetic behaviour of the mutants and double mutants together with the interpretations of further genetic tests is given elsewhere [1, 2].

3. PROTEOMIC AND MICROARRAY ANALYSIS

The recently established *proteomic* technology has become crucial for comprehensive studies of the function of expressed proteins for a particular growing stage of a plant [6]. The separation of a cellular protein set by sensitive 2 D-gel electrophoresis followed by the characterization of isolated protein spots by mass spectrometry and the biometric evaluation of the obtained data generates a unique image of the active proteins in the cell. This approach can for example be used for the comparison of a tissue carrying a mutation with its wild-type complement. This has been done in the case of the primary root by comparing this tissue from *lrt1* and wild-type showing that the absence of lateral roots has great impact on the overall composition of the active proteins of the primary root.

Microarray based RNA profiling techniques have the capacity to display the transcriptome of a particular tissue or even of a particular cell-type if the corresponding cell-layer can be isolated in pure form [7]. This has recently been achieved in the case of maize roots for the pericycle cell layer by using the recently established laser capture micro dissection method for the isolation of the pericycle layer. Submitting pericycle tissue isolated in this manner to an RNA analysis by microchip (carrying the complement of 12,160 different maize cDNAs) tests allowed a comparison of the transcriptome from wild-type pericycle cells to non-pericycle cells. This technique allows also for the monitoring of the RNA diversity present between two different genotypes such as a mutant and its corresponding wild-type as shown in the case of pericycle cells from the mutant *rum1* and the corresponding wild-type.

4. OUTLOOK

The work described here on the genetic and molecular analysis of root formation in maize although still incomplete can be considered as a case study to be adapted for the genetic study of the root system of other cereals. The work with maize will be continued among others along the following lines:

Work in progress on the isolation and analysis of the genes affected in the mutants *rtcs* and *lrt1* will initiate further studies towards an identification and understanding of the molecular basis for stem derived root formations in maize. Comparable studies in other cereals will profit from the maize data if used for the screening of other genomes by comparative genomics. The use of DNA sequences of the isolated gene structures will furthermore be very helpful for the establishment of advanced mapping tools in support of breeding efforts and for attempts of allelic mining concerning these mutants.

The ongoing proteomics studies as well as the microarray based RNA profiling experiments will continue to be used for connecting the genes impaired in the mutants to the complex mechanism of interacting genes and their coordinated expression during root formation and to identify target genes of the mutated gene structure.

Root specific genes identified from cDNA libraries or from the proteomics or microarray chip approaches may be used for the isolation of new root mutants by reverse genetic procedures using for example the Trait Utility System of Corn (TUSC) with its genetic knock-out approach.

An improved assessment and knowledge of developmental and environmental influences on the timing and extent of the synthesis of crown roots and higher order lateral root structures will become relevant for breeding efforts concerning agronomic traits such as the root lodging resistance and the extent of the root stock branching with its influence on preferential deep/or shallow rooting.

REFERENCES

[1] Feix, G., Hochholdinger, F., Park W.J. “Maize root system and genetic analysis of its formation”, Plant roots – the hidden half, (Waisel, Y., Eshel, A., Kafkafi, U., Eds), Marcel Dekker, New York (2002) 239-248.

[2] Hochholdinger, F., Woll, K., Sauer, M., Dembinsky, D. Genetic dissection of root formation in maize (*Zea mays*) reveals root-type specific developmental programmes, Ann. Bot. (2004) 93:359-368.

[3] Hetz, W., Hochholdinger, F., Schwall, M., Feix, G. Isolation and characterization of *rtcs* a mutant deficient in the formation of nodal roots, Plant J. (1996) 10:845-857.

[4] Hochholdinger, F., Feix, G. Early post-embryonic root formation is specifically affected in the maize mutant *lrt1*, Plant J. (1998a) 16:247-255.

[5] Woll, K., Hochholdinger, F. Isolation of a new root mutant *rum1* affected in lateral and seminal root initiation. Maize Gen. Coop. Newsletter 78 (in press).

[6] Rose J.K., Bashir S., Giovannoni J.J., Jahn M.M., Saravanan R.S. Tackling the plant proteome: practical approaches, hurdles and experimental tools. Plant J. (2004) 39:715-33.

[7] Schnable P.S., Hochholdinger F., Nakazono M. Global Expression Profiling Applied to Plant Development. Current Opin. Plant. Biol. (2004) 7:50-56.

IDENTIFICATION OF MUTANTS WITH TOLERANCE TO RHIZOSPHERIC STRESSES AND INHERITANCE AND QTL MAPPING OF RELATED ROOT TRAITS IN SOYBEAN

J. GAI, Y. LIU, H. LÜ, T. ZHAO, D. YU
National Center for Soybean Improvement,
Nanjing Agricultural University, Nanjing, China

Abstract

A sample of soybean accessions (*Glycine max* (L.) Merr.) from Huanghe-Huaihe- Haihe and Middle-Lower Changjiang Valleys was evaluated for their tolerance to rhizospheric stresses; namely; drought, aluminium toxicity and low phosphorus. Fifteen accessions with Rank 1 abiotic stress tolerance were screened out. Drought tolerance was found to be significantly (0.01) correlated with the relative values of total root length (RL), root volume (RV) and dry root weight (DW) per plant. The same relationship was found for aluminium toxicity tolerance with the stressed to unstressed ratios of number of lateral roots, tap RL, total RT, RV and DW. Segregation analysis indicated that between the two parents of the RIL population Kefeng 1×Nannong 1138-2 (Rank 1×Rank 4), the relative values of DW, total RL and RV were respectively controlled by two major genes (linked together for the latter two traits with recombination value 4.30% and 1.93%, respectively) plus modifiers with their major gene heritability values 62.26~91.81%. Between the two parents of the RIL population Bogao×NG94-156 (Rank 4×Rank 2), the ratio values of lateral root number (RN), tap RL, total RL and DW were controlled by three major genes plus modifiers with their major gene heritability values being 80.22~91.81%, while the ratio value of RV was controlled by three major genes with a heritability value of 93.44%. The Kefeng 1×Nannong 1138-2 RIL population was also used for mapping QTLs of relative DW, RL and RV related to drought tolerance. Five, three, and five QTLs located on Linkage group N6-C2, N8-D1b+W, N11-E and N18-K for each of the three traits, respectively, were identified. Each of them appeared to have one locus (*Dw1, Rl1, and Rv1*) with relatively large effect in comparison with their other loci, and the above three loci were closely linked to one another on the linkage group N6-C2. The results between segregation analysis and QTL mapping appeared to be consistent, therefore could be used for verifying each other.

1. INTRODUCTION

Abiotic stresses are a group of non-biological factors limiting the yield potential of a soybean cultivar in addition to biotic stresses [1–4]. In the arid and semi-arid northern parts of China soybean production is often affected by drought stress throughout the growing season, this is further compounded by high temperatures which affect pod setting and seed filling, resulting in low yields. In southern China there is a problem of aluminium toxicity that is related with low phosphorous and acidic red soils. In the coastal areas there is a problem of soil salinity. In the past research on tolerance to individual stress factors used to be conducted separately, now there is a need to work on multiple tolerances to the different stresses.

The first plant organ to be affected by rhizospheric stresses is the under ground part or root. It is the root that then conveys the stress signal to the above ground parts [5–8]. Consequently, root traits should be considered in addition to the above-ground parts when tolerance to stresses in the rhizosphere is concerned. Unfortunately, very few studies on soybean root traits, especially related with rhizospheric stresses are reported. The present paper is a summary of identification of mutants with tolerance to rhizospheric stresses and inheritance and QTL mapping of related root traits in soybeans.

2. MATERIALS AND METHODS

2.1. Test Materials

A total of 301 accessions were sampled from various eco-regions in Huanghe-Huaihe-Haihe and Middle-Lower Changjiang Valleys in China and surveyed for their root morphological performances around R4 stage. Obvious variation of soybean root structure was found among the accessions. An overall classification of soybean root architecture was conducted as follows:

- Type 1, taproot type, with long dominant taproot with thinner basal roots emerging from the taproot, accounted for about 12~37% of the tested population,
- Type 2, fibrous root type, without obvious difference between the taproot and basal root in length and diameter, accounted for about 8~18 %,
- Type 3, the intermediate root type, with taproot and basal roots both relatively dominant although the former a little thicker, accounted for about 45~80%.

Both Type 1 and Type 2 tended to be drought tolerant type. According to the root type, 62 entries from 10 provinces in Huanghe-Huaihe-Haihe and Middle and Lower Changjiang Valleys were selected for further stress tolerance study (Table I).

2.2. Indexing and Evaluation of Tolerance to Abiotic Stresses

2.2.1. Tolerance to drought and aluminium toxicity

2.2.2. Drought tolerance at seedling stage

For the valuation of drought tolerance at seedling stage, 62 accessions were grown in soil (sand: clay = 85:15) in pots (ϕ25×h28 cm) in the greenhouse in 2001 and 2002. Two plants were grown per pot in three replications, using a split plot design (major-plot treatment was water amount (Table II) and sub-plot treatment was the accession). Water treatment was started when the first pair of leaves unrolled.

The plant height, number of leaves, above-ground (above cotyledon node) dry weight and under-ground (below cotyledon node) dry weight were measured and evaluated at the 18th day after water treatment.

For each trait per accession, the membership index F_{ij} value was calculated from the ratio of values obtained in drought stress to normal conditions, and then the mean membership index averaged over all the traits (F_i) was used as an indicator of tolerance to a given stress.

$$F_{ij} = \frac{X_{ij} - X_{\min}}{X_{\max} - X_{\min}} \; ; \quad F_i = \Sigma F_{ij} / n$$

TABLE I. MATERIAL USED IN TESTS FOR STRESS TOLERANCES

Variety	Source	Tests*	Variety	Source	Tests*
HZ Bayuehuang	Shaanxi	D A	GC Yishuhou	Hubei	D A P
LY Yaoheidou	Shaanxi	D A	You 91-11	Hubei	D A P
NQ Laoshupi	Shaanxi	D A	Zhongdou 19	Hubei	D
ZB Xiaobaihuangdou	Shaanxi	D	Zhongdou 8	Hubei	D
Bianheidou	Shanxi	D A	1138-2×86-53	Jiangsu	D A
Jinda 53	Shanxi	D A	NG94-156	Jiangsu	A
Jindou 14	Shanxi	D A	Bogao	Jiangsu	A
Jindou 16	Shanxi	D	FX Sunlouzihuangdou	Jiangsu	D A P
Jindou 19	Shanxi	D	JR Dabianqingdou	Jiangsu	D A P
Yuanheidou	Shanxi	D	LH Dafengqing	Jiangsu	D A
Kefeng 1	Beijing	D A P	Lvbaozhu	Jiangsu	D A
3-29	Hebei	D A	Nannong 1138-2	Jiangsu	D A P
5-5	Hebei	D A P	Nannong 18-6	Jiangsu	D A
6-13	Hebei	D	Nannong 86-4	Jiangsu	D A P
Dawudou	Hebei	D A P	Nannong 88-29	Jiangsu	D A
Naiyinheidou	Hebei	D A	Nannong 88-31	Jiangsu	D A P
Yixianheidou	Hebei	D A P	Nannong 96B-2	Jiangsu	D A P
CS Xianheidou	Shandong	D A P	QD Heidou	Jiangsu	D A
WM Tiezhugan	Shandong	D A P	SH Dalihuangdou	Jiangsu	D A
Qihuang 10	Shandong	D A P	Suxie 1	Jiangsu	D A
Qihuang 1	Shandong	D A P	HF Guizilan	Jiangxi	D A
WS Gunlongzhu	Shandong	D A P	NC Zaibuyao	Jiangxi	D A P
CF Xiaotianedan	Henan	D A P	TG Xiazhidou	Jiangxi	D
RN Pingdingdou	Henan	D A P	YG Zaowudou	Jiangxi	D A
XX Xiaolihuang	Henan	D A P	CM Tiegendou	Shanghai	D A
XX Taipingzihuadou	Henan	D A P	SH Daqingdou (xuan)	Shanghai	D A P
XS Dalihuang	Henan	D A	DQ Xianzhudou	zhejiang	D A
CX Nongjiawuming	Anhui	D A	NH Wanhuangdou	zhejiang	D A
Liufeng	Anhui	D A P	XJ Xiaomaodou	zhejiang	D A P
SC Qujiahuangdou	Anhui	D	XC Liuyuedou	zhejiang	D A P
GY Heidou	Anhui	D A P	PI416937	USA	A

* D, A and P represent the material used for identification of drought, aluminium and low phosphorus stress tolerance, respectively

TABLE II. WATER APPLICATION IN THE 18 DAY DROUGHT TOLERANCE TEST IN 2001 AND 2002

Year	Water (ml/pot/day)		Soil moisture (%)	
	Stress	Control	Stress	Control
2001	30	100	6.7 ± 0.86	9.4 ± 1.08
2002	30	100	6.3 ± 1.22	9.1 ± 1.13

Where X_{ij} is the ratio of the ith accession, jth trait, X_{max} and X_{min} are the maximum and minimum ratio of the trait, F_{ij} is the membership index value of the ith accession, jth trait, and

F_i is the mean membership index averaged over n traits of the ith accession. The rank of an accession's drought tolerance is classified according to the following criteria:

Rank 1: $F_i > 0.8$, high tolerant type

Rank 2: $0.6 \leqslant F_i < 0.8$, tolerant type

Rank 3: $0.4 \leqslant F_i < 0.6$, medium type

Rank 4: $0.2 \leqslant F_i < 0.4$, susceptible type

Rank 5: $F_i < 0.2$, high susceptible type

2.2.3. Drought tolerance at reproductive stage

Evaluation of drought tolerance at the reproductive stage was done in 2001 using plant growth conditions similar to the seedling stage experiment. Eight traits were used in the experiment, these were, plant height, number of nodes on main stem, number of branches, number of pods per plant, number of seed per plant, 100 seed weight, dry root weight and above ground weight.

2.2.3. Aluminium Toxicity

Six concentrations of aluminium ions, i.e. 0 ppm, 7 ppm, 14 ppm, 21 ppm, 28 ppm and 35 ppm, were tested for PI 416937 in a randomized block design experiment with three replications under sandy culture in $\phi 25 \times h28$ cm pot watered with 1/5 Steteinburg nutrition solution adjusted to pH 4.1[9, 10]. Plant height, number of leaves, above ground dry weight and under ground dry weight were measured and evaluated after two weeks of treatment. The turning point for most of the traits was found to be 28 ppm, therefore, it was used for evaluation of aluminium toxicity tolerance and its inheritance study.

2.2.3.1. Aluminium toxin tolerance at seedling stage

The experimental design and evaluation procedure were the same as those of drought tolerance at seedling stage except that it was only tested in one year, 2002 and the major plot treatment being 28ppm aluminium concentration and subplot treatment being accession. Plant height, number of leaves, above-ground dry weight and under-ground dry weight were measured and evaluated after two weeks. The calculation of mean membership index and classification of tolerance rank were similar to those of drought tolerance at seedling stage.

2.2.4. Tolerance to low phosphorous

The experiment was similar to the above except major plot treatment being high phosphorus 1000μM/L ($Ca(H_2PO_4)_2$ 500μM/L) and low phosphorus 0.2 μM/L ($Ca(H_2PO_4)_2$ 0.1μM/L). The other nutrition prescription was K_2SO_4 0.75mM/L, KCl 0.1mM/L, $Ca(NO_3)_2$ 2mM/L, H_3BO_3 1μM/L, $MnSO_4$ 1μM/L, $CuSO_4$ 0.1μM/L, $(NH_4)_5Mo_7O_{24}$ 0.005μM/L, $ZnSO_4$10μM/L, and FeEDTA 10mM/L [11]. After eight days of treatment, the plants were analyzed for their phosphorus content by using the method of H_2SO_4-H_2O_2 Digestion - Colorimetric Vanadium-Molybdate and classified into 5 tolerance ranks based on the ratio of P content of low phosphorus treatment to that of high phosphorus treatment according the following criteria:

Rank 1: ratio value >0.85

Rank 2: 0.70 ⩽ ratio value < 0.85

Rank 3: 0.55 ⩽ ratio value < 0.70

Rank 4: 0.40 ⩽ ratio value < 0.55

Rank 5: ratio value < 0.40

2.3. Correlation between stress tolerance and root traits

In both experiments on tolerance to drought and tolerance to aluminium toxicity, the following root traits were measured and evaluated on per plant base: number of lateral roots (> 1cm), tap root length, dry root weight; total root length and root volume. The latter two were obtained from scanned data and processed with WinRhizo software. Based on that, relative dry root weight, relative root volume and relative total root length were calculated from the counterparts under drought treatment divided by dry weight of the whole plant; and root weight ratio, root volume ratio, total root length ratio, tap root length ratio and lateral root number ratio were calculated from the related measurements under aluminium toxicity stress to their respective counterparts under non-stress condition.

2.4. Genetic Analysis

The 184 RILs derived from Kefeng 1×Nannong 1138-2 (Rank 1×Rank 4) were used in analyzing the inheritance of drought tolerance using the relative values of total root length, root volume and dry root weight per plant. The segregation analysis under major gene plus polygene mixed inheritance model was used [12]. The data were further analyzed for QTL mapping using the QTL Cartographer V2.0 software [13] and the genetic map constructed according to [14] with a threshold LOD value of 3.0.

The 164 RILs derived from Bogao×NG94-156 (Rank 4×Rank 2) were used to analyze the inheritance of aluminium toxicity tolerance in relation to root weight ratio, root volume ratio, total root length ratio, tap root length ratio and lateral root number ratio, using segregation analysis.

3. RESULTS

3.1. Identification and evaluation of germplasm tolerant to rhizospheric stresses

3.1.1. Drought tolerance

The membership index values for each trait in Table IV were pretty close to each other in both years, which indicated that the mean member index value averaged over the four traits could be used as a good indicator for all the traits in tolerance to drought. Among the tested accessions, the Genetic Coefficient of Variation (GCV) of drought tolerance at seedling stage was about 42.6~43.3%, indicating the existence of great genetic variation in the tested population, thus an indication of its potential for drought tolerance breeding.

Five (HZ Bayuehuang, Jindou 14, Yuanheidou , Kefang 1, and Yixianheidou) and eight (LY Yaoheidou, NQ Laoshupi, Jindou 14, Jindou 16, Yuanheidou, 6-13, CS Xianheidou, and GY Heidou) accessions were highly tolerant to drought (rank 1) at the

seedling and reproductive stages respectively; and only two of these (Jindou 14 and Yuanheidou) were tolerant at both stages (Table III).

3.1.2. Aluminium toxicity tolerance

The membership index values for each trait in Table V were not significantly different from each other, which indicated that the mean member index value averaged over the four traits could be used as a good indicator for tolerance to aluminium toxicity using all the traits. Among the tested accessions, the Genetic Coefficient of Variation (GCV) of tolerance to aluminium toxicity at the seedling stage was about 41.1%, indicating the existence of great genetic variation in the tested population that could be used for aluminium toxicity tolerance breeding.

Seven accessions (Tiezhugan, Qihuang 1, Xiaotianedan, Pingdingdou, You 91-11, SH Daqingdou (xuan) and XC Liuyuedou) were highly tolerant to aluminium toxicity (rank 1) at the seedling stage.

3.1.3. Low phosphorus stress tolerance

Three accessions (WM Tiezhugan, Qihuang 1 and SH Daqingdou (xuan)), were highly tolerant to low phosphorus stress at seedling stage (Table III). It was very interesting that all the three accessions were also tolerant to aluminium toxicity. The materials are relevant to breeding of new cultivars for the southern areas with red acidic soils.

3.1.4. Correlation among rhizospheric stress tolerances

Table VI shows the existence of significant correlations among the rhizospheric stress tolerances except for drought tolerance and aluminium toxicity tolerance at the reproductive stage. More meaningful correlations existed between drought tolerance at both stages and between tolerance to aluminium toxicity and to low phosphorus stress.

Table VII shows that the selected accessions having at least one rank 1 tolerance for drought had medium tolerance to aluminium toxicity and low phosphorus stress, but the accessions with high aluminium toxicity and low phosphorus stress tolerance were more susceptible to drought stress.

3.1.5. Correlation among above and under-ground traits

The correlation values among above and under-ground traits are listed in Table VIII. Dry plant weight and dry weight of aboveground parts were significantly correlated with dry weight of under-ground, total root length, and root volume under both normal and drought conditions; so were for the correlations among the latter three traits. But the number of lateral roots only had some correlation with dry plant weight, dry weight above ground, dry weight under ground, total root length, and root volume under both environmental conditions, while length of taproot had no meaningful correlation with the five traits except with total root length and root volume under drought conditions. Since biomass usually was related with yield potential, the three root traits, dry weight under-ground, total root length and root volume which reflected the development of root system should be more meaningful for yield improvement than the number of lateral roots and tap root length.

TABLE III. EVALUATION OF INDICES AND RANKS OF TOLERANCE TO STRESSES OF THE MATERIALS TESTED

Variety	Drought Tolerance								Aluminium		Low Phosphorus	
	Seedling Stage						Reproductive		Seedling		Seedling	
	2001		2002		2 Year Average		2002		2002		2002	
	F	Rank	F	Rank	F	Rank	F	Rank	F	Rank	Ratio of P	Rank
HZ Bayuehuang	0.822	1	0.854	1	0.838	1	0.532	3	0.708	2	0.650	3
LY Yaoheidou	0.865	1	0.629	2	0.747	2	0.803	1	0.478	3		
NQ Laoshupi	0.446	3	0.408	3	0.427	3	0.829	1	0.454	3		
ZB Xiaobaihuangdou	0.624	2	0.412	3	0.518	3	0.721	2				
Bianheidou	0.809	1	0.642	2	0.726	2	0.697	2	0.744	2	0.620	3
Jinda 53	0.628	2	0.614	2	0.621	2	0.516	3	0.385	4		
Jindou 14	0.955	1	0.906	1	0.931	1	0.874	1	0.679	2	0.836	2
Jindou 16	0.674	2	0.443	3	0.559	3	0.861	1				
Jindou 19	0.841	1	-	-	-	-	0.589	3				
Yuanheidou	0.875	1	0.807	1	0.841	1	0.856	1				
Kefeng 1	0.872	1	0.891	1	0.882	1	0.749	2	0.555	3	0.825	2
3-29	0.434	3	0.638	2	0.536	3	0.753	2	0.575	3		
5-5	0.570	3	0.251	4	0.411	3	0.783	2	0.167	5	0.347	5
6-13	0.603	2	0.792	2	0.698	2	0.886	1				
Dawudou	0.633	2	0.507	3	0.570	3	0.534	3	0.090	5		
Naiyinheidou	0.830	1	0.630	2	0.730	2	0.756	2	0.362	4		
Yixianheidou	0.796	2	0.904	1	0.850	1	0.500	3				
CS Xianheidou	0.874	1	0.610	2	0.742	2	0.824	1	0.703	2	0.814	2
WM Tiezhugan	0.652	2	0.467	3	0.560	3	0.536	3	0.875	1	0.896	1
Qihuang 10	0.405	4	0.341	4	0.373	4	0.516	3	0.351	4	0.497	4
Qihuang 1	0.760	2	0.727	2	0.744	2	0.498	3	0.873	1	0.973	1
WS Gunlongzhu	0.480	3	0.468	3	0.474	3	0.617	2	0.723	2	0.598	3
CF Xiaotianedan	0.690	2	0.908	1	0.799	2	0.521	3	0.907	1	0.826	2
RN Pingdingdou	0.244	4	0.405	3	0.325	4	0.221	4	0.921	1	0.673	3
XX Xiaolihuang	0.579	3	0.774	2	0.677	2	0.311	4	0.207	4	0.346	5
XX Taipingzihuadou	0.431	3	0.628	2	0.530	3	0.724	2	0.461	3	0.671	3
XS Dalihuang	0.480	3	0.614	2	0.547	3	0.758	2	0.621	2		
CX Nongjiawuming	0.417	3	0.610	2	0.514	3	0.616	2	0.555	3		
liufeng	0.417	3	0.439	3	0.428	3	0.131	5				
SC Qujiahuangdou	0.540	3	0.565	3	0.553	3	0.288	4				
GY Heidou	0.787	2	0.446	3	0.617	2	0.891	1	0.798	2	0.836	2
GC Yishuhou	0.474	3	0.362	4	0.418	3	0.226	4	0.497	3	0.555	3
You 91-11	0.325	4	0.338	4	0.332	4	0.543	3	0.851	1	0.571	3
Zhongdou 19	0.533	3	0.435	3	0.484	3	0.534	3				
Zhongdou 8	0.423	3	0.442	3	0.433	3	0.192	5	0.456	3		
1138-2×86-53	0.108	5	0.235	4	0.172	5	0.373	3	0.185	5		
NG94-156									0.767	2		
Bogao									0.314	4		
FX Sunlouzihuangdou	0.725	2	0.823	1	0.774	2	0.568	3	0.573	3	0.531	4
JR Dabianqingdou	0.357	4	0.399	4	0.378	4	0.073	5	0.346	4	0.332	5
LH Dafengqing	0.153	5	0.171	5	0.162	5	0.443	3	0.292	4		
Lvbaozhu	0.507	3	0.260	4	0.384	4	0.112	5	0.222	4		
Nannong 1138-2	0.248	4	0.282	4	0.265	4	0.288	4	0.461	3	0.810	2
Nannong 18-6	0.485	3	0.416	3	0.451	3	0.296	4	0.464	3		
Nannong 86-4	0.441	3	0.481	3	0.461	3	0.461	3	0.725	2	0.835	2
Nannong 88-29	0.315	4	0.434	3	0.375	4	0.391	4	0.379	4		
Nannong 88-31	0.434	3	0.406	3	0.420	3	0.587	3	0.423	3	0.630	3
Nannong 96B-2	0.242	4	0.326	4	0.284	4	0.554	3	0.554	3	0.675	3
QD Heidou	0.324	4	0.403	3	0.364	4	0.605	2	0.477	3		
SH Dalihuangdou	0.450	3	0.507	3	0.479	3	0.203	4	0.212	4		
Suxie 1	0.342	4	0.188	5	0.265	4	0.445	3	0.379	4	0.509	4
HF Guizilan	0.289	4	0.153	5	0.221	4	0.073	5	0.753	2		
NC Zaibuyao	0.415	3	0.360	4	0.388	4	0.467	3	0.669	2	0.666	3
TG Xiazhidou	0.233	4	0.238	4	0.236	4	0.397	4				
YG Zaowudou	0.241	4	0.404	3	0.323	4	0.421	3	0.689	2	0.671	3
CM Tiegendou	0.665	2	0.647	2	0.656	2	0.411	3				
SH Daqingdou (xuan)	0.183	5	0.306	4	0.245	4	0.450	3	0.927	1	0.937	1
DQ Xianzhudou	0.438	3	0.225	4	0.332	4	0.084	5	0.598	3		
NH Wanhuangdou	0.112	5	0.178	5	0.145	5	0.154	5	0.274	4	0.459	4
XJ Xiaomaodou	0.650	2	0.829	1	0.740	2	0.714	2	0.681	2	0.721	2
XC Liuyuedou	0.194	5	0.295	4	0.245	4	0.478	3	0.922	1	0.727	2
PI416937									0.677	2		

TABLE IV. THE PERFORMANCE AND GENETIC VARIATION OF DROUGHT TOLERANCE AT SEEDLING STAGE AND ROOT TRAITS AMONG ACCESSIONS

Year	Statistic	Drought tolerance membership index value					Root trait		
		Plant height	No. leaves	Dry stem and leave weight	Dry root weight	Mean	Dry root weight/ Plant weight	Total root length/ plant weight	Root volume/ plant weight
2001	$\bar{x}$	0.530	0.565	0.454	0.485	0.514	0.262	568.113	0.597
	Min.	0.000	0.000	0.000	0.000	0.108	0.215	415.246	0.431
	Max.	1.000	1.000	1.000	1.000	0.955	0.339	739.960	0.797
	s	0.238	0.248	0.203	0.297	0.223	0.025	76.038	0.077
	GCV (%)	44.9	43.8	44.6	61.2	43.3	16.3	21.3	21.8
2002	$\bar{x}$	0.540	0.544	0.488	0.439	0.503	0.262	614.883	0.626
	Min.	0.000	0.000	0.000	0.000	0.155	0.215	445.071	0.451
	Max.	1.000	1.000	1.000	1.000	0.918	0.351	905.188	0.836
	s	0.212	0.213	0.248	0.270	0.214	0.027	102.524	0.094
	GCV (%)	39.2	39.2	50.8	61.5	42.6	17.7	28.3	22.6

TABLE V. THE PERFORMANCE AND GENETIC VARIANCE OF THE TOLERANCE TO ALUMINIUM TOXICITY AND ROOT TRAITS AMONG ACCESSIONS

Statistic	Membership index value					Root trait				
	Plant height	No. leaves	Dry root weight	Dry stem and leave weight	Average	Number of lateral root ratio	Tap root length ratio	Total root length ratio	Root volume ratio	Dry root weight ratio
$\bar{x}$	0.583	0.608	0.409	0.531	0.548	0.75	0.73	0.69	0.65	0.700
Min.	0.000	0.000	0.000	0.000	0.000	0.397	0.486	0.330	0.365	0.560
Max.	1.000	1.000	1.000	1.000	1.000	0.984	0.897	0.968	0.958	0.902
s	0.319	0.337	0.248	0.220	0.225	0.160	0.080	0.150	0.130	0.085
GCV (%)	54.8	55.4	60.7	41.3	41.1	0.210	0.110	0.220	0.200	0.121

TABLE VI. CORRELATION ANALYSIS AMONG STRESS TOLERANCES

Trait	Drought tolerance at seedling stage	Drought tolerance at reproductive stage	Aluminium toxin tolerance at seedling stage
Drought tolerance at reproductive stage	0.56**		
Aluminium toxin tolerance at seedling stage	0.23*	0.20	
Low phosphorous tolerance at seedling stage	0.29*	0.36**	0.74**

*, ** represent significant at 0.05 and 0.0, respectively.

TABLE VII. THE SELECTED ACCESSIONS WITH RANK 1 STRESS TOLERANCE

Name	Maturity Group	Source	Stress tolerance*			
			SD	RD	A	P
Yuanheidou	III	Shanxi	**1**	**1**	-	-
Jindou 14	III	Shanxi	**1**	**1**	2	2
Jindou 16	III	Shanxi	3	**1**	-	-
HZ Bayuehuang	VI	Shaanxi	**1**	3	2	3
Kefeng 1	III	Beijing	**1**	2	3	2
NQ Laoshupi	IV	Shaanxi	3	**1**	3	-
6-13	III	Hebei	2	**1**	-	-
GY Heidou	V	Anhui	2	**1**	2	2
XC Liuyuedou	IV	Jiangxi	4	3	**1**	2
You 91-11	V	Hubei	4	3	**1**	3
CF Xiaotianedan	V	Anhui	2	3	**1**	2
RN Pingdingdou	IV	Henan	4	4	**1**	3
WM Tiezhugan	V	Shandong	3	3	**1**	**1**
Qihuang 1	IV	Shandong	2	3	**1**	**1**
SH Daqingdou (xuan)	VII	Shanghai	4	3	**1**	**1**

* SD, RD, A, and P represent tolerance to drought tolerant at seedling stage and at reproductive stage, aluminium toxicity, and low phosphorus, respectively.

3.1.6. Correlation between drought tolerance and root traits

Table IX shows that tolerance to drought at seedling stage (mean membership index) is not correlated with the three first order root traits, i.e. dry root weight, total root length, and root volume, but is significantly correlated with the three second order root traits or the relative root values, i.e. dry root weight relative to dry plant weight, total root length relative to dry plant weight and root volume relative to dry plant weight. This might be due to the difference in the development of the first order root traits among eco-types or groups. Therefore, the relative values seem to be better than the first order root traits as indicators of drought tolerance.

TABLE VIII. CORRELATION AMONG ABOVE AND UNDER GROUND TRAITS

Treatment (water)	Trait	2001					2002			
		DPW	DWA	DWU	TRL	RV	DPW	DWA	TRL	RV
Non-stress	NLR	0.54	0.56	0.44	0.54	0.59				
	LTR	0.24	0.25	0.17	0.19	0.10				
	DWU	0.94	0.89		0.86	0.90	0.95	0.91	0.85	0.84
	TRL	0.81	0.77			0.91	0.81	0.78		0.87
	RV	0.88	0.85				0.82	0.79		
Stress	NLR	0.33	0.34	0.25	0.36	0.35				
	LTR	0.13	0.12	0.16	0.35	0.27				
	DWU	0.90	0.82		0.81	0.89	0.92	0.87	0.82	0.85
	TRL	0.72	0.67			0.85	0.65	0.57		0.86
	RV	0.83	0.77				0.75	0.77		

DPW = dry plant weight, DWA = dry weight above ground, DWU = dry weight under ground, TRL = total root length, RV = root volume, NLR = number of lateral roots, LTR = length of tap root. $r_{0.05} = 0.250$, $r_{0.01} = 0.325$

TABLE IX. CORRELATION BETWEEN DROUGHT TOLERANCE AND ROOT TRAITS UNDER DROUGHT STRESS

Year	Dry root weight	Total root length	Root volume	Dry root weight/plant weight	Total root length/plant weight	Root volume/ plant weight
2001	-0.21	0.10	-0.05	0.63**	0.81**	0.77**
2002	-0.23	0.17	0.07	0.66**	0.79**	0.81**

Note: ** represents significant at 0.01 level.

3.1.7. Correlation between aluminium toxicity tolerance and root traits

Table X shows significant correlations between aluminium toxicity tolerance (mean membership index) and the second order root traits, namely; the ratios of number of lateral roots, tap root length, total root length, root volume, and dry root weight. There was some correlation between aluminium toxicity tolerance and first order root traits, but not high enough except with tap root length. Therefore, the ratio values also seem to be better than the first order root traits as indicators of aluminium toxicity tolerance.

TABLE X. CORRELATION BETWEEN ALUMINIUM TOXICITY TOLERANCE AND ROOT TRAITS

Number of lateral Roots	Tap root length	Total root length	Root volume	Dry root weight	Ratio of number of lateral roots	Ratio of tap root length	Ratio of total root length	Ratio of root volume	Ratio of dry root weight
-0.26*	0.55**	0.34**	0.20	-0.01	0.67**	0.74**	0.81**	0.81**	0.93**

Note: **represents significant at 0.01 level.

3.2. Inheritance of root traits related with tolerance to rhizospheric stresses

Table XI shows the existence of significant differences between the two parents, Kefeng 1 and 1138-2, and among the RILs in dry root weight relative to dry plant weight, total root length relative to dry plant weight and root volume relative to dry plant weight.

By using the segregation analysis of quantitative traits under the mixed major gene plus polygene genetic model, the optimum genetic models for the three relative root traits were detected through the comparison of AIC values and a set of tests for goodness of fit as indicated in Table XII. The models showed that between the two parents (Rank 1 and Rank 4) all the three relative root traits, dry root weight relative to dry plant weight, total root length relative to dry plant weight and root volume relative to dry plant weight, were controlled by two major genes plus polygenes, with the latter two traits having the two major genes linked to each other with a recombination value 4.30% and 1.93%, respectively.

TABLE XI. ESTIMATES OF GENETIC PARAMETERS OF THE RIL POPULATION UNDER DROUGHT STRESS

Root trait	Kefeng 1	1138-2	RIL				
			Mean	Range	Genetic variance	Genetic CV	h^2
Dry root weight/plant weight	0.30	0.24	0.284	0.180~0.392	0.0015	13.64	90.60
Total root length/plant weight	875.32	586.26	756.10	407.75~1220.12	15098.75	16.25	79.11
Root volume/plant weight	0.70	0.55	0.673	0.374~0.932	0.0087	13.71	77.00

All the root traits were significantly different between parents and among RILs at 0.01 level.

TABLE XII. GENETIC MODELS OF THE ROOT TRAITS RELATED TO DROUGHT TOLERANCE

Root traits	Optimum model	Major gene	Additive polygene	Recombination (%)
Dry root weight/plant weight	E-1-0	2	+	
Total root length/plant weight	E-2-5	2 linked	+	4．30
Root volume/plant weight	E-2-4	2 linked	+	1．93

The estimates of genetic effects of major genes and heritability values of both major gene and polygene are listed in Table XIII. For all the three relative root traits, between their two major genes, the additive effects of one were much larger than the other one. The major gene heritability value of relative dry root weight was 91.81%, much larger than the other two traits (75.35% and 62.26%), but the polygene heritability value of relative dry root weight was only 2.99%, much smaller than the other two traits (11.15% and 24.75%), indicating the relative importance of major gene for the former trait and the relative importance of minor genes for the latter two traits.

TABLE XIV. ESTIMATES OF GENETIC PARAMETERS OF ROOT TRAITS RELATED TO DROUGHT TOLERANCE UNDER MAJOR GENE PLUS POLYGENE MODEL

Genetic effect		Dry root weight /plant weight	Total root length /plant weight	Root volume /plant weight
Additive effect	d_a	0.023	199.472	0.029
	d_b	0.014	114.950	0.148
Epistasis effect	i	-0.007	-	-
Genetic variance of major gene σ_{mg}^2		0.001	25944.713	0.012
Heritability of major gene h_{mg}^2 (%)		91.81	75.35	62.26
Genetic variance of polygene σ_{pg}^2		0.000	3840.163	0.005
Heritability of polygene h_{pg}^2 (%)		2.99	11.15	24.75

3.2.2. Inheritance of tolerance to aluminium toxicity

Table XV shows that there are also significant differences between the two parents, Bogao (Rank 4) and NG94-156 (Rank 2), and among the RILs in lateral root number ratio, tap root length ratio, total root length ratio, root volume ratio, and dry root weight ratio.

Segregation analysis and the optimum genetic models for the five ratios of root traits and a set of tests for goodness of fit (Table XVI) revealed that between the two parents (Rank 4 and Rank 2) four root traits, lateral root number ratio, tap root length ratio, total root

length ratio, and dry root weight ratio were controlled by three major genes plus polygenes, while root volume ratio was controlled by three major genes without polygene detected.

The estimates of genetic effects of major genes and heritability values of both major gene and polygene were listed in Table XVII. For all the five ratios of root traits, among their three major genes, the additive effects of one were much larger than the other two. There existed additive × additive first order and second order interaction effects among major genes for total root length ratio and root volume ratio, but not for the other three root traits. The major gene heritability values of the five ratios of root traits were 80.22%~93.44%, much larger than their polygene heritability values, 3.52%~11.39%, indicating the relative importance of major gene in comparison with the minor genes.

TABLE XV. ESTIMATES OF GENETIC PARAMETERS OF THE RIL POPULATION UNDER ALUMINIUM TOXICITY STRESS

Root trait	Parent		RIL				
	Bogao	NG94-156	Mean	Range	Genetic variance	Genetic CV(%)	Heritability (%)
Lateral root number ratio	0.528	0.770	0.658	0.363~0.962	0.010	15.43	77.72
Tap root length ratio	0.519	0.795	0.658	0.350~0.993	0.011	15.94	83.94
Total root length ratio	0.537	0.792	0.653	0.360~0.914	0.010	15.31	89.65
Root volume ratio	0.546	0.771	0.645	0.381~0.912	0.011	16.26	88.52
Dry root weight ratio	0.563	0.737	0.658	0.360~0.963	0.015	18.59	90.32

Note: All the root traits were significantly different between parents and among RILs at 0.01 level, respectively.

TABLE XVI. THE OPTIMUM GENETIC MODELS OF ALUMINIUM TOLERANT ROOT TRAITS FROM SEGREGATION ANALYSES

Root traits	Optimum model	Major gene	Additive polygene
Number of lateral root ratio	G-2	3	+
Tap root length ratio	G-2	3	+
Total root length ratio	G-1	3	+
Root volume ratio	F-1	3	-
Dry root weight ratio	G-2	3	+

3.3. QTL mapping of root traits related with drought tolerance

Table XVIII showed the results of QTL mapping of the three root traits related to drought tolerance, i.e. dry root weight relative to dry plant weight, total root length relative to dry plant weight and root volume relative to dry plant weight from the data of the (Kefeng No.1×1138-2) RIL population by using composite interval mapping procedure with QTL Cartographer V 2.0. The QTLs of the three traits were mapped on four linkage groups, i.e. N6-C2,N8-D1b+W, N11E and N18-K (Table XVIII, Fig. 1).

TABLE XVII. ESTIMATES OF GENETIC EFFECTS AND HERITABILITY VALUES OF MAJOR GENE AND POLYGENE OF ALUMINIUM TOLERANCE RELATED ROOT TRAITS

Genetic parameters		Number of lateral root ratio	Tap root length ratio	Total root length ratio	Root volume ratio	Dry root weight ratio
Major gene additive effect	d_a	-0.095	-0.030	-0.074	-0.067	-0.100
	d_b	-0.026	-0.043	0.002	-0.028	-0.019
	d_c	-0.028	-0.097	0.056	-0.066	-0.067
Major gene epistasis effect	i_{ab}	-		0.008	0.024	-
	i_{ac}	-	-	0.013	-0.010	-
	i_{bc}	-	-	0.003	0.025	-
	i_{abc}	-	-	0.048	0.020	-
Major gene genetic variance σ_{mg}^2		0.014	0.013	0.013	0.012	0.014
Major gene heritability h_{mg}^2		81.02	87.13	91.81	93.44	80.22
Polygene genetic variance σ_{pg}^2		0.001	0.001	0.001	-	0.002
Polygene heritability h_{pg}^2		7.79	6.18	3.52	-	11.39

From the above results, the major loci of each of the relative root traits, *Dw1, Rl1* and *Rv1* were all located on N6-C2, while the others scattered on the four linkage groups. There is one major locus for each of the three traits and all of them were located on N6-C2, which related with both root traits and drought tolerance. Furthermore, *Dw1, Rl1,* and *Rv1* were located in the same STAS8_3T-STAS8_6T region within a 1.7–0.7 cm distance. Therefore, *Dw1, Rl1,* and *Rv1* may indeed be one gene Table XVIII).

TABLE XVIII. QTL MAPPING OF ROOT TRAITS RELATED TO DROUGHT TOLERANCE OF THE (KEFENG NO.1×1138-2) RIL POPULATION

Root trait	Linkage group	Locus	Marker	Distance（cm）	LOD	Variance Explained (%)	Addictive effect
Dry root weight/ plant dry weight	*N6-C2*	*DW1*	STAS8_3T-STAS8_6T	1.7–0.7	18.7	24.7	0.028
	N8-D1b+w	*Dw2*	LC5T-Rn1	10.2–5.6	3.1	4.0	-0.011
		Dw3	Rn3-Rsa	16.1–5.4	3.0	3.9	-0.011
	N11E	*Dw4*	LC20B-Sat_112	0.7–12.9	4.6	5.9	0.016
		Dw5	A86H	0.0	4.2	5.2	0.014
Total root length/ plant dry weight	N6-C2	*RL1*	STAS8_3T-STAS8_6T	1.7–0.7	13.6	22.9	85.978
		Rl2	A676I-Satt316	3.8–15.9	3.0	6.8	53.065
	N8-D1b+w	*RL3*	LC5T-Rn1	12.2–3.6	3.5	4.1	-45.044
Root volume/ plant dry weight	N6-C2	*Rv1*	STAS8_3T-STAS8_6T	1.7–0.7	14.7	22.0	0.164
		Rv2	A131I-K455D	17.9–0.4	3.1	3.6	0.031
		Rv3	A676I	0.0	5.4	6.5	0.046
	N11E	*Rv4*	LC20B-Sat_112	0.7–12.9	4.5	6.2	0.038
	N18-K	*Rv5*	K401H	0.0	3.2	4.2	0.031

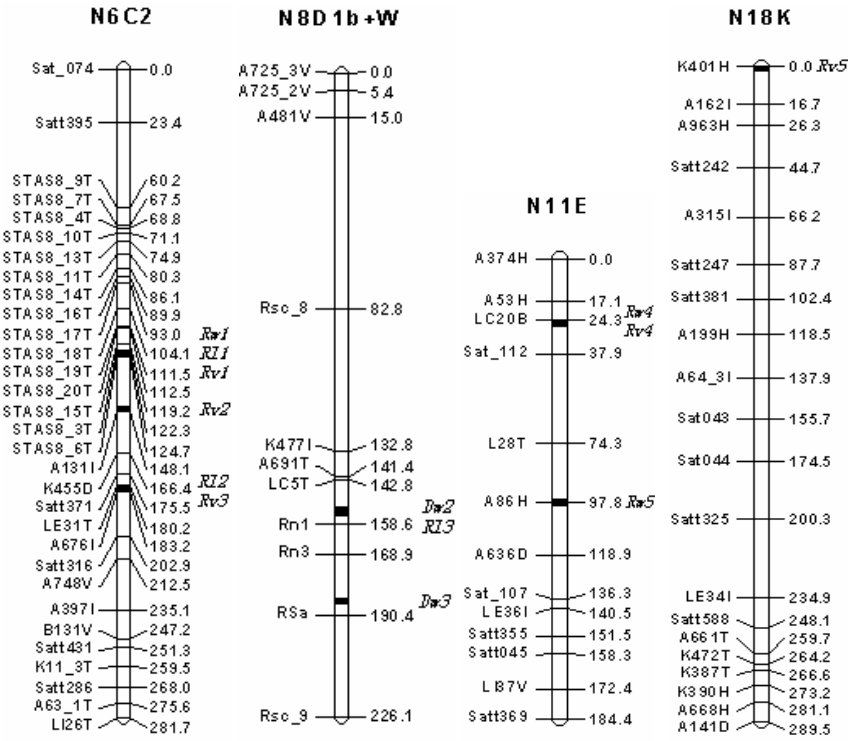


Fig. 1. Linkage groups with QTLs of three root traits related to drought tolerance.

4. DISCUSSION

4.1. Ecological properties of the screened germplasm tolerant to rhizo-spheric stresses

The present study indicated that there is variation in tolerance to rhizospheric stresses which might result from spontaneous mutation, among the landraces of soybean. From the elite accessions screened out for rhizospheric stress tolerance, it seems that the tolerant accessions might have their specific ecological characteristics. Those tolerant to drought were mainly from northern arid and semi-arid area, while those tolerant to aluminium toxicity and low phosphorus stress were mainly from southern acid red soil area. The stress factor(s) of the environment caused the adaptive variation. However, due to the same reason, the materials from the stressed environment might lack the desired yield potential. Accordingly, it is especially important to choose the other parent to be complementary to the stress tolerant material used as one of the parents.

4.2. Root traits related to stress tolerances

It is interesting that the first order measurement of root traits, dry root weight, total root length, and root volume, did not correlate with drought tolerance, but the three second order root traits or the relative root values very significantly correlated with drought tolerance. The same situation was for the correlation between ratio values of root traits and aluminium toxicity tolerance. Unfortunately, the two sets of relative root traits were obtained in different ways; the set for drought tolerance was the values relative to the whole plant dry weight under stress condition, while the set for aluminium toxicity tolerance was the stressed values relative to their corresponding unstressed values. However both kinds of the relative root traits were correlated with stress tolerances, which might be due to the relative values reflected the whole plant performance or the normal performance and the first order values were not comparable

among accessions, especially different eco-types of accessions were involved. Therefore, important root traits are to be explored for stress tolerance studies.

4.3. Relative consistency of the genetic information from segregation analysis and QTL mapping

It was found that the results from segregation analysis and those from QTL mapping for the three root traits related to drought tolerance were relatively consistent. The number of major gene(s) that could be detected with segregation analysis currently is only three and needs to be extended, while it is not limited for QTL mapping. However, in the present study, both procedures detected a major gene with obviously larger effect than the other(s) for each of the traits. In segregation analysis, the two major genes for relative dry root weight as well as relative root volume were tightly linked each other, respectively, while in QTL mapping, *Rl1* and *Rl2* as well as *Rv1* and *Rv3* were located on a same linkage group N6-C2. Therefore, in this case, the results from both procedures were comparable. Since the segregation analysis procedure of quantitative trait costs much less, it can be used broadly, especially when the genetic map is not saturated enough and could not offer accurate mapping information.

4.4. The relative importance of linkage groups

In the present study, the major genes with the largest additive effect for each of the three relative root traits related to drought tolerance, *Dw1, Rl1, and Rv1,* were located on the same linkage group N6-C2, even in the same marker region STAS8_3T-STAS8_6T with same distances 1.7–0.7 cm. Possibly, the three major genes of the three traits might be the same gene with pleiotropic effects that should be further studied. However, it seems that the linkage group N6-C2 is important to root traits. On another linkage group N8-D1b+W were located some other loci of root traits related to drought tolerance in the present study, on which also were located six genes resistant to soybean mosaic virus [15]. Therefore, it indicated the linkage group N8-D1b+W might be important for both biotic and abiotic stress tolerance. Anyway, to know the detailed relationship among various traits through gene mapping needs to be further studied since the present study is limited only to a few traits.

REFERENCES

[1] Sloane, R.J., Patterson, R.P., Carter, T.E. Field drought tolerance of soybean plant introduction, Crop Science (1990) 30:118-123.

[2] Von Uexkull, H.R. Global extent, development and economic impact of acid soil, Plant and soil, (1995) 171(1):1-15.

[3] Carter, T.E. Jr., Rufty, T.W., Kuo, C.G. Soybean Plant introductions exhibiting drought and aluminium tolerance, in Proceedings of International Symposium on Adaptation of Food Crops to Temperature and Water Stress, Taiwan (1992) 335~346.

[4] Hudak, C.M., Patterson, R.P. Vegetative growth analysis of a drought-resistant soybean plant introduction, Crop Sci., (1995) 35:464~471.

[5] Hoogenboom, G.M., Huck, C.M., Peterson, R.P. Root growth rate of soybean as affected by drought stress, Agron J. (1987) 79:607~614.

[6] Garay, A.F., Wilhelm, W.W. Root system characteristics of two soybean isolines undergoing water stress conditions, Agron J. (1983) 75(6):973~977.

[7] Liu, F.L., Andersen, M.N., Jensen, C.R. Root signal controls pod growth in drought-stressed soybean during the critical, abortion-sensitive phase of pod development, Field Crops Research, (2004) 85:159-166.

[8] Hudak, C.M., Patterson, R.P. Root distribution and soil moisture depletion pattern of a drought-resistant soybean plant introduction, Agronomy J. (1996) 88:478-486.

[9] Sartain, J.B., Kamprath, E.J. Aluminium tolerance of soybean cultivars based on root elongation in solution culture compared with growth in acid soil. Agron J. (1998) 70:17-23.
[10] Spehar, C.R. Aluminium tolerance of soybean genotypes in short term experiments, Euphytica, (1994) 76:73-80.
[11] Yan, X., Zhang, F.S. Plant Nutrition Genetics, China Agriculture Press, Beijing (1997): p22，pp40-41, pp193-196.
[12] Gai, J.Y., Zhang, Y.M., Wang, J.K. Genetic System of Quantitative Traits in Plants, Science Press, Beijing (2003).
[13] Wang, S., Basten, C.J., Zeng, Z.B. Windows QTL Cartographer 2.0, Department of Statistics, North Carolina State University, Raleigh, NC, (2001-2004).
[14] Wang, Y.J., Wu, X.L., Yu, D.Y., Zhang, Y.M., Chen, S.Y., Gai, J.Y. Method of evaluation and adjustment of recombinant inbred line population and its application to the soybean RIL population NJRIKY, Agronomica Sinica (2004) 30:5413-418.
[15] Wang, Y.J., Dongfang, Y., Wang, X.Q., Yang, Y.L., Yu, D.Y., Gai, J.Y., Wu, X.L., He, C.Y., Zhang, J.S., Chen, S.Y. Mapping of five genes resistant to SMV strains in soybean, Acta Genetica Sinica (2004) 31 1:87~90.

ON THE ROAD TO QUANTITATIVE GENETIC / GENOMIC ANALYSES OF ROOT GROWTH AND DEVELOPMENT COMPONENTS UNDERLYING ROOT ARCHITECTURE

X. DRAYE, S. DE DORLODOT, T. LAVIGNE
Université catholique de Louvain,
Louvain-la-Neuve, Belgium

Abstract

The quantitative genetic and functional genomic analyses of root development, growth and plasticity will be instrumental in revealing the major regulatory pathways of root architecture. Such knowledge, combined with in-depth consideration of root physiology (e.g. uptake, exsudation), form (space-time dynamics of soil exploration) and ecology (including root environment), will settle the bases for designing root ideotypes for specific environments, for low-input agriculture or for successful agricultural production with minimal impact on the environment. This report summarizes root research initiated in our lab between 2000 and 2004 in the following areas: quantitative analysis of root branching in bananas, high throughput characterisation of root morphology, image analysis, QTL mapping of detailed features of root architecture in rice, and attempts to settle a Crop Root Research Consortium.

1. INTRODUCTION

Roots contribute largely to crop performance and environmental impact. They have indeed a determinant influence on the exploitation efficiency of soil resources (water and nutrients) [1]. They also effect their environment through the induction of physico-chemical and microbiological processes acting on important aspects such as the bioavailability of nutrients, the cycling of organic matter, weathering of soil minerals and the quality of water [2]. Despite this functional importance, the attention to roots in crop improvement remains limited, for obvious practical reasons but also because the establishment of particular root features as favourable traits is in most cases lacking.

The design of root ideotypes for specific environments, for low-input agriculture or for successful agricultural production with minimal impact on the environment requires the consideration of root physiology (e.g. uptake, exsudation), form (space-time dynamics of soil exploration) and ecology (including root environment). One way to progress in these domains is to achieve a genetic dissection of root traits, using quantitative genetics and functional genomics, in order to reveal the major regulatory pathways of root growth, activity and plasticity. This will allow a better understanding of plant-autonomous processes and will benefit both plant/soil interactions research and crop improvement. It can also be argued that the discovery of genes/alleles that can be used to enhance root function remains a major challenge in the future, contrary to the introduction of favourable genes/alleles into elite cultivars, for which knowledge and technology are widely available.

The dissection of root architecture in traits amenable to functional and genetic analysis poses an important scientific challenge because root architecture is extremely plastic and it reflects the simultaneous activity of a large number of meristems that responds individually to internal and environmental stimuli [3]. For these reasons, we feel important to formulate root architecture in terms of underlying dynamic processes (growth and development) and not as a static picture. This opens new avenues related to the use of space-time models of root architecture to understand how root architecture relates to these dynamic processes and to help gaining better insights of allocation rules within a root system [4–5]. It is most likely that

architectural models will play a significant role in the connection between genomics and ecophysiology [6].

This report summarizes root research initiated in our lab between 2000 and 2004. This work consisted of a characterisation of the polymorphism of root architecture (unpublished data) and an histological study of lateral root initiation in bananas [7–8] , and a QTL analysis for fine root architecture in rice [9]. It also involved the development of image analysis software (XD, in preparation) and the design of a growth system suitable for observation and quantification of root growth and development in large numbers of rice/barley plants beyond the seedling stage [9]. It is thought that this system may be amenable for high-throughput screening of root architecture, and that this would enable the use of mutagenesis to resolve the genetics of complex facets of root architecture in crop plants. In addition, the possibility to phenotype the plant architecture of large number of mutated plants (mutation grids, for ex.) would provide large number of scenarios of C allocation that will be most useful to improve architectural models which rely heavily on rules of allocation within the plant.

2. RESULTS AND DISCUSSION

2.1. Genetic polymorphism for root architecture in *Musa*

The root system architecture of five *Musa* varieties (‘Grande Naine’, ‘Pisang Lilin’, ‘Yangambi Km5’, ‘Obino l'Ewai’ and ‘Agbagba’) was completely characterized at 3 time intervals in water and in sand cultures. Significant genetic variation was observed at the level of root axes and lateral roots for the rate of new root production, the root branching frequency, the elongation rate, the diameter and the senescence. The most striking difference was the type of growth of laterals, which was indeterminate for ‘Grande Naine’ and determinate for the other cultivars. The rate of increase of total leaf area was closely related to that of the total root volume, but not to that of the total root length, the latter relationship being obscured by differences of root diameter. The nature of the substrate affected traits related to growth (elongation, diameter) but not those related to development (production of root axes). The classical responses to mechanical impedance, viz. reduced elongation rate and increased diameter, were observed in sand, despite the near absence of substrate compaction. The lack of significant gene-environment (sand-water) interactions in the investigated parameters suggested that hydroponics in bananas might be a good candidate system to characterize part of the polymorphism of root architecture.

2.2. Distribution of lateral root primordia in *Musa*

In order to evaluate the extent to which lateral root initiation might be involved in the polymorphism for root architecture, the distribution of lateral root primordia in the root tips of four *Musa* varieties (‘Grande Naine’, ‘Pisang Berlin’, ‘Ngok Egome’ and ‘Yangambi Km5’) grown in the field has been investigated. In banana (*Musa* sp.), lateral roots are initiated in the root tip, 0.6–4 mm behind the root/cap junction and arise in several protoxylem-based longitudinal rows or 'ranks'. Significant differences were observed among varieties for the position of the most distal primordium; however the longitudinal spacing between successive primordia along the ranks was similar for all varieties. These results suggested that the acropetal initiation of lateral roots in *Musa* is a built-in process specified as part of the general process of cell division and differentiation in the parent root tip and that the genetic variations of root branching density in the field are not mediated by lateral root initiation.

This study stressed the need to consider the spacing between laterals in the same ranks instead of the spacing between laterals irrespective of their rank. Indeed, the number of ranks

varied significantly among varieties and was proportional to the stelar diameter. Finally, because all ranks were found to contribute to lateral root initiation, the density of lateral roots (roots/cm) was positively and linearly related with stelar diameter.

These conclusions should not be overemphasised as they apply only to polyarch roots. Species like Arabidopsis, tomato or, to some extent, cereals, whose roots have a constant number of protoxylem poles, would be expected to display a much lower level of variability in lateral root spacing and might be less amenable to genetic analysis of branching.

2.3. Consequences of root growth kinetics and vascular structure on the distribution of lateral roots

The inter-lateral distance along the parent root has been investigated using three banana varieties (*Musa* spp) grown in two substrates. It was found that the pattern of lateral root initiation (see above: conservation of spacing within ranks) was obscured by variations of root growth patterns and vascular structure among roots, genotypes and substrates. A framework model has been proposed showing the influence of growth pattern and vascular structure on branching density [8]. The model raises a distinction between growth components which should not affect the branching density (i.e. rate of cell division) and those which may affect it (i.e. size of mature cells and number of transverse divisions performed by cells executing their trajectory in the meristem). According to this model, lateral root density and root growth rate might even be independently modulated by appropriate changes of root growth patterns, and this would occur in banana and presumably many other crops.

We are currently testing this model in barley using various mutations / conditions that affect root growth patterns.

2.4. QTL analysis for root architecture

A segregating population of rice (*Oryza sativa*) has been used in a QTL analysis for detailed root architecture. The population is comprised of 135 doubled haploid lines derived from a cross between the cultivars IR64 and Azucena. Under field conditions, IR64 develops thin short roots while Azucena develops long thick roots. The genotype of each line is known at 130 RFLP marker loci selected from a saturated genetic map. Because of the need to recover complete and intact root systems, the phenotyping of all lines has been performed in aeroponics. Roots of IR64 were thinner than those of Azucena, but the differences in length nearly vanished. We hypothesize that oxygen availability in the root apex is a limiting factor of IR64 root growth in field conditions, as a result of the small diameter of IR64 roots which limits oxygen flow in the aerenchyma. This limitation would be absent in aeroponics conditions. The QTL analysis of marker and phenotypic data (under way) reveals a number of putative QTLs, some of which co-locate with QTLs for root traits observed in the field. According to this result, aeroponics conditions, despite their marked effect on the phenotype (GxE interactions), may be suitable to identify genomic regions containing genes involved in the variation of root system architecture in the field. However, the resolution of QTL analysis in the IR64×Azucena population (~15cm) does not allow to infer that the genes responsible for the QTL effect in field and aeroponics conditions are the same.

A similar study is under way with a segregating population of barley (*Hordeum vulgare*), in collaboration with the Scottish Crop Research Institute.

In parallel, we are initiating a genomic approach of lateral root initiation. In this context, we seek to identify a number of candidate genes involved in lateral root initiation in

barley (using a combination of microarray (gene expression) and *in silico* approaches) and to relate this information to QTL analysis data using positional information (genetic map). This novel intuitive approach is sought to be promising compared to the positional cloning of QTL, which has proven to be a difficult process, limited to rare QTL that explain a significant fraction of the genetic variance.

2.5. The SmartRoot image analysis software

The measurement of root architecture can sometimes be simplified with the use of image analysis. The root material usually consists of washed roots from the soil (usually contaminated with soil or other foreign particles), clean water-grown roots or root systems (hydroponics, aeroponics), or roots in situ (rhizotrons, minirhizotrons).

The first step in image-based root measurement is the acquisition of an image. Images are captured with a camera or scanner, exploiting visible (grayscale or colour) or UV light, or using an intermediate x-ray processing. The camera has a lower maximal resolution than the scanner and may require geometric correction of the image. However, it allows image acquisition in a few seconds, compared to a minute or more to obtain a scanned image. In the future, 3D acquisition systems (NMR or tomography) which offer unprecedented possibilities to analyse the space distribution of roots may become more and more available, but they currently remain restricted to a limited number of facilities.

The second step is the analysis of the image. Several packages are available, either commercially (MacRhizo/WinRhizo, DeltaT-SCAN) or freeware (RootEDGE) [10–13]. These software implement morphological operations to separate root objects from the background (segmentation, traditionally done by thresholding pixel values), sort them according to their shape to erase soil debris, reduce the remaining root objects to a one-pixel width line (skeletonisation) and estimate the root diameter along the skeleton (distance function) [14]. Root length is then estimated from the root skeleton as a whole or for individual roots or root segments, allowing the calculation of root length as a function of diameter classes. The MacRhizo/WinRhizo system also computes topological indices as a measure of the branching patterns of the root system [15].

The system which we are developing in our lab comes into a completely different context. SmartRoot is a system to the intention of users whose interest is not a whole root length or diameter or topology estimate, but rather a number of specific measurements like the position of specific features (hairs, clusters) along roots, the diameter/angle at given location, the length of particular roots, etc. It is much like a drawing software that “knows" something about roots (it can draw roots automatically) and allows the user to correct root tracings and place different kind of annotations along the roots. It also has a number of features to facilitate the comparison of time-sequences of images (registration, window focus, named roots), typically for the estimation of root growth rates, or to compare the location of features as a function of time. Among other features, SmartRoot uses an adaptive thresholding method that facilitates the segmentation of low quality images for which normal thresholding would not work, and maintains a data connection with SQL-compliant DB software (MS Access, Oracle, The SAS System). SmartRoot has been implemented as a plug-in of the ImageJ software (http://rsb.www.nih.gov/imageJ) and comes as a Windows self-installing bundle freely available on request from XD. In principle, SmartRoot should work on Mac, Linux and UNIX platforms, but this has not been tested yet.

In a near future, we are considering to make our system evolve towards a high-throughput system that would be able to trace complete root systems without user intervention

and record the root growth rate and branching patterns of most roots on a time-series of images. Obviously, such a tool would be of interest for the screening of mutant populations for more complex features than primary root length or presence/absence of lateral roots. But this will be another story.

2.6. The Crop Root Research Consortium (CRRC) initiative

Roots have been a neglected area of research, as being underground they are difficult to evaluate. Root research is entering a new era where functional genomics and proteomics supply powerful tools to help the scientific community investigate root function and structure. Information on roots is of direct relevance to agricultural/environmental issues, such as crop production with minimal impact on the environment, and crop production in low input and stressed environments. The recent surge of interest in roots has stimulated the Crop Root Research Consortium (CRRC) initiative. The mission of the CRRC is to coordinate research efforts of an ever-increasing number of research groups involved in root screening techniques, development of molecular markers, root architecture modelling, assessment of genetic potential, environmental impact and plant breeding. The CRRC platform aims to extend and facilitate exchange of information and material, to promote concerted development of new resources (populations / libraries) and to foster collaboration through voluntary networking within thematic sub-consortia, and joint research proposal submission under bi-lateral or multilateral cooperation. The consortium web-site: http://www.crop-roots.org.

REFERENCES

[1] Berntson, G.M. 1994. Modelling root architecture: are there tradeoffs between efficiency and potential of resource acquisition? New Phytologist 127:483-493.

[2] Hinsinger, P. 1998. How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. Advances in Agronomy 64:225-265.

[3] Eshel, A., Waisel, Y. 1996. Multiform and multifunction of various constituents of one root system. In: Waisel, Y., Eshel, A., Kafkafi, U., eds. Plant roots: the hidden half. 2nd Ed. New York: Marcel Dekker.

[4] Pagès, L., Asseng, S., Pellerin, S., Diggle, A. 2000. Modelling root system growth and architecture. In: Smit, A.L., Bengough, A.G., Engels, C., van Noordwijk, M., Pellerin, S., van de Geijn, S.C., eds. Root Methods a Handbook.

[5] Drouet, J.L., Pagès, L. 2003. GRAAL: a model of GRowth, Architecture and carbon ALlocation during the vegetative phase of the whole maize plant - Model description and parameterisation. Ecological Modelling 165:147-173.

[6] Xinyou, Y., Struik, P.C., Kropff, M.J. 2004. Role of crop physiology in predicting gene-to-phenotype relationships. Trends in Plant Science 9:426-432.

[7] Draye, X., Delvaux, B., Swennen, R. 1999. Distribution of lateral root primordia in root tips of *Musa*. Annals of Botany 84:393-400.

[8] Draye, X. 2002. Influence of root growth and vascular structure on the distribution of lateral roots. Plant Cell and Environment 25:1463-1474.

[9] de Dorlodot, S., Bertin, P., Baret, P., Draye, X. 2005. Scaling up quantitative phenotyping of root system architecture using a combination of aeroponics and image analysis. Aspects of Applied Biology 73:41-54.

[10] Kirchhof, G. 1992. Measurement of root length and thickness using a hand-held computer scanner. Field Crops Research 29:79-88.

[11] Arsenault, J.-L., Pouleur, S., Messier, C., Guay, R. 1995. WinRHIZO, a root-measuring system with a unique overlap correction method. HortScience 30:906.

[12] Kaspar, T.C., Ewing, R.P. 1997. ROOTEDGE: Software for measuring root length from desktop scanner images. Agronomy Journal 89:932-940.
[13] Himmelbauer, M.L., Loiskandl, W., Kastanek, F. 2004. Estimating length, average diameter and surface area of roots using two different Image analyses systems. Plant and Soil 260:111-120.
[14] Soille, P. 1998. Morphological image analysis. Berlin, Germany: Springer-Verlag.
[15] Fitter, A.H. 1987. An architectural approach to the comparative ecology of plant root systems. New Phytologist 106:61-77.

DEVELOPMENT AND IDENTIFICATION OF WHEAT MUTANTS WITH SOME NEW ROOT CHARACTERISTICS AND RESISTANCE TO STRIPE RUST

X. YE[1], L. DU[1], Y. WEI[2], Z. LIN[1], J. LI[1], H. XU[1]
[1] Institute of Crop Sciences,
Chinese Academy of Agricultural Science, Beijing, China
[2] Institute of Crop Research,
Ningxia Academy of Agri-forestry Sciences, Ningxia, China

Abstract

Wheat production is greatly affected by biotic factors and abiotic stresses. Therefore, it is necessary to develop some new wheat lines with disease resistance, abiotic stress tolerance and other characteristics using mutation techniques and biotechnology. In this study, twelve wheat materials with different backgrounds were tested for root characteristics *in vitro* and in the field. The results demonstrated that the intermediate materials derived from interspecies crosses between common wheat and *Th.intermedium* such as Zhong 4 Awnless and L1, Linkang 1 and Ningchun 4, had stronger root system, and showed better resistance to PEG than other wheat varieties. These materials might contain some genes controlling root traits or drought tolerance, and these characteristics might be derived from *Th.intermedium*. In addition, mature seeds of some materials and the calli derived from immature interspecies hybrid embryos were irradiated with γ-rays. Through field selection and laboratory identification, some new lines were developed from the offspring. Mutant TC2001-16 with a larger root system proved to be tolerant to drought stress, mutant TC2001-31 was resistant to stripe rust.

1. INTRODUCTION

Wheat is the second most cultivated crop in China, covering 30 million hectares every year. Of this wheat area, about one-third is in arid or semi-arid regions, mainly in central and northwest China. Drought tolerance of wheat varieties in these areas is very limited; as a result production is very low in most years. Breeding for root performance has been neglected for many years. Root characteristics such as size and water absorbing efficiency vary widely among species or varieties and they have been proved to play a very important role in drought tolerance in plants [1–4].

Generally, tall-stemmed crops have deeper root systems, but, some short-statured crops, such as wheat, peanut, sugar beet, also bear deeper roots [5–7]. Absorption of more Ca^{2+} and active mobilization of phosphate are the basic characters of efficient wheat genotypes [8]. In artificial circumstances, such as in the glass box, the type and the distribution range of wheat roots is different among genotypes [9]. Related research indicated that the root size of wheat is controlled by one or a few genes and is highly heritable.

Wheat stripe rust is another problem for wheat production, especially in northwest and northeast China, causing about 10-30% grain loss every year. Commercial wheat varieties either don't have the disease resistance or lose the resistance in a few years after release because of the shortage of the resistance genes in wheat species. Some disomic addition lines (2n = 22II = 44) between wheat and *Th.intermidum* such as Z_1, Z_2, Z_4 and L_1 were found to contain some new stripe rust resistance genes. In addition, *Agrobacterium* mediated wheat transformation is still very difficult in spite of great efforts [1–13], thus our approach to target the genotype directly for radiation mutation induction.

2. MATERIALS AND METHODS

2.1. Wheat materials

In this study one partial amphidiploid between wheat and *Thinopyrum intermidum,* Zhong 4 Awnless (2n = 28II = 56), five wheat — *Th.intermidum* disomic addition lines (2n = 22II = 44) Z_1, Z_2, Z_4, Z_6, and L_1, and six common wheat varieties(2n = 21II = 42) Zhong 8423, Zhong 7606, Wan 7107, Fengkang 8, Linkang 1, and Ningchun 4 were evaluated for their root systems and PEG resistance, and crossed and irradiated for the development of new lines.

2.2. Root examination

Ten plants with whole roots from each material or line were dug out carefully at the seedling and maturity stages, rinsed in water and root length, plant height, root dry weight, plant dry weight and other agronomic characteristics were measured.

2.3. Irradiation treatment

Mature seeds were irradiated with 200–250 Gy of r-rays, and the calli were irradiated with 25 Gy of r-rays at the Institute of Atomic Energy Application of Chinese Academy of Agricultural Sciences.

2.4. PEG test

The mature seeds and immature seeds (18 days post anthesis) were sterilized in 70% ethanol for 30 seconds followed by 10% sodium hypochlorite solution for 20 minutes, and were finally rinsed four times with sterile water. The sterilised mature embryos and immature embryos were isolated and cultured onto the 1/2MS medium with 15% PEG and 10% PEG respectively.

2.5. Cytogenetic analysis

Squash slides were made from root tips and young spikes pre-treated with ice cold water for 24 hours, stained in Carnoy solution for 2–4 days and then stained with 1% aceto carmine. Chromosome number and pairing were observed under a microscope. For chromosome in situ hybridization, the root tips were pre-treated with cellulose and pectinase before making chromosome slides. Available slides with clear chromosome division were treated with 0.2M HCI for denaturation and with 0.5M NaOH for renaturation, respectively. The genomic DNA of *Th.intermedium* labelled with fluorescein was used as probe to be hybridized with the chromosome DNA on the slides, in which the genomic DNA extracted from Chinese Spring, was used as block DNA. Finally, the slides were observed under fluorescent microscope after two washes**.**

2.6. Hybrid immature embryo culture for callus induction

Four commercial wheat varieties Ningchun 4, Ningchun 16, Linkang 1, and Jing 411 were used as females for crossing with four addition lines of L_1, Z_2, Z_4 and Z_6 in the greenhouse. One hundred and sixty-six immature hybrid embryos were obtained from eight crosses. The immature embryos (at 15–18 days after pollination) were excised from sterilized developing seeds and cultured on SD_2 medium (MS+2.0mg/l 2,4-D) for callus induction. Good calli were selected and sub-cultured on SD_1 medium (MS+1.0mg/l 2,4-D) every four weeks. After 3–4 subcultures, the embryonic calli were irradiated with 25 Gy of r-rays. Irradiated hybrid calli were sub-cultured on MS medium with 1.0mg/l 2,4-D and 150.0mg/l Asp for six weeks, only embryonic callus was transferred onto MS medium with 1.0mg/l KT and 0.5mg/l NAA for regeneration.

3. RESULTS

3.1. Characterisation of root system and drought tolerance

3.1.1. Characteristics of the seedling roots

Root lengths of the evaluated materials varied from 12.38 cm for Zhong 8423 to 18.24 cm for Linkang 1 (Table I). The partial amphidiploid line (Zhong 4 Awnless), all the addition lines (Z_1, Z_2, Z_4, Z_6 and L_1), and three commercial varieties (Linkang 1, Ningchun 4, and Wan 7107) had roots longer than 15 cm, but the other two commercial varieties of Zhong 8423 and Zhong 7606 had roots shorter than 15 cm (Table I). Root dry weights varied from 24.0 mg of Zhong 7606 to 46.7mg of Linkang 1. Zhong 4 Awnless, the four addition lines of Z_2, Z_4, Z_6 and L_1, and two commercial varieties Linkang 1 and Ningchun 4, had root weights of more than 27.0 mg. The ration between root length and seedling height ranged from 0.527 (Zhong 8423) to 1.173 (Linkang 1), and that of six materials, Zhong 4 Awnless, Z_1, Z_4, L_1, Linkang 1 and Ningchun 4 was more than 0.800. The weight ration between under and above ground parts ranged from 0.148 of Wan 7107 to 0.224 of Zhong 4 Awnless, and that of Z_1, Z_2, Z_4, L_1, Linkang 1, Ningchun 4 and Zhong 4 Awnless was more than 0.160.

3.1.2. Embryo resistance to PEG in vitro

The resistance of the mature and immature embryos to PEG on culture medium are summarized in Table II. On the medium containing 10% PEG, the mature embryos survived well, but the growth of immature embryos was suppressed heavily for some but not for Zhong 4 Awnless, L_1, Z_2, Linkang 1 and Ningchun 4. On the medium containing 15% PEG, the growth of the mature embryos of some varieties was suppressed significantly, but that of Zhong 4 Awnless, L_1, Z_2, Linkang 1 and Ningchun 4 still attained a survival rate of more than 80%. Moreover, the immature embryos of L_1, Linkang 1 and Ningchun 4 survived well on the medium containing 15% PEG, with a survival rate of around 50% (Fig. 2).

TABLE I. ROOT CHARACTERISTICS OF THE WHEAT MATERIALS

Genotypes	Chromosome number	Root length (cm)	Root weight (mg)	Ratio of root to seedling:	
				Length	Weight
Zhong 4 Awnless	56	16.50	31.0	0.853	0.224
Z_1	44	15.98	26.8	0.852	0.160
Z_2	44	16.58	29.0	0.760	0.162
Z_4	44	16.19	32.0	0.886	0.167
Z_6	44	15.38	27.5	0.686	0.156
L_1	44	16.96	29.5	0.877	0.165
Lingkang 1	42	18.24	46.7	1.173	0.189
Ningchun 4	42	16.19	32.5	0.987	0.171
Zhong 7606	42	14.00	24.0	0.737	0.152
Zhong 8423	42	12.38	26.0	0.527	0.151
Wan 7107	42	15.22	24.7	0.653	0.148
Fengkang 8	42	14.35	25.8	0.769	0.162

TABLE II. THE GROWING OF WHEAT MATURE AND IMMATURE EMBRYOS ON PEG CONTAINING MEDIUM

Genotypes	Explant	PEG % tration(%)	Inoculating number	Survival number	Percentage (%)
Zhong 4 Awnless	ME	15	60	58	96.7
L_1	ME	15	48	48	100.0
Z_2	ME	10	45	36	80.0
Z_2	ME	15	50	19	38.0
Zhong 8423	ME	10	38	29	76.3
Zhong 8423	ME	15	42	12	28.6
Linkang 1	ME	15	40	40	100.0
Ningchun 4	ME	15	49	48	98.0
Fengkang 8	ME	15	40	17	42.5
Zhong 4 Awnless	IME	10	72	72	100.0
L_1	IME	10	68	68	100.0
L_1	IME	15	58	30	51.7
Z_2	IME	10	36	33	91.7
Z_2	IME	15	22	0	0
Zhong 8423	IME	10	20	0	0
Linkang 1	IME	10	41	41	100.0
Linkang 1	IME	15	47	26	55.3
Ningchun 4	IME	10	66	66	100.0
Ningchun 4	IME	15	74	41	55.4
Fengkang 8	IME	15	54	5	9.3

ME: mature embryo IME: immature embryo

Fig. 1. Root characteristics of wheat; Left-Z_2; Middle-Zhong 8423;Right-L_1.

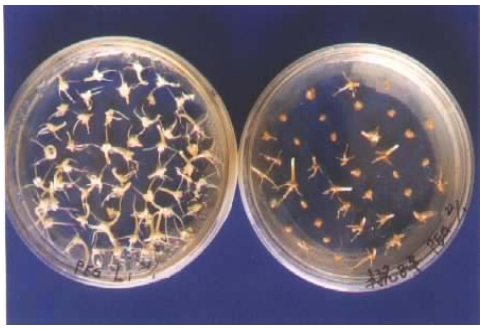


Fig. 2. Wheat embryos on PEG medium; Left-L_1; Right-Zhong 8423.

3.2. Plantlet regeneration from irradiated calli

The seed set of crosses between commercial variety and addition lines ranged from 4.5% to 76.4% for different combinations (Table III). The callus induction frequency was more than 80% for the immature embryos from every cross.

TABLE III. CROSSES AND IMMATURE EMBRYO CULTURE OF WHEAT AND ADDITION LINES

Crosses	Florets Pollinated	Hybrid embryos	Seed-set (%)	Callus forming embryos	Callus quality
Ningchun 4 × L_1	61	44	72.1	44	good
Ningchun 16 × Z_2	18	2	11.1	2	average
Ningchun 4 × Z_2	74	55	74.3	55	good
Linkang 1 × Z_4	25	6	24.0	6	bad
Linkang 1 × Z_6	26	6	23.1	5	average
Ningchun 16 × Z_4	20	2	10.0	2	good
Jing 411 × L_1	55	42	76.4	40	good
Jing 411 × Z_2	22	1	4.5	1	average

In total, three hundred and seventy two plantlets were regenerated from the irradiated calli (Table IV).

TABLE IV. PLANT REGENERATION FROM IRRADIATED CALLUS

Crosses	Irradiated callus	Callus quality	Callus for regeneration	Plantlets	Frequency (%)
Ningchun 4× L_1	116	Good	241	107	44.4
Ningchun 16× Z_2	14	Bad	36	5	13.9
Ningchun 4× Z_2	132	Good	302	98	32.5
Linkang 1× Z_4	26	Bad	29	13	44.8
Linkang 1× Z_6	18	Average	33	10	30.3
Ningchun 16× Z_4	15	Bad	18	4	22.2
Jing 411× L_1	93	Good	235	119	50.6
Jing 411× Z_2	10	Average	34	16	47.1

3.3. Mutant selection by PEG test and cytogenetic analysis

In total, 59 plants resistant to PEG were obtained during *in vitro* screening, most embryos died gradually, only the ones resistant to PEG survived.

The root tip and the young spikes of the PEG resistant plants were collected and their chromosome number and pairing were observed under a microscope. As a result, six plants namely; TC 2001-24, TC 2001-3, TC 2001-8, TC 2001-16, TC 2001-31 and TC 2001-36 with 42 chromosomes or 21 bivalents in pollen mother cells were identified (Figs 3 and 4). The other 53 PEG plants were found to be having 43 chromosomes.

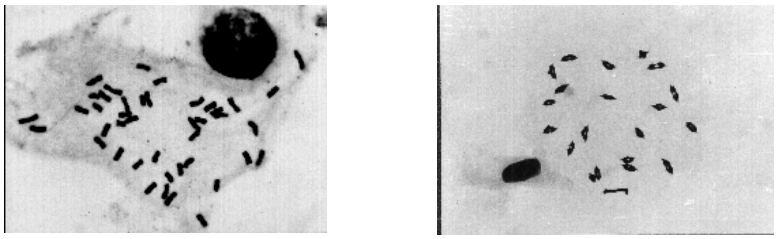
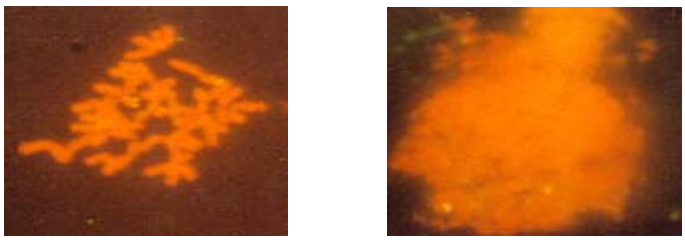


Fig. 3. Chromosome in root tips (left) and pollen mother cells (right).

In situ hybridization test of the six PEG resistant mutants with 42 chromosomes showed that only two lines (TC 2001-16 and TC 2001-31) were translocation lines and the other four were substitution lines, in which two yellow fluorescent segments or whole chromosomes from *Th.intermedium* occurred (Fig. 4).



1 **2**

Fig.4. Identification of the translocation by in situ hybridisation;
1. At middle stage of mitosis 2. At early stage of mitosis.

3.3. Drought tolerance and rust resistance of mutant lines.

The mature seeds of the mutant lines and a control variety Ningchun 4 were germinated on filter papers in plates containing 15% PEG solution and then planted in the field with limited water supply. All the mutant lines had germination rates higher than the control variety by 11.3% to 24.7%, and showed more drought tolerance than the control during the whole growing period in field. Also, the mutant lines had taller stalks, longer roots and higher yields compared to the control (Table V).

TABLE V. SOME AGRONOMIC CHARACTERISTICS RELATED TO DROUGHT TOLERANCE AND YIELD OF THE TRANSLOCATION LINES AND SUBSTITUTION LINES

Material	Germination frequency of mature seed on 15% PEG medium (%)	Root length (cm)	Plant height (cm)	Yield (kg/m^2)
TC 2001-3	91.4	16.6	83.3	0.424
TC 2001-8	86.1	17.5	85.2	0.461
TC 2001-16	79.8	16.9	78.0	0.430
TC 2001-24	93.2	18.0	81.5	0.432
TC 2001-31	84.3	15.8	80.2	0.448
TC 2001-36	90.0	16.1	77.5	0.425
Ningchun 4 (CK)	68.5	14.9	71.8	0.412

Two translocation lines TC2001-16 and TC2001-31 were also found to be resistant to wheat stripe rust in the nursery field. TC2001-31 showed very good resistance to the all races of the pathogen tested, with no disease symptom observed, while TC2001-16 and the two controls were infected by races to different extents (Table VI, Fig. 6).

TABLE VI. REACTION CLASSES OF THE TWO MUTANT TRANSLOCATION LINES TO STRIPE RUST*

	Tiao 29	Tiao 30	Tiao 31	Tiao 32	Shuiyuan
TC2001-16	3	3	3	1	0
TC2001-31	0	0	0	0	0
Ningchun 4 (CK_1)	3	4	3	3	3
Huixianhong (CK_2)	4	4	4	4	4

* 0 = resistant; 4 = susceptible

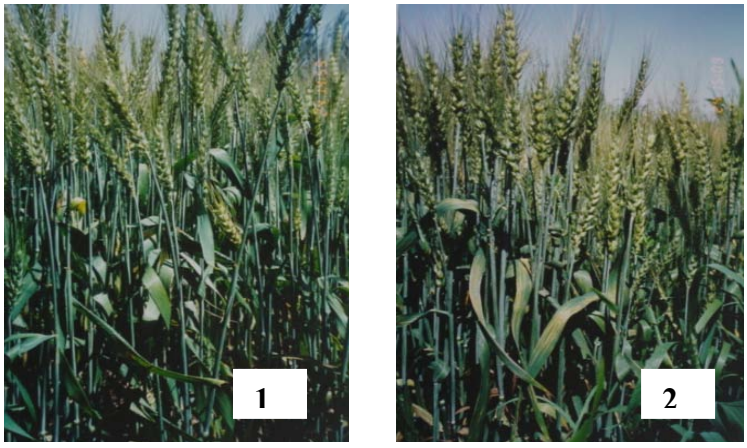


Fig. 5. Field resistance test to stripe rust of the mutant developed; 1. TC2001-16 2. Ningchun 4 (CK).

4. DISCUSSION

The intermediate materials between common wheat and *Th.intermedium*, such as the partial amphidiploid of Zhong 4 Awnless, the addition lines of Z_1, Z_2, Z_4, Z_6 and L_1, had more robust root systems when compared with the four normal wheat varieties of Wan 7107, Fengkang 8, Zhong 7606 and Zhong 8423. In addition, the intermediate materials were more

resistant to PEG than normal wheat varieties *in vitro*. The results demonstrated that the mediate materials might contain some genes controlling root traits or drought tolerance, and the observed characteristics might have been derived from *Th.intermedium*. Irradiating the hybrid embryogenic callus (cross between wheat and the intermediate materials) resulted in six mutant lines; 2001-24, TC 2001-3, TC 2001-8, TC 2001-16, TC 2001-31 and TC 2001-36 with more resistance to PEG. TC 2001-16 and TC 2001-31 were found to be translocation lines and the other four lines are substitution lines. When tested in the field with limited water supply, the six lines showed more pronounced drought tolerance, longer roots, taller stems and higher yields than the control. The result demonstrated that the drought tolerance can be transferred from the intermediated materials and that variation in these traits can be enhanced by use of mutation induction. In addition, the two translocation lines of TC 2001-16 and TC 2001-31 were tested for the resistance to wheat stripe rust, and the former line showed more resistance to the five main strains of the pathogen tested. This result also suggested that the disease resistance was also from the intermediate materials.

REFERENCES

[1] Cheng M, Joyce E F, Pang S.Z., Zhou, H., Hironaka, C., Duncan, D.R., Conner, T.W., Wan, Y. 1997, Genetic transformation of wheat mediated by *Agrobacterium tumefacien*s. Plant Physiol, 115: 971-980.

[2] Garkavy, P.F. 1970, The selection of malting varieties of spring barley according to the vigor of root development, Vop. Genet., pp.53-65.

[3] Hu, T., Metz, S., Chay, C., Zhou, H.P., Biest, N., Chen, G., Cheng, M., Feng, X., Radionenko, M., Lu, F. 2003, *Agrobacterium*-mediated large-scale transformation of wheat (*Triticum aestivum L.*) using glyphosate selection. Plant Cell Rep. 21: 1010-1019.

[4] Hurd, E.A. 1964, Root study of three wheat varieties and their resistance to drought and damage by soil cracking, Can. J. Plant Sci., 44:240-248.

[5] Hurd, E.A. 1968, Growth of roots of seven varieties of spring wheat at high and low moisture, Agron. J., 60:201-205.

[6] Hurd, E.A., Patterson, L.A., Mallough, D., Townley-Smith, T.F., Owen, T.F. 1972, Techniques used in producing Wascana wheat, Can. J. Plant Sci., 52:689-691.

[7] Kirichenko, F.G. 1963, The effect of plant selection according to root strength on increasing the yield and improving its quality in the progeny, J. Vestvik Sel Skokhoyaistvennoi Nauki No. 4, 3-30.

[8] Liu, G.D., Li, Z.S., Li, J.Y. 1998, A root system split pot experiment with various wheat genotypes for studying their differential utilization of phosphate in liquid and solid phase, Acata Agronomica Sinica, 24(1):78-83.

[9] Miao, G.Y., Yin, J., Zhang, Y.T., Zhang, A.L. 1998, Study on root growth of main crops in north China, Acata Agronomica Sinica, 24(1):1-6.

[10] Raper, C.D. Jr, Barber, S.A. 1970, Rooting systems of soybeans. I. Differences in root morphology among varieties, Agron. J., 62:581-584.

[11] Sullivan, C.Y., Ross, W.M. 1979, Selecting for drought and heat resistance in grain sorghum, Stress Physiology in Crop Plants (Eds H. Mussell and R. C. Staples, Wiley: New York), pp. 263-281.

[12] Ye, X.G., Shirley, S., Xu, H.J., Du, L.P., Clemente, T. 2002, Regular production of transgenic wheat mediated by *Agrobacterium tumefaciens*. Agricultural Sciences in China, 3: 239-244.

[13] Xia, G.M., Li, Z.Y., He, C.X., Chen, H.M., Brettell, R. 1999, Transgenic plant regeneration from wheat (*Triticum aestivum L.*) mediated by *Agrobacterium tumefacien*s. Acta Phytophysiologica Sinica, 25(1): 22-28.

WHEAT AND BARLEY MUTANTS SELECTED IN HYDROPONICS BY ROOT AND/OR COLEOPTILE CHARACTERISTICS AND THEIR RELATIONSHIP WITH WATER STRESS

A.R. PRINA, A.E. MARTINEZ, M.C. ARIAS, D.G. DIAZ, L. MANGUERS, M.G. PACHECO
Instituto de Genética "Ewald A. Favret",
CICVyA – INTA, Castelar, Argentina

Abstract

In order to produce a collection of lines with putative mutations related with response to water stress and root characteristics, of two bread wheat varieties were treated with three different mutagens. Approximately 50000 M_2 seeds per variety were germinated in plastic boxes with high osmotic NaCl solutions. Putative mutant seedlings with changes in morphological characteristics of roots and coleoptile were selected and further tested in M_3 and M_4 generations. Nine M_5 families with increased tolerance to NaCl or artificial drought (polyethylene glycol solutions) at four leaf stage were selected. Three families which presented the highest tolerance to drought and the highest capacity for maintaining water content under temporary drought conditions are considered the most interesting for further studies. Additionally, a barley mutant that shows a differential root behavior under hydroponic conditions is briefly described.

1. INTRODUCTION

It is widely recognized that water stress, in its broadest sense encompassing both deficit (caused by drought and salinity) and excess (caused by water-logging), is one of the major constraints for Agriculture [1, 2]. On the other hand, root genetics in crop plants is a subject little known in comparison to that of the aerial parts and efficient selection procedures for improving root systems, as well as well-defined indicators for improving water stress tolerance are needed [3, 4]. New genetic variability, most desirably controlled by single genes, of these characteristics is critical for understanding the genetics and physiology of root development and response to stress. In this study a collection of putative mutants were identified through a massive screening exercise of mutagenized wheat in the M_2 generation. The differences in germination capacity root and coleoptile characteristics were checked on advanced generations by germinating seeds in high osmotic solutions. The most promising progenies were additionally tested for longer periods using ionic and non-ionic osmolytes and artificial drought conditions. Besides, a single gene controlled barley mutant showing a different root growth pattern in hydroponics, which is potentially related with a differential water-logging response, is briefly described.

2. MATERIAL AND METHODS

2.1. Experimental material

Seeds of two spring bread wheat (hexaploid) cultivars, ProINTA-Isla Verde (PIV) and ProINTA-Elite (PEL) were mutagenized and their progenies were used in experiments of mutagenesis and selection for drought tolerance. In addition, seeds of a barley root mutant, (MC169) and of its parental line (MC182) were used for studies on root behavior in hydroponics.

2.2. Mutagenic treatments

Experiments of dosimetry were carried out with both wheat cultivars mentioned above. Samples of 40 seeds each were treated with several doses of X-rays, ethyl methane sulphonate (EMS) and sodium azide (SA). Treated seeds were sown in hydroponics using the sandwich method [5] which enabled the estimation of physiological damage induced by the treatments by measuring the length of the first fully grown leaf [6]. From these results (data not shown) we choose the treatments to be applied in each variety. They are presented in Table I.

TABLE I. MUTAGENIC TREATMENTS*

Variety	X-Rays (Gy)	EMS (% v/v)	Sodium Azide (10^{-4} M)
ProINTA-Isla Verde	1.2	0.25	2
	1.6	0.30	10
ProINTA-Elite	1.2	0.20	2
	1.6	0.25	10

*X-rays treatments were carried out at 15 mA and 120 kW and were applied on seeds with 12% moisture. EMS solutions were made in tap water, while sodium azide solutions were made in distilled water with buffer phosphate pH 6. Both chemicals were applied under constant shaking for 18 h.

2.3. Management of the experimental material

Two main management schemes (a) and (b) were followed as indicated in Table II. Management scheme (a) was followed in order to estimate the mutagenic effects of the treatments. Five hundred seeds per treatment were sown at a rate of 25 seeds per meter in the nursery field. At maturity four spikes per individual plant were harvested. M_2 plants were evaluated for the frequency of chlorophyll mutants in the green house. Management scheme (b) was followed in screening for putative mutants showing differences when germinated in high osmotic solution of NaCl. Initially 4500 seeds per treatment were sown at a density of 50 seeds per meter. At maturity M_1 plants were harvested in bulks per row (one bulk per row, 90 bulks per treatment). In addition to treated-seeds sown, 400 seeds per variety were sown without any treatment.

2.4. Estimation of mutagenic effects

2.4.1. Somatic mutations

The percentage of M_1 plants carrying well defined chlorophyll deficient stripes was used as a rough estimation of the mutagenic effects of each treatment. This analysis was done before heading on at least 200 M_1 plants per treatment.

2.4.2. Germinal mutations

The percentage of the M_1 spike-progenies carrying M_2 chlorophyll deficient seedlings was used as an estimation of the mutagenic effects on the germinal line. For this test, four spikes per M_1 plant were harvested at maturity and analyzed in the greenhouse. Each spike was individually sown in 5 cm diameter clay pots and pots containing spike progenies originated in the same M_1 plant were placed together. This methodology was used in order to distinguish newly induced mutants, which are waited to occur only in one, or at most two, of

the spike progenies of the chimeric M_1 plant, from variants originated in pre-existing variability or in accidental seed contaminations which would appear in all the spike progenies [7].

TABLE II. MANAGEMENT OF EXPERIMENTAL MATERIAL

Year	Generation	Activities	a) Mutagenic effects estimations	b) Screening for tolerant mutants
2000	M_1	Sowing at the nursery field	500 seeds (25 seeds/row)	4500 seeds (50 seeds/row)
		Harvesting	4 Individual spikes per M_1 plant	1 bulk per row (90 bulks per treatment)
2001	M_2	M_2 seedlings analysis	M_2 chlorophyll deficiencies in the greenhouse	M_2 seed bulks germinating in high osmotic solution
		Transplanting	—	Selected seedlings transplanted to the nursery field
		Harvesting	—	Individual M_2 plants
2002 - 2003	M_3 - M_4	M_3 and M_4 seedlings analysis	—	M_3 and M_4 seeds germinating in high osmotic solution
		Transplanting	—	Selected seedlings transplanted to the nursery field
2004	M_5	M_5 seedlings analysis	—	ITS, ITP and hydric stress tests

Original 5.000 seeds/ treatment
(2 doses x 3 mutagens x 2 varieties = 60.000 treated seeds)

2.5. Selection procedures

2.5.1. Massive screening of seedlings tolerant to NaCl

The methodology previously applied in barley [8] was adopted in this experiment. Seeds were first soaked in osmotic solution (3% NaCl) in an Erlenmeyer flask with constant shaking, for 20 hours, and then germinated on blotting paper soaked in a similar solution in plastic boxes. The germination capacity and morphological characteristics of the root and/or the coleoptile were observed. It is important to remark that these experiments were carried out in closed boxes that behaved as humid chambers; this probably enabled us to perform selections in a relatively high NaCl concentration when compared with similar experiments done by other authors.

This methodology was applied on M_2 seeds harvested in bulks per row. This kind of management has the following advantages: 1) In comparison with screening individual spikes or individual plant progenies, increase the capability of testing a good deal of M_2 seeds. 2) In comparison with making only one big bulk per treatment, offers the possibility to distinguish different mutational events, when similar mutants are found in different bulks. Besides it offers the possibility of recurrence to a relatively small quantity of seeds of the selected bulk. Samples of approximately 350 seeds per bulk were soaked in an Erlenmeyer with 2,5% NaCl,

under constant shaking for 20 h. Thereafter, the seeds were sown in plastic boxes on blotting paper sheet soaked in a similar 2.5% NaCl solution.

The boxes were kept in darkness, under room temperature for seven days. The length of the root and/or the coleoptile, and abnormalities observed in root growth were taken into account in this screening. Twenty four bulks, of approximately 350 seeds each, per treatment were analyzed, summing up more than 50.000 seeds per variety. The putative mutants were transplanted into pots in the greenhouse and later on, they were transplanted to the nursery field. A similar procedure was also applied in the M_3 and M_4 generations in order to confirm if the observed characteristics breed true, but using in this case 30 seeds per individual plant progeny. Six of those progenies were analyzed simultaneously in each plastic box.

2.5.2. Index of tolerance to salinity (ITS)

The index of tolerance to salinity (ITS) methodology was previously used in barley [9], it tests the capacity of growing biomass under NaCl osmotic stress conditions and has been successful to distinguish among tolerant and susceptible barley cultivars. It is the ratio of fresh weight of the aerial part of seedlings grown in a high osmotic solution to that of those grown in normal tap water for 14 days. In barley 50 seeds per treatment and a NaCl solution of 1% (171mM) were used. In our experiments with wheat we noticed a high heterogeneous effect of NaCl on seedlings of the same container, which depended on the position of the seeds in the container. As these effects are thought to be mainly due to draft, some modifications are proposed. The first one involves discarding the two sandwiches located at the borders of the container. In order to minimize draft effects, a second modification proposed involves placing the container carrying the growing seedlings into a case with 4 glass walls, (30 cm high) with its top covered with a thin cloth.

2.5.3. Index of tolerance to polyethylene glycol (ITP)

The methodology for determining ITS described above was followed in determining ITP, but with a 10% aqueous solution of the non-ionic osmolyte polyethylene glycol (PEG, PM 6000) was used instead of NaCl. Mutant lines previously selected in NaCl solutions were analyzed for their capacity to grow in a non ionic osmolyte. In this way, it could be possible to have an approach to distinguish if the differential response of the putative mutants would be due to osmotic stress or to sodium toxicity.

2.5.4. Index of tolerance to temporary drought (ITD)

Thirty seeds per putative mutant line were individually sown in clay-pots with 80 cm^3 of soil. Seedlings were grown in the green house for 21 days. On the 21st day, when all plants were at fourth leaf stage, 20 normal and healthy plants per mutant were selected for further testing. Two groups of five plants each were left without watering for six days, while the other two groups were maintained with normal watering. After that period, each individual plant was weighed (fresh weight) and, after being dried for 20 hrs in an oven at 65°C, the dry weight of each group of five plants was measured.

2.5.5. AFLP analysis

AFLP analysis [10] was carried out in order to check the genetic background of the putative mutants by comparing their DNA patterns with their corresponding parental varieties. Primers *Eco*-AAT + *Mse*-ACA and *Eco*-ACG + *Mse*-ATG were used.

3. RESULTS AND DISCUSSION

3.1. Somatic mutations

Similar frequencies of striped plants were observed in M_1 populations treated with different chemical mutagens, lower frequencies were observed in populations treated with X-rays Table III).

TABLE III. PERCENTAGE OF M_1 PLANTS CARRYING CHLOROPHYLL DEFICIENT STRIPES

Variety	X–Rays (Gy)		E M S (%v/v)			S A(10^{-4}M)		Control
	1.2	1.6	0.20	0.25	0.30	2	10	
ProINTA-Isla Verde	0.6 (347)[a]	0.3 (340)	—	4.6 (260)	4.0 (273)	7.0 (327)	5.7 (265)	0.0 (349)
ProINTA-Elite	0.0 (278)	0.0 (246)	7.5 (266)	7.6 (210)	—	2.5 (478)	5.1 (315)	0.0 (216)

[a] In brackets is the number of plants analyzed.

3.2. Germinal mutations

The percentage of the M_1 spike-progenies carrying M_2 chlorophyll deficient seedlings was used as an estimation of the mutagenic effects of the treatments on the germinal line. EMS applied at 0.3% on PIV variety behaved as the most powerful treatment (Table IV).

The spectrum of M_2 chlorophyll mutants is presented in Table V. In comparison with our previous results with diploid barley [11], a low frequency of albino mutants was obtained with the chemical treatments, contrasting with the high frequency of mutants carrying discontinuous patterns of chlorophyll deficiencies.

In Table VI a general comparison between present results in wheat with those of previous experiments in barley are presented.

Present results in wheat show the higher rates of somatic mutations in EMS- and SA-treatments. They contrast with our previous results in barley which showed X-rays and EMS as inducing a much higher frequency of somatic mutations (per M_1 leaf) in comparison with sodium azide [12]. It suggests that damages induced by the different agents would have differential expression between hexaploid wheat and diploid barley. A comparative analysis between somatic and germinal mutations using data from different genotypes and mutagenic agents has been previously made in barley [13].

In relation with germinal mutations induction, sodium azide treatments, which have been previously observed with contradictory results in wheat (Maluszinsky, personal communication) showed high levels of germinal chlorophyll mutations which are comparable with those of EMS in both barley and wheat experiments.

TABLE IV. PERCENTAGE OF M_1 SPIKE PROGENIES CARRYING CHLOROPHYLL DEFICIENT MUTANTS

	X-Rays(Gy)		EMS (%v/v)			SA (10^{-4}M)		Control
Variety	1.2	1.6	0.20	0.25	0.30	2	10	
ProINTA-	0	0.13	—	0.76	1.38	0.63	0.38	0
Isla	(799)[a]	(798)	—	(794)	(797)	(799)	(783)	(795)
Verde	(37)[b]	(39)	—	(31)	(34)	(34)	(39)	(39)
ProINTA-	0	0.13	0.63.	0.25	—	0.63	0.88	0
Elite	(797)	(800)	(798)	(796)	—	(798)	(797)	(730)
	(36)	(38)	(35)	(33)	—	(29)	(28)	(36)

[a] M_1 spikes progenies.
[b] Average of M_2 seedlings per M_1 spike progeny.

TABLE V. SPECTRUM OF CHLOROPHYLL DEFICIENT TYPES OBSERVED IN DIFFERENT M_1 SPIKE PROGENIES

	Viridis	Albino	Tigrina	Maculata	Striata	Discontinuous
EMS	10	0	5	2	0	8
SA	4	1	3	2	1	9
X-rays	0	1	1	0	0	0

TABLE VI. COMPARISON OF MUTATION FREQUENCIES OBTAINED IN WHEAT AND BARLEY BY MUTAGENIC TREATMENTS

	Wheat (present results)	Barley (previous results)
Somatic mutations	X-rays < EMS ≅ SA	X-rays ≅ EMS > SA
Germinal mutations	X-rays < EMS ≅ SA	X-rays < EMS ≅ SA
Spectrum of M_2 chlorophyll deficiencies	Discontinuous (37.8%) > Albina (2.2%)	Discontinuous (12.5%) < Albina (24.5%)
	n = 45	n = 490

3.3. Screening at germination in NaCl-solutions

After screening over 50000 M_2 seeds per variety, we obtained a total of 190 progenies in the M_3 generation, each one derived from a different M_2-selected plant by self pollination. As mentioned in *2.5.1.*, the same procedure, but using 30 seeds per plant progeny, was applied to the subsequent generations in order to select the families in which the observed differences breed true. In this way we obtained only 15 selected families to be tested more in detail in the M_5 generation. They corresponded to seven different origins of PIV (PIV-1 to -7), and seven of PEL (PEL-1 and -3 to -8). Five of them presented both, roots and coleoptiles longer than the control, two presented longer roots, six longer coleoptiles and one longer coleoptiles but shorter roots (Figs 1 and 2; Table VII). One family (PEL-2), was selected from observations in water and presented some deleterious characteristics, thus was not further tested as a potentially favorable mutant.

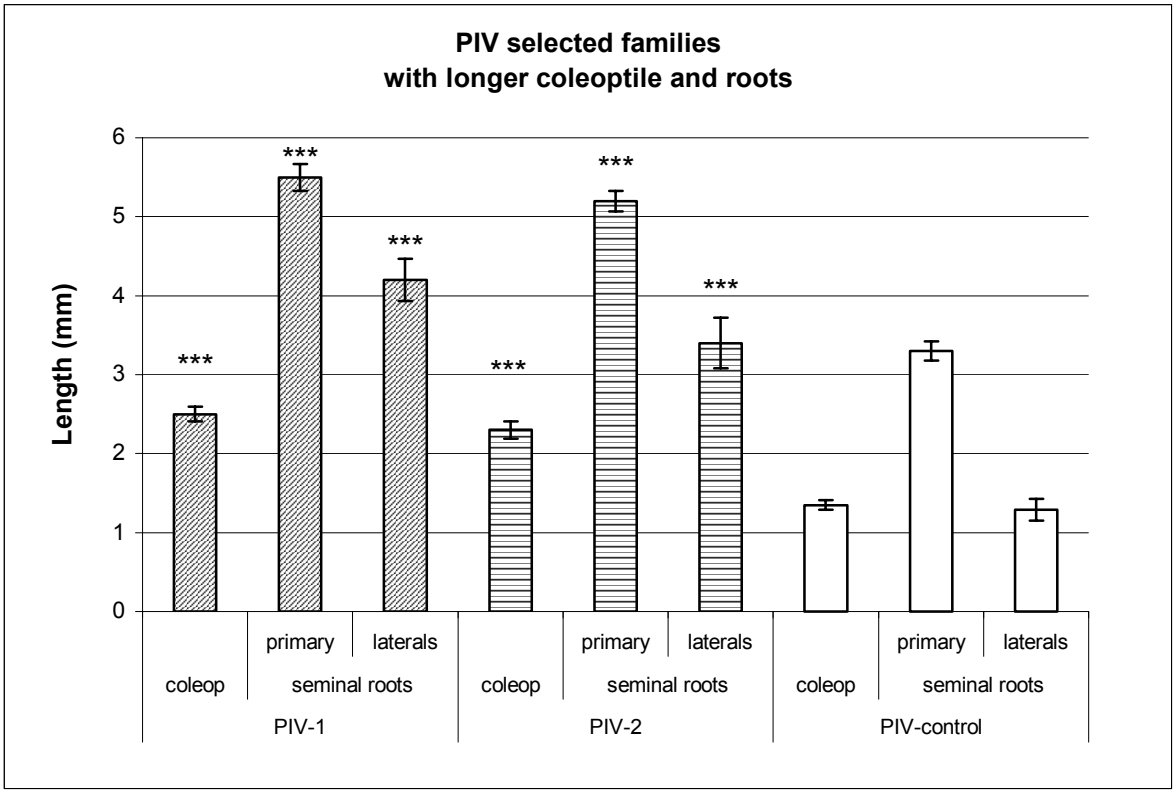


Fig. 1. Examples of PIV-families selected in 2.5% NaCl solutions.

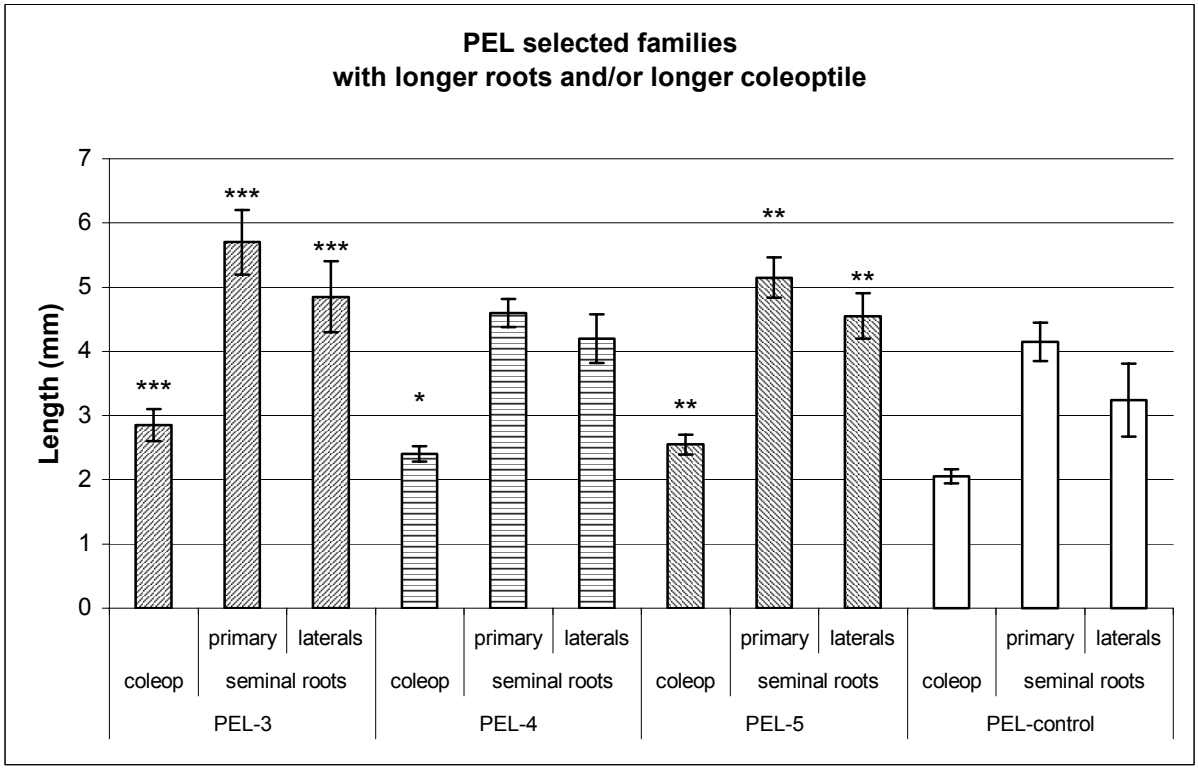


Fig. 2. Examples of PEL families selected in 2.5% NaCl solutions.

TABLE VII. MASSIVE SCREENING AT GERMINATION IN NaCl-SOLUTIONS

	Mutant Characteristics			
	Longer coleoptile & Longer roots	Longer roots	Longer coleoptile	Longer coleoptile & Shorter roots
Number of selected Families & their names	5	2	6	1
	PIV-1, -2 PEL-3, -5, -8	— PEL-6, -7	PIV-3, -4, -5, -6, -7 PEL-4	— PEL-1

3.4. AFLP analysis and the origin of the selected families

Fifty six fragments were observed using *Eco*-AAT + *Mse*-ACA and 70 in the case of *Eco*-ACG + *Mse*-ATG. The seven PEL-families presented the same banding patterns as the control, while only two of the seven selected PIV-families had the pattern of the control. All these families were isolated from different M_2 bulks, five of them originated from progenies of sodium azide treated seeds, while two were isolated from EMS and two from X-rays-treated materials.

3.5. Tests of seedling growth capacity in high osmotic solutions

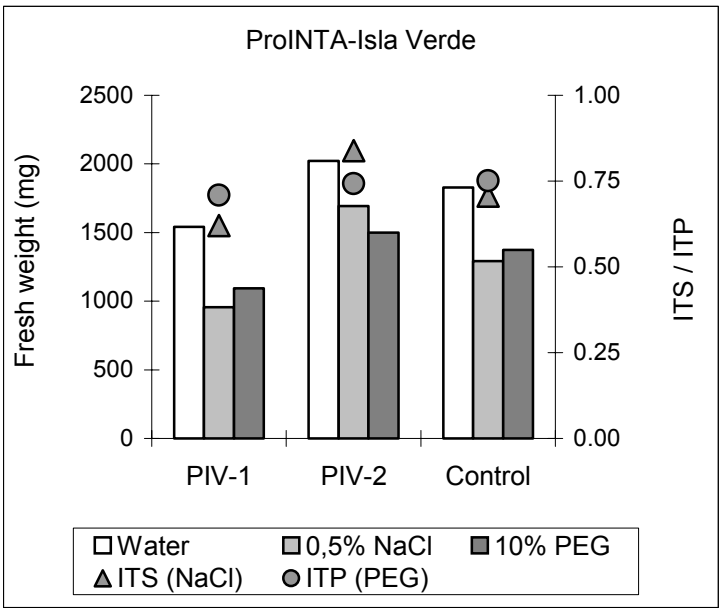
Tests of tolerance to grow in saline (ITS) or polyethylene glycol solutions (ITP) were applied on the M_5 selected families that presented similar AFLP patterns of their respective controls. Results are presented in Fig. 3 (A, B).

Only a few of the previously selected families showed marked differences with the corresponding controls when grown in these conditions, suggesting that the characteristics observed at germination in NaCl solutions do not necessarily correlate with the capacity of the seedlings to grow for a longer period in NaCl or PEG solutions. Only one PIV family (PIV-2) and two PEL families (PEL-6 and -7) presented ITS values over the controls. However, only one of them, PIV-2, produced more biomass than the control in NaCl solution. ITP coefficient was similar for the three PIV mutants, while a low correlation coefficient (0.015) was found between ITS and ITP values in PEL mutants. Only one PEL selected family had a higher ITP than the control, but considering these results in terms of biomass no one of the selected families was superior to the PEL-control.

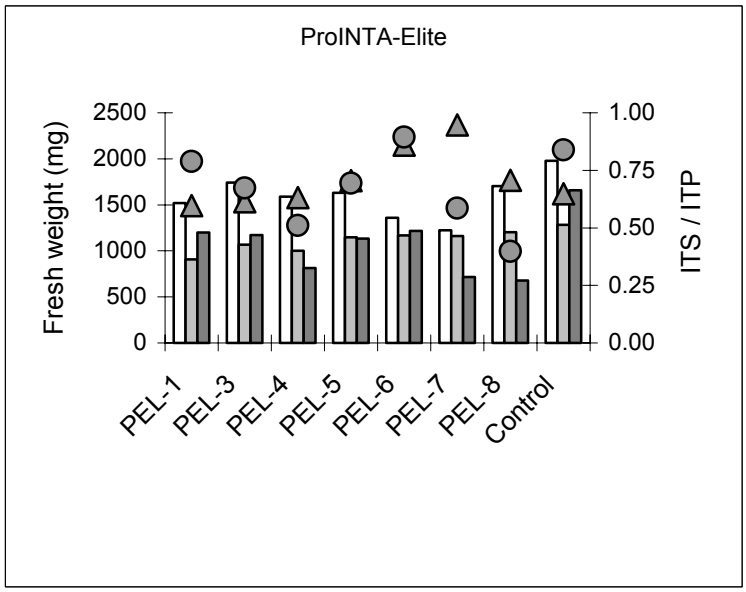
Both indexes, ITS and ITP, could be related with osmotic stress tolerance, meanwhile ITS could also have a component of sodium toxicity. Results previously obtained at germination with barley using NaCl or PEG solutions with comparative osmotic pressure showed a tight correlation. Those results suggest that salt inhibits seed germination primarily by osmotic effect. Additionally, in barley cultivars no correlation was observed between salt tolerance at germination or at seedling stage, suggesting different mechanisms acting at different stages [14]. Among several wheat, triticale and barley cultivars, small differences in biomass production in salinity were found and it was pointed out that for actual evaluation of salt tolerance rather than short-term water stress, plants must be grown for a prolonged period of about one month [15].

As mentioned in section *2.5.2,* in our experiments of growing wheat seedlings in osmolyte solutions we noticed that seedlings in the same container were heterogeneously affected by NaCl solutions. For this reason we propose to modify ITS and ITP procedures, by

cultivating seedlings inside a glass case, as described in *2.5.2.*, in order to avoid probable effects of draft. In one experiment carried out with the two genotypes growing in 1% NaCl, we observed that germination percentage increased two fold in both genotypes when the material is grown in the glass-case, meanwhile the variation coefficient of seedling weight decreased by several times in these conditions as it can be seen in Table VIII below.



A



B

Fig. 3. Biomass produced by seedlings of PIV (A) and PEL (B) selected families grown in high osmotic solutions.

TABLE VIII. VARIATION COEFFICIENT OF SEEDLING WEIGHT AND PERCENT OF GERMINATION OF PROINTA-WHEAT CULTIVARS

Variety	Variation coefficient		%Germination	
	Uncovered	Covered	Uncovered	Covered
ProINTA-Isla Verde	0.410	0.022	40.0 ± 15.0	95.0 ± 5.0
ProINTA-Elite	0.470	0.037	45.0 ± 15.0	90.0 ± 10.0

3.6. Temporary-drought tolerance experiments

M_5 plants of the previously mentioned selected families were subjected to temporary drought stress at four leaf stage in the greenhouse. Fresh and dry weights were measured after six days of growth under drought conditions (Figs 4 and 6). Based on these data, water content per group of 5 plants was calculated (Figs 5 and 7).

In normal watering conditions, fresh weight differed between the two control varieties and among the mutant families from the same variety (Figs 4 and 6), meanwhile all families showed water contents similar to the control (Figs 5 and 7). In temporary-drought conditions, water contents were similar among PIV-mutants but, varied among PEL-mutants. The same trend was observed for the relationship between the fresh weights obtained in the two different watering regimes. Based on plant fresh weight and water content data, the most promising mutants for tolerance to temporary-drought are PEL-1, -4 and -5. Taking into consideration that temporary-drought does occur occasionally during the life span of the crop, the simplicity of this methodology makes it adequate for testing materials stressed during several different periods of development, including adult plants.

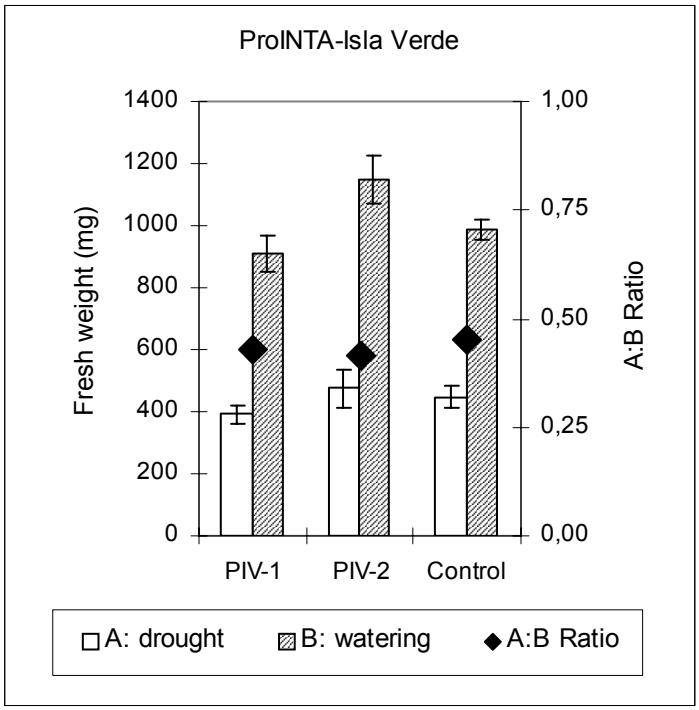


Fig. 4. Biomass produced by PIV-selected families cultivated in normal watering conditions or after six days without watering at fourth leaf stage.

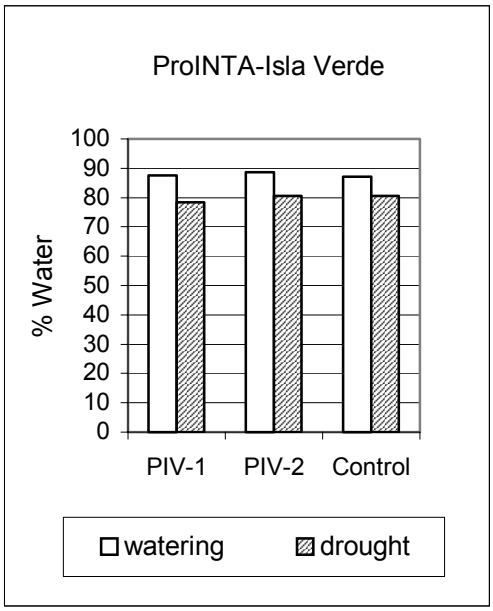


Fig. 5. Water content in PIV-selected families cultivated in normal watering conditions or after six days without watering at fourth leaf stage.

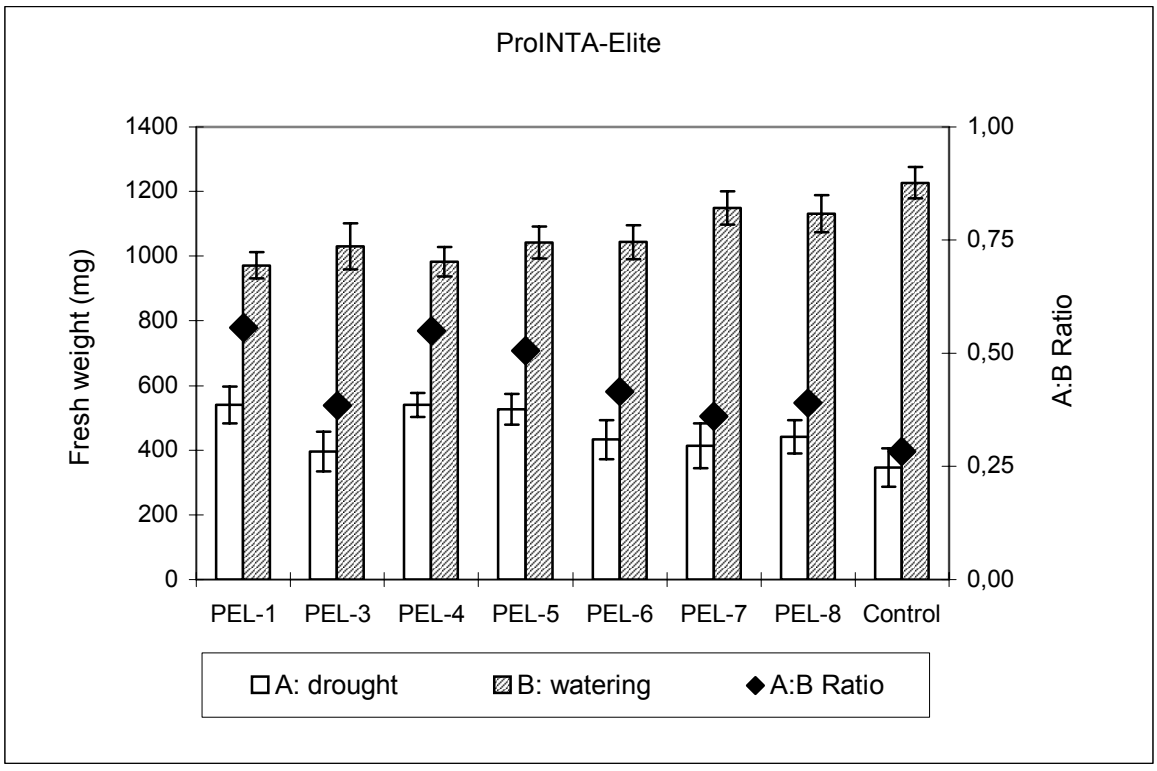


Fig. 6. Biomass produced by PEL-selected families cultivated in normal watering conditions or after six days without watering at fourth leaf stage.

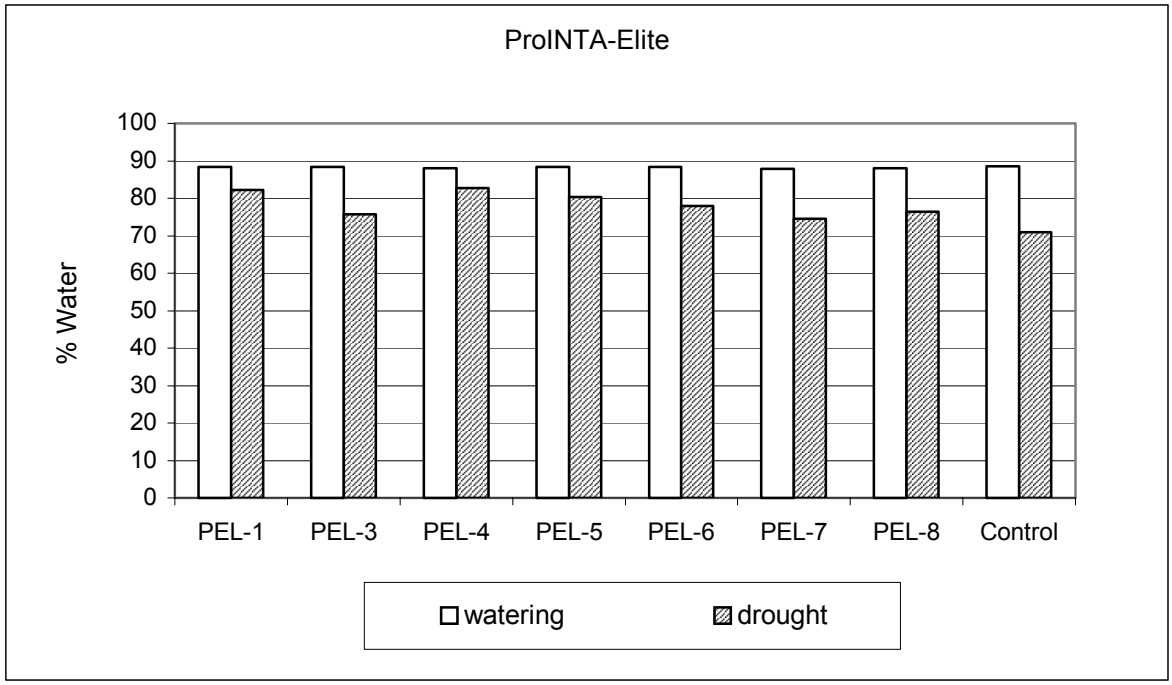


Fig. 7. Water content in PEL-selected families cultivated in normal watering conditions or after six days without watering at fourth leaf stage.

3.7. A barley mutant with longer roots

This mutant was identified in hydroponics using the sandwich method [5]. In addition to longer roots it did not present the characteristic growth pattern with windings and turnings usually observed on wild type barley roots in these conditions. Mutant roots grew straight down into the water surface without displaying any pronounced bending before they submerged. Genetic analysis indicated that mutant root characteristics were controlled by a single nuclear gene which showed semi-dominant expression. Ethylene diffusion from wild type roots was higher than that from the mutant. A phenocopy of this mutant was obtained by treating wild type seedlings with silver ions which are known to be antagonists of ethylene perception [16]. New studies about the response to the ethylene-related plant growth regulators aminoethoxyvinil glicine and aminocyclopropane carboxylic acid and to different environments for growing roots suggest that an additional production of ethylene is induced under certain environmental circumstances in roots of the wild type, but not in the mutant, and confirm that ethylene plays a central role in eliciting the observed wild type roots response when barley seedlings are grown by the sandwich method [5]. It was interpreted as an adaptative response of wild type barley seedlings to avoid water-logging [17].

CONCLUSIONS

The mass screening methodology described in this work, involving the germination of M_2 seeds in 2.5% NaCl solutions in closed plastic boxes was useful for selecting mutants with new seminal roots and/or coleoptile characteristics. No clear relationships were observed between the selected mutants and their capacity to grow in NaCl or PEG, but some of them seem to improve temporary drought tolerance. Differential effects, depending on the genotype, were observed when the selected mutants were germinated in NaCl or PEG solutions, suggesting that these osmolytes can affect genotypes by different mechanisms. Analysis of a barley root mutant showing no tropic response to submergence indicated that the typical growth with windings and turnings, usually observed in wild type barley seedlings

when grown in hydroponics by the "blotting paper sandwich method", is controlled by a single semi-dominant nuclear gene. The wild type barley root behavior is associated with an overproduction of ethylene in the sandwich assay and is interpreted as an adaptative response to avoid water-logging.

ACKNOWLEDGEMENTS

The technical assistance of Irlando García, Abel Moglie and Jorge Cayo Salinas is gratefully acknowledged.

REFERENCES

[1] Zhu, J-K. Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. 53 (2000) 247.

[2] Visser, E.J.W., Voesenek, L.A.C., Vartapetian, B.B., Jackson, M.B. Flooding and Plant Growth. Ann. Bot. 91 (2003) 107.

[3] Maluszinsky, M. Crop germplasm enhancement through mutation techniques (Proc. Intl. Symp. on Rice Germplasm Evaluation and Enhancement, 1998), (Rutger, J.N., Robinson, J.F., Dilday, R.H., Eds.), AAES Special Reports 195 Arkansas 72701 (1998) 74-82.

[4] Ashraf, M., Harris, P.J.C. Potential biochemical indicators of salinity tolerance in plants. Plant Science 166 (2004) 3.

[5] Mihyll, R.R., Konzak, C.F. A new technique for culturing and measuring barley seedlings. Crop Sci. 7 (1967) 275.

[6] IAEA. Manual on Mutation Breeding (1977).Tech. Rep. Series 119, FAO/IAEA, Vienna.

[7] Prina, A.R, Favret, E.A. Parabolic effect of sodium azide mutagenesis in barley. Hereditas 98 (1983) 89.

[8] Prina, A. R., Saione, H., Manghers, L., Ríos, R., Díaz, D., Robredo, C., Acevedo, A., Dieguez, M.J., Colombo, N., Arias, M.C. The barley chloroplast genome as a source of genetic variability for herbicide and abiotic stress tolerance. (Barley Genetics VIII, 2000), (LOGUE, S., Ed.), ISBN 0-086396-680-2, Vol. 3 (2000) 282-283.

[9] Manguers L., Diaz D.G., Herencia de la tolerancia a la salinidad en cebada ("Actas de Genética", (XXIX Congreso Argentino de Genética, 1999), (UNR Editora, Rosario, Argentina), Proc. XXIX Congreso Argentino de Genética (2000) 372.

[10] Voss, P., Hogers, R., Bleeker, M., Reijans, M., De Lee, T.V., Hones, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M. AFLP: a new technique for DNA fingerprinting. Nucleic Acid Res**.** 23 (1995) 4407.

[11] Prina, A.R. Efecto mutagénico y mutacromosómico de la azida sódica en cebada, Magister Scientiae Thesis, Escuela para Graduados en Ciencias Agropecuarias, INTA-UNLP (1978), pp 115.

[12] Prina, A.R., Favret, E.A. Comparative analysis of the somatic mutation process in barley. (Barley Genetics IV, 1981), (Asher, M.J.C., Ed.), Edinburgh University Press (1981) 886-891.

[13] Prina, A.R., Favret, E.A. Influence of marker genes on the expression of somatic mutations in barley. The Journal of Heredity 79 (1988) 371.

[14] Stanca, A.M., Romagosa, I., Takeda, K., Lundborg, T., Terzi, V., Cattivelli, L. Diversity in abiotic stress tolerances. In: Diversity in barley, (R. von Bothmer, T., van Hintum, H., Knüpffer, K., Sato, Eds.), Elsevier Science B.V. (2003) pp 179-199.

[15] Munns, R. Salt tolerance in wheat and barley. (Barley Genetics VIII, 2000), (Logue, S., Ed), ISBN 0-086396-680-2, Vol.1 (2000) 238-245.

[16] Martinez, A.E., Franzone, P.M., Aguinaga, A., Polenta, G., Murray, R., Prina, A. R. A nuclear gene controlling seminal root growth response to hydroponic cultivation in barley. Environ. Exp. Botany 51 (2004) 133.
[17] Martinez, A.E., Landau, A., García, P.T., Arias, M.C., Murray, R., Pensel, N., Prina, A.R. Two mutants affecting adaptative responses to abiotic stresses in barley seedlings. Czech J. Genet. & Plant Breed. 41 (2005) 1.

TRANSLOCATED SIGNALS REGULATING ROOT MERISTEM ACTIVITY IN LUPINS (*Lupinus albus* AND *L. angustifolius*)

C.A. ATKINS
School of Plant Biology,
The University of Western Australia, Crawley, Australia

Abstract

Pluripotent stem cells in flowering plants occur at the root and shoot apices, at the cambium of shoot organs and the root pericycle. These meristematic cells provide sites for cell division and post-embryonic organ differentiation. Their activity responds to environmental and endogenous cues that determine rate and direction of growth, developmental pattern and change in organ function. Recent analysis of gene expression in the shoot apical meristem (SAM) of *Arabidopsis* has revealed close cell/cell interactions and an exchange of signals between differentiating cell types. However, it is clear that the long distance translocation channels of vascular plants, phloem and xylem, also provide regulatory signals that influence the course of events in the SAM, such as the transition from vegetative to floral development. These channels serve as pathways for translocation of assimilates providing the vascular link between 'sources' and 'sinks' on the plant. Similarly, the below ground meristems responsible for root growth, lateral root initiation and branching as well as the initiation of nodules on legumes receive translocated shoot-derived 'signals' as well as assimilates in phloem. Physiological studies have established that such signals are integral components of meristem activity but their nature has not been clearly established.

1. INTRODUCTION

The principal xylem pathway is the transpiration stream that moves solutes and water taken up by roots to the shoot. This stream also bears products of root metabolism, such as the products of N_2 fixation in legume nodules or assimilation of soil N, and compounds that 'signal' features of the internal and external root environment to the shoot. Phloem provides the means for translocation of assimilates (principally sugars and amino acids) to heterotrophic organs like roots and root nodules, vegetative and reproductive apices, flowers and fruits. Analysis of phloem exudates collected in many ways from a range of species indicates a complex mixture of mineral nutrients, organic solutes of C, N and S, larger molecules such as polysaccharides, proteins, peptides and nucleic acids. Phloem not only links but also potentially communicates between assimilate sources, principally the leaves, and sinks for assimilates. Both xylem and phloem exudates contain the five, common, so-called plant hormones or plant growth regulators- cytokinins, auxin, gibberellins, the ethylene precursor ACC and abscisic acid. Although each of these has been assigned signalling or regulatory roles either alone or with others of the 'group of five' in complex networks in an array of plant processes these 'roles' largely remain unproven [1]. However, there is a wealth of evidence that has predicted the existence of other signalling molecules, mostly yet to be characterised, that have been inferred as translocated from one organ to another, particularly in phloem. Among the possibilities for these roles are steroids, sterols, other lipids, oligosaccharides, proteins and small peptides together with a range of RNAs.

This study was initiated to discover the role(s) that novel signal compounds in phloem, specifically low molecular weight bioactive peptides and small RNA species, have in gene regulation in shoot and root meristems of lupin. Lupin was chosen as a test plant because uncontaminated phloem exudate can be collected readily and in sufficient volume to detect potential signal solutes that may be present in very small amounts.

2. POTENTIAL NOVEL TRANSLOCATED SIGNALS

2.1. Peptides

A large number of potential peptide transporter genes have been revealed in the *Arabidopsis* genome. These belong to a number of transporter families of genes and it has been suggested that their abundance indicates a diversity of peptide substrates and reflects a central role for peptide transport in plant growth and development [2]. Given the diversity of peptide hormones or 'signals' that regulate growth and development in animals their occurrence, with similar signalling roles in plants is to be expected. Both xylem and phloem contain a small proportion of their N as peptides and there has been speculation that these fulfil a nutritional role or that they are hydrolysed from larger proteins within the translocation channels. However, if some are loaded onto phloem or xylem from surrounding cells then their membrane transport will need to be mediated. Similarly if they are formed from larger protein precursors in sieve tubes then a means for their unloading at sites of action will be critical.

Although evidence for a number of classes of endogenous plant signalling peptides has been assembled [3] only one has been shown to be translocated in phloem and to perform a signalling role at a distance from its source. Systemin, an 18 amino acid peptide, activates a signalling cascade that produces jasmonic acid and results in expression of enhanced resistance to pathogens/herbivores in target leaves distant from the actual site of damage. In keeping with the high level of bioactivity of peptides in animal systems, systemin is active at femto molar concentrations. Leaves of solanaceous spp receiving the peptide in phloem are induced to express and accumulate a series of systemic wound response proteins just as if they were wounded. The systemin cDNA encodes a 200 amino acid precursor that is cleaved to provide the 18 C-terminal residues for the bioactive translocated signal. Both the larger precursor and the translocated peptide are active in generating the response [4]) and putative high affinity receptors have been purified from tomato plasma membrane that will bind both. Recent analysis of systemin expression in tobacco has identified two bioactive 18 amino acid peptides, each of which is cleaved from the same 165 amino acid precursor, one from the C-terminal end, and the other from the N-terminus [5].

A similar bioactive peptide, also elicited by an oligosaccharide, is the 12-13 amino acid product of the *ENOD40* gene induced by rhizobial nod factors (lipochito-oligosaccharides) in initiating a nodule primordium in legume roots [6]. Unlike the systemin peptides that are cleaved from a protein precursor the ENOD40 peptides are encoded as such, each by a small ORF within a larger transcript. There has been some controversy regarding the molecular basis for the broader activity of these peptides but their action appears to be in inducing cell division together with other 'hormone' factors in the inner cortex of the legume root. As well as *ENOD40* transcripts being found in other tissues homologous genes have been identified in non-legumes [7] and, while there is no direct evidence for translocation of this extremely active peptide away from nodulation sites, its structure indicates it could be a substrate for ABC transporters [2].

A third group of bioactive peptides are the phytosulfokines (PSK), first isolated because of their ability to stimulate cell division in embryo cultures of *Asparagus*. These are sulphated tetra- or pentapeptides that have now been isolated from cell cultures of a number of plants. Like systemin, these peptides are processed products of a larger precursor protein, and while sufficiently small to be loaded and translocated in phloem or xylem long distance mobility of PSK in intact plants has not been studied.

The *CLAVATA* genes (*CLV1* and *CLV3*) of *Arabidopsis* are expressed in the shoot apical meristem (SAM) and function to regulate proliferation and differentiation of stem cells [8, 9]. *CLV3* encodes a peptide that appears to be a ligand for CLV1, a receptor kinase-like protein. CLV3 is a 96-amino acid peptide with a putative 18 amino acid N-terminal secretion signal [10]. There is no evidence that the CLAVATA peptide or a smaller cleaved bioactive product is translocated away from the SAM. However, a long distance signalling role has been proposed for a CLAVATA1-like receptor kinase in soybean [11]. Supernodulation mutants (*nts*) are defective in their ability to regulate the initiation of nodule primordia and as a consequence exhibit a dramatic increase in nodules [12]. Grafting experiments demonstrate clearly that initiation of both nodule and lateral root primordia is auto regulated by a phloem translocated shoot signal. It has been shown that the mutation is in *GmNARK* (*Glycine max* autoregulation receptor kinase), which is a receptor kinase similar to CLV1 [11]. Their data indicate that GmNARK functions in the leaf but exerts long distance control of nodulation with no detectable phenotype in the leaf itself, the SAM or floral tissues. While they have speculated that this receptor kinase is likely to interact with a ligand, analogous to the CLV extracellular peptide, there is no evidence for such a peptide yet. The homologous gene (*HAR1-1*) has now been isolated from *Lotus japonicas* using similar mutants [13].

A receptor kinase (S-locus receptor kinase or *SRK*) has also been identified as one of the 2 genes involved in the pollen recognition system in Brassicas. The kinase is expressed in the stigmatal papillae and the pollen borne determinant of self-incompatibility is a family of small polypeptides encoded by SCR (S-locus cysteine-rich) genes [14]. These have been shown to form a receptor-ligand pair during pollen self-recognition [15]. The *Arabidopsis* genome reveals a gene family of similar SCR proteins and it is possible that they have a diversity of functions with other receptor kinases.

A number of the phytotoxins produced by pathogens are also peptides [16] and there is good evidence that these are translocated and distributed throughout the plant. Some are linear, others are cyclic, and in a number of cases the transported peptide is hydrolysed to release a disease causing toxic component. Although a role for transporters that load these peptides or their precursors into translocation channels can be envisaged [2] there is no evidence yet to support the notion.

The few peptides now associated with signalling roles are likely to be joined by many others as this area develops [17], and among these will be a new suite of translocated peptide signals.

2.2. RNAs

Like the long held view that phloem contains peptides, the occurrence of nucleic acids in phloem has been well documented [18] and it was accepted that these were a minor translocated component. However no real significance was attached to RNA in sieve tube sap. Its origin was not established, though it was thought to be transferred or was a contaminant from the adjacent, nucleate, companion cells, and no specific function was attached to its ‘apparent’ translocation. This view has been completely revolutionised by the appreciation that plants have evolved supracellular control mechanisms that rely on directed systemic trafficking of macromolecular signals in phloem [19]. Among these are mRNAs that act as mobile transcripts. These transcripts are functional [20], transferring a ‘gain-of-function’ leaf trait from a mutant rootstock to a wild type scion in tomato. Importantly the mobile transcripts were localised and expressed in the SAM. The extent to which phloem traffics transcripts that function at a distance from their initial site of transcription has yet to be explored, but it is likely that many more examples that relate to normal plant development will be revealed.

However, there is a class of very small RNAs, micro RNAs, which may be equally important in establishing developmental patterns in plants. Posttranscriptional gene silencing (PTGS) is a mechanism that defends against infections by double stranded RNA (dsRNA) viruses. Replication intermediates of these viruses are detected and cleaved by a special RNase (Dicer) to produce short interfering RNAs (siRNAs) around 22 nucleotides long (reviewed in Ref. [21]). These short sequences are also formed by from endogenous dsRNA transcripts [22] and there is a growing body of evidence suggesting a role for these in developmental regulation, both in plants and animals [23]. RNA silencing in this way can lead to transmission of the silenced state throughout the organism. Plants establish resistance to viral replication at distant sites and in advance of infection [24] and there is a general view that the mobile signal in phloem is one of the siRNAs. This idea has been tested by biolistic delivery of a range of transcripts, including siRNAs, into leaves of transgenic tobacco that carried GUS as a positive marker for silencing [25]. This led to the conclusion that siRNAs or small intermediates induced by their detection provide the mobile silencing signals that cause systemic PTGS. The *Arabidopsis* genome reveals an abundance of microRNAs [22] and it has been suggested that among these will be long distance regulatory signals that form part of the molecular mechanism linking environmental or developmental cues in one part of the plant with responses elsewhere [23].

2.3. Examples of stem cell regulation by uncharacterised phloem-mobile signals

One of the most studied examples of phloem communication in transmitting a 'signal' is in the floral transition of SAM's. A wealth of physiological data has established the existence of a graft transmissible signal that originates as a consequence of the plant perceiving external stimuli in leaves. At different times members of the 'group of five' have been implicated in mediating the transition but definitive proof that one is the signal transmitted from leaves to the SAM has been elusive. Thus the nature of 'florigen', the substance proposed originally as the universal flowering hormone [26], has defied biochemical definition. Current thinking varies from the signal being an unknown plant growth regulator, yet to be identified, to a complex interaction of a number of the group of five hormones.

Analysis of flowering time genes in *Arabidopsis* has led to the identification of a complex regulatory network that comprises pathways for perception of day-length, for internal developmental signals and for a GA mediated response. A triple mutant in which genes for each of these pathways has been knocked out is completely unable to flower [27]). Despite this level of knowledge, genes and signals involved in communication between leaves and the SAM have not so far been revealed.

The possibility that the floral stimulus causes endogenous gene silencing in the SAM has led to speculation that 'florigen' may exist among phloem mobile RNAs, especially among microRNAs [23]. However detailed analysis of the RNA complement of the phloem of flowering and non-flowering *Arabidopsis* or other species have yet to be undertaken.

Some studies [28, 29] have sought the floral stimulus among the proteins and low molecular weight peptides in phloem exudate collected from flowering and non-flowering *Perilla*. Using HPLC fractionation followed by MALDI-TOF-MS more than 100 proteins and peptides were detected. Most were common between the two types of plant but four of the peptides (1-9 kDa) were specific to the phloem of flowering plants. Their sequences showed some similarity to protein kinases and purine permeases, others showed no similarity to known sequences. However, there have been no further reports of the nature of these peptides or their likely functional significance.

Two further examples involve basipetal transmission of a signal or signals that alter the rate and pattern of meristem initiation in roots. Autoregulation of nodulation (AON) revealed by physiological experiments on supernodulating soybean are most easily interpreted in terms of a phloem mobile signal, likely to be an inhibitor of further primordial progression, translocated from the shoot. Although the connection between a CLAVATA-like receptor kinase in leaves and a peptide ligand for the kinase that is also translocated to the root meristem is yet to be established, the newly discovered relationship between autoregulation and *GmNARK* ([11]) may represent a universal 'shoot-to-root' communication mechanism. A second example for a process that appears to rely on a phloem mobile basipetal signal is the response of roots of species that form 'cluster roots' to low nutrient supply, especially to low Pi [30]. Cluster roots are determinate, short lateral roots that proliferate in localised clusters as a result of initiating many new meristems. Split root experiments using *L. albus* indicate that development of cluster roots is systemically regulated by the shoot [30]. Changes in phloem-Pi levels following application of P to deprived plants was correlated with suppressed cluster development suggesting that the systemic signal may be associated with retranslocation of Pi. The possibility that a phloem mobile shoot signal similar to that postulated for *GmNARK* is involved, probably in addition to phloem Pi level, is worth considering. However, it has been suggested that a central signalling (hormonal) cascade accounted for the synchronous initiation of a cluster of new meristems on the lateral root [31]. Consistent with this idea, it has been reported [32] that cytokinin (CK) reduces expression of several Pi starvation responsive genes and that *Arabidopsis* mutants uncoupled in this respect are allelic to the *CRE1/WOL* locus. Thus a translocated signal may be linked to the two-component transduction mechanism associated with *CRE1* expression.

There is no doubt that many other examples of plant organ communication through regulatory translocated signals exist. Although the signals involved in the three examples chosen above are yet to be found each has a sufficiently well characterised physiological basis to justify their further investigation.

2.4. Phloem as a signalling conduit

While phloem accounts for the bulk flow of assimilate it also engages in specific solute exchanges, i.e. transfers that are not accounted for by mass flow. For example, asparagine is selectively transferred from xylem to phloem in the minor vein network of leaves and in stems [33]. Specific solute transfers are also a feature of xylem in lupin. The transpiration stream is selectively enriched with asparagine but not other amino compounds that are carried from the nodulated root system with the acropetal flow of water [34]. These transfers literally 'fashion' the C/N of phloem to meet the nutritional demands of different assimilate sinks [35]. Similar transfers in lupins have been noted for CK in stems [36] and for transfer of ABA from phloem to xylem in roots [37]. Thus the processes of solute transfer into and exchange between translocation channels that are essential for signals to move from their source to specific cellular sites of activity exist. Furthermore, the phloem especially has the potential to control the rate of transfer of solutes, probably through the activity of strategic groups of transfer cells, and this too is likely to be an essential component in regulating signal transmission. The concept of a supramolecular signalling network in plants [38] relies on regulated differential gating of plasmodesmata in sources and at sinks. Such a mechanism, inferred from early studies in this area involving viral transmission, has now been demonstrated [39]. The phloem network is thus likely to accommodate a range of signals that includes small bioactive molecules, peptides, oligosaccharides, proteins and nucleic acids, and to have the endogenous means to regulate their flux independently of mass flow.

Phloem exudates contain significant levels of protein that for many years were simply regarded as the metabolic machinery of the sieve tubes or structural components of the translocation apparatus. It is now clear that phloem-mobile proteins are a diverse group [40, 41] many of which are synthesised in the sieve tube-companion cell complex (ST/CCC) [42]. Some are quite large with MW as high as 100kDa but most are less than 20 kDa with a few as small as 2 kDa [41, 43]. Polypeptides of this size are no doubt able to pass readily through the plasmodesmata at their source of translation in the ST/CCC and at sinks following translocation. An initial attempt was made to use MALDI-TOF/MS to analyse the sieve tube proteins from white lupin. Crude phloem exudate was used with no attempt to separate the proteins before MS. The data obtained revealed many proteins ranging in size down to peptides of 1-2 kDa [43]. Interestingly analysis of exudates collected from racemes at anthesis and from fruits 13 and 31 days after anthesis indicated great variation in the low MW peptides. However, there appears to have been no attempt to characterise these peptides and small proteins further. As noted above bioactive peptides are likely to be components of this low MW group. PAGE as well as MALDI-TOF MS was used to analyse phloem exudate collected from a number of sites and from different ages of *Cucurbita maxima* plants [44]. Their data also indicate a large number of low MW proteins that varied in composition between phloem of sink and source tissues and with plant development. A more recent study [45] has identified a number of the soluble proteins in the 5–50 kDa range in cucurbit phloem. In addition to the prominent P-proteins that have been described many years earlier for this particular plant they identified a number of proteinase inhibitor proteins, ubiquitin and peroxidase. Recently a complete antioxidant defence system has been associated with functional sieve tubes [46]. Based on phloem sap from cucumber and pumpkin, superoxide dismutase, dehydroascorbate reductase and peroxidase proteins were detected. Furthermore their level of activity increased in response to drought stress. Although much more detailed analyses are required the application of PAGE together with TOF-MS to determine peptide mass fingerprints and partial sequence analysis as tools to identify proteins and peptides from phloem is now well established.

Functional genomic and proteomic approaches are being applied to discover genes and gene products associated with plant responses to both biotic and abiotic factors as a means to gain new information about response networks, and to identify traits for genetic manipulation or generate molecular markers to assist plant breeding. A subset of these genes is that encoding pathways for synthesis of signal molecules, for the transduction mechanisms they set in train and particularly for the translocation mechanisms involved in resultant plant responses. These same approaches are applicable to gene expression in and associated with phloem. The use of highly complex MS analyses for metabolite screening (metabolomics) in tissue extracts is also beginning to be applied to plants and should be directly applicable to transport fluids.

We already have a wealth of biochemical and physiological data that relate the source sink relations of white lupin (*Lupinus albus*), narrow-leafed lupin (*L. angustifolius*) and the Andean lupin (*L. mutabilis*) to the translocation of specific solutes of C and N in xylem and phloem (reviewed- in Ref. [35]) and more recently to the translocation of cytokinins [47]. This information is not available for most other species because unlike most other species the phloem stream of lupin does not occlude rapidly and can be sampled easily and reliably; shallow incisions made in the vasculature at most sites on the plant yield phloem exudate in volumes up to 0.1 ml within minutes. None of the ‘model plants’ display this feature. That does not mean that phloem contents cannot be sampled from these species, it is just much more difficult, and in some cases more susceptible to artefact [48]. For example the brown plant hopper stylets were used to collect phloem contents from rice [49] and aphid stylets

were used to collect sufficient sieve-tube contents from wheat [42], to separate and detect phloem-mobile proteins following 2D gel electrophoresis. A number of laboratories have used these techniques to sequence and identify separated phloem proteins [41]. However, the volumes required necessitated many collections from many stylets and it is unlikely that these techniques could be used for repeated routine sampling of phloem from the many plants required to establish changes in translocated solutes. The methods used [28, 29] to collect phloem solutes from *Perilla* involved the use of EDTA to prevent sieve tube occlusion at wound sites. Such methods lead to extensive cellular leakage and the composition of solutes must be interpreted with caution. Proteins have been characterised in the exudate from castor bean that, like lupin, bleeds freely from wounded phloem [50] and from species of *Cucurbita* that also bleed phloem exudate profusely from severed fruit stalks and other tissues [44, 45].

3. POTENTIAL SIGNIFICANCE

Phloem-mediated transport of assimilates together with translocation of water and nutrients in xylem are among the most critical processes in plants. The emerging concept that these channels are much more than conduits to deliver nutritional commodities but participate directly in transmitting signals that regulate organ development, or are transduced as responses to intrinsic or external change, provides a new framework in which to view translocation. These communication functions lie at the heart of plant responses to both biotic and abiotic stresses, transmitting information through the transfer of signal molecules that link sites of perception, as well as those that generate assimilates, to targets or sinks. The identity of some of the types of signal molecule in phloem is beginning to emerge from initial attempts to characterise translocated macromolecules. However, it is clear that a much more detailed analysis of a wide range of solutes, both of low MW as well as macromolecules, in both xylem and phloem is essential to detect and identify a broader range of signals. Because these signals represent the mechanisms that govern both rate and pattern of growth they are determinants of yield, harvest index and adaptation to environmental change.

Most plant breeding programs, whether through conventional means or assisted by molecular approaches are aimed at discovering traits, genes or markers that can be exploited to alter these responses and in so doing enhance the value and utility of crops. The tools of functional genomics will no doubt identify a myriad of genes that reflect adjustment of plant processes associated with these responses. However, knowledge of and the ability to alter aspects of signalling networks and the transduction pathways that respond to translocated signals, both of which precede physiological adjustment, would provide a means to intervene at a much earlier and more critical point in the response. Such knowledge and the genes that regulate signal flow would be new and powerful tools in the plant breeder's armoury. The first step in developing this approach is to detect and identify the translocated 'signals' involved. High throughput proteomics and metabolic profiling assisted by modern methods for MS analysis offer new and innovative tools to achieve this step. Their application to translocation channels in lupin is soundly based on a detailed knowledge of the underlying structural and biochemical features that account for the source sink relations of the species [35]. Such detailed knowledge is not available for other crops. Lupins are the most important pulse crop in Australia and, despite a continuing breeding effort through both conventional and marker assisted means, a new tool that links plant response to newly identified phenotypic traits could provide a way to further enhance their utility in agriculture.

Autoregulation of nodule initiation and the formation of cluster roots are two examples of root-based processes that appear to be regulated by phloem borne systemic

signals. Each is a feature of lupin and provides a useful starting point for studies aimed at revealing the nature and role of novel translocated signals.

ACKNOWLEDGEMENTS

Financial assistance from the Australian Research Council to CAA is gratefully acknowledged.

REFERENCES

[1] Rock, C.D., Quatrano, R.S. 1995. The role of hormones during seed development. In: Davies, P.J. (Ed.), Plant Hormones: Physiology, Biochemistry and Molecular Biology, pp. 671-697, Kluwer Academic Publishers, Dordrecht.

[2] Stacey, G., Koh, S., Granger, C., Becker, J.M. 2002. Peptide transport in plants Trend Plant Sci 7:257-263.

[3] Lindsey, K., Casson, S., Chilley, P. 2002. Peptides: new signalling molecules in plants. Trends Plant Sci 7:78-83.

[4] Dombrowski, J.E., Pearce, G., Ryan, C.A. 1999. Proteinase Inhibitor-Inducing Activity of the Prohormone Prosystemin Resides Exclusively in the C-Terminal Systemin Domain Proc Nat Acad Sci (USA) 96:12947-12952.

[5] Pearce, G., Moura, D.S., Stratmann, J., Ryan, C.A. 2001. Production of multiple plant hormones from a single polyprotein precursor. Nature 411:817-820.

[6] Hirsch, A.M., 1992. Development biology of legume nodulation. New Phytol 122:211-237.

[7] Kouchi, H., Takane, K., So, R.B., Ladha, J.K., Reddy, P.M. 1999. Rice ENOD40: isolation and expression analysis in rice and transgenic soybean root nodules. Plant J 18:121-129.

[8] Brand, U., Fletcher, J.C., Hobe, M., Meyerowitz, E.M., Rudiger, S. 2000. Dependence of stem cell fate in Arabidopsis on a feedback loop regulated by CLV3 activity. Science 289:617-619.

[9] Schoof, H., Lenhard, M., Haecker, A., Mayer, K.F.X., Jürgens, G., Laux, T. 2000. The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. Cell 100:635-644.

[10] Fletcher, J.C., Brand, U., Running, M.P., Simon, R., Meyerowitz, E.M. 1999, Signalling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems. Science 283:1911-1914.

[11] Searle, I.R., Men, A.E., Laniya, T.S., Buzas, D.M., Iturbe-Ormaetxe, I., Carroll, B.J., Gresshoff, P.M. 2002. Long-distance signalling in nodulation directed by a CLAVATA1-like receptor kinase. Science 299:109-112.

[12] Carroll, B.J., McNeil, D.L., Gresshoff, P.M. 1985. Isolation and Properties of Soybean [Glycine max (L.) Merr.] Mutants that nodulate in the presence of high nitrate concentrations. Proc Nat Acad Sci (USA) 82:4162-4164.

[13] Krusell, L., Madsen, L.H., Sato, S.I., Aubert, G., Genua, A., Szczyglowski, K., Duc, G., Kaneko, T., Tabata, S., de Bruijn, F., Pajuelo, E., Sandal, N., Stougaard, J. 2002 Shoot control of root development and nodulation is mediated by a receptor-like kinase. Nature 420:422-426.

[14] Schopfer, C.R., Nasrallah, M.E. 1999. The male determinant of self-incompatibility in Brassica. Science 286:1697-1799.

[15] Takayama, S., Shimosato, H., Shiba, H., Funato, M., Che, F-S., Watanabe, M., Iwano, M., Isogai, A. 2001. Direct ligand–receptor complex interaction controls Brassica self-incompatibility Nature 413:534-538.

[16] Walton, J.D. 1990. In: H. Kleinkauf and H.V. Doehren (Eds.), Biochemistry of Peptide Antibiotics. pp 179-203, Walter and de Gruyter, Germany.
[17] Miklashevichs, E., Czaja, I., Rohrig, H., Schmidt, J., Michael, J., Schell, J., Walden, R. 1996 Do peptides control plant growth and development. Trend Plant Sci 1:411.
[18] Ziegler, H. 1975. Nature of transported substances. In: A. Pirson & M.H. Zimmerman (Eds.), Encyclopedia of Plant Physiology, pp. 59-100, Springer-Verlag, Germany.
[19] Haywood, V., Kragler, F., Lucas, W. 2002. Plasmodesmata: Pathways for protein and ribonucleoprotein signaling. Plant Cell Suppl 2002: S303-S325.
[20] Kim, M., Canio, W., Kessler, S., Neelima, S. 2001. Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. Science 293:287-289.
[21] Hannon, G.J. 2002. RNA interference. Nature 418:244-251.
[22] Llave, C., Kasschau, K.D., Rector, M.A., Carrington, J.C. 2002 Endogenous and silencing-associated small RNAs in plants. Plant Cell 14:1605-1619.
[23] Jorgensen, R.A. 2002. RNA traffics information systemically in plants. Proc Nat Acad Sci (USA) 99:11561-11563.
[24] Klahre, U., Crete, P., Leuenberger, S.A., Iglesias, V., Meins, F. Jr. 2002. High molecular weight RNAs and small interfering RNAs induce systemic post-transcriptional gene silencing in plants. Proc Nat Acad Sci (USA) 99:11981-11986.
[25] Palauqui, J-C., Elmayan, T., Pollien, J.M., Vaucheret, H. 1997 Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted from silenced stocks to non-silenced scions. EMBO J 16:4738-4745.
[26] Chailakhyan, M.K. 1936. New facts in support of the hormonal theory of plant development. C.R. (Dokl) Acad Sci URSS 13:79-83
[27] Colasanti, J., Sundaresan, V. 2000. 'Florigen' enters the molecular age: long-distance signals that cause plants to flower. Trends Biochem Sci 25:236-240.
[28] Hoffmann-Benning, S., Kende, H., Zeevaart, J.A.D. 2000. Analysis of phloem sap of flowering and non-flowering *Perilla* plants. Plant Physiol Meeting Supp., p30.
[29] Hoffmann-Benning, S., Gage, D.A., McIntosh. L., Kende, H., Zeevaart, J.A.D. 2002 Comparison of peptides in the phloem of flowering and non-flowering Perilla and lupine plants using microbore HPLC followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Planta 216:140-147.
[30] Shane, M.W., deVos, M., de Roock, S., Lambers, H. 2003. Shoot P status regulates cluster-root growth and citrate exudation in *Lupinus albus* grown with a divided root system. Plant Cell Environ 26:265-273.
[31] Watt, M., Evans, J.R. 1999. Proteoid roots, physiology and development. Plant Physiol 120:705-716.
[32] Franco-Zorrilla, J.M., Martin, A.C., Solano, R., Rubic, V., Levva, A., Pas-Ares, J. 2002 Mutations at CRE1 impair cytokinin-induced repression of phosphate starvation responses in Arabidopsis. Plant J 32:353-360.
[33] McNeil, D., Atkins, C.A., Pate, J.S. 1979. Uptake and utilisation of xylem borne amino compounds by shoot organs of a legume. Plant Physiol 63:1076-1081.
[34] Layzell, D.B., Pate, J.S., Atkins, C.A., Canvin, D.T. 1981 Partitioning of carbon and nitrogen and nutrition of root and shoot apex in a nodulated legume. Plant Physiol 67:30-36.
[35] Atkins, C.A. 2000. Biochemical aspects of assimilate exchange along the phloem path: Exchange of N-solutes in lupin. Aust Jl Plant Physiol 27:531-537.
[36] Taylor, J.S., Thompson, B., Pate, J.S., Atkins, C.A., Pharis, R.P. 1990. Cytokinins in the phloem sap of white lupin (*Lupinus albus* L.) Plant Physiol 94:1714-1720
[37] Wolf, O., Jeschke, W.D., Hartung, W. 1990. Long distance transport of abscisic acid in NaCl-treated intact plants of Lupinus albus. JExp Bot 41:593-600.

[38] Lucas, W.J., Ding, B., van der Schoot, C. 1993. Plasmodesmata and the supracellular nature of plants. New Phytol. 125:435-476.
[39] Baluska, F., Cvrckova, F., Kendrick-Jones, J., Volkmann, D. 2001. Sink plasmodesmata as gateways for phloem unloading. MyosinVIII and calreticulin as molecular determinants of sink strength? Plant Physiol 126:39-46.
[40] Crawford, K.M., Zambryski, P.C. 1999. Phloem Transport: are you chaperoned? Curr Biol 9: R281-R285.
[41] Hayashi, H., Fukuda, A., Suzui, N., Fujimaki, S. 2000. Proteins in the sieve element-companion cell complexes: their detection, localization and possible functions. Aust J Plant Physiol 27:489-496.
[42] Fisher, D.B., Wu, Y., Ku, M.S.B. 1992. Turnover of soluble proteins in the wheat sieve tube. Plant Physiol 100:1433-1441.
[43] Marentes, E., Grusak, M.A. 1998. Mass determination of low-molecular-weight proteins in phloem sap using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. JExp Bot 49:903-911.
[44] Kehr, J., Haebel, S., Blechschmidt-Schneider, S., Willmitzer, L., Steup, M., Fisahn, J. 1999. Analysis of phloem protein patterns from different organs of *Cucurbita maxima* Duch. by matrix-assisted laser desorption/ionization time of flight mass spectrometry combined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Planta 207:612-619.
[45] Haebel, S., Kehr, J. 2001. Matrix-assisted laser desorption/ionization time of flight mass spectrometry peptide mass fingerprints and post source decay: a tool for the identification and analysis of phloem proteins from *Cucurbita maxima* Duch. separated by two-dimensional polyacrylamide gel electrophoresis. Planta 213:586-593.
[46] Walz, C., Juenger, M., Schad, M., Kehr, J. 2000. Evidence for the presence and activity of a complete antioxidant defence system in mature sieve tubes. Plant J 31:189-197.
[47] Emery, R.J.N., Leport, L., Barton, J.E., Turner, N.C., Atkins, C.A. 1998. Cis-Isomers of cytokinins predominate in chickpea seeds throughout their development. Plant Physiol 117:1515-1523.
[48] Atkins, C.A. 1999. Spontaneous phloem exudation accompanying abscission in *Lupinus mutabilis* (Sweet). J Exp Bot 50:805-812.
[49] Nakamura, S., Hayashi, H., Mori, S., Chino, M. 1993. Protein phosphorylation in the sieve tubes of rice plants. Plant Cell Physiol 34:927-933.
[50] Schobert, C., Baker, L., Szederkenyi, J., Grossmann, P., Komor, E., Hayashi, H., Chino, M., Lucas, W.J. 1998. Identification of immunologically related proteins in sieve-tube exudate collected from monocotyledonous and dicotyledonous plants. Planta 206:245-252.

DEVELOPMENT AND EVALUATION OF DROUGHT RESISTANT MUTANT GERMPLASM OF *Vigna unguiculata* AND *Vigna subterranea*

M.H. SPREETH, J.A. DE RONDE
Agricultural Research Council,
Roodeplaat Vegetable and Ornamental Plant Institute, Pretoria, South Africa

Abstract

The aim of this project was to select cowpea and bambara groundnut plants with improved levels of drought resistance without alteration to the colour of the testa or the growth form. M_2 to M_5 generation plants were tested. The M_2 to M_4 plants were evaluated in the wooden boxes in the greenhouse and the field. Six cowpea mutant lines and seven bambara groundnut lines were included in a physiological screening experiment that was conducted in the greenhouse. One cowpea line exhibited high yield under watered conditions, and three under drought stress conditions. Three bambara groundnut mutants yielded more than the parent, and one showed relatively high yield under drought stress. It proved possible to examine mutant plants at the seedling stage in wooden boxes, mature plants were screened in rain-out shelter and physiological traits were distinguished among the tested lines for drought stress. Roots of mature plants were also assessed and variation could be correlated with drought tolerance. Chlorophyll fluorescence was found to be a good predictor of plant performance in drought conditions.

1. INTRODUCTION

According to the New Agriculturist the pulses are known as the "Poor man's meat" because they are often grown in areas where the availability of animal protein is limited. The high protein content as well as the carbohydrates and lipids in the legumes make it an important part of a nutritious diet [1]. These crops are also very versatile in that the seeds, immature pods and also leaves can be consumed while the plant residues that remain after harvesting can be used as a cattle fodder. Although cereals provide the bulk of the world's food produce the intensive cropping has resulted in problems with soil fertility, pests and diseases. Introducing pulses could break the pest and disease cycle as well as improve the soil fertility because of their nitrogen fixation qualities.

The crops that were selected for this study were *Vigna unguiculata* (cowpea) and *V. subterranea* (bambara groundnut). Both these crops are indigenous in Africa and have been used for many years as a source of protein, vitamins and minerals for people as well as cattle fodder and green manure. The subsistence farmers in semi-arid parts of Africa are the main producers of cowpeas and bambara groundnuts and the countries that produce the bulk of the crop are Nigeria, Niger, Mali, Myanmar and Malawi [2]. Bambara groundnuts are mainly produced for household purposes while cowpeas are often produced as a cash crop. Although both these crops are regarded as drought resistant, a lot of variation occurs within each species. The objective of this project was to improve the drought tolerance and yield of these neglected crops to enable their production in marginal areas where rainfall is either scarce or unreliable.

Existing cultivars and lines of these crops were evaluated to identify plants with favourable traits. Seeds of the selected cowpea line IT93K129-4 and bambara groundnut line SB1-1, were irradiated with gamma rays and their progenies were screened for drought tolerance and yield. Screening was in the greenhouse and the field using physiological methods and phenotypical observations. The roots systems of plants that exhibit drought tolerant characteristics were evaluated using the root architecture box technique described in Ref. [3].

2. MATERIALS AND METHODS

2.1. Plant material

2.1.1. Cowpea (Vigna unguiculata)

Over the duration of the research project various cowpea mutant lines were screened in comparison with control lines from the International Institute of Tropical Agriculture (IITA) in Nigeria. These lines included the control line IT96D-602 (drought tolerant), TVu7778 (susceptible) and parent line of the mutants, IT93K129-4. This line was selected for its light beige to white coloured seeds, an upright growth form and good yield.

The initial irradiation dosages used for IT93K129-4 ranged between 0 and 300 Gy. The irradiated seeds (n = 100) were evaluated in pots in the greenhouse, in wooden boxes and some seeds were also germinated *in vitro*. After these irradiation dosage trials, 180 Gy was found to be the optimal dosage and was thus selected for all subsequent irradiation for the bulk of the seeds.

The M_1 seeds were planted in the field and the M_2 seeds were harvested from these plants. Each plant was allocated a number that was used for that specific plant and its offspring. Some of the M_2 seeds were subsequently planted in the greenhouse, rain-out shelter and in the field to select lines with higher levels of drought tolerance (Table I). The selected M_3 and M_4 lines were replanted in the wooden boxes in the greenhouse and the six most promising lines were selected for the physiological screening experiments.

2.1.2. Bambara groundnut (Vigna subterranea)

Bambara groundnuts are normally cultivated by small scale farmers for household purposes. So far very little or no development has been done on this crop. It was therefore not possible to identify bambara groundnut lines or selections with known levels of drought tolerance. The bambara groundnut line SB1-1 was selected for the colour of the hilum which is widely accepted by the consumers and also for the availability of seed. The determination of the irradiation dosage and screening was performed in a similar fashion as with cowpea (Table I).

2.2. Screening methods

2.2.1. Rain out shelters and Field experiments

Hundred and forty six cowpea lines were planted in the field in Stellenbosch, South Africa and the control lines IT96D-602 and IT93K129-4 were planted as border rows. The plants were watered for the first two weeks after which no water was applied. Very little rain fell during the stress period (17 mm in January) and the plants experienced severe moisture stress. Seeds were harvested from all the plants that survived the stress treatment

Cowpea seeds were also planted in two rain-out shelters at Roodeplaat, Pretoria, South Africa. The first rain-out shelter contained 120 mutant lines while 70 mutant cowpea lines were planted in the second shelter. The seeds were planted and watered until they germinated and the first trifoliate leaves of the seedlings were fully extended. The plants did not receive any water for two months, re-watering took place at this stage as the leaves were severely wilted. Plants were allowed to form seeds.

M_3 bambara groundnut seeds were also planted in a rain-out shelter at Roodeplaat, selection was based on the number of seeds formed during the previous season. These seeds were planted and allowed to germinate and when the first trifoliate leaves were fully extended all watering were stopped and the plants were left to dry out. The plants did not receive any water for a period of four and a half months, where after the plants were re-watered. The plants were then left to form seeds and the yield of each line was determined.

TABLE I. NUMBER OF COWPEA AND BAMBARA GROUNDNUT PLANTS FROM DIFFERENT GENERATIONS SCREENED DURING THE COURSE OF THIS PROJECT

Crop	Seeds irradiated	Survived Progenies	Number screened in wooden boxes	Number screened in field	Number subjected to physiological screening	Total number screened
Cowpea	17 000	M2 = 8230	891	1239		2130
		M3	294	190	3	487
		M4	48		3	51
Bambara	3200	M2 = 913	40	913	-	913
		M3 = 309	158	144	7	309

2.2.2. Wooden box

The wooden box procedure was used for the 2004 screening of M_3 and M_4 cowpea seedlings. Seeds were sown in rows directly on a soil mixture of peat/vermiculite/sand (5:2:2) in the wooden boxes (approximately 800mm x 150 mm x 200 mm), and thinned out to ten seedlings of each line per treatment. Eleven boxes were planted with 26 plants each. Plants were allowed to grow for 2 weeks, where after water was withheld. Observations for moisture stress symptoms were made until 75% of the plants had reached the permanent wilting point. Plants were then re-watered and the survival and recovery of the plants were also noted. The number of plants that survived the stress was noted and the plants were allowed to re-grow and to form seeds.

2.2.3. Physiological screening of M_4 mutants

Before planting, the seeds were treated with rhizobium innoculum (*Bradyrhizobium* sp.*)* The plants were planted in 25 cm pots containing a soil mixture described above. The plants were kept in the greenhouse whose night - day temperature regime was 18–28°C, and were watered twice a week. After a 45 - 50 day growth period, the plants of the stress treatment received one last watering after which the soil was allowed to dry out. Measurements were taken every second day as the stress intensified. Just before the plants reach permanent wilting point, the plants were re-watered and one last measurement to assess the recovery potential of the plants was taken two or three days later.

Apart from the two control lines, IT96D-602 and IT93K129-4, six other mutant lines were included in the physiological screening of the cowpea trials (Table II) and for the bambara groundnut trials the control line SB1-1 and seven mutant lines were included (Table II).

TABLE II. COWPEA LINES AND MUTANTS SELECTED FOR PHYSIOLOGICAL SCREENING EXPERIMENTS

Crop	Plant name or number	Remarks
Cowpea	IT96D-602	Drought tolerant control line
	IT93K129-4	Mother material of the mutants
	164	M_3 generation
	217	Mutant line with short growth season (M_4 generation)
	46	M_4 generation
	447	M_4 generation
	MA_1	M_3 generation
	MA_2	M_3 generation
Bambara groundnut	SB1-1	Control line and mother material of the mutants
	1	M_3 generation; high seed production
	9	M_3 generation; high seed production
	79	M_3 generation; high seed production
	104	M_3 generation; high seed production
	120	M_3 generation; high seed production
	134	M_3 generation; high seed production
	144	M_3 generation; high seed production

2.2.3.1. Chlorophyll fluorescence

Chlorophyll-a fluorescence transients were measured using a Plant Efficiency Analyser (PEA, Hansatech Ltd. King's Lynn, Norfolk, UK). The fluorescence transients were induced by a red light of 600 W m^{-2} intensity (excitation intensity) provided by 6 light-emitting diodes. Leaves were covered for 1 hour, using leaf clips and PEA measurements taken. During the first second of illumination, the following data were stored: F_m (maximal fluorescence intensity when all the reaction centres (RC's) are closed); F_O (fluorescence intensity at 50 µs when all RC's are open); F_J (fluorescence intensity at 100 µs, 300 µs and 2 ms); and F_I (fluorescence intensity at 30 ms); the time t_{Fmax} to reach F_M and the area between the fluorescence transient and the level of $F_{M.}$ These transients were quantified using the Biolizer program. This data was used to calculate the phenomenological and biophysical expressions. The JIP-test [4] refers to the main steps for F_O-J-I-P. The energy fluxes of absorption (ABS), trapping (TR) and electron transport (ET) through photosystem II (PSII as well as the flux ratios and yields were calculated.

Four plants of each treatment were measured and three measurements were taken on each plant. The leaves that were used for this experiment were marked at the beginning of the experiment and all the measurements were taken on the same leaves throughout the trial. The measurements for the cowpeas were taken between 10H00 and 12H30 on day 10, 12, 14, 17, 19, 21 and 24 after the watering stopped, and also three days later when the plants have been re-watered, and the data were transferred to the computer. For Bambara groundnuts the measurements were taken on the 10^{th}, 12^{th} , 14^{th} , 17^{th} , 20^{th} , 24^{th} and 27^{th} day after the watering stopped and recovery was measured on the 31^{st} day.

2.2.3.2. Changes in the Free Proline Concentration

The calorimetric method described in Ref. [5] was used to determine the proline concentrations. Samples of 50µg freeze-dried leaves were pulverised in liquid nitrogen before 5ml sulphosalicylic acid was added. The pulverised mixture was centrifuged and the supernatant was combined with equal volumes of acid ninhydrin and acetic acid. The samples were mixed well and placed in a water bath at 100°C for one hour, where after the reaction was terminated on ice. Toluene was added to the reaction solution, vortexed before the toluene phase were transferred to heat resistant ELISA plates. Solution absorbance was determined with a multiscan reader at 520nm. The proline concentration was determined using a standard curve and the concentration was expressed as µg proline/g dry weight.

2.2.3.3. Relative Water Content

Leaves were collected early in the morning from stressed and control plants on similar days as for the proline determination. Using a cork borer, leaf disks were cut and weighed immediately after harvest (within 30 minutes) to obtain the fresh weight (W). Five disks were used for each replicate. The samples were re-hydrated by putting the disks into small glass bottles and adding distilled water to allow the disks to float at room temperature. After 4 hours the leaf disks were patted dry with paper towels, and weighed again to obtain the turgid weight (TW). The samples were then oven dried overnight at 70ºC, cooled in a desiccator, and weighed again to obtain the dry weight (DW). The RWC was calculated using the equation:

$$RWC = [(W-DW)/(TW-DW)] \times 100$$

2.2.3.4. Yield determination

Seed yield of the mutant as well as the control lines was measured. The cowpea seeds were allowed to dry on the plant before it was harvested, while the bambara groundnut seeds were harvested when the whole plant had dried out. The seeds were then shelled and the dry weight and number of seeds was determined.

2.2.3.5. Root architecture determination in 2-D rooting boxes

It is not always possible to form a good impression of the three-dimensional development of plant's root system. Plants were therefore grown in a flat box, where they were able to develop a two-dimensional root system to get an impression of the root distribution. The root architecture screening boxes consisted of two flat sides (800 mm X 600 mm) separated by a piece of wood, 50 mm thick. The one side of the box was firmly attached with long nails while the other side was attached in such a way that it could be removed. The box was lined with a thick plastic sheet and filled with a sandy soil mixture. Three healthy seeds were planted in each box and the plants were thinned to one healthy plant shortly after germination. The plants were watered daily. Once the plants have developed a good rooting system (approximately 4 weeks), the box was opened, sandy soil washed away and the root system examined. A nailboard was used to keep the roots in their original position while the sand was being washed away. The total length of the roots was measured.

3. RESULTS AND DISCUSSION

3.1. Cowpea

Although the cowpea plants in the field experienced severe stress, most of the 146 mutant lines planted in Stellenbosch survived the stress period and produced seeds. The best nine lines were selected for further screening based on their survival, seed size and number of seeds produced. Of the 190 lines screened in the rain out shelter, 22 promising lines were identified (Table III).

One Hundred and seventy two M_3 cowpea plants were planted in wooden boxes in the greenhouse. The drought tolerant line, IT96D-602, proved to be drought tolerant along with 36 mutant lines (Table III). Seeds were harvested from the plants for further screening.

TABLE III. MUTANT COWPEA LINES PLANTED IN DIFFERENT LOCALITIES DURING THE LAST GROWING SEASON

Location	Planting date	Number of lines	No. of lines selected
M_2 Generation			
Field (Stellenbosch)	Dec. 2003	146	9
M_3 Generation			
Rain shelter 1 (Roodeplaat)	Dec. 2003	120	10
Rain shelter 2 (Roodeplaat)	Dec. 2003	70	12
Wooden box (Stellenbosch)	Oct. 2003	172	36

Eight cowpea lines were identified for physiological screening experiments in the greenhouse. These lines included six mutant lines that had been selected through trials during previous growth seasons, the parent line IT93K129-4 and the drought tolerant control line IT96D-602. From the data that was recorded during the first second of illumination using the chlorophyll fluorescence technique several phenomenological and biophysical values could be calculated. From all the energy fluxes, quantum efficiencies, rate constants and vitality indexes, some values were selected to give a description of the condition of the photosynthetic process in the leaves during the stress period.

When the specific and phenomenological fluxes were compared, it was found that the lines compare very favourably because they are all proven to be drought resistant (Table IV). The line MA_2 had the highest value for the number of reaction centres per cross section (RC/CS_o) of all the selected cowpea lines. The lines that had the lowest number of activated RCs were 164, IT96D-602 and MA_1. In the cowpea lines where a lower number of RCs were activated the remaining RCs have to absorb, trap and transport much more energy just to have the same output as the RCs of the line MA_2. This is evident in the high value registered for the absorption per reaction centre (ABS/RC) for 164 (Table IV).

When the rate of absorption of photons by the antenna in the cross section of the tested sample (ABS/CS_o) and the trapping of the electrons (TR/CS) were examined it was found that the line MA_2 also had the highest absorption and trapping rate while 364 had the lowest rate (Table IV). Despite the low absorption and trapping rate the line 364 had the lowest loss of energy (DI/CS) while MA_2 lost the most energy. The values of the drought resistant control line, IT96D-602, were somewhere in the middle for the ABS and TR but this line was most effective to move the electrons into the electron transport chain.

TABLE IV. RANKING OF EIGHT COWPEA LINES ACCORDING TO THE SPECIFIC AND PHENOMENOLOGICAL FLUXES AFTER 24 DAYS WITHOUT WATER

Line	RC/CSo	ABS/CSo	TRo/CSo	ETo/CSo	DIo/CSo	ABS/RC	TRo/RC	ETo/RC	DIo/RC	Ranking
IT96D602	0.829	1.004	0.993	1.546	1.034	1.213	1.198	1.853	1.252	1
IT93K1294	0.878	1.169	1.076	1.153	1.473	1.335	1.229	1.312	1.683	1
164	0.779	1.079	0.992	1.052	1.390	1.402	1.283	1.337	1.828	5
217	0.868	1.052	0.984	1.101	1.274	1.200	1.128	1.279	1.432	7
346	0.887	0.875	0.897	1.020	0.804	0.990	1.012	1.142	0.914	8
447	0.862	1.062	0.993	1.078	1.302	1.233	1.152	1.253	1.511	5
MA1	0.830	1.017	0.976	1.165	1.115	1.180	1.163	1.400	1.219	4
MA2	0.918	1.185	1.090	1.115	1.520	1.293	1.188	1.211	1.664	3

The specific energy fluxes of each individual RC of most of the lines were higher than that of the control line IT96D-602. The exceptions were the three lines 217, MA_1 and 346, which had lower ABS/RC values. For the line 346 this can be explained by the fact that this line had the 2nd highest number of active RC/CS and therefore each individual RC does not have to "work" so hard to trap the same amount of light energy. This is just the opposite of the line 164 where the individual RCs must trap, absorb and transport more energy because fewer of the RCs are activated. The TR/RC values of the lines were similar to the ABS/RC values (Table IV).

Contrary to what was found in some other crops, in cowpea the more drought tolerant plants start producing proline in the latter part of the stress period and at lower concentrations. The level of the proline gives an indication of the level of stress the plant is experiencing. In all the cowpea lines that were tested the proline levels of the stressed plants increased over time, although there were differences in the time of the onset of the proline production and also the levels of proline that were produced. The lines that produced the lowest levels of free proline were 217 and IT96D-602 followed by MA1, 164 and IT93K129-4 (Table IV). The highest proline production was from the line 447. The other two mutant lines that produced high levels of free proline were 346 and MA_2. In the line MA_2 this increase was already visible from the 21st day without water while the levels in the other lines only increased after 24 days without water.

The free proline levels of up to 1890µg proline /g dry weight is much higher than the levels registered during the previous season but the stress period was also longer than during previous experiments. The ranking of the lines was not only determined by the levels of free proline at the height of the stress but also the time when the proline started to increase.

After 12 days without water (w.o.w) the plants started to react visibly to the drought stress condition. Some plants starts to lose chlorophyll in their lower leaves and others changed the inclination of their leaves away from the sun. The relative water content (RWC) of the stressed plants was between 80 and 90% at this stage. As the stress condition intensified the RWC dropped further and after 24 days w.o.w. the RWC of the mutant line 346 was as low as 65% (Table VI). The two lines that were able to keep the RWC highest were MA_1 and IT96D-602. In these two lines the RWC was still around 75% even though the plants have not been watered for 24 days (Table VII). In the parent line IT93K129-4 the RWC dropped to 69.5% but these plants recovered well after re-watering, as did all the other lines.

The ability of the cowpea plants to keep the RWC so high is one of the contributing factors to their drought resistance. This can be attributed to very sensitive stomata control.

TABLE V. THE RANKING OF EIGHT COWPEA LINES ACCORDING TO THE FREE PROLINE PRODUCTION UNDER STRESS CONDITIONS

Cowpea line or mutant	Free proline concentration after 24 days without water (µg proline/g dry weight)	Ranking
IT96D-602	276.51	2
IT93K129-4	788.75	5
164	690.00	3
217	98.56	1
346	1053.86	6
447	1892.08	8
MA_1	585.97	3
MA_2	1106.76	8

TABLE VI. THE RWC (%) OF EIGHT COWPEA LINES AS IT WAS REGISTERED AT THE HEIGHT OF THE STRESS AND ALSO AFTER RE-WATERING

	24^{th} day without water		Recovery after re-watering		
Cowpea line	Control	Stress	Control	Stress	Ranking
IT96D-602	87.95	74.15	89.44	85.43	2
IT93K129-4	92.26	69.51	91.45	87.57	5
164	89.18	72.12	91.73	86.70	2
217	92.45	73.60	92.82	89.37	2
346	91.08	65.27	90.79	88.00	7
447	90.52	70.62	90.02	89.47	5
MA_1	91.61	76.98	90.44	89.03	1
MA_2	90.14	70.90	92.27	87.22	7

The yield of both the stressed and control cowpea plants were determined. The plants were ranked according to the number of seeds produced and also the weight of the seeds (Table VIII). This was done to distinguish between plants that produced only a few big seeds and the plants that produced large numbers of small seeds. The lines that produced the most seeds were lines 217, IT93K129-4 and 447. The stressed plants of the line 346 produced highest number of seeds of all the stressed plants and the 6^{th} highest over all, followed by 217 and 447. This is an excellent trait for a drought resistant plant to be able to produce good yields even under stress conditions (Table VII).

When the seed weight of the control plants was compared, IT93K129-4 produced the heaviest seeds with MA_2 ranked 2^{nd} and 447, 3^{rd}. The plant that produced the most seeds under control conditions, 217, was only ranked 6^{th} in terms of seed weight. Under drought conditions was it lines IT93K129-4, 447 and 164 that produced the heaviest seeds. The drought tolerant line IT96D-602 that normally performs well in terms of yield produced much fewer seeds than what was expected. This line was ranked 2^{nd} last, both in terms of seed number and seed weight. However, the stressed plants produced more seeds than the control

plants which is a very good characteristic. MA_2 did not perform well under the drought conditions. Assessment of the yield of the plants used in all the experiments is very important. It is not worthwhile to have a plant that can survive adverse conditions but which produces no yield. The ideal plant for subsistence farmers will be one capable of producing a moderate to good yield under optimal conditions and also produce approximately the same yield under adverse conditions.

TABLE VII. THE YIELD OF EIGHT COWPEA LINES AS IT WAS PRODUCED BY STRESSES AND CONTROL PLANTS. THE PLANTS WERE RANKED ACCORDING TO THE MEAN NUMBER OF SEEDS AS WELL AS THE MEAN SEED WEIGHT PER PLANT

Cowpea line	Treatment	Ranking according to mean number of seeds		Ranking according to mean seed weight (g)	
		No. of Seed	Ranking	Weight (g)	Ranking
IT96D-602	Control	88.5	8	17.3	8
	Stress	108.8	7	22.6	7
IT93K129-4	Control	245.3	2	45.9	1
	Stress	188.8	4	36.4	1
164	Control	178.3	7	33.1	4
	Stress	146.8	6	27.8	3
217	Control	250.8	1	29.5	6
	Stress	205.8	2	25.0	5
346	Control	221.3	4	24.5	7
	Stress	212.3	1	24.2	6
447	Control	239.8	3	36.9	3
	Stress	199.3	3	30.4	2
MA_1	Control	181.0	6	31.1	5
	Stress	158.0	5	27.7	4
MA_2	Control	214.0	5	37.0	2
	Stress	80.5	8	14.8	8

TABLE VIII. BAMBARA GROUNDNUT LINES THAT PRODUCED THE MOST SEEDS IN THE RAIN OUT SHELTER

Line	No of plants that survived	Seed weight (g)
1	7	50.1
4	5	15.8
6	8	13.4
79	1	6.8
101	9	20.9
111	5	27.7
134	6	42.5
144	9	14.5
174	2	14.9
190	6	52.3
335	4	16.7
504	5	21.4
588	5	18.4

When the root architecture of the tolerant (IT96D-602) and susceptible line (TVu7778) were examined it became clear that there were a marked difference in the distribution of the roots of the drought tolerant plants. This was done in order to see if it would be possible to identify the mechanisms used by cowpeas to survive adverse conditions. When the length of the roots was determined it was evident that the difference between these two lines was not in the total length of the roots but in the distribution of the roots (Table IX; Fig.1). When the mutants root lengths were compared with the root architecture of the control plants it was found that the distribution of the roots were more similar to that of the drought tolerant control line, IT96D-602, than to the susceptible line (Table IX).

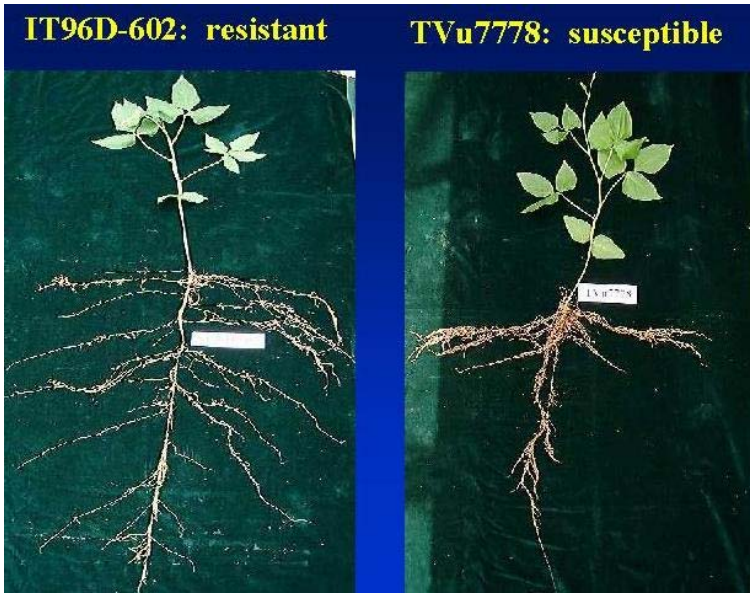


Fig. 1. Root patterns of the drought tolerant control line IT96D-602 and the more susceptible line TVu7778.

TABLE IX. TOTAL ROOT LENGTH OF DIFFERENT COWPEA LINES AND MUTANTS

Line	Length		Total length
	Top 25 CM	Below 25 CM	
IT96D-602	452 MM	586 MM	1038 MM
TVU7778	557 MM	475 MM	1032 MM
MUTANT NO. 217	519 MM	676 MM	1194 MM
MUTANT NO. 164	520 MM	659 MM	1178 MM

3.2. Bambara groundnut

Bambara groundnuts are still being regarded as a neglected crop and although a lot of people in rural areas of Africa rely on this crop for food security, the potential of this crop is still largely unexploited. The aim of this study was to determine the levels of drought tolerance in this specie and to compare the known bambara groundnut lines with the mutant lines. The status of the bambara groundnuts lines in terms of drought tolerance has not been determined before this study and no reference lines were available. The irradiation dosage of

180 Gy was found to be optimum for bambara groundnut. The mutant lines were evaluated from M1 to M5 generation.

Most of the mutant bambara lines that were planted in the rain shelter at Roodeplaat recovered after the stress condition and produced some seeds, but of the 165 lines only 26 produced more than 10 g of seeds. This emphasise the problem of yield - although the plants can survive adverse conditions it has a detrimental effect on the yield and this impacts on the choice of the crop which the farmers will cultivate. The mutants that produced the most seeds are listed in Table X.

TABLE X. THE YIELD OF EIGHT BAMBARA GROUNDNUT LINES AS IT WAS PRODUCED BY STRESSES AND CONTROL PLANTS. THE PLANTS WERE RANKED ACCORDING TO THE MEAN NUMBER OF SEEDS AS WELL AS THE MEAN SEED WEIGHT PER PLANT

	Treatment	Ranking according to mean number of seeds		Ranking according to mean seed weight (g)	
Bambara groundnut line		No. of Seed	Ranking	Weight (g)	Ranking
SB1-1	Control	11.8	2	4.22	1
	Stress	7.0	3	2.28	3
1	Control	6.3	7	2.32	7
	Stress	2.8	16	0.65	8
9	Control	7.3	6	1.86	8
	Stress	5.3	14	2.03	4
79	Control	10.8	3	2.96	5
	Stress	5.0	6	1.18	7
104	Control	13.5	1	3.90	2
	Stress	6.8	4	1.46	6
120	Control	11.8	2	2.95	6
	Stress	11.0	2	2.78	2
134	Control	9.3	5	3.07	4
	Stress	12.3	1	4.36	1
144	Control	10.0	4	3.10	3
	Stress	5.5	5	1.76	5

The control line, SB1-1, together with seven mutant lines that were identified for physiological screening experiments, were planted and evaluated in the greenhouse. Chlorophyll fluorescence, free proline and RWC measurements were taken from the 10th day without water, but only the data at the height of the stress (27 days without water) will be incorporated in this paper.

By using the chlorophyll fluorescence parameters it become apparent that line 104 had the highest density of active reaction units per leaf area (RC/CS), with no 1 in the 2nd place (Table XI). When the lines were compared in terms of the activity per CS, the lines 144, 120 and 9 gave the best results. The two lines, 144 and 120 did however lose a lot of energy not used in the electron transport chain. The line no. 9 performed well in terms of the single RS's as well as for the CSs. This line was therefore ranked 1st. The lines SB1-1 en 134 suffered in terms of the active RC/CS, ABS, TR, ET and dissipation (DI) per CS but these lines

performed well in terms of the ABS, TR, ET and DI per RS. This is an indication that the individual RSs of these lines were "working" very hard to try and keep the energy levels high but because less RSs were functional these lines were not able to trap a lot of energy.

The free proline levels of three of the bambara groundnut lines, 120, 144 and 104, were much higher than that of the rest of the lines, while the levels of lines no. 9, SB1-1 and 79 stayed very low (Table XII). As was the case with cowpea, bambara groundnuts also does not use proline accumulation as a method to survive adverse conditions and therefore the lines with lower levels of free proline are regarded as more drought tolerant.

TABLE XI. RANKING OF EIGHT BAMBARA GROUNDNUT LINES ACCORDING TO THE SPECIFIC AND PHENOMENOLOGICAL FLUXES AFTER 27 DAYS WITHOUT WATER

Line	RC/CSo	ABS/CSo	TRo/CSo	ETo/Cso	DIo/CSo	ABS/RC	TRo/RC	ETo/RC	DIo/RC	Ranking
SB1-1	0.871	1.13	1.005	0.932	1.527	1.307	1.158	1.068	1.784	7
No 1	1.062	1.079	1.06	0.98	1.132	1.008	0.994	0.926	1.045	6
No 9	0.959	1.115	1.071	1.094	1.238	1.166	1.118	1.142	1.301	1
No 79	1.018	1.119	1.065	0.982	1.284	1.103	1.047	0.963	1.272	5
No 104	1.129	1.021	1.058	1.14	0.922	0.906	0.934	1.005	0.828	4
No 120	1.049	1.331	1.178	1.013	1.892	1.247	1.105	0.962	1.754	2
No 134	0.939	1.049	1.002	0.961	1.185	1.125	1.071	1.024	1.286	8
No 144	1.013	1.178	1.085	1.055	1.439	1.164	1.069	1.055	1.424	2

The RWC of most bambara groundnut lines were remarkably stable over the stress period. In only three of the lines (SB1-1, 134 and 9) did the RWC fall below 80%, the rest of the lines maintained extremely high RWC (Table XIII). This is normally achieved by closing the stomata and by slowing down all the processes involved in photosynthesis. All the lines recovered well when the plants were re-watered. Seeds were only harvested below soil when the whole plant had dried out, resulting in germination of some of the earlier seeds before the mother plant died down. The number of seed and seed weight were generally very low (Table XVI). The lines 104,120 and SB1-1 yielded the most seeds under control conditions, lines 134, 120 and SB1-1 yielded the most seed under stressed conditions. The seed weight was the highest again in SB1-1 followed by 104 and 144 under control conditions and 134, 120 and SB1-1 under drought conditions. Line 134 yielded under drought stress even more than all the other lines under control conditions. It was noted that some lines (1, 71) that maintained high RWC during the stress period paid the price for healthy biomass in terms of yield loss. Line 134 on the other hand had a much lower RWC but these plants were able to form seeds even under stress conditions. The yield performance of the lines 104, 120 and the control line SB1-1 was very good under well watered conditions but the line 120 also did very well under stress conditions where this line was ranked 2nd after the line 134.

Bambara groundnut seeds were planted in root architecture boxes and were allowed to grow undisturbed for four weeks. The boxes were opened and the root architecture was examined. The different lines and selections did not form such regular root patterns as was seen with the cowpea plants. The control line SB1-1 for instance usually formed a traditional taproot system with side roots but root system patterns with 5 or 6 long strong roots with no definite tap root has also been observed (Table XIV).

The roots did however cover most of the surface of the root architecture box. The examination of the root systems of these plans might still provide an answer to the high levels of drought tolerance of these plants. Bambara groundnuts normally produce seeds with

varying sizes. Some of the Bambara groundnut growers have the tendency to eat the larger seeds and plant only the small seeds. For this reason one big and one small Bambara seed were planted in the same root analysis box to compare the development of the two plants. In the four-week period that the plants were in the box the plant that originated from the big seed managed to produce roots with a total length of 1.11m while the plant from the small seeds only produced roots with a length of 0.052 m (Table XIV).

TABLE XII. THE RANKING OF EIGHT BAMBARA GROUNDNUT LINES ACCORDING TO THE FREE PROLINE PRODUCTION UNDER STRESS CONDITIONS

Bambara groundnut line	Free proline accumulation after 27 days without water (μg proline/g dry weight)	Ranking
SB1-1	8.67	2
1	37.96	5
9	8.18	1
79	9.85	3
104	231.52	6
120	972.59	8
134	13.95	4
144	581.29	7

TABLE XIII. THE RWC (%) OF EIGHT BAMBARA GROUNDNUT LINES AS IT WAS REGISTERED AT THE HEIGHT OF THE STRESS AND ALSO AFTER RE-WATERING

Bambara groundnut line	24^{th} day without water		Recovery after re-watering		Ranking
	Control	Stress	Control	Stress	
SB1-1	90.72	64.18	93.45	94.01	7
1	93.37	90.49	94.29	94.43	1
9	93.91	73.82	94.02	93.56	6
79	93.36	91.72	93.24	92.22	1
104	93.67	91.29	94.19	93.88	1
120	93.55	82.24	93.16	93.51	5
134	91.67	64.40	93.20	94.14	7
144	93.45	90.35	93.81	94.03	1

TABLE XIV. TOTAL ROOT LENGTH OF DIFFERENT BAMBARA GROUNDNUT LINES AND MUTANTS AFTER FOUR WEEKS OF GROWTH

Line	Length (mm)		Total length
	Top 25 cm	Below 25 cm	
SB1-1	599	565	1164
SB1-1	567	610	1177
SB1-1 Big seed	562	544	1106
SB1-1 Small seed	433	52	485
Mutant no. 596	613	664	1277
Mutant no. 596	562	607	1169

The depth of the roots from the plant from the big seeds was 60 cm while the plant that originated from the small seed only managed to form roots that was 30 cm deep (Fig. 2). The big seeded plant also produced double the amount of leaves. This shows clearly that plants that originated from bigger seeds have an advantage over plants that originates from smaller seeds. The practice to cultivate only the smaller seeds should therefore be discouraged and the farmers should rather plant a mixture of seeds sizes or only the bigger seeds.

Fig. 2. A plant that originated from a small bambara ground nut seed (left) and a big seed (right) was planted in the same box to see if there was a difference in the development of the plants.

4. CONCLUSIONS

The aim of this project was to use mutation breeding as a tool to improve the drought tolerance of cowpea and bambara groundnut lines with favourable traits to such an extent that these plants can survive and produce a good yield even under adverse conditions.

The final results indicated that 6 mutant cowpea lines proved to be of similar tolerance as the parent line. In some the screening test the mutant lines even performed better. The data demonstrated that the mutant lines 217 performed very well in terms of the RWC, free proline concentration and yield. The yield performance of the mutant lines 447, 346 and 217 proved to be outstanding under well watered conditions and under drought stress conditions. We have thus succeeded to obtain drought tolerant lines with good yields and favourable traits that are adapted to local conditions by using mutation technology.

Seeing that there was no prior knowledge about the drought tolerance status of the bambara groundnut lines, this study created the opportunity to understand the systems used by bambara groundnut slightly better and to start to identify plant with better yield and higher levels of drought tolerance. Seven mutant lines were selected through out the study as a result of their performance under drought stress. Lines 79 and 104 performed well in terms of the physiological parameters. The mutant lines 134 and 120 yielded exceptionally well under drought stress conditions.

ACKNOWLEDGEMENTS

The authors would like to thank the International Atomic Energy Agency and the Agricultural Science for the funding of this project, as well as the following people for their invaluable contributions towards this project: R Slabbert, T Caetano, P Fourie, H Phasha, J Mojela, J Lebese, J Maisela and L Mokobi.

REFERENCES

[1] Duke, J.A. 1981. Handbook of legumes of world economic importance. pp. 302-333. Plenum Press New York.

[2] Fowler, C. 2000. Establishing the scope of a multilateral system for plant genetic resources for food and agriculture: implications of crop exclusions. Biopolocy 3(2) Online Journal, available at: http://bioline.bdt.org.br/py

[3] Singh, B.B., Mai-Kodomi, Y. and Terao, T. 1999. A simple screening method for drought tolerance in cowpea. Indian Journal of Genetics 59(2):211-220.

[4] Strasser, B.J. and Strasser, R.J. 1995. Measuring fast fluorescence transients to address environmental questions: the JIP-test In: Photosynthesis: From light to Biosphere P. Mathis (ed.) Vol. 5: 977-980. Kluwer Academic Publishers, Dordrecht. ISBN 0-7923-3862-6.

[5] Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39:205-207.

MUTATIONS IN THE GENETIC ANALYSIS OF ROOT TRAITS IN BARLEY AND THEIR ASSOCIATIONS WITH PLANT PERFORMANCE IN STRESSED AND NON-STRESSED CONDITIONS

B.P. FORSTER[1], O. CHLOUPEK[2], S. CLARK[3], H. AL-MENIAE[1+4], W.T.B. THOMAS[1]

[1] Scottish Crop Research Institute,
Invergowrie, Dundee, Scotland, United Kingdom

[2] Mendel University of Agriculture and Forestry, Brno, Czech Republic

[3] University of Abertay,
Science and Engineering Dept.,
Dundee, Scotland, United Kingdom

[4] Kuwait Institute for Scientific Research,
Safat, Kuwait

Abstract

Mutations have been used to study the genetic control of root traits in controlled environment and field experiments of barley. The investigations can be divided into three groups: 1) genome scanning, using the Bowman mutation backcross lines, 2) effects of mutant dwarfing genes (*sdw*1 and *ari-e*.GP), to investigate associations with root traits and plant performance, and 3) the development of a barley mutation grid for forward and reverse genetics. The work has been dependent on the development of reliable phenotypic screens for seedling and mature plant root traits. Measurements of the seminal root system (root number, length and spread) were obtained from 10-day-old seedlings grown in a 2-dimensional root observation chamber. Root lengths of older seedlings (3-week old) were obtained from rhizo-trunking experiments grown in sandy soil. Data on adult plant root system size (RSS) were obtained from field trials at various sites and growth stages using electrical capacitance. The genome scanning exercise indicated several regions of the barley genome with significant effects on seedling root number, length and spread. Root trait associations with plant performance in stressed and non-stressed conditions were generated from both controlled environment studies and field trials. Field testing for drought tolerance was carried out in Egypt, Morocco and Tunisia, and salinity tolerance was tested in Kuwait. Field testing in the UK and the Czech Republic provided data for contrasting environments. Quantitative trait loci (QTLs) for seedling and adult plant root traits were mapped along with QTLs for plant performance in stressed and non-stressed environments. The *ari-e*.GP mutation was found to be associated with short seminal roots and a reduced RSS, whereas the *sdw*1 mutation was found to have no effect on the seminal root system, but was associated with a large RSS. Both these mutations had strong pleiotropic effects on a number of plant performance traits in both stressed and non-stressed environments. A barley mutation grid has been constructed for forward and reverse genetics. The grid is composed of over 20,000 M_3 families and high throughput germination and seedling tests are being developed to screen for mutations affecting root traits.

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is a classic species for mutation studies. Several hundred well characterised mutations have been induced in barley [1]. Many morphological mutants have been backcrossed individually into the cultivar Bowman and loci genetically mapped [2]. The Bowman backcross mutant lines (BBMLs) provides a resource for investigating mutant gene effects in a common genetic background, as well as allelism testing for new mutations. Since the BBMLs represent introgressions covering all seven barley chromosome, selected lines can be exploited in a step-wise scan of the whole barley genome.

The *ari-e*.GP (*Gpert*, located on chromosome 5H) and *sdw*1 (*denso*, located on 3H) are induced semi-dwarf mutants in barley. Both are commercially important genes. Semi-dwarf cultivars have dominated the spring barley crop in north/west and central Europe for the past 40 years. About 70% of current spring barleys of north/west Europe carry one or other of these genes. A doubled haploid (DH) genetic mapping population has been created from the cross Derkado (*sdw*1) and B83-12/21/5 (*ari-e*.GP) and provides a resource in which the effects of both mutant genes can be studied in a common genetic background [3]. Both *sdw*1 and *ari-e*.GP confer a semi-dwarf stature, but have pleiotropic effects on a number of yield, quality and agronomic traits, as well as tolerance to abiotic stress [4–7]. Several physiological traits associated with stress have been linked to these dwarfing genes, but most are derived from above ground tissues/measurements, and root traits have been neglected.

A mutation grid for forward and reverse genetics has been developed in the barley cultivar Optic [8]. A population of over 20,000 M_3 families has been developed. These have been scored for visible mutant phenotypes and the data is archived in a searchable database (URL:http://bioinf.scri.sari.ac.uk/distilling/distilling.html). The Optic mutants provide a valuable resource to investigate gene function of many traits, including those of roots.

2. METHODS AND RESULTS

2.1. Phenotypic screening for root traits

2.1.2. Seedling root traits

A simple 2-dimensional (2-D) observation chamber was devised to study seedling roots (Fig. 2). Germinated seed, from a standard sieve fraction, are sandwiched in airspace between two gel-coated plates and incubated in controlled environment conditions (10 or 12ºC in the dark) for 10 days. One plate is transparent and allows the non-destructive observation and image analysis of seminal root development over time. Traits measured include root number, root length and root angle. The method has been described in Ref. [9]. The test was used to score seedling root architecture in barley genetic stocks: the BBMLs and the Derkado x B83-12/21/5 DH genetic mapping population.

Root length of older seedlings (3 week old) was measured from rhizo-trunking experiments using 1 m lengths of electrical conduit trunking with a square cross-section (50 mm x 50 mm). The bottom was closed with plastic and the trunking filled with soil. Three germinated seed of one genotype are placed on top of the soil column, covered with a layer of soil and topped with a mulch of synthetic pebbles (to minimise surface evaporation). In the experiments on salt tolerance the rhizo-trunking was filled with 2.5 kg of moist sieved (0.2 mm) Muslan soil from Al Afra experimental station in Kuwait. A wide range of barley germplasm, including semi-dwarf mutant lines was tested. Seedlings were watered with 50 ml of either fresh or brackish water (EC 6.75 dS/m) twice a week. The experiment was replicated in a randomised block design and conducted in a growth room at 16ºC with a 16h light period. After 3 weeks the plants were harvested, soil was removed and root lengths were measured. Root lengths were then correlated with plant performance in fresh water and brackish water irrigated field trials at Al Wafra, Kuwait [10].

2.2. Root system size of adult plants

Root system size (RSS) was evaluated by electrical capacitance where the plant provides the first plate and the soil the second plate of a capacitor. Electrical capacitance has been shown to be associated to RSS and can be used as a non-destructive surrogate [11]. The

Derkado x B83-12/21/5 DH population was sown in replicated trials at two sites in the Czech Republic, and RSS measured at three times during the life cycle, stem elongation, heading and grain filling. Data were then subject to quantitative trait locus (QTL) analysis.

2.3. Genome scanning for loci controlling seedling root traits

Over 100 Bowman backcross mutant lines (BBMLs) were selected that represented a range of barley mutant phenotypes that sampled the barley genome. Ninety-one of these mutant loci have been assigned to chromosomes and relative chromosomal positions have been estimated for many, but the remainder have not been mapped (Table I). The selected BBMLs were subject to replicated and randomised tests using the 2-D observation chamber. When scores for total root length where plotted against the mutant locations associations were detected between genomic regions and root lengths that were significantly longer (>500mm) and shorter (<300 mm) than the population mean (Fig. 1). These were located on five of the seven chromosomes (1H, 2H, 3H, 5H and 6H). Mutant introgresssions on chromosomes 4H and 7H were inert, but four with negative associations are unmapped (unknown). The BBML genome scan provides a means of estimating the number and location of genes controlling root traits in barley. Thus, for example, backcross introgressions in genomic areas around the *Lax-c* locus on the long arm of chromosome 6H (GSHO2086 in Fig. 1) are associated with long root length, and those around *lzd* (GSHO1938) on the short arm of 3H are associated with short seedling roots.

In addition to the genome scan, certain BBMLs were found to have distinctive seedling root traits. The Bowman control typically gave rise to 5-6 seminal roots, BBMLs with significantly fewer roots (typically 4) included accessions BGS59 (*gpa*, on 2H), BGS102 (*uzu1*, 3H), BGS124 (*vrs4*, 3H) and BGS323 (*nld*, 5H). Accessions BGS1 (*brh1*, 7H) and BGS226 (*rvl*, un-mapped) had the most roots (up to 7), but this was not significant. Accessions with significantly shorter total root lengths (compared to the overall mean) included: BGS460 (*cur4*, 2H), BGS59 (*gpa*, 2H), BGS124 (*vrs4*, 3H), BGS559 (*ari-r*, un-mapped), BGS409 (*cer-o*, un-mapped) and BGS235 (*lel1*, 1H), whereas BGS227 (*sls1*, 1H), BGS592 (*yhd2*, 1H) and BGS343 (*Lfb1*, unmapped) had significantly longer seminal roots. A distinctive phenotype was found for BGS460 (*cur4* mutation, 3H) this had curly roots as well as the already defined phenotype of curly leaves, culms and awns (Fig. 2).

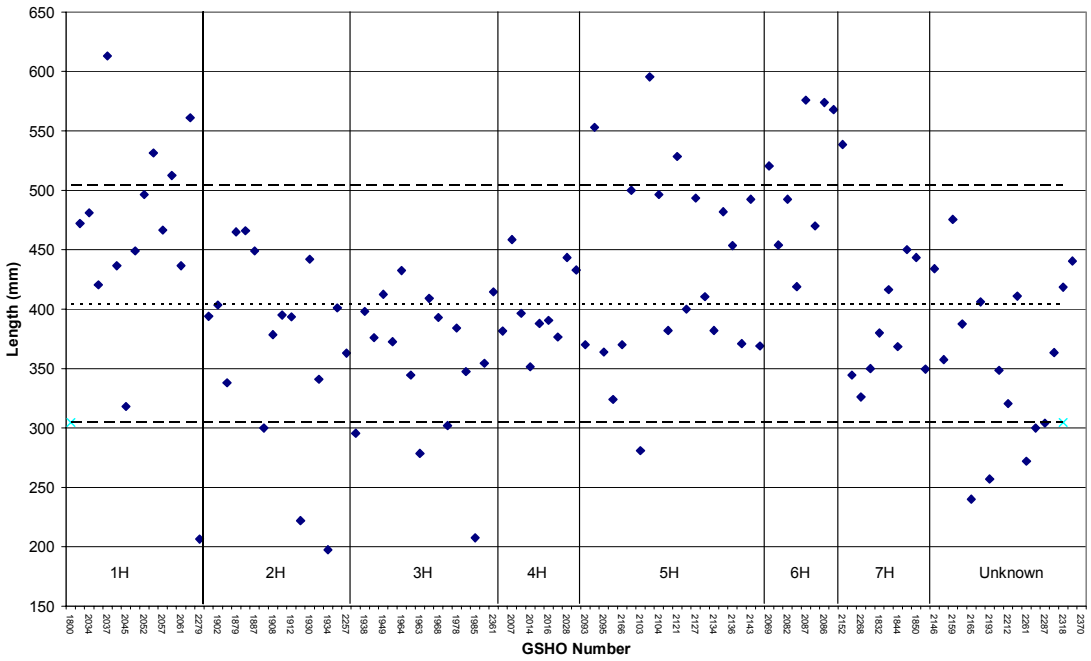


Fig. 1. Genome scanning for total root length of 10 day old seedlings using mapped mutants in the Bowman backcross mutant lines. Dotted line indicates the population mean and dashed lines indicate 95% confidence limits.

2.4. The effect of mutant, semi-dwarfing genes on root characters and performance

Extensive genetic studies have been carried out on the Derkado x B83-12/21/5 doubled haploid (DH) populations. The DH population has been used to construct a genetic marker map of barley, which in turn has led to QTL mapping of numerous traits (yield, quality, agronomic, physiological, stress and recently root characters). QTL data from various studies [4, 6, 7, 12](Chloupek 2005, Ellis 2002, Forster 2004, Thomas, 2003) are summarised here with special attention to associations with the two dwarfing genes (Fig. 3). From Fig. 3 it can be seen that the two dwarfing genes *sdw*1 and *ari-e*.GP are associated with clusters of QTLs. Note that in Fig. 3 the effects of Derkado alleles are given, since Derkado is wild type at *Ari-e*, the effects of the mutant allele (*ari-e*.GP) are opposite to those given in the chromosome map. The associated traits include: RSS in adult plants, seedling root traits (2-D test), and plant performance in drought (Morocco, Tunisia and Egypt) and non-stressed (Czech Republic and UK) field environments as well other performance traits. No such clustering was found elsewhere in the genome where QTLs were more dispersed. The data therefore show strong associations of the semi-dwarf mutant genes with root traits and performance.

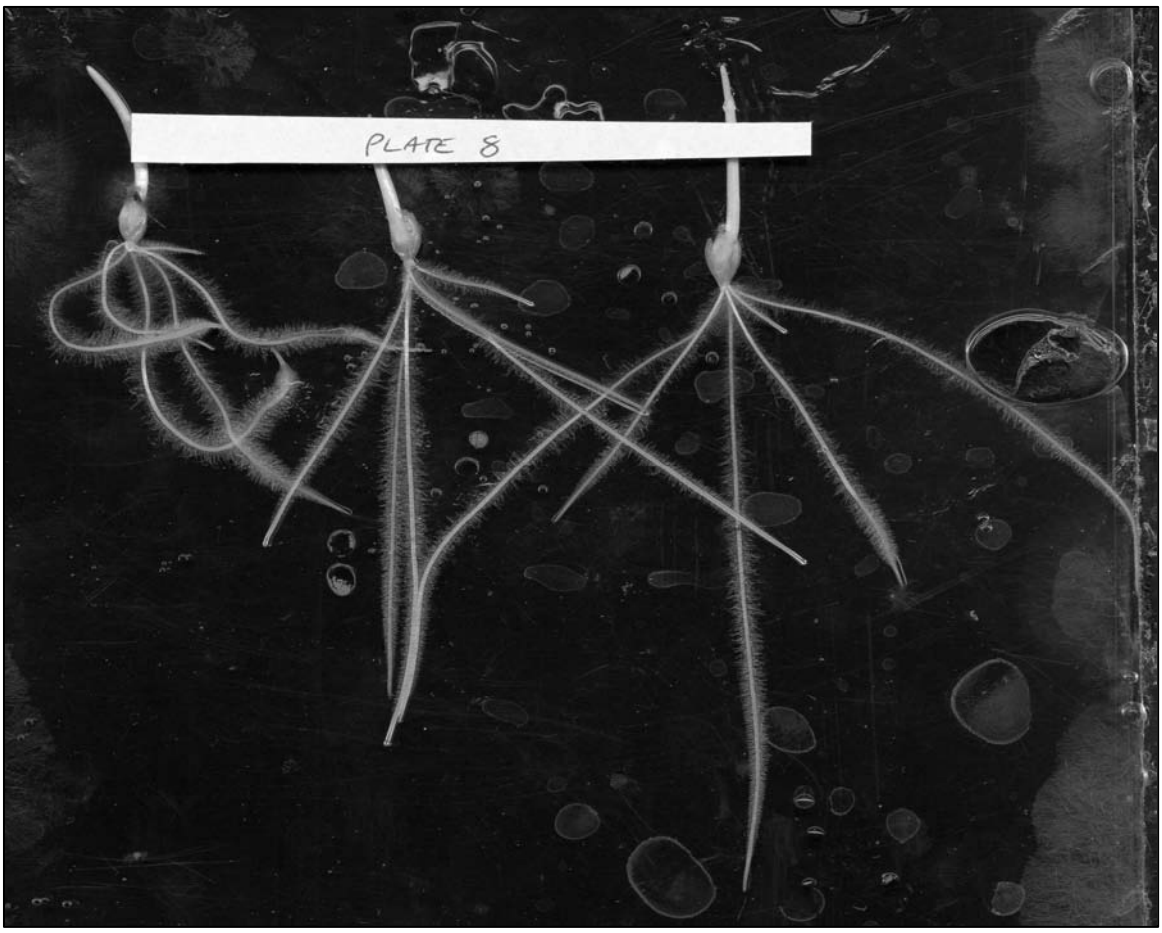


Fig.2. Curly seminal root phenotype of cur4 *(left) compared to normal seedlings in the BBMLs in the 2-D test.*

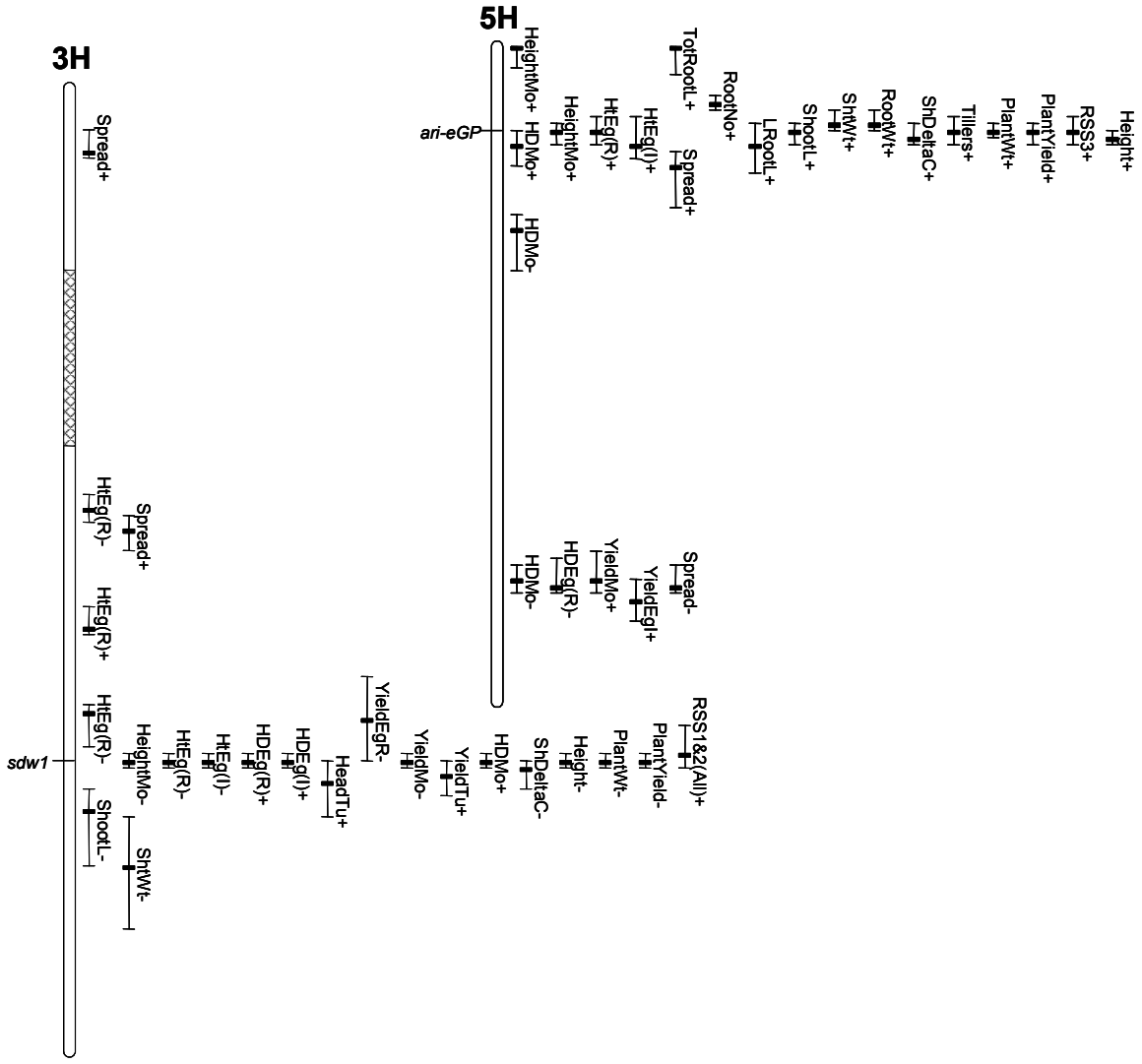
TABLE I. LIST OF THE BOWMAN BACKCROSS MUTANT LINES, ORDERED BY CHROMOSOMAL LOCATION, AND WHERE POSSIBLE, ESTIMATED DISTANCE FROM THE CENTROMERE (0 POSITION), POSITIVE VALUES ARE IN THE SHORT ARM, NEGATIVE POSITIONS ARE IN THE LONG ARM

Chromosome Barley Genetic Stock (Genetic Stock, Hordeum)	Mutant	Gene symbol	Estimated position
1H			
BGS228 (GSHO2032)	Subcrown internode length 1	*Sil1*	
BGS227 (GSHO2034)	Small lateral spikelet 1	*sls1*	40
BGS220 (GSHO2033)	Chlorina seedling 3	*fch3*	30
BGS592 (GSHO2037)	Yellow head 2	*yhd2*	
BGS519 (GSHO2038)	Many-noded dwarf 1	*mnd1*	
BGS231 (GSHO2045)	Curly 5	*cur5*	
BGS224 (GSHO2049)	Erectoides-b	*ert-b*	18
BGS222 (GSHO2052)	Necrotic leaf spot 1	*nec1*	0
BGS221 (GSHO2053)	White streak 5	*wst5*	-35
BGS223 (GSHO2057)	Zebra stripe 3	*zeb3*	
BGS201 (GSHO2058)	Chlorina seedling 7	*fch7*	-75
BGS202 (GSHO2061)	Third outer glume 1	*trd1*	-80
BGS214 (GSHO2063)	Early maturity 8	*eam8*	-91
BGS235 (GSHO2279)	Leafy lemma 1	*lel1*	
2H			
BGS97 (GSHO1899)	Accordian rachis 1	*acr1*	
BGS90 (GSHO1902)	Erectoides-j	*ert-j*	
BGS83 (GSHO1876)	Slender dwarf 2	*sld2*	30
BGS72 (GSHO1879)	Globosum-c	*glo-c*	
BGS57 (GSHO1891)	Elongated outer glume 1	*eog1*	0
BGS472 (GSHO1887)	Accordian basal rachis internode 1	*abr1*	-6
BGS56 (GSHO1905)	White streak 4	*wst4*	-17
BGS58 (GSHO1908)	Six-rowed spike 1	*vrs1*	-30
BGS87 (GSHO1911)	Chlorina seedling 14	*fch14*	
BGS75 (GSHO1912)	Awnless 1	*Lks1*	-44
BGS460 (GSHO1915)	Curly 4	*cur4*	
BGS60 (GSHO1930)	Liguleless 1	*lig1*	-76
BGS82 (GSHO1931)	Zeocriton dwarf 1	*Zeo1*	-81
BGS59 (GSHO1934)	Grandpa 1	*gpa1*	-94
BGS61 (GSHO1936)	Triple awned lemma 1	*trp1*	-142
BGS566 (GSHO2257)	Brachytic 3	*brh3*	
3H			
BGS115 (GSHO1937)	Brittle rachis 1	*brt1*	39
BGS125 (GSHO1938)	Lazy dwarf 1	*lzd*	37
BGS109 (GSHO1939)	Yellow streak 2	*yst2*	21
BGS618 (GSHO1949)	Many noded dwarf 3	*mnd3*	
BGS108 (GSHO1953)	Albino lemma 1	*alm1*	20
BGS120 (GSHO1964)	Zebra stripe 1	*zeb1*	6
BGS134 (GSHO1960)	Erectoides-c	*ert-c*	5
BGS102 (GSHO1963)	Uzu 1	*uzu1*	0
BGS107 (GSHO1966)	White stripe 1	*wst1*	0
BGS129 (GSHO1968)	White streak 6	*wst6*	-11

Chromosome Barley Genetic Stock (Genetic Stock, Hordeum)	Mutant	Gene symbol	Estimated position
BGS126 (GSHO1970)	Slender dwarf 1	*sld1*	-14
BGS124 (GSH01985)	Six-rowed spike 4	*vrs4*	-30
BGS518 (GSHO1978)	Semidwarf 1	*sdw1*	-47
BGS133 (GSHO1965)	Semidwarf 2	*sdw2*	-54
GSHO2241	Pyramidatum ai	*pyr.ai*	
BGS617 (GSHO2361)	Uniculm 4	*cul4*	
4H			
BGS73 (GSHO2005)	Extra floret-a	*flo-a*	
BGS152 (GSHO2007)	Hooded lemma	*Kap*	33
BGS171 (GSHO2011)	Light green 4	*lgn4*	20
BGS172 (GSHO2014)	Short awn 5	*lks5*	17
BGS155 (GSHO2015)	Glossy leaf 1	*glf1*	7
BGS157 (GSHO2016)	Brachytic 2	*brh2*	2
BGS161 (GSHO2023)	Semi-minute dwarf 1	*min1*	-7
BGS158 (GSHO2028)	Yellow head 1	*yhd1*	-78
GSHO2363	Vascular bundle number 1	*vbn1*	
5H			
BGS323 (GSHO2093)	Narrow leaf dwarf 1	*nld1*	50
BGS313 (GSHO2097)	Chlorina seedling 6	*fch6*	48
BGS336 (GSHO2095)	Globosum-b	*glo-b*	46
BGS324 (GSHO2094)	Curly dwarf 1	*cud1*	43
BGS326 (GSHO2166)	Broad leaf 1	*blf1*	22
BGS304 (GSHO2102)	White streak 2	*wst2*	5
BGS474 (GSHO2103)	Laxatum-a	*lax-a*	5
BGS473 (GSHO2098)	Compositum 1	*com1*	3
BGS328 (GSHO2104)	Breviaristatum-e	*ari-e.*	2
BGS321 (GSHO2108)	Short rachilla hair 1	*srh1*	0
BGS306 (GSHO2121)	Variegated 1	*var1*	-26
BGS312 (GSHO2124)	Smooth awn 1	*raw1*	-30
BGS448 (GSHO2127)	Eceriferum-ye	*cer.ye*	
BGS350 (GSHO2132)	Brachytic 6	*brh6*	
BGS 334 (GSHO2134)	Smooth awn 6	*raw6*	
BGS347 (GSHO2135)	Many noded dwarf 4	*mnd4*	
BGS323 (GSHO2136)	Narrow leaf dwarf	*nld1*	
BGS303 (GSHO2143)	Variegated 3	*var3*	-70
BGS633 (GSHO2235)	Many noded dwarf 6	*mnd6*	
6H			
BGS 254 (GSHO2069)	Orange lemma 1	*rob1*	0
BGS253 (GSHO2075)	Uniculm 2	*cul2*	-4
BGS260 (GSHO2082)	Chlorina seedling 11	*fch11*	-6
BGS251 (GSHO2089)	Multiforous 2	*mul2*	-27
BGS263 (GSHO2087)	Curly 3	*cur3*	-29
BGS262 (GSHO2088)	Curly 1	*cur1*	-39
BGS475 (GSHO2086)	Laxatum-c	*lax-c*	-63
BGS266 (GSHO2091)	Erectoides-e	*ert-e*	-84
7H			
BGS619 (GSHO2183)	Bracteatum-a	*bra-a*	
GSHO2268	Globossum-h	*glo-h*	

Chromosome Barley Genetic Stock (Genetic Stock, Hordeum)	Mutant	Gene symbol	Estimated position
BGS1 (GSHO1820)	Brachytic 1	*brh1*	61
BGS23 (GSHO1832)	Winding dwarf	*wnd*	32
BGS30 (GSHO1843)	Erectoides-m	*ert-m*	22
BGS28 (GSHO1844)	Erectoides-a	*ert-a*	18
BGS9 (GSHO1833)	Dense spike 1	*dsp1*	8
BGS10 (GSHO1850)	Short awn 2	*lks2*	-7
BGS380 (GSHO1853)	Shrunken endosperm 4	*seg4*	-35
Unmapped			
BGS343 (GSHO2152)	Leafy bract 1	*Lfb1*	
BGS325 (GSHO2146)	Curly lateral 1	*crl1*	
BGS549 (GSHO2147)	Long glume awn 1	*Lga1*	
BGS552 (GSHO2159)	Breviaristatum-j	*ari-j*	
BGS554 (GSHO2161)	Breviaristatum-m	*ari-m*	
BGS559 (GSHO2165)	Breviaristatum-r	*ari-r*	
BGS586 (GSHO2185)	Bracteatum-d	*bra-d*	
BGS409 (GSHO2193)	Eceriferum-o	*cer-o*	
BGS434 (GSHO2202)	Eceriferum-zq	*cer-zq*	
BGS449 (GSHO2212)	Eceriferum-yf	*cer-yf*	
BGS529 (GSHO2220)	Eceriferum-yp	*cer-yp*	
BGS571 (GSHO2261)	Erectoides-za	*ert-za*	
BGS463 (GSHO2267)	Gigas 3	*gig3*	
BGS585 (GSHO2287)	Praematurum-i	*mat-i*	
BGS634 (GSHO2313)	Premature ripe 2	*pmr2*	
BGS624 (GSHO2318)	Opposite spikelets 1	*ops1*	
BGS226 (GSHO2319)	Revoluted leaf	*rvl*	

Data from Barley Genetics Newsletter (special issue) 1996, volume 26, and J Franckowiak (pers. commun.)



Legend: Eg - Egypt, Height - plant height, HD, heading date, Head - heading date, Ht - height, I - irrigated, LrootL - length of the longest seedling root, Mo - Morocco, PlantWt - plant weight, PlantYield - total plant aerial biomass, R-rainfed, RootNo - seedling root number, RootWt - root weight, RSS1&2 - total root system size at stem elongation and heading, RSS3 - root system size at grain filling, ShDeltaC - shoot $\delta^{13}C$, ShootL - shoot length, ShtWt - shoot weight, Spread - seedling root spread, Tillers - number of tillers, TotRootL - total seedling root length, T - Tunisia, Yield - grain yield

Fig. 3. QTL clustering for root and other traits at the semi-dwarf mutant loci (sdw1 and ari-e.GP). Thick lines indicate QTL peaks and whiskers indicate 1 LOD confidence intervals. '+' and '-' after a variate name indicate the effect of the Derkado QTL allele on the expression of the character.

It is interesting to note that whereas both dwarfing genes are associated with short plant stature, they have different effects on seedling root growth: *ari-e*.GP was associated with short seedling roots, but no such associations were found for *sdw*1. Data on seminal roots were reflected in the adventitious root system of older plants as QTL analysis of RSS showed that *sdw*1 was associated with increased RSS over the first two growth stages, but that *ari-e*.GP was associated with negative effects at the third growth stage (Fig. 3, [12]). The data

suggest that *sdw*1 genotypes have a more extensive root system than *ari-e*.GP genotypes throughout the life cycle (at seedling, stem elongation, heading and grain filling). The effects of *ari-e*.GP (and possibly *sdw*1) may be constitutive, affecting seminal and adventitious roots in a similar manner.

The effect of brackish water irrigation on root length and field performance was studied in a range of barley genotypes. The experiments were set up at the Kuwait Institute for Scientific Research (KISR) in a study to match soil, water and genotype for barley cultivation in Kuwait [10]. The material tested (141 genotypes) included cultivars, landraces, wild barley lines and semi-dwarf mutants and their parental lines. Root length was measured in soil-filled rhizo-trunking with two treatments (fresh and brackish water irrigation). The four mutants at the *Ari-e* locus (*ari-e*.1, *ari-e*.156, *ari-e*.226 and *ari-e*.GP) had significantly shorter roots than their parental lines (Bonus, Foma, Foma and Maythorpe, respectively) in both treatments. The four *ari-e* mutant lines had the shortest root lengths in fresh water irrigation of all the 141 lines tested, and significantly their root lengths were little changed by brackish water treatment (Fig. 4). When root length data were compared to field performance under fresh and brackish water irrigation short roots were found to have positive associations, but the effects were more pronounced in the brackish treatment. Short roots were associated with higher number of seed/spike, higher number of seed/plant, greater spike weight and greater plant weight. Three reasons are given for the positive effects of the semi-dwarf *ari-e* mutant lines:

- *ari-e* mutants are known to have enhanced salt tolerance [13–15],
- short rooted plants do not penetrate the salt pan (at 1 m in Al Wafra soil) and escape toxic effects, and
- a shallow rooting system is better suited to scavenge water supplied from surface irrigation.

2.4. The development of a barley mutation grid

The barley cultivar Optic is currently one of the top malting quality barleys in the UK. This cultivar has been subject to induced mutagenesis using ethylmethanesulphonate (EMS) to produce a mutant population for both forward and reverse genetics (Caldwell 2004). Over 20,000 M_3 families have been developed. These have been subject to classical phenotypic characterisation (variation for seed, seedling, leaf, stem, ear, maturity etc) see, http://bioninf.scri.sari.ac.uk/distilling/distilling.html, but not for root traits. There is a great lack of root mutants in barley, and many have been lost, e.g. the 'few roots' mutant is now thought to be extinct. Root hairless mutants are available and have been extremely valuable in studies of nutrient uptake in barley [16]. One of our aims is therefore to trawl among the M_3 Optic families for root mutations. A problem here is one of size, as several thousand families need to be assessed. The 2-D test was successful in detecting novel root phenotypes in the BBMLs. We are therefore attempting to scale up the 2-D seedling test so that thousands of seedlings can be screened to reveal mutant root phenotypes. Optic families carrying putative mutations identified in the BBMLs will be among the first to be screened. Initial field observations in the M_3 Optic families have also given some indication of variation in root traits in the barley mutation grid. For example, one family possesses normal coloured plants in beds lacking fertiliser where all other families and controls turn pale green. This is a putative nitrogen-use efficient or stay-green family. In addition several lines exhibit poor germination and seedling establishment and weak development which may be indicative of abnormal root development.

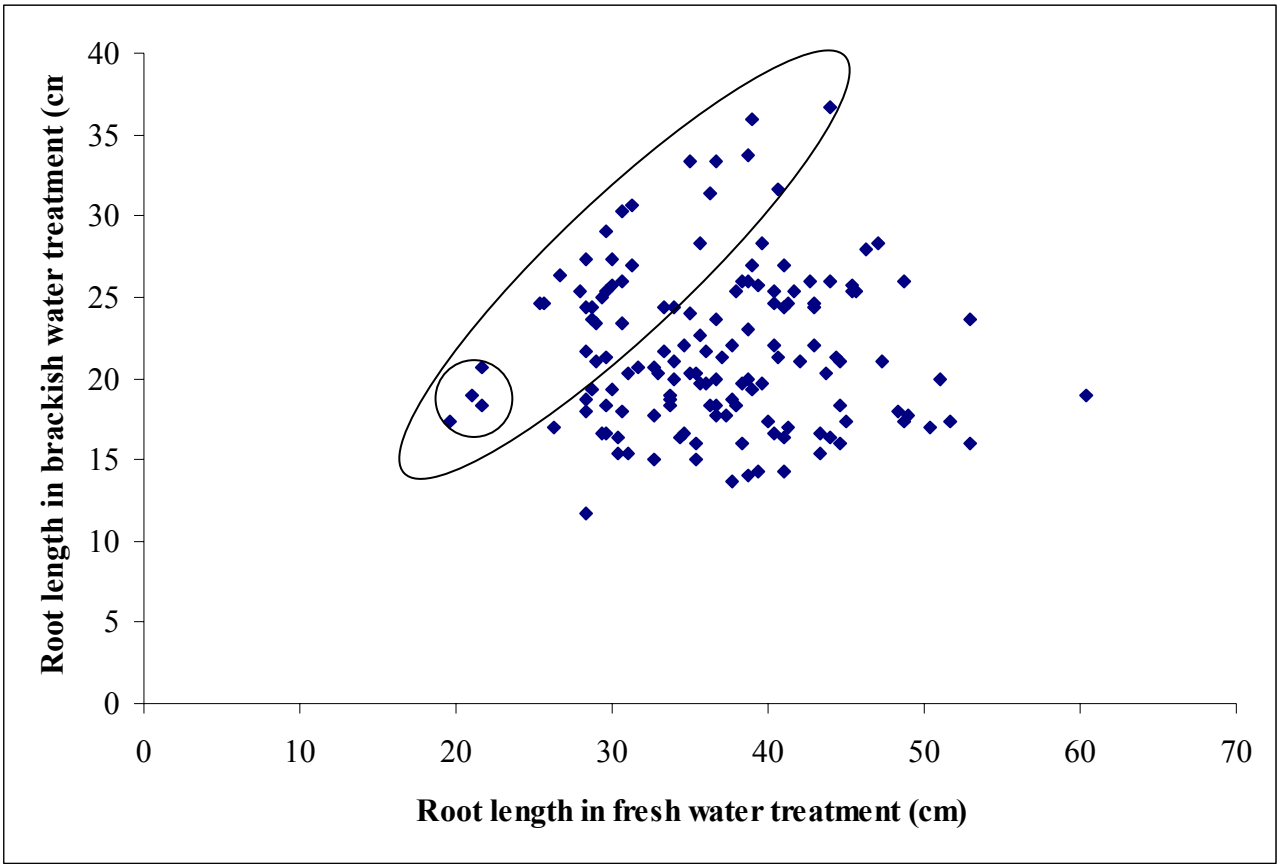


Fig. 4. Root length in brackish water versus root length in fresh water treatment of 141 barley genotypes grown in soil-filled rhizo-trunking. Genotypes contained in the ellipse are relatively un-affected by treatment, the four ari-e *mutants are circled.*

ACKNOWLEDGEMENTS

SCRI receives grant in aid from the Scottish Executive Environment and Rural Affairs Department.

REFERENCES

[1] Davis, M.P., Franckowiak, J.D., Konishi, T., Lundqvist, U. 1997. Barley Genetics Newsletter, special issue, pp553.

[2] Franckowiak, J.D., Barr, A.R., Moody, D.B. 2000. Morphological markers for barley in 'Bowman' backcross-derived lines. Barley Genetics VIII: 148-150.

[3] Thomas, W.T.B., Baird, E., Fuller, J.D., Lawrence, P., Young, G., Russell, J.R., Ramsay, L., Waugh, R., Powell, W. 1998. Identification of a QTL decreasing yield in barley linked to Mlo powdery mildew resistance. Molecular Breeding 4:381-393.

[4] Ellis, R.P., Forster, B.P., Gordon, D.C., Handley, L.L., Keith, R.P., Lawrence, P., Meyer, R., Powell, W., Robinson, D., Scrimgeour, C.M., Young, G., Thomas, W.T.B. 2002. Phenotype/genotype associations for yield and salt tolerance in a barley mapping population segregating for two dwarfing genes. Journal of Experimental Botany 53: 1163-1176.

[5] Forster, B.P. 2001. Mutation genetics of salt tolerance in barley: An assessment of Golden Promise and other semi-dwarf mutants. Euphytica 120:317-328.

[6] Forster, B.P., Ellis, R.P., Moir, J., Talamè, V., Sanguineti, M.C., Tuberosa, R., This, D., Teulat-Merah, B., Ahmed, I., Mariy, S.A.E.E., Bahri, H., El Ouahabi, M., Zoumarou-Wallis, N., El-Fellah, M., Ben Salem, M. 2004. Genotype and phenotype associations with drought in barley tested in North Africa. Annals of applied Biology 144:157-168.

[7] Thomas, W.T.B. 2003. Prospects for molecular breeding. Annals of applied Biology 142:1-12.

[8] Caldwell, D.G., McCallum, N., Shaw, P., Muehlbauer, G.J., Marshall, D.F., Waugh, R. 2004. A structured mutant population for forward and reverse genetics in barley (*Hordeum vulgare* L.). The Plant Journal 40:143-150.

[9] Bengough, A.G., Gordon, D.C., Al-Menaie, H., Ellis, R.P., Allan, D., Keith, R., Thomas, W.T.B., Forster, B.P. 2004. Gel observation chamber for rapid screening of root traits in cereal seedlings. Plant and Soil 262:63-70.

[10] Al-Meniae, H.S. 2003. Matching soil, water and genotype for barley cultivation in Kuwait. PhD thesis, The University of Reading, Reading, UK.

[11] Chloupek, O. 1977. Evaluation of the size of a plant's root system using its electrical capacitance. Plant and Soil 48:525-532.

[12] Chloupek, O., Forster, B.P., Thomas, W.T.B. 2005. The effect of semi-dwarf genes on root system size in field grown barley. Theoretical and Applied Genetics (in preparation).

[13] Pakniyat, H., Handley, L.L., Thomas, W.T.B., Connolly, T., Macaulay, M., Caligari, P.D.S., Forster, B.P. 1997a. Comparison of shoot dry weight, Na^+ and $\delta^{13}C$ values of ari-e and other semi-dwarf barley mutants under salt-stress. Euphytica 94:7-14.

[14] Pakniyat, H., Thomas, W.T.B., Caligari, P.D.S., Forster, B.P. 1997b. Comparison of salt tolerance of GPert and non-GPert barleys. Plant Breeding 116:189-191.

[15] Wenxue, W., Bilsborrow, P.E., Hooley, P., Fincham, D.A., Lombi, E., Forster, B.P. 2003. Salinity induced differences in growth, ion distribution and partitioning in barley between the cultivar Maythorpe and its derived mutant Golden Promise. Plant and Soil 250:183-191.

[16] Gahoonia, T.S., Nielsen, N.E. (2003) Phosphorus (P) uptake and growth of a root hairless barley mutant (bald root barley, brb) and wild type in low- and high-P soils. Plant Cell and Environment 26:1759-1766.

DETERMINATION OF ROOT/SHOOT CHARACTERS IN BARLEY MUTANT AND IMPROVED LINES AT EARLY STAGES

M. İLHAN ÇAĞIRGAN, M. ONUR ÖZBAŞ
Field Crops Department, Faculty of Agriculture,
Akdeniz University, Antalya, Turkey

Abstract

This study was carried to determine root/shoot traits in barley mutants, their wild types and improved lines selected from the crosses among the wild types and mutants. Barley mutants were selected for above-ground characters and two groups of genetic material were grown in two different experiments in coca-peat in the greenhouse and in sand culture in outdoor conditions, respectively. The longest root length, root dry weight, shoot length, shoot dry weight, and root/shoot dry weight ratios were measured at an early stage in both experiments. There were statistically significant differences among genotypes for the longest root length and shoot length in barley mutant and improved lines. Orthogonal contrasts were used to compare subgroups for the characters showing significant F values. A mutant, M-Q-54, selected from Quantum for tallness, high biomass, and ABA insensitivity and early heading mutants from Kaya and improved lines derived from early heading mutants were superior for root length and shoot length compared to the other genotypes. Specifically early heading lines with longer roots should be useful for direct and indirect use in breeding programs aiming at improving drought tolerance.

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) ranks as the world's fourth major cereal crop after wheat, rice, and maize. Turkey is one of the most important barley growing countries in the world, with 3.5 million ha area of cultivation and 8.1 million tons of production [1]. Barley is grown in diverse environments mostly rainfed, water-limited conditions and rainfall is usually irregular during the growing season. In these areas, barley yields are highly variable and sensitive to numerous abiotic and biotic factors such as early and late drought stress [2], which is one of the major environmental factors limiting its productivity. To avoid the severe effects of drought, breeders have successfully selected mutants with drought escape traits such as early heading as well as drought avoidance traits such as longer roots and thicker epicuticles, among other traits such as fast germination [3–6].

Plant biomass production depends on the amount of water available for growth as well as on water use efficiency [7]. Root traits are key components of plant adaptation to drought environments, it has been shown that water uptake under drought conditions depends on root size, its activity and spatial distribution, especially depth [8–9]. It has also been suggested that root length can be used as a selection criterion for developing drought-tolerant barley [10] and turfgrass [11]. Because of the difficulty in assessing underground-traits, roots are generally omitted in breeding programs aiming at improving tolerance to drought. The main objective of this study was therefore to determine the root and shoot characteristics of barley mutants selected for above-ground characters and other improved lines to provide germplasm for drought tolerance research.

2. MATERIALS AND METHODS

2.1. Experiment 1

2.1.1. Genetic material

Barley mutants were selected from two malting cultivars, namely Kaya and Quantum, irradiated with gamma rays of 15 krad and 30 krad and designated M-K and M-Q, respectively [12]. One exotic cultivar (Baronesse), two wild-types (Kaya and Quantum) and 17 mutants were used in the study. Important features of the genetic material were summarized in Ref. [4].

2.1.2. Growth conditions

Genetic material was grown from December 2001 to January 2002 in a container in the rooting greenhouse of Agricultural Faculty, Akdeniz University, Antalya, Turkey. The container (width 0.90 m, length 3.40 m, and depth 0.23 m) was filled with perlite and cocopeat [electrical conductivity of 250–500 s/cm max; pH 6.1; 96% total organic matter; 0.5% nitrogen ; 2.8 % K_2O; and 2.8 % P_2O_5]. The experimental design used was a randomised complete blocks design with 2 replicates. Each plot consisted of 1 row, 45 cm long. The distance between the rows was 15 cm and each row consisted of 9 plants. Irrigation was applied using the fogy system at 0.4 litre 1 h^{-1}. Minimum and maximum temperatures (ºC) of the greenhouse during the growing period are shown in Fig. 1.

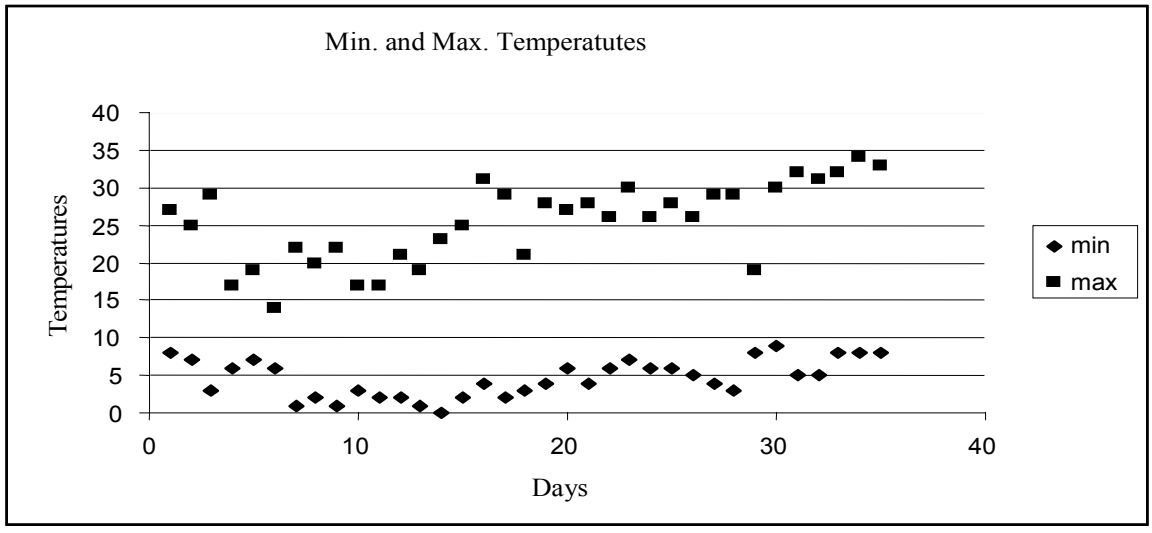


Fig. 1. Minimum and maximum temperatures of the greenhouse during growing period

2.1.3. Traits studied

The longest root length (mm), root dry weight (g), shoot length (mm), and shoot dry weight (g) were measured in three plants on the 35th day after sowing. The uprooted plants were dried for 48 h at 70ºC to measure root and shoot dry weight and calculate the root to shoot ratio.

2.1.4. Statistical analysis:

Analysis of variance was applied to the data for each character using MSTAT-C software package program [13]. Genotypic differences in measured traits were analysed using

Duncan's Multiple New Range Test. Orthogonal contrasts were also described to test the significance of the difference of sub-groups of genotypes for the characters showing significant F values for genotypes.

2.2. Experiment 2

2.2.1. Genetic material

Thirty-five improved mutant lines and five parents were used in this study. Improved lines were randomly chosen from a F_6 nursery for the study of root and shoot traits. Kaya and Quantum are two rowed, spring type, malting variety among parents. Tokak 157-37 is a land race cultivar and national check for dry areas specifically in stored moisture conditions. M-K-88 is an early heading, drought tolerant mutant selected from Kaya. M-Q-73 is an erectoid mutant with large seed size selected from Quantum.

2.2.2. Growth conditions

Trials were conducted in the experimental area of the Faculty of Agriculture, at the campus of Akdeniz University, Antalya, in the West Mediterranean Region of Turkey in 2002 and 2004. Plants were grown in an out-door system composed of benches 25 m long, 1.7 m wide and 0.3 m deep. In the first year, the experimental material, consisted of forty genotypes, was sown in sand culture in two sets of experiments arranged in randomized complete blocks design with three replicates. Genotypes were sown on 7 November 2002. Each entry was grown in one row, 10 seeds sown with 15 cm distance in each row and 20 cm apart between the rows. In the second year, twenty-eight genotypes selected according to the carbon isotope discrimination values and the results of the trial in the first year, were grown in a randomised complete block design with three replications. The growing sand media was improved by adding small and soft particles and organic matter. The same sowing system was carried out on 17 March 2004 as in the previous year described above.

2.2.3. Traits studied

The screening was at twenty-one days after sowing in each set of experiments in the first year. The longest root length (mm), root dry weight (g), shoot length (mm), and shoot dry weight (g) were measured. Samples were then dried at 72°C for 72 h, root dry weight and shoot dry weight were determined. In the second year, the same traits were measured after six weeks of growth.

2.2.4. Statistical analysis

Data were analysed using MINITAB, version12 software. Analyses of variance were performed to study the differences between genotypes.

3. RESULTS AND DISCUSSION

3.1. Experiment 1

There were statistically significant differences among the genotypes for the longest roots ($p<0.01$) and shoots ($p<0.05$) (Table I). M-Q-54, Quantum and M-Q-73 were the genotypes with the longest roots while M-K-8, Baronesse, Kaya and M-K-6 had the shortest roots. The other mutants selected from Kaya had greater root lengths than their wild type.

Early heading mutants, M-K-1, M-K-2, M-K-29 and M-K-88, and thicker cuticle mutants, M-K-55 and M-K-49, exhibited the longer root lengths than the other mutants of Kaya.

Shoot length varied between 130.3 mm and 219.5 mm, with the Quantum mutant M-Q-54 having the highest value followed by a six rowed mutant of Kaya, M-K-37 with a shoot length of 211.1. Genotypes M-K-8, M-K-1, Kaya and Baronesse had the shortest shoots.

TABLE I. MEAN VALUES AND DUNCAN MULTIPLE RANGE TEST FOR THE CHARACTERS MEASURED IN THE GENOTYPES AT 35 DAYS AFTER SOWING

Genotypes	The longest root length (mm)	Root dry weight (g)	Shoot length (mm)	Shoot dry weight (g)	Root/shoot dry weight ratio
Baronesse	140.0 e[1]	0.022 bc	141. 5 e	0.027 abc	0.87
Kaya	161.0 de	0.027 abc	136.5 e	0.025 c	1.18
M-K-1	188.3 bcd	0.029 abc	136.0 e	0.032 abc	0.91
M-K-2	209.6 bc	0.029 abc	150.8 cde	0.031 abc	1.03
M-K-29	194.0 bcd	0.032 abc	148.5 cde	0.038 abc	0.88
M-K-88	198.8 bcd	0.032 abc	157.1 cde	0.038 abc	0.83
M-K-8	137.3 e	0.023 bc	130.3 e	0.023 c	0.96
M-K-23	192.5 bcd	0.036 abc	196.6 abc	0.047 abc	0.77
M-K-24	180.1 cd	0.028 abc	176.4 abcde	0.043 abc	0.70
M-K-12	206.5 bc	0.035 abc	189.3 abcd	0.046 abc	0.79
M-K-85	185.1 bcd	0.043 a	145.1 de	0.037 abc	1.16
M-K-6	162.6 de	0.034 abc	156.8 cde	0.033 abc	1.03
M-K-49	191.5 bcd	0.022 c	165.5 bcde	0.027 bc	1.01
M-K-55	204.5 bc	0.024 bc	154.1 cde	0.027 abc	0.96
M-K-38	191.1 bcd	0.027 abc	168.8 bcde	0.035 abc	0.77
M-K-37	204.5 bc	0.039 ab	211.1 ab	0.055 a	0.71
Quantum	224.0 ab	0.036 abc	162.0 bcde	0.037 abc	0.98
M-Q-54	256.8 a	0.036 abc	219.5 a	0.053 ab	0.68
M-Q-73	220.8 b	0.036 abc	140.3 de	0.039 abc	1.04
M-Q-80	202.5 bc	0.027 abc	181.8 bcde	0.036 abc	0.80
F values	6.10**	1.46^{ns}	2.90*	1.90^{ns}	0.37^{ns}

*, ** and ns; significant at $p<0.05$, $p<0.01$ and no significant, respectively

[1] Values within a group followed by the same letter or letters are not significantly different at the 5% level (Duncan's multiple new range)

To compare statistically the similar sub-groups with each other, 6 orthogonal contrasts were described on the basis of similarity or genotypic relatedness of the entries and were tested in variance analyses (Table II). Of the 6 contrasts, 4 were statistically significant for root length and only 2 were significant for shoot length, suggesting that root length differed much more among the sub-groups than shoot length. The Baronesse mean was inferior to all the other genotypes' mean for root length ($p<0.01$), but for shoot length the difference was not significant ($P<0.05$). This result may be interpreted as the better adaptability of the domestic varieties and their mutants selected under semi-dry conditions. Quantum and its mutants were superior to Kaya and Kaya mutants in the mean performance of root length ($P<0.01$). While Kaya mutants as a group were greater in root length than wild type Kaya, there were no statistically significant differences in root length between early heading mutants of Kaya and the other Kaya mutants but there were differences in shoot length. In contrast

Quantum *vs.* Quantum mutants revealed no statistically significant difference for either root or shoot length. However, the tall Quantum mutant, M-Q-54, was higher than the semi dwarf mutants, M-Q-73 and M-Q-80, for root length and shoot length.

TABLE II. ORTHOGONAL CONTRASTS FOR THE LONGEST ROOT LENGTH AND SHOOT LENGTH

Contrasts	The longest root length (mm)	Shoot length (mm)
Baronesse ***vs.*** others	22.853**	2.140^{ns}
Kaya+Kaya mutants ***vs*** Quantum+Quant. Mutants	37.499**	1.285^{ns}
Kaya ***vs.*** Kaya mutants	5.755*	3.118^{ns}
Early Hed.Kaya Mutants ***vs.*** all other Kaya Mutants	3.285^{ns}	6.033*
Quantum ***vs.*** Quantum mutants	3.256^{ns}	4.235^{ns}
Tall Quantum mutant ***vs*** semidwarf Quantum mutants	10.692**	14.49**

*, ** and ns; significant at $p<0.05$, $p<0.01$ and no significant, respectively

3.2. Experiment 2

The mean values of root length, root dry weight, shoot length, shoot dry weight and root to shoot ratio are given in Table III. In the first year there was significant ($p<0.01$) variance among entries for shoot length measured on the 21st day after sowing. Coefficient of variation (C.V.) value for experimental error was 9.9% for this trait (Table III).

Relatively well-controlled experimental error was for root length, which is 14.2 but there was no significant variance for this character. However, it is evident that “weight” related traits had very high C.V. values, i.e., 91.5 and 155.5, for root dry weight and shoot dry weight respectively, suggesting that it is difficult to control the experimental error for these traits despite of 6 replicates in 2 sets of experiments. “Length” traits are easier to measure and there is no need to dry before measuring and they are quite informative.

The mean values of genotypes for root length, root dry weight, shoot length, shoot dry weight and root to shoot dry weight ratio in second year are given in Table IV. While the C.V. value for experimental error was 12.06 and 21.39% for root length and shoot length, respectively, “weight” related traits had high C.V. values, i.e., 73.66 and 71.80% for root dry weight and shoot dry weight, respectively. The C.V. values for experimental errors in the second year of the trials, which was carried out in improved growing media by adding small and soft particles and organic matter, were lower than in the first year.

TABLE III. MEAN AND F VALUES FOR ROOT AND SHOOT TRAITS IN IMPROVED MUTANT LINES OF BARLEY IN THE FIRST YEAR

Genotypes	Root length (cm)	Root dry weight (g)	Shoot length (cm)	Shoot dry weight (g)	Root/shoot ratio
Kaya	17.7	0.18	13.4	0.04	6.03
Quantum	17.9	0.08	13.1	0.03	2.91
Tokak	19.7	0.08	11.5	0.04	2.24
MK88	18.3	0.06	11.6	0.03	2.05
MQ73	17.5	0.09	14.2	0.04	2.36
8716123-1	20.3	0.06	14.7	0.12	1.18
8716171-2	17.8	0.15	14.0	0.12	3.00
8717362-8	18.4	0.25	15.2	0.13	4.25
8717274-14	20.3	0.08	12.9	0.04	2.35
8717354-22	17.3	0.06	14.4	0.04	1.74
8717275-29	18.3	0.09	12.7	0.04	2.42
8717367-34	17.6	0.11	14.9	0.04	3.15
8718583-45	18.3	0.06	11.3	0.03	2.57
8718667-53	17.1	0.14	12.6	0.03	5.10
8718572-54	18.0	0.08	12.4	0.04	2.15
8718572-58	18.0	0.06	12.2	0.12	1.02
8718572-59	19.1	0.17	12.6	0.03	6.38
8718644-69	20.2	0.11	13.7	0.13	1.49
8718659-75	18.5	0.09	13.6	0.08	2.15
8718659-79	18.5	0.13	13.5	0.08	2.44
8718659-81	19.4	0.12	12.6	0.12	3.12
87221572-97	18.9	0.08	14.1	0.05	1.64
87221562-101	21.6	0.12	14.9	0.05	2.67
87221552-106	18.7	0.13	14.9	0.09	2.76
87231669-109	17.9	0.10	14.7	0.04	2.63
87231653-114	19.4	0.10	15.1	0.05	2.06
87231653-115	18.9	0.13	13.9	0.04	2.83
87231667-118	19.7	0.13	14.1	0.05	2.77
87231704-121	20.0	0.11	14.2	0.04	2.53
87241892-124	19.3	0.11	13.1	0.04	2.66
87241873-127	20.1	0.12	13.2	0.05	2.64
87241875-131	18.6	0.20	12.6	0.13	2.66
87241875-134	20.4	0.12	13.0	0.04	2.92
87431120-136	20.0	0.10	14.7	0.04	2.27
87431082-137	20.1	0.08	14.6	0.04	1.88
87461471-138	18.0	0.07	14.8	0.05	1.34
87461490-139	16.9	0.15	13.4	0.13	1.63
8702386-152	16.7	0.06	14.5	0.12	1.47
8702386-154	18.0	0.11	15.4	0.04	2.39
8702386-155	18.5	0.07	16.0	0.04	1.67
Grand Mean	18.7	0.108	13.7	0.062	2.59
F value	1.07	1.06	4.24	0.85	1.03
Probability	0.368	0.386	0.000	0.716	0.426
C.V. (%)	14.2	91.5	9.9	155.5	105.5

TABLE IV. MEAN AND F VALUES FOR ROOT AND SHOOT TRAITS IN IMPROVED MUTANT LINES OF BARLEY IN THE SECOND YEAR

Genotypes	Root length (cm)	Root dry weight (g)	Shoot length (cm)	Shoot dry weight (g)	Root/shoot ratio
Kaya	24.8	0.89	38.0	1.81	0.51
Quantum	23.5	1.30	47.2	2.97	0.47
Tokak	23.9	0.92	35.4	1.95	0.52
MK88	23.5	0.44	33.3	0.92	0.55
MQ73	23.6	0.90	33.6	1.40	0.69
8716171-2	28.2	1.75	40.4	3.46	0.58
8717274-14	22.9	0.73	34.7	1.45	0.59
8717367-34	21.6	0.47	35.3	0.73	0.65
8718668-47	25.0	0.93	43.5	2.24	0.44
8718667-49	24.5	1.46	42.3	2.77	0.54
8718659-79	25.8	1.38	45.4	3.30	0.48
8718659-81	26.5	1.54	49.5	2.82	0.49
87221572-97	26.3	1.76	41.4	2.74	0.64
87231669-109	26.9	0.50	33.9	0.95	0.50
87231653-115	24.0	1.34	34.6	2.18	0.69
87231653-116	24.4	1.97	43.4	3.35	0.60
87231667-118	25.9	2.21	42.6	2.99	0.75
87241892-124	25.2	1.05	36.9	2.14	0.52
87241892-125	25.6	1.12	36.8	2.85	0.42
87241873-127	24.6	1.32	42.2	2.81	0.50
87241875-131	25.7	0.81	29.1	2.52	0.38
87241875-134	25.4	0.73	33.1	2.31	0.39
87431082-137	23.0	1.12	39.9	2.52	0.49
87461471-138	30.4	0.84	36.7	2.30	0.45
8711227-142	19.9	1.31	40.8	2.33	0.53
8702386-154	26.9	1.01	45.7	2.27	0.48
Baronesse	25.7	0.76	36.7	1.78	0.44
BNS	26.4	1.12	36.9	2.08	0.54
Grand mean	25.003	1.131	38.897	2.284	0.529
F values	1.81	1.14	2.49	1.07	1.24
Probability	0.032	0.330	0.002	0.401	0.249
C.V. values (%)	12.06	73.66	21.39	71.80	31.67

There were statistically significant ($p<0.05$) differences among the genotypes for root length. The root length ranged from 19.9 cm to 30.4 cm in the sixth week. Kaya, among the parents, had the greatest root length with 24.8 cm. Improved line, 87461471-138 derived from M-K-88 x M-Q-73 mutant crosses, had the longest root length, it was followed by improved lines, 8716171-2 derived from Kaya x M-K-88 having a root length of 28.2 cm. Significant ($p<0.01$) genotypic differences were also found for shoot length. The mean values of genotypes for shoot length ranged from 29.1 cm to 49.5 cm. Improved line, 8718659-81 derived from Tokak x M-K-88 showed the highest shoot length at 49.5 cm followed by 47.2 cm, 45.7 cm and 45.4 cm obtained from Quantum, improved lines, 8702386-154 and 8718659-79, respectively. Improved lines derived from M-K-88 crosses exhibited generally above mean values for root and shoot length, thus, M-K-88, an early heading mutant from

Kaya, can be used to improve root length of barley by using it as a parent in breeding programs.

4. CONCLUSION

In conclusion, the tall, high biomass, ABA insensitive and fast germinating, M-Q-54, selected from Quantum, was superior for the longest roots and shoots compared to other genotypes. Root and shoot characteristics of barley mutants measured on the 35th day were similar to those previously obtained on the 7th, 14th and 21st day [14]. Since screening and measurement of root characteristics gets complicated as the plant grows older, studies on root characters can be performed in the early developmental stages of barley. They also reported that an induced mutation could also exhibit pleiotropic effects of the mutated gene. Wide genetic variability was observed for root traits in barley mutant and improved lines (F_7 and F_8) that were derived from a number of mutants. Early heading mutants were superior for root traits compared with the rest of the entries in both experiments. These mutants can be used to improve crops having a good and fast root growth at the beginning of development of barley. The lower experimental error associated with the improved outdoor sand culture system makes this system more suitable for screening root traits.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of the International Atomic Energy Agency with the Research Contract No 10964 as part of the Co-ordinated Research Project on "Mutational Analysis of Root Characters in Annual Food Plants Related to Plant Performance".

REFERENCES

[1] FAO 2003. FAOSTATS. http://www.fao.org/

[2] Rezgui, S., Yahyaoui, A., Cecceralli, S. 2000. Yielding ability and stability of selected barley cultivars in semi-arid areas of Tunisia. *The 8th International Barley Genetic Symposium*, Australia, 22-27 October, Volume III. pp:39-41.

[3] Mahar, A.R., Hollington, P.A., Virk, D.S., Witcombe, J.R. 2003. Selection for early heading and salt-tolerance in bread wheat. *Cereal Research Communications,* 31 (1):81-88.

[4] Çağırgan, M.I., Gorham, J., Toker, C., Uzun, B., Visser, K. 2002. Evaluation of barley mutants for drought tolerance: a physiology – breeding approach. Mutations, In Vitro and Molecular Techniques for Environmentally Sustainable Crop Improvement In Kluwer Academic Publishers Edited by M. Maluszynski and K.J. Kasha pp.246 Dordrecht/Londan.

[5] Shindo, C., Sasakuma, T. 2001. Early heading mutants of T-monococcum and Ae. squarrosa, A- and D-genome ancestral species of hexaploid wheat. *Breeding Science,* 51 (2):95-98.

[6] Katsura, M. 1999. Summer-sown cultivation of forage oats and breeding in Japan. *Jarq-Japan Agrıcultural Research Quarterly*, 33 (1):57-63.

[7] Martin, B., Tauer, C.G., Lin, R.K. 1999. Carbon isotope discrimination as a tool to improve water-use efficiency in tomato. *Crop Science*, 39:1775-1783.

[8] Huang, B., Gao, H. 2000. Root physiological Characteristics associated with drought resistance in tall fescue cultivars. *Crop Science*, 40:196-203.

[9] Richards, R.A., Rebetzke, G.J., Condon, A.G., van Herwaarden, A.F. 2002. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. *Crop Science*, 42:111-121.

[10] Kafawin, O.M. 1998. Seed size and water potential effect on germination and early growth two barley cultivars. *Dirasat Agriculture Sciences*, 25 (3):335-342.
[11] Huang, B., Duncan, R.R., Carrow, R.N. 1997. Drought-resistance mechanisms of seven warm-season turfgrasses under surface soil drying: II. Root aspects. *Crop Science*, 37:1863-1869.
[12] Çağırgan, M.I., Yıldırım, M.B. 1989. Arpa mutant populasyonundaki genotipik varyasyonun belirlenmesi ve seleksiyon yoluyla değerlendirilmesi üzerinde araştırmalar. E. Ü. Fen Bilimleri Enstitüsü Doktora Tezi, Bornova, İzmir.
[13] Freed, R., Einensmith, S.P., Guetz, S., Reicosky, D., Smail, V.W., Wolberg, P. 1989. User's guide to MSTAT-C, an analysis of agronomic research experiments. Michigan State University, USA.
[14] Özbaş, M.O., Çağırgan, M.I. 2004. Variability and relationships of root traits in a subset of induced barley mutant collection. *Cereal Research Communications*, 32 (1):119-126.

ECOLOGY AND GENETICS OF ROOT ARCHITECTURE AND SOIL WATER EXTRACTION

L.E. JACKSON
Department of Land, Air and Water Resources,
University of California, Davis, United States of America

Abstract

Merging the study of plant ecophysiology and genetics has great potential for improving crops for stressful environments. Root systems play a key role in acquiring resources and stress avoidance, but they also represent a major cost to plants in terms of carbon expenditures. Additional allocation to roots potentially affects crop growth and yield, either positively in the case of stress avoidance, or negatively in situations where the costs of root allocation outweigh the benefits in yield. Knowledge of these relationships are necessary for optimizing plant breeding for root system characteristics, and suggest that breeding goals for root systems may vary by species, resource availability, and local agricultural environments. This research project focused on the deep acquisition of water by a vegetable crop from the soil profile, as a means to reduce water stress and irrigation inputs. The goal was to determine the type of root architectural system that was conducive to rapid shoot growth and deep soil water extraction in lettuce, a shallow-rooted crop with high water demand. Genes, i.e. Quantitative Trait Loci (QTL), for a deeper taproot and for more laterals at the tip of the taproot were compared in recombinant inbred lines from an initial cross between cultivated (*Lactuca sativa*) and wild (*Lactuca serriola*) lettuce. Lines with the QTL for more deep laterals at the tip of the taproot appear to benefit from greater water availability, i.e., they had higher shoot biomass, deeper soil water extraction, and greater discrimination for ^{13}C. A marker-assisted breeding program was initiated to introgress this QTL region in *L. sativa*, with the potential for allowing irrigation less frequently and with less water without a negative impact on yield. Information on approaches and strategies for breeding for crop root systems is also presented here and in a review article, including comparison of screening methodologies.

1. INTRODUCTION

Root architecture and root growth are important for soil exploration for water and nutrients, since the acquisition of these resources drives plant growth [1, 2]. There are many types of root forms, ranging from woody roots to root hairs, and many of their various functions still remain unknown. Construction and maintenance costs of roots should be considered in the assessment of different root architectural patterns. An optimization approach suggests that total plant growth will be greatest when the root system maximizes nutrient and water acquisition per unit resource supplied to the shoot. Allocation of large amounts of carbohydrates and nutrients to roots may detract from shoot growth, leaf area, and photosynthetic carbon gain, thereby reducing total plant growth, especially if the increased root allocation does not achieve increased rates of nutrient or water uptake. By considering these issues, plant breeding for root systems can be directed toward both stress avoidance and crop yield. Due to the complex genetics of root architectural traits, root plasticity, and the difficulty of studying roots in soil, it is often hard to use quantitative trait locus (QTL) analysis to identify the key root traits for stress tolerance. This project addresses some of these issues.

In this project, the focus is on how deep roots may enable deeper acquisition of soil moisture in irrigated farming systems, and thereby maintain yield with reduced frequency and amount of irrigation. A comparison was made between two types of deep rooting structures, a deep taproot vs. deep lateral roots at the tip of the taproot, and their differences in shoot growth and deep water extraction from the soil profile. These are root architectural types

which compose the root systems of many horticultural and agronomic crops, and thus are of interest to this RCM in terms of their role in resource acquisition. The specific goal was to determine the best strategy for breeding for deeper root systems in lettuce, a shallow-rooted vegetable crop that requires frequent irrigation in the Mediterranean climate of California, where this research was conducted.

As a foundation for breeding lettuce to increase rooting depth and deep water extraction from the soil profile so that irrigation can be applied less frequently and in lesser amounts, the main questions addressed in a broad sense are:

- What are the genetics of root architecture, root allocation and water uptake in lettuce?
- Can root architecture increase uptake of deep moisture without altering biomass allocation and yield?

Our previous work on root architectural traits described differences between wild (*Lactuca serriola*) and cultivated lettuces (*L. sativa*) [3]. Wild lettuce has a deeper taproot and more branching at the tip of the taproot, which increases utilization of water deep in the soil profile [3, 4]. Wild (*L. serriola*) and cultivated lettuce (*L. sativa* cv. 'Salinas') were crossed, and the resulting $F_{2:3}$ populations were used to create a genetic linkage map. Genes controlling complexly inherited traits, *i.e.*, quantitative trait loci (or QTL), were identified using this map. The QTL indicate that specific regions of the lettuce genome control taproot length, number of lateral roots produced near the taproot tip, and the ability to extract water from deep in the soil [5]. The deep rooting genes came from the wild lettuce parent. A QTL for taproot length/plant biomass co-localizes in the genome with a QTL for water content deep in the soil profile[5]. The map location of this QTL is on linkage group 2d-f. A QTL for the number of laterals at the bottom 5 cm of the taproot/root biomass nearly co-localizes with a QTL for water content deep in the soil profile. The map location of this QTL is on linkage group 4c-f.

Wild lettuce alleles are potentially useful in breeding cultivated lettuce with root systems that can mine the soil for deep water and nutrients, reducing water use, and production inputs and costs. Recombinant inbred lines (F_7 and F_8 RILs) were generated from the original cross described above to use in further studies on how these QTL may be useful in improving cultivated lettuce. Indirect selection based on molecular markers with known linkages to genes for root architectural characters would facilitate the generation of lettuce cultivars with a modified root system.

2. PROCEDURES AND RESULTS

2.1. Confirmation of QTL for deep rooting traits and soil water extraction in lettuce

To confirm that the QTL regions consistently affected soil water extraction, we examined F_7 recombinant inbred lines (RILs) that were derived from the original cross of cultivated lettuce (*L. sativa* cv. 'Salinas') by wild lettuce (*L. serriola*). The F_7 RILs are very different in size and form from one another, and provide a good opportunity to test the effect of a given gene on growth and water acquisition. F_7 RILs were identified that were homozygous for cultivated and wild parent alleles at the two map regions of interest (2d-f and 4c-f) based on AFLP markers. These were grown in a field experiment at UC Davis on silt loam soil. The plants were grown with adequate moisture for 6 weeks, and then drought was imposed for 11 days before sampling. This level of drought is typical for plants in commercial fields between furrow irrigations. Shoot dry weight and soil cores (0–10 cm, 10–25 cm, 25–

50 cm, 50–75 cm, and 75–100 cm) were taken within a 30-hour period. In each plot, four plants were taken for aboveground dry weight, and one 3.5 cm diameter core was taken and separated into the five depth increments for gravimetric moisture analysis at each depth.

The goal was to determine if F_7 RILs for *L. serriola* alleles for 2d-f and 4c-e were associated with greater moisture extraction at depth, and had greater yield compared to F_7 RILs with *L. sativa* genes. If a deeper root system requires more belowground allocation of carbon, then shoot production may suffer, causing slower growth rates of lettuce.

When plants had the wild lettuce (*L. serriola*) alleles for the QTL associated with deep lateral roots in the lowest 5 cm of the taproot and deep soil water extraction at 75–100 cm depth (4c-f) [5], shoot biomass was greater, and more water was extracted deep in the profile per amount of shoot biomass compared to plants with the cultivated lettuce (*L. sativa*) alleles (Fig. 1). Also, they had decreased water use efficiency, based on $d^{13}C$ measurements, implying higher transpiration rates, and thus greater water availability (Table I). Deep laterals at the tip of the taproot may increase water use from the deep profile at relatively low construction costs, which if introgressed into cultivated lettuce could yield higher shoot biomass production under typical irrigation regimes.

TABLE I. CARBON ISOTOPE DISCRIMINATION IN F_7 RILS WITH EITHER *L. serriola* OR *L. sativa* ALLELES IN THE QTL REGIONS FOR ROOT ARCHITECTURAL TRAITS AND DEEP SOIL WATER EXTRACTION. THE PARENTAL VALUES WERE -27.4 FOR *L. serriola* AND -26.4 FOR *L. sativa*

	$d^{13}C$ (n = 257)		Shoot dry wt (g/plant) (n = 365)	
	L. serriola alleles	*L. sativa* alleles	*L. serriola* alleles	*L. sativa* alleles
QTL 4c-f (deep lateral roots/root biomass & deep water extraction)	-26.91a	-26.39b	6.36	6.57
QTL 2d-f (taproot length/biomass & deep water extraction)	-26.63	-26.79	6.47	6.82

Unexpectedly, the deep taproot alleles from *L. serriola* at QTL 4c-f did not give similar results. Plants with wild lettuce alleles for QTL 2d-f (deep taproot/ biomass & deep soil water extraction at 50–100 cm depth) [5] had decreased shoot biomass, increased soil water present per amount of shoot biomass deep in the profile, and no difference in $d^{13}C$, compared to plants with *L. sativa* alleles at this QTL region (Fig. 2, Table I). Under these growing conditions, *L. serriola* alleles for this QTL resulted in lower shoot growth, and thus, reduced shoot water use. An explanation may be that allocation of root biomass to a storage taproot may be more costly in terms of shoot growth than the construction of fine lateral roots at the tip of the taproot. Construction of a deep taproot per amount of shoot biomass may only increase uptake of deep soil water in some circumstances, e.g., more severe drought than imposed by the typical irrigation regime for lettuce in California.

A marker-assisted selection program was initiated to introgress the *L. serriola* alleles in the 4c-f QTL region into cultivated lettuce to produce near-isogenic lines of cultivated lettuce with the trait for many laterals at the tip of the taproot, and deep soil water extraction. This was done using RAPD and microsatellite markers that were integrated with the existing

AFLP genetic map that was originally produced for this cross [5]. This is now at the BC_3 stage. Since data from the RILs experiment suggest that introgression of the *L. serriola* QTL for many lateral roots near the tip of the taproot could increase lettuce yield and increase the uptake of water from deep in the soil profile, this could potentially allow growers to irrigate lettuce less frequently and with less water without negative impact on yield.

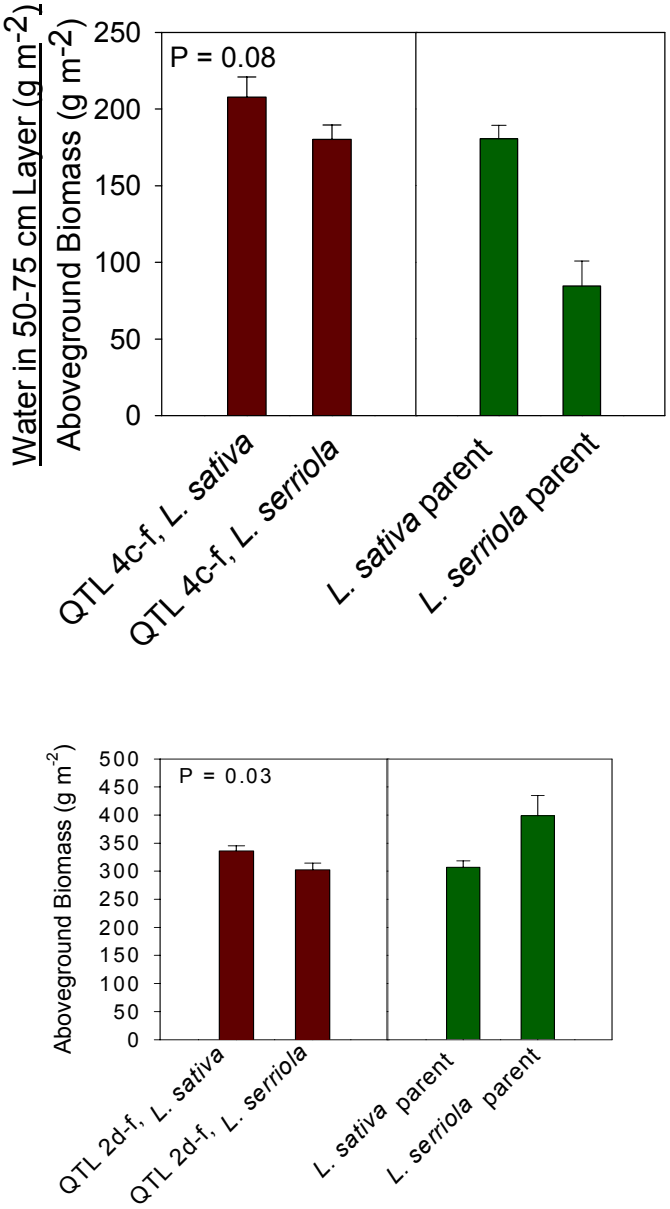


Fig. 1. F_7 RILS: Verifying QTL 4c-f (deep laterals/root biomass & depth of soil water extraction). A comparison was made between F_7 RILS with either L. serriola or L. sativa alleles in the QTL region (n = 93). The field was well-watered until 11 days before harvest. The L. sativa parent is cultivated lettuce and the L. serriola parent is wild lettuce.

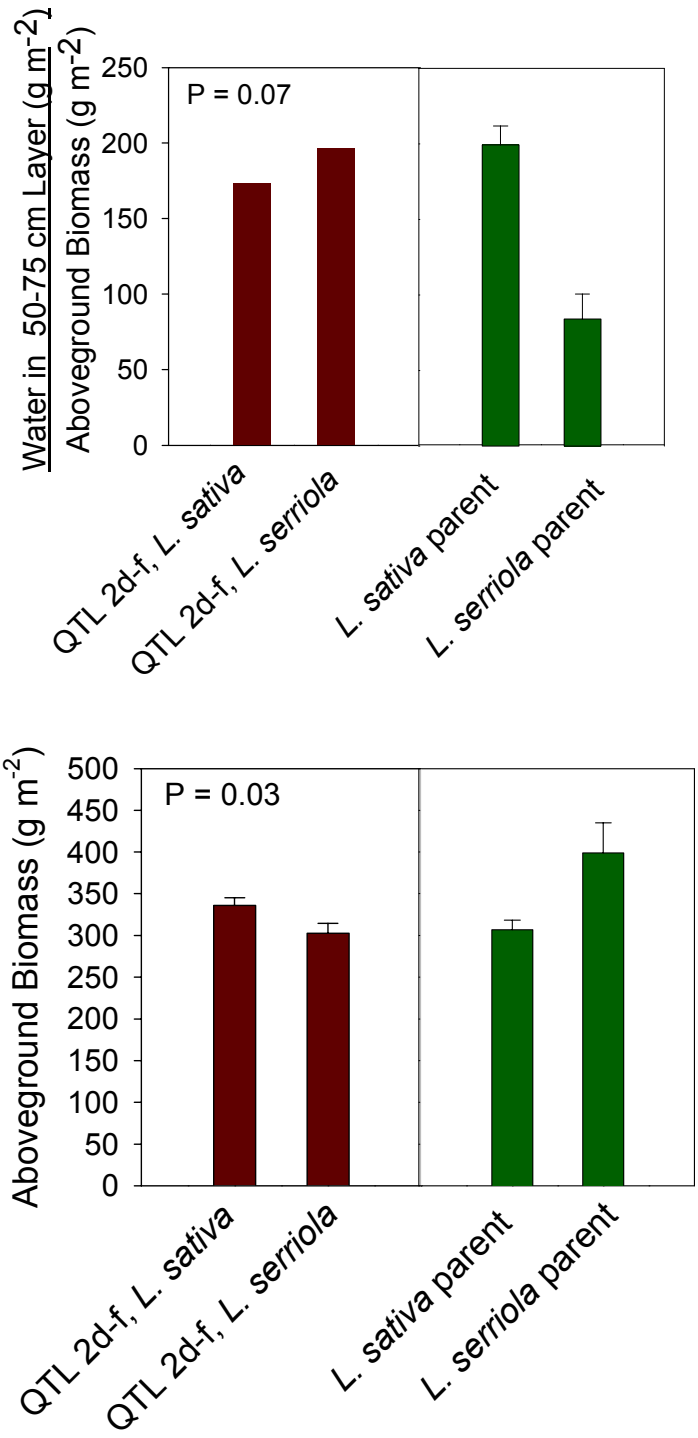


Fig.2. F_7 RILS: Verifying QTL 2d-f (deep taproot/biomass & depth of soil water extraction). A comparison was made between F_7 RILS with either L. serriola or L. sativa alleles in the QTL region (n = 81). The field was well-watered until 11 days before harvest. The L. sativa parent is cultivated lettuce and the L. serriola parent is wild lettuce.

2.2. Approaches and methods for root system analysis for crop breeding

In the course of this research, several methods were used to screen plants for root architecture. Our initial work [3] was conducted in large containers of silt loam soil (60 cm deep x 25 cm diameter.) that closely portrayed actual growing conditions for young plants. A time point (4–6 weeks) was determined when the cultivated lettuce and wild lettuce species, had similar root and shoot biomass allocation, and similar growth. This was considered a valuable period for evaluation of root architectural traits since the plants were well-matched in terms of age, size, and allocation. Later, plants were grown in sand in tall pots (30 cm x approx. 10 cm diameter.) with generally similar results [5].

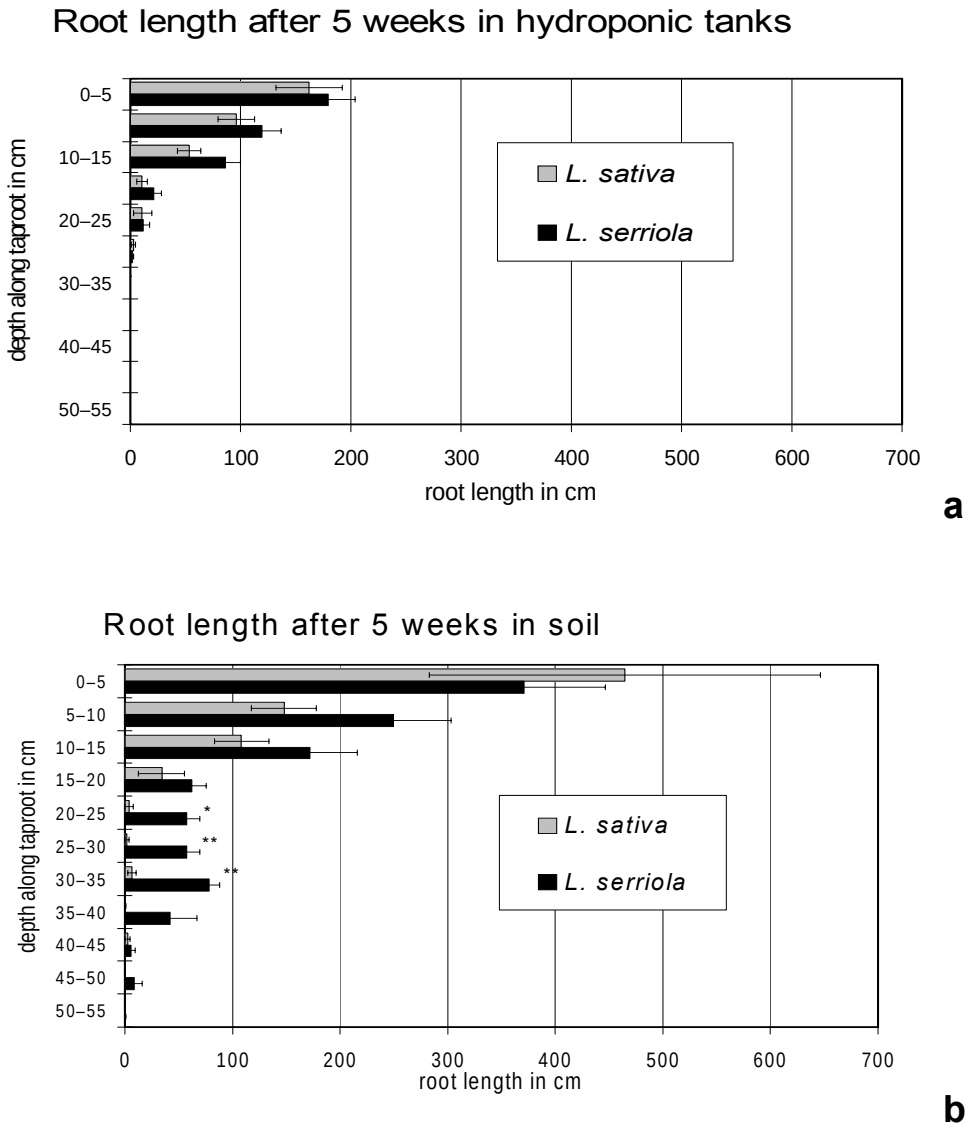


Fig. 3. Results of two experiments on 5-week old lettuce plants on depth distribution of root length. One experiment was conducted in solution culture in hydroponics tanks(a) and the other was in large soil cores (60 cm deep x 25 cm diameter)(b). In a, the mean taproot length for cultivated and wild lettuce is 34 cm and 42 cm, respectively. In b, the mean taproot length for cultivated and wild lettuce is 35 cm and 45 cm, respectively.

Recently, an experiment in hydroponics tanks revealed that lettuce plants in solution culture have very different root architecture than plants grown in soil. Taproots grew very long, and had almost no laterals near the tip (Fig. 3). Thus, hydroponics screening was not a viable method for screening for our traits of interest. New methods for root assessment are obviously needed for effective screening large populations of plants, e.g. root in-growth cores, isotope uptake from depth, etc.

In a larger sense, a methodological improvement for this research would have been to screen other accessions of *L. serriola* before beginning the program on root genetics. A recent comparison of our research population of *L. serrriola*, which was collected in a wheat field in California's Central Valley, was made with three other accessions of this species (Fig. 4).

TABLE II. COMPARISON OF FOUR ACCESSIONS OF *L. serriola* FOR ROOT DISTRIBUTION AND SHOOT DRY WEIGHT IN FIELD SOILS, AND THEIR LOCATION OF COLLECTION. * INDICATES THE RESEARCH POPULATION USED IN THE GENETIC STUDIES DESCRIBED ABOVE

	Wheat field (CA)*	Grass-land (CA)	Wheat field (Spain)	Olive orchard (Spain)
Taproot length (cm)	93a	144b	152b	147b
No. laterals cm^{-1} in deepest 30 cm of taproot	2.7	2.5	2.6	2.5
Shoot dry wt. (g)	159	150	131	150

Fig. 5. The root profile of L. serriola in the field.

The field comparison showed that the research population had a shorter taproot than the other accessions, but equal numbers of laterals in the deepest 5 cm of the taproot. Although this bodes well for the traits of interest in our breeding program, other sets of root traits may also play a role in deep water acquisition, and a more comprehensive analysis of starting populations would have been useful.

REFERENCES

[1] Waisel, Y., Eshel, A., Kafkafi, U. (2002) *Plant Roots: The Hidden Half*. Marcel Dekker, Inc., New York, NY, USA.

[2] Jackson, L.E. Soil biology: root architecture and growth. Ed. Hillel, D., Rosenzweig, C., Powlson, D., Scow, K., Singer, M., Sparks, D. In press, *Encyclopedia of Soils in the Environment,* Academic Press.

[3] Jackson, L.E. 1995. Root architecture in cultivated and wild lettuce (*Lactuca* spp.). *Plant Cell and Environment* 18:885-894.

[4] Gallardo, M., Jackson, L.E., Thompson, R.B. 1996. Shoot and root physiological responses to localized zones of soil moisture in cultivated and wild lettuce (*Lactuca* spp.). *Plant, Cell and Environment* 19:1169-1178.

[5] Johnson, W.C., Jackson, L.E., Ochoa, O., Peleman, J., Van Wijk, R., St.Clair, D.A., Michelmore, R.W. 2000. A shallow-rooted crop and its wild progenitor differ at loci determining root architecture and deep soil water exploitation. *Theoretical and Applied Genetics* 101:1066-1073.

MORPHOLOGICAL CHARACTERISATION OF WHEAT MUTANTS AND ITS RELATIONSHIP WITH THE TOLERANCE TO DROUGHT

M. CABRERA, S. PEREZ, C. DIAZ, L. GUTIERREZ, M. ESQUIVEL, C. PEREZ, E. LAGO, M. PEREZ, M.J. MENDOZA, H. PENICHET, S. MARRERO
Institute of Fundamental Research in Tropical
Agriculture "Alexander von Humboldt" (INIFAT),
Santiago de las Vegas, Havana, Cuba

Abstract

Seven wheat (*Triticum aestivum* L.) mutants (RM-26, RM-29, RM-30, RM-31, RM-32, RM-36 and RM-37) derived from the cultivar Cuba C-204 was characterized for their root morphology, anatomy and yield. The mutants were found to differ to various extents from the parent variety and among themselves. Under drought conditions in the field, RM 30 had the longest roots followed by RM-37, RM-26, RM-36 and RM-31. Root anatomy varied greatly among the mutants, the more drought tolerant mutants RM-26 and RM-30 found to have more meta xylem vessels, 6 and 3 respectively, compared with only one found in the parent variety. These mutants need to be further characterized in order to understand the role of their unique anatomy in water absorption and transport. The different mutants evaluated in this study can be used in future wheat breeding programmmes in Cuba.

1. INTRODUCTION

Breeding work on bread wheat *Triticum aestivum* L., started many years ago at the Agronomic Experimental Station of Santiago de Las Vegas, now "Institute of Fundamental Research in Tropical Agriculture "*Alexander von Humboldt*" (INIFAT), in Havana

There is a need to increase genetic variability of the local wheat cultivars. Radiation has been used to induce mutations in selected breeding materials adapted to Cuban conditions such as cultivar Cuba C-204 [1, 2]). Six promising genotypes (RM-26, RM-29, RM-30, RM-31, RM-36 and RM-37) with good agronomic characteristics and adaptability were obtained from this work. However, the root morphology and physiology and adaptability to salinity and drought of these mutants have not been characterized. Water deficit and salinity are major limiting factors in wheat production [3]. Different physiological, morphological and biochemical indexes have been used to measure drought tolerance in plants, these include morphological parameters such as leaf length, leaf area, root length and volume as well as photosynthetic rates, tissue water potential and accumulation of free proline in leaves [4]. Here we report only the results from our mutants root studies.

2. MATERIALS AND METHODS

The selected mutants, RM-26, RM-29, RM-30, RM-31, RM-36 and RM-37, and a non– irradiated control, Cuba C-204 were sown in laboratory, using the modified sandwich method, and in the field.

These materials were evaluated and characterized by means of several morphological and yield characteristics as: plant height, number of tillers, fertile tillers, spike length, 1000 grains weight, total grain weight per spike, total grain weight per plant, and through protein electrophoresis (esterase and peroxidase).

2.1. Experiment in Greenhouse conditions

Seeds of mutants and the parent variety Cuba C-204, were planted on Red Ferralitic soil mixture with organic matter in pots in November 2002 and 2003. After germination two levels of soil moisture were established: 85% and 50% field capacity (FC). From vegetative period to the beginning of anthesis, the plants were watered once in 2 days, thereafter, they were watered on a daily basis until harvest.

Plant height was measured once a week, at the end of development cycle the roots were harvest and their length, diameter and weight were determined.

2.2. Experiment in Field conditions.

Seeds of the 7 lines were sown in continuous rows in four replications randomly placed in the field, on red ferralitic soil in November 2002 and 2003 at the Institute of Fundamental Research in Tropical Agriculture "Alexander *Von Humboldt*". The seeds were watered to facilitate germination, there-after there was no supplementary irrigation, the only source of water was from the rain. At 60 days after germination (anthesis period) semi-fine transverse sections (1–1.5μm thick), were prepared from on roots between the apical and the insertion zone of the cotyledon.

Ten plants samples were processed through the conventional technique used for transmission electron microscopy. Samples were observed under an optical microscope at 20X magnification. Photos were taken with a camera coupled to the microscope.

Also similar transverse sections of the leaf were prepared. This study was carried out on mutants RM-26, RM-30 and RM-37 which had shown some tolerance to drought, in the germination screening. Plant height, number of tillers, spike length, 1000 grains weight, total grain weight per spike, total grain weight per plant, were measured, and the yield was determined. Plants were harvested through traditional methods between March and April.

3. RESULTS AND DISCUSSION

3.1. Experiment in greenhouse conditions

The response of root dimensions of the mutants and the parent variety to soil moisture stress are shown in Tables I and II. The mutants exhibited longer roots than the parent variety in under soil both moisture conditions (50%–85% FC). Longer roots were observed under 85% FC than 50% (stress condition).

Some evidence suggests that shoot-to-root ratios are lower in dry than in wet habitat. Total root-system size, however, is greater in none-stressed than stressed plants [5], due to the interaction between root and shoot, because roots require assimilates from the shoot, while the shoot requires water and nutrients from the root.

The transport of photosynthetic assimilates from the source in leaves to sink in other plant parts are reduced by water stress. The reduction is produced either by an indirect effect due to stress-induced reductions in photosynthesis, and hence assimilate availability. The stress reduces assimilates translocation more than it reduces photosynthesis.

TABLE I. LENGTH, WIDTH AND WEIGHT OF MUTANTS AND DONOR VARIETY ROOTS UNDER TWO SOIL MOISTURE CONDITIONS

	50%			85%		
Varieties	Length (cm)	Width (cm)	Dry weight(g)	Length (cm)	Width (cm)	Dry weight(g)
CubaC-204	36.0 c	3.5 b	0.96c	39.0 c	3.6b	2.02b
RM-26	43.0 b	5.5 a	1.56b	50.0 a	5.3a	2.78a
RM-29	31.0 c	5.0 a	1.00c	52.0 a	5.0a	2.43b
RM-30	49.0 a	5.5 a	1.70a	55.0 a	5.4a	2.55b
RM-31	40.0 b	6.0 a	1.27b	43.0 b	6.2a	2.41b
RM-36	38.0 b	6.0 a	1.00c	45.0 b	6.0a	1.5c
RM-37	44.0 b	4.0 b	1.23c	45.0 b	4.0a	1.67c

Root length is greater in RM-26, RM-30, RM-31, and RM-37 in stress conditions, showing that its roots were able to grow littler more that those of the other mutants and the parent variety. The development of roots under drought stress is of particular significance to plants, since an extensive and efficient root system is an important drought-tolerance mechanism. There were no significant differences in root width between the moisture levels among varieties

TABLE II. THE HEIGHT AND NUMBER OF TILLERS OF PLANTS GROWN IN 50% AND 85% OF SOIL CONDITIONS

Variety	Height		Tiller Number	
	50%	85%	50%	85%
CubaC-204	48.73 b	60.20 a	8.0 b	13.6 a
RM-26	49.60 b	62.00 a	8.6 b	13.4 a
RM-29	41.20 b	52.10 a	7.8 b	11.75 a
RM-30	50.90 b	63.20 a	8.0 b	15.2 a
RM-31	54.00 b	60.70 a	8.8 b	13.0 a
RM-36	47.20 b	61.30 a	8.0 b	10.0 a
RM-37	49.40 b	63.00 a	10.0 b	15.4 a

3.2. Experiment in field condition

3.2.1. Root Anatomy study

For all mutants and the parent variety in the first experiments, the root anatomy follows a primary structure pattern, with a radial alternate distribution having: epidermis, cortex, and central vascular cylinder where the metaxylem is located in it centre. However, at 60 days after germination some differences among the lines emerged, in the number and size (diameter) of meta-xylem vessels (Table III and Fig. 1). These larger xylem vessels may be responsible for the observed tolerance to moisture stress in the mutants. Studies in rice had similar findings that a major portion of drought avoidance mechanism can be attributed to a

large number of xylem vessels, as the increased xylem area provides more effective water and nutrient conductivity from the roots to the shoots [6].

TABLE III. NUMBER AND DIAMETER OF VESSELS IN THE CENTRAL VASCULAR CYLINDER IN WHEAT MUTANTS AND PARENT VARIETY

Varieties	Number of vessels	Dameter of vessels (μ)
Cuba C-204	1 Big central vessel	6.2
RM-26	4 big vessels and 2 smellers	(4,03 - 3,72) & (2.48 - 1.86)
RM-30	2 big vessels and 1 smaller	(5.89-4.65) and (2.17)

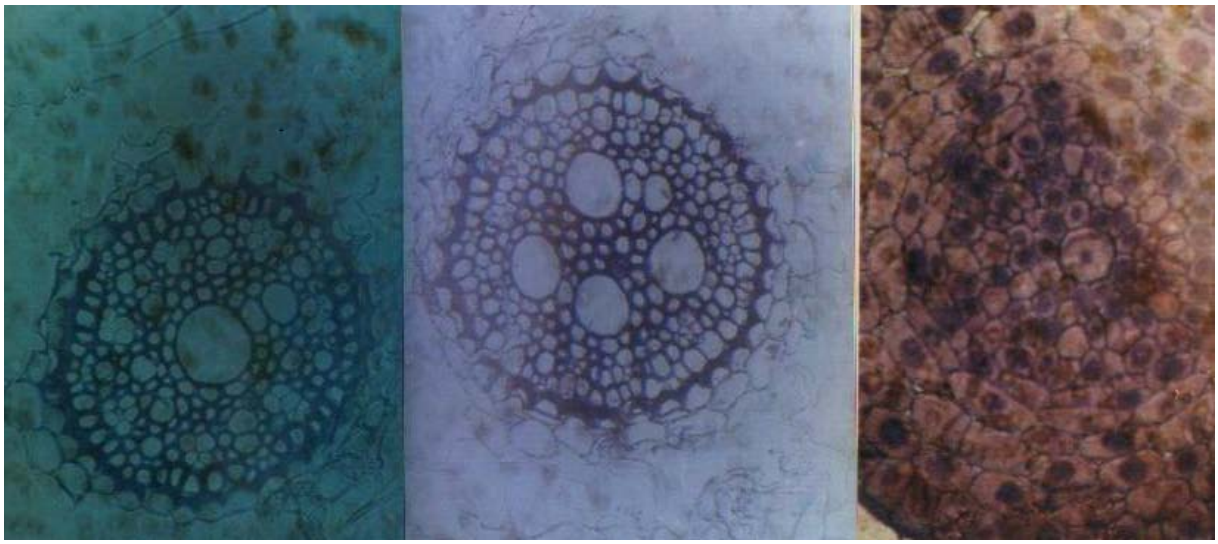


Fig. 1. Differences in the root central cylinder vessels among parent variety Cuba C-204 and mutans RM-26 and RM-30.

3.2.2. Yield indexes

Table V shows the yield indexes of the parent variety and the mutants. In general, there were no significant differences among mutants and the parent variety, however there was some variation among some mutants, with the mutants RM-26, RM-30 and RM-37 having higher yields compared with the other mutants and Cuba C-204. These mutants presented tolerance to drought by other morphological and physiological characteristics studied.

TABLE V. YIELD INDEXES OF THE PARENT VARIETY AND THE MUTANTS

Characteristics	Cuba C-204	RM-26	RM-29	RM-30	RM-31	RM-32	RM-36	RM-37
Plant Height (cm)	82.0	81.04	74.3	77.7	74.8	70.8	72.6	82.7
No of Spike/plant	3. 00	3.47	2.94	3.85	2.75	2.85	3.24	3.64
Spike length (cm)	7.48	7.23	6.09	7.66	6.85	6.57	6.79	7.02
No grains/spike	23.0	26.0	21.8	29.7	24.8	22.4	23.9	27.4
Total weight of grains/plant (g)	2.12	3.02	2.00	3.97	2.20	2.14	2.25	2.85
1000 grains weight (g)	33.4	35.6	30.6	35.4	33.6	34.0	32.6	35.6
Yield T/ha	1.59	1.72	1.57	1.69	1.49	1.56	1.42	1.86

4. CONCLUSIONS

The results presented in this paper demonstrate that some mutant lines are tolerant to drought conditions, and this may be related to the changes in root morphology and anatomy. These mutants can be used in future wheat breeding programs in Cuba. Although yields obtained in these trials were low when compared with those obtained worldwide, these mutants are promising to give better yields to small-scale farmers growing wheat under warm and dry climatic conditions in Cuba.

ACKNOWLEDGEMENTS

We thank Dr. Miroslaw Maluszynski, Former Head, Plant Breeding and Genetics Section, and Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture for the financial support of the contract 10963/CUB. Also to all my colleagues who participed in the development of this contract.

REFERENCES

[1] Pérez Talavera, S. 1998. Estudio de la radiosensibilidad de variedades de especies de importancia agrícola cultivada en Cuba. Tesis presentada en opción al grado científico de Doctor en Ciencias Agrícolas. INIFAT. La Habana. 148pp.

[2] GITANA. 1995. Informe de balance. V Taller "Las radiaciones y los isótopos en la agricultura".

[3] Larbin, A., Mekliche, A., Abed, R., Badis, M. 2000. Effect of water deficit on the production of two varieties of durum wheat in a semi-arid region. Options Mediterraneennes. Serie A.

[4] Ykhief, N., Dje Koun, A. 1999: Photosynthetic adaptation and drought resistance in durum wheat: analysis of genotypic variability. Options Mediterraneennes. Serie A. Seminaires Mediterranneens. N^{o} 40, 227-330. 1.

[5] Clarke, J.M., Durley, R.C. 1984. Water stress on plants. The response of plants to drought stress. Tata McGraw Hill Press.

[6] IRRI (1987). International Rice Research Institute (IRRI) Highlights. Philippines. 1987.

EFFECT OF SALT AND PHOSPHORUS CONCENTRATIONS, PH AND TEMPERATURE OF THE NUTRIENT SOLUTION ON WHEAT PRIMARY ROOT GROWTH

C.E. CAMARGO, A.W.P. FERREIRA FILHO
Instituto Agronômico,
Centro de Análise e Pesquisa Tecnológica do Agronegócio de Grãos e Fibras,
Campinas, SP, Brazil

Abstract

Extensive primary root growth is very important for field set up in wheat (*Triticum aestivum* L.) production in upland conditions in the State of São Paulo, Brazil. Fourteen wheat genotypes (mutant lines and cultivars) were each evaluated for their primary root growth at 7 and 15 days after germination in aerated nutrient solutions. The effects of salt concentrations, temperature, pH and phosphorus concentration on primary root growth were studied. High genetic variability of primary root growth was observed. The genotype BH-1146 presented the best genetic potential for root growth at all solution salt concentrations and temperatures, while genotypes BH-1146, KAUZ"S" / IAC-24 M2, KAUZ"S" / IAC-24 M8, IAC-24 / TUI"S" M2, TUI"S" / IAC-24 M1 and TUI"S", showed better potential over the others at all P concentrations and pHs. Separate heritability studies were also conducted and they revealed that primary root growth was influenced by a few genes exhibiting partial dominant behavior.

1. INTRODUCTION

In Brazil, wheat is cultivated in upland acidic soil conditions, in sequence to soybeans or (and) maize crops, in the period of April to August. In addition to high yielding potential, semi-dwarf stature, resistance to diseases, tolerance to aluminium toxicity and good nutritive and technological qualities, longer primary roots for field establishment proved to be very important in wheat breeding because of the short sowing period (April) that is often characterized by water stress associated with high temperatures [1].

The early wheat cultivar BH-1146, highly Al^{3+} tolerant in acid soils, has also shown high tolerance to drought when compared to other cultivars, under field conditions. Previous studies in Al-free nutrient solution have demonstrated that it had the longest root system compared to other 26 wheat genotypes [2, 3]. Some studies have shown that root growth of wheat seedling at early development stages in the presence of Al^{3+}, is affected by temperature, pH, salts and the phosphorus concentrations of the nutrient solutions [3–8].

The objective of this research was to investigate the effect of salt and phosphorus concentration, temperature, and pH on primary root growth in the absence of Al^{3+}.

2. MATERIAL AND METHODS

Four experiments on wheat seedling primary root growth were conducted in the laboratory: the first experiment was set with different nutrient solution salt concentrations, the second, with different nutrient solution temperatures, the third with different nutrient solution pHs and the fourth with different nutrient solution phosphorus concentrations.

The genotypes used in this experiment (Tables I- IV) were selected from 45 genotypes for root growth characteristics in a previous study [9]. Except for four genotypes i.e. Anahuac, Anahuac M3, KAUZ“S” and TUI“S”, all the others showed tolerance to Al^{3+}-toxicity, according Ref. [3].

Seeds (originated from Tatuí, State of São Paulo - latitude 23°20'S, longitude 47°52'W and altitude 600 m - harvested in 1999 and preserved in a cold and dry chamber) were rinsed in 10% sodium hypochloride solution and germinated in Petri dishes in the growth chamber at 12°C during 72 hours. After root emergence, 25-selected germinated seeds of each genotype were put on top of a nylon netting, placed over four 8.3 L-plastic containers, each containing four different salt concentrations (1/1, 1/2, 1/5 and 1/10 strength of the salt concentration of the complete nutrient solution).

The treatment solutions were arranged in a randomized complete block design with two replications. The nylon netting with the seeds on top was kept in contact with the treatment nutrient solutions. The concentration of various salts of the complete nutrient solution were: $Ca(NO_3)_2$ 4 mmol L^{-1}, $MgSO_4$ 2 mmol L^{-1}, KNO_3 4 mmol L^{-1}, $(NH_4)_2SO_4$ 0.435 mmol L^{-1}, KH_2PO_4 0.5 mmol L^{-1}, $MnSO_4$ 2 μmol L^{-1}, $CuSO_4$ 0.3 μmol L^{-1}, $ZnSO_4$ 0.8 μmol L^{-1}, NaCl 30 μmol L^{-1}, Fe-CYDTA 10 μmol L^{-1}, Na_2MoO_4 0.10 μmol L^{-1} and H_3BO_3 10 μmol L^{-1}.

The nutrient solutions in the containers were continuously aerated and maintained in water-baths at 24 ± 1°C, and their pH was adjusted on a daily basis to 4.0 using 0.5 mol L^{-1} H_2SO_4 or 1 mol L^{-1} NaOH solutions. After 7-days of growth in the solutions, 10 seedlings of each genotype were removed from the containers and their central primary root lengths were measured. The remaining seedlings were grown for an additional 8 days under the same conditions, after that ten other seedlings (15-days-old) were removed for root length measurements.

Similarly, the effect of solution temperature (18°C±1°C, 24°C±1°C and 30°C±1°C; maintained by keeping the plastic containers in water baths with temperature control), the solution's pH (4 and 6; adjusted on a daily basis) and the phosphorus concentrations (3.875, 7.75, 15.5 and 31.0 mg.L^{-1} of P) on primary root growth were studied by analysis of root length of 7 and 15 day old seedlings. However, due to similarity in growth trends at both stages, only data for 15 day old seedlings is reported in this paper.

All data were subjected to statistic analysis of individual and interactive effects of the above factors.

3. RESULTS AND DISCUSSION

3.1. Effect of salt strength

Significant effects of genotypes and salt concentrations were detected on primary root growth, but their interactive effects were insignificant.

For most lines root growth in complete nutrient solutions was faster than in other solutions (Table I), these results are in line with the observation discussed in Ref. [10].

TABLE I. EFFECT OF SALT STRENGTH AND GENOTYPE ON WHEAT PRIMARY ROOT LENGTH (mm) OF 15 DAY OLD SEEDLINGS GROWN IN NUTRIENT SOLUTIONS

Salt strength	1/1	1/2	1/5	1/10
1- BH-1146 M1	234b-d	207a-d	181c-f	141a-d
2- BH-1146 M2	229b-d	186c-e	157e-h	127a-d
6- BH-1146	292a	244a	217a	168a
8- Anahuac M1	252ab	235ab	211ab	160a-c
10- Anahuac M3	248ab	207a-d	183c-e	163ab
11- Anahuac	213b-d	166de	168d-g	132a-d
12- IAC-17 M	236bc	221a-c	198a-c	164ab
13- IAC-17	250ab	249a	187b-d	161ab
14- IAC-24 M	182cd	178c-e	150gh	115cd
15- IAC-24	191cd	187c-e	139hi	120b-d
17- (MON"S" / ALD "S") x IAC-24 M2	182cd	168de	135hi	130a-d
18- (MON"S" / ALD "S") x IAC-24 M3	179d	147e	132hi	108d
22- KAUZ"S" / IAC-24 M1	216b-d	194b-e	153gh	126a-d
24- KAUZ"S" / IAC-24 M3	181d	153e	121i	109d
Means	220A	196B	167C	137D

Data followed by the same letters are not significantly different by Duncan's test (0.05).
Solution pH = 4, Temperature 24±1°C.

3.2. Effect of Temperature on root growth

Both genotype and temperature had significant effects on root growth (Table II). Higher temperature appeared to be beneficial for root growth for most genotypes. These results are in line with the observations in Ref. [11] that at lower temperatures wheat root growth usually surpasses the one of aerial parts, but as temperature rises, both plant parts have increased growth. However, shoots grow at a faster than roots under no aluminum toxicity conditions. The obtained data disagree from those published in Ref. [5] that showed a general trend of root growth reduction of wheat genotypes with the increase in the solution temperature from 22 to 34°C, in nutrient solution with varying aluminum concentrations. These results also do not agree with the ones presented in Ref [12] for the rye cultivar 1443 that showed root growth in nutrient solution with 35 mg L^{-1} of Al^{3+} at 25°C and did not repeat this performance with 20 mg L^{-1} of Al^{3+} at 30°C. According to Ref. [12], the higher temperature increased the proportion of aluminum uptake induced by metabolic processes.

TABLE II. PRIMARY ROOT LENGTH (mm) OF WHEAT GENOTYPES AFTER 15-DAY-GROWTH IN COMPLETE NUTRIENT SOLUTIONS WITH PH 4.0 AND VARIED TEMPERATURES

Temperature	18°C±1°C	24°C±1°C	30°C±1°C
1- BH-1146 M1	205de	226c-e	270b-e
2- BH-1146 M2	211cd	230cd	247e-g
6- BH-1146	253a	284a	349a
8- Anahuac M1	226bc	248b-d	295bc
10- Anahuac M3	229bc	240b-d	288b-d
11- Anahuac	197d-f	214d-f	259c-f
12- IAC-17 M	231a-c	254a-c	285b-d
13- IAC-17	235ab	270ab	306b
14- IAC-24 M	176f	193ef	224fg
15- IAC-24	185ef	194ef	211g
17- (MON"S" / ALD "S") x IAC-24 M2	182f	192ef	222fg
18- (MON"S" / ALD "S") x IAC-24 M3	189d-f	188f	218g
22- KAUZ"S" / IAC-24 M1	211cd	225c-e	256d-f
24- KAUZ"S" / IAC-24 M3	181f	194ef	212g
Means	208A	225B	260C

Data followed by the same letters are not significantly different by Duncan's test (0.05).
Solution pH = 4, Salt concentration = full-strength.

3.3. Effect of pH on root growth

There were significant pH and genotype effects on root growth (Table III). Root growth in pH 4.0 solutions was higher compared with the pH 6.0. These results are consistent with those in Ref. [6] reporting a general reduction trend in the wheat root growth as pH increased from 4.0 to 6.0 when cultivated in complete nutrient solution.

TABLE III. PRIMARY ROOT LENGTH (mm) OF WHEAT GENOTYPES AFTER 15-DAY-GROWTH IN COMPLETE NUTRIENT SOLUTIONS WITH TEMPERATURE OF 24°C±1°C AND VARIED PHS

pH	4.0	5.0	6.0
6- BH-1146	310a	307a	268a
23- KAUZ"S" / IAC-24 M2	272b	256b	227b
25- KAUZ"S" / IAC-24 M4	193ef	179d-f	157e-g
27- KAUZ"S" / IAC-24 M6	229cd	201c-f	184d-f
29- KAUZ"S" / IAC-24 M8	252bc	236bc	224bc
32- KAUZ"S"	201de	196d-f	184d-f
34- IAC-24 / TUI"S" M2	240bc	213c-e	199b-d
36- TUI"S" / IAC-24 M1	243bc	204c-f	188c-e
37- TUI"S" / IAC-24 M2	176ef	178d-f	159e-g
39- TUI"S"	232cd	217b-d	192b-e
40- IAC-287 / IAC-24 M1	169ef	170f	145g
41- IAC-287 / IAC-24 M2	173ef	182d-f	156e-g
42- IAC-287 / IAC-24 M3	157f	177ef	148fg
44- IAC-287 / IAC-24 M5	179ef	184d-f	175d-g
Means	216A	207B	186C

Data followed by the same letters are not significantly different by Duncan's test (0.05).
Temperature 24±1°C.; Salt concentration = full strength

The lower root growth at higher pH, in the absence of Al^{3+} may be due to a lower phosphorus uptake and lower availability of iron and other micronutrients. This conflicts with the situation where a constant amount of aluminum is supplied in the nutrient solution and pH is changed from 4.0 to 6.0, and where reasonable root growth of wheat seedlings takes place in pH 6.0 [12]. The hydrolysis theory may elucidate this, since a 10-fold trivalent aluminum (Al^{3+}) compared to divalent form ($AlOH^{2+}$) occurs under pH 4.0; 3.1-fold at pH 4.5; while at pH 5.0 the trivalent and bivalent ionic species are practically equivalent [13].

3.4. Effect of Phosphorus on root growth

Significant effects of genotypes and nutrient solution P concentrations on root growth were observed. These results also showed that adequate amounts of P result in rapid growth in young plants because their rapid growth makes greater demands on the available supply [14] and are consistent with those in Ref. [15].

TABLE IV. PRIMARY ROOT LENGTH (mm) OF WHEAT GENOTYPES AFTER 15-DAY-GROWTH IN NUTRIENT SOLUTIONS WITH PH 4.0 AND TEMPERATURE OF 24°C±1°C COMBINED WITH FOUR PHOSPHORUS CONCENTRATIONS

P concentration ($mg.L^{-1}$)	3.875	7.75	15.5	31.0
6- BH-1146	250a	295a	290a	282a
23- KAUZ"S" / IAC-24 M2	203b	233b	246b	236b
25- KAUZ"S" / IAC-24 M4	155c-e	181c-e	197d-f	191cd
27- KAUZ"S" / IAC-24 M6	157c-e	191cd	208c-e	188cd
29- KAUZ"S" / IAC-24 M8	172b-e	206bc	224b-d	205bc
32- KAUZ"S"	141de	156de	182ef	153d
34- IAC-24 / TUI"S" M2	175b-e	207bc	225b-d	208bc
36- TUI"S" / IAC-24 M1	190bc	230b	235bc	207bc
37- TUI"S" / IAC-24 M2	142de	205bc	179ef	171cd
39- TUI"S"	179b-d	207bc	218b-d	211bc
40- IAC-287 / IAC-24 M1	142de	157de	169f	158d
41- IAC-287 / IAC-24 M2	144de	151e	173f	159d
42- IAC-287 / IAC-24 M3	134e	160de	165f	167cd
44- IAC-287 / IAC-24 M5	157c-e	180c-e	178ef	169cd
Means	167C	197AB	206A	193B

(1) Means followed by the same letters are not different by Duncan's test (0.05).
Temperature 24±1°C.; Salt concentration = full strength; Solution pH =4

4. ACKNOWLEDGEMENTS

To the 'Fundação de Amparo à Pesquisa do Estado de São Paulo' (FAPESP), to the International Atomic Energy Agency (IAEA) Vienna, Austria and to the 'Conselho Nacional de Desenvolvimento Científico e Tecnológico' (CNPq) for the financial support and scholarships.

REFERENCES

[1] Munstock, C.M. Cultivo dos cereais de estação fria: trigo, cevada, aveia, centeio, alpiste e triticale. Gráfica e Editora NBS Ltda, Porto Alegre (1983) 265pp.

[2] Camargo, C.E. de O., Felicio, J.C., Tulmann-Neto, A., Ferreira-Filho, A.W.P., Pettinelli-Júnior, A., Castro, J.L. de. Melhoramento do trigo: XXVIII. Novos

genótipos obtidos por seleções em população segregante interespecífica submetida à irradiação gama. Bragantia 54 (1995) 51-65.
[3] Camargo, C.E. de O., Oliveira, O.F. de. Tolerância de cultivares de trigo a diferentes níveis de alumínio em solução nutritiva e no solo. Bragantia 40 (1981a) 21-31.
[4] Ali, S.M.E. Influence of cations on aluminum toxicity in wheat (*Triticum aestivum* Vill., Host), PhD Thesis, Oregon State University, Corvallis (1973).
[5] Camargo, C.E. de O. Efeito da temperatura da solução nutritiva na tolerância ao alumínio de cultivares de trigo. Bragantia 42 (1983) 51-63.
[6] Camargo, C.E. de O. O pH das soluções nutritivas no comportamento de cultivares de trigo à toxicidade de alumínio. Bragantia 43 (1984) 327-335.
[7] Moore, D.P. "Physiological effects of pH on plant roots", The plant root and its environment (Carson, E.W., Ed.) University Press of Virginia, Charlottesville (1974) 135-151.
[8] Moore, D.P., Kronstad, W.E., Metzger, R.J. "Screening wheat for aluminum tolerance" Plant Adaptation to Mineral Stress in Problem Soils (Proc. Workshop Beltsville, 1976) (Wright, M.J., Ed.) Cornell University, Ithaca, NY (1976) 287-295.
[9] Camargo, C.E. de O., Ferreira-Filho, A.W.P., Ramos, L.C. da S., Felicio, J.C., Tulmann-Neto, A. "Primary root growth: genetics and differential among wheat mutant lines in nutrient solutions" Mutational Analysis of Root Characters in Annual Food Plants Related to Plant Performance (Report of the First Research Co-ordination Meeting of FAO/IAEA Co-ordinated Research Project held in Viena, Austria 2000). Viena, Austria (2002) 43-46.
[10] Camargo, C.E. de O., Oliveira, O.F. de, Lavorenti, A. Efeito de diferentes concentrações de sais na solução nutritiva na tolerância de cultivares de trigo à toxicidade de alumínio. Bragantia 40 (1981) 93-101.
[11] Evans, L.T. Crop physiology some case histories. Cambridge University Press, Cambridge (1975) 374pp.
[12] Benitez, A.L. Influence of aluminum toxicity in intergeneric crosses of wheat and rye, PhD Thesis, Oregon State University, Corvallis (1977).
[13] Foy, C.D., Fleming, A.L. "The physiology of plant tolerance to excess available aluminum and manganese in acid soils" Crop tolerance to suboptimal land conditions (Proc. Symposium Houston, 1976). American Society of Agronomy, Madison (1978) 301-328.
[14] Lamb, C.A. "Physiology", Wheat and wheat improvement (Quisenberry, K.S., Reitz, L.P., Ed.) American Society of Agronomy, Madison (1973) 181-223.
[15] Camargo, C.E. de O., Felicio, J.C. Trigo, triticale e centeio: avaliação da eficiência ao fósforo e tolerância à toxicidade ao alumínio. Bragantia 46 (1987) 203-215.

LIST OF PUBLICATIONS PRODUCED WITHIN THE CRP

Al-Hammadi, A.S.A, Sreelakshmi, Y., Negi, S., Siddiqi, I. and Sharma, R. 2003. The polycotyledon mutant of tomato shows enhanced polar auxin transport, Plant Physiol 133: 113-125.

Cabrera Lejardi, M., Gutiérrez, L., Pérez, C., Lago, E., Pérez, M., Mendoza, M.J., Díaz, M. and Marrero, S. Caracterización fisiológica de radiomutantes de trigo. En : Taller Fisiología Vegetal y Bioquímica. Congreso Científico del INCA (14: 2004, Nov. 9-12, La Habana). Memoria CD-ROM, ISBN 959-7023-27-X.

Cabrera Lejardi, M., Gutiérrez, L., Pérez, C., Lago, E., Pérez, M., Mendoza, M.J., Díaz, M. and Marrero, S. Tolerancia de radiomutantes de trigo a la sequía y salinidad. En: Convención Trópico (2004 April 4-8, La Habana). Memoria CD-ROM, ISBN 959-7167-02-6.

Çağırgan, M.I., Özbaş, M.O., Heng, L. and Afza, R. 2005. Genotypic Variability for Carbon Isotope Discrimination in the Mutant and Improved Lines of Barley. Isotopes in Environment and Health Studies (In press, issue 3, September).

Camargo, C.E., Tulmann-Neto, A., Ferreira-Filho, A.W.P. and Felicio, J.C. Genetic control of aluminum tolerance in mutant lines of the wheat cultivar Anahuac. Euphytica **114** (2000a) 47-53.

Draye, X., 2002. Influence of root growth and vascular structure on the distribution of lateral roots, Plant Cell and Environment 25: 1463-1474.

Feix, G., 2002. "Maize root system and genetic analysis of its formation", Plant roots – the hidden half, (Waisel, Y., Eshel, A., Kafkafi, U., Eds.), Marcel Dekker, New York. 239-248.

Forster, B.P., 2001. Mutation genetics of salt tolereance in barley: An assessment of Golden Promise and other semi-dwarf mutants. Euphytica 120: 317-328.

Forster, B.P., Ellis, R.P., Moir, J., Talamè, V., Sanguineti, M.C., Tuberosa, R., This, D., Teulat-Merah, B., Ahmed, I., Mariy, S.A.E.E., Bahri, H., El Ouahabi, M., Zoumarou-Wallis, N., El-Fellah, M. and Ben Salem, M. 2004. Genotype and phenotype associations with drought in barley tested in North Africa. Annals of applied Biology 144: 157-168.

Gutierrez, L., Cabrera Lejardi, M. y colaboradores: Algunas consideraciones para el cultivo del trigo en Cuba. En: Taller Alternativas para la producción Agrícola. Congreso Científico del INCA (14: 2004, Nov. 9-12, La Habana). Memoria CD-ROM, ISBN 959-7023-27-X.

Gutierrez, L., Cabrera Lejardi, M. y colaboradores: Comportamiento de la variedad de trigo RM-26, frente a la aplicación de bioproductos. En: Fitogen (2003, La Habana).

Gutierrez, L., Cabrera Lejardi, M. y colaboradores: El cultivo del trigo en Cuba, un siglo de trabajos. En: Convención Trópico (2004, April 4-8, La Habana). Memoria CD-ROM, ISBN 959-7167-02-6.

Jackson, L.E. 2005. Soil biology: root architecture and growth. Encyclopedia of Soils in the Environment. Elsevier Ltd. Pp. 411-421.

Jackson, L.E., Miller, D. and Smith, S.E. 2002. Arbuscular mycorrhizal colonization and growth of wild and cultivated lettuce in response to nitrogen and phosphorus. Scientia Horticulturae 94: 205-218.

Johnson, W.C., Jackson, L.E., Ochoa, O., Peleman, J., van Wijk, R., St. Clair, D.A. and Michelmore, R.W. 2000. A shallow-rooted crop and its wild progenitor differ at loci determining root architecture and deep soil water exploitation. Theoretical and Applied Genetics 101:1066-1073.

Martínez A., Landau, A., García, P.T., Polenta, G., Arias, M.C., Murray, R., Pensel, N. and Prina, A.R.Two Mutants Affecting Adaptative Responses to Abiotic Stresses in Barley Seedlings. Czech J. Genet. Plant Breed. 41 (1): 1-10, 2005.

Martínez, A.E., Franzone, P.M., Aguinaga, A., Polenta, G., Murray, R. and Prina, A.R. A nuclear gene controlling seminal root growth response to hydroponic cultivation in barley. Environmental and Experimental Botany 51(2): 133-144, 2004.

Ozbas, M.O. and Cagirgan, M.I. 2004. Comparison of Root/Shoot Traits between Certain Mutants and Parents in Sesame. Sesame and Safflower Newsletter. 19: 28-31.

Ozbas, M.O. and Cagirgan, M.I. 2004. Variability and Interrelationships for Root Traits in a Subset of Induced Barley Mutant Collection. Cereal Res. Commun. 32(1):119-126.

Szarejko, I, Kwasniewski, M., Janiak, A., Chmielewska, B., Karcz, J., Nawrot, M. and Maluszynski, M. 2004. Mutational analysis to study root hair formation in barley. The International Conference on the Status of Plant and Animal Research. PAG XII. San Diego, CA. p. 65.

Wenxue, W., Bilsborrow, P.E., Hooley, P., Fincham, D.A., Lombi, E. and Forster, B.P. 2003. Salinity induced differences in growth, ion distribution and partitioning in barley between the cultivar Maythorpe and its derived mutant Golden Promise. Plant and Soil 250: 183-191.

Ye, X. 2002. Regular production of transgenic wheat mediated by Agrobacterium tumefaciens. Agricultural Sciences in China, 3: 239-244.

LIST OF PARTICIPANTS

Atkins, C.	The University of Western Australia Department of Botany, 35 Stirling Highway Crawley WA 6005, Australia Fax: +618-9380 1001 E-mail: catkins@cyllene.uwa.edu.au
Cabrera Lejardi, M.	Instituto de Investigaciones Fundamentales en Agrigultura Tropical "Alejandro de Humboldt" del Ministerio de la Agricultura Calle 1 y 2 Santiago de las Vegas, Ciudad de la Habana, Cuba Fax: +53-7-579014 E-mail: mcabrera@inifat.co.cu
Cagirgan, M.I.	Akdeniz University, Faculty of Agriculture Field Crops Department, P.O. Box 510, 07070 Antalya, Turkey Fax: +90-242-2274564 E-mail: cagirgan@agric.akdeniz.edu.tr
Camargo, C.E.	Rice and Winter Cereal Department, Instituto Agronomic (IAC) Av. Barao de Itapura, 1481, Caixa Postal 28 CEP: 13020-902, Campinas, Sao Paulo, Brazil Fax: +19-2314 943 E-mail: ccamargo@cec.iac.br
De Ronde, K.	Agricultural Research Council, ARC-Roodeplaat Private Bag X293, Pretoria, 0001 Fax: +27-12-808 0844 E-mail: mspreeth@infruit.agric.za
Draye, X.	University of Louvain, Plant Breeding - Crop Science AGRO/BAPA/ECOP-GC, Croix du Sud, 2/11 1348 Louvain-la-Neuve, Belgium Fax: +32-10-472021 E-mail: draye@ecop.ucl.ac.be
Feix, G.	University of Freiburg, Institute of Biologie III Schaenzlestr. 1, D-79104 Freiburg, Germany Fax: +49-761-203 2745 E-mail: feix@biologie.uni-freiburg.de
Forster, B.P.	Scottish Crop Research Institute, Invergowrie Dundee DD2 5DA, Scotland United Kingdom Fax: +44-1382-562426 Email: bforst@scri.sari.ac.uk

Gai, J.	Nanjing Agricultural University National Center for Soybean Improvement Nanjing 210095, China, P.R. Fax: +86-25-439 5110 or 4431492 E-mail: nausri@public1.Ptt.js.cn; sri@njau.edu.cn
Nawrot, M.	Silesian University, Department of Genetics Jagiellonska 28, 40032 Katowice, Poland Fax: +48-32-251 5446 E-mail:mnawrot@us.edu.pl
Prina, A.R.	Centro Nacional de Investigaciones Agropecuarias Instituto de Genética "Ewald A. Favret" CNIA-INTA C.C. 25, 1712 Castelar, Argentina Fax: +54-11-4450 0805 1876 E-mail: aprina@cnia.inta.gov.ar
Sharma, R.	University of Hyderabad, School of Life Sciences Central University PO, Hyderabad- AP 500 046, India Fax: +91-40-3010 120 E-mail: rpssl@uohyd.ernet.in
Shu, Q.Y.	Plant Breeding and Genetics Section International Atomic Energy Agency Wagramer Strasse 5, P.O. Box 100, A-1400 Vienna, Austria Fax: +43-1-26007 E-mail: q.y.shu@iaea.org
Waisel, Y.	Tel Aviv University, Department of Plant Sciences Tel Aviv 69978, Israel Fax: +972-3-640 9380 E-mail: waisel@post.tau.ac.il
Ye, X.	Chinese Academy of Agricultural Science Institute of Crop Sciences, Haidian District Beijing 100081, China, P.R. Fax: +86-10-68975212 E-mail: yexg@mail.caas.net.cn