WORKING MATERIAL

Selection and Evaluation of Food Crop Genotypes Tolerant to Low Nitrogen and Phosphorus Soils Through the Use of Isotopic and Nuclear-related Techniques (D1-50.10)


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Selection and Evaluation of Food Crop Genotypes Tolerant to Low Nitrogen and Phosphorus Soils Through the Use of Isotopic and Nuclear-related Techniques

Report of the First Research Coordination Meeting of the FAO/IAEA Coordinated Research Project

Vienna, Austria

16-20 October 2006

CRP D1.50.10

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2006
EDITORIAL NOTE

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1. SUMMARY

The Coordinated Research Project (CRP) on “Selection and Evaluation of Food Crop Genotypes Tolerant to Low Nitrogen and Phosphorus Soils Through the Use of Isotopic and Nuclear-related Techniques” was approved by the IAEA in 2005, and implementation started in 2006 with an anticipated duration of 5 years (2006-2010). The overall objective of this CRP is to develop integrated crop, soil and nutrient management practices to increase crop production in marginal lands by identifying and promoting the development of food (cereal and legume) crop genotypes with enhanced nitrogen (N) and phosphorus (P) use efficiency and greater productivity in low fertility soils.

The first RCM of the CRP was held at IAEA HQ during 16-20 October 2006. Eleven research contract holders from Burkina Faso, Brazil, Cameroon, China Peoples Republic, Cuba, Ghana, Malaysia, Mexico, Mozambique and Sierra Leone, five Agreement Holders from Australia (UWA), Benin (WARDA), Kenya (TSBF-CIAT), Nigeria (IITA), France (INRA), and two Technical Contractors from Germany (University of Hanover) and USA (University of Pennsylvania) attended the meeting. In addition one observer from Turkey and staff of the Soil and Water Management & Crop Nutrition (SWMCN) sub-program were among the participants.

During the opening address, the Head of the SWMCN Section, Mr M-L Nguyen stressed the importance of the current CRP to the sub-program and the need for effective networking and to generate quantitative data for the production of success stories at the end of the CRP. In an introductory lecture, the Head of the Plant Breeding and Genetics Section, Mr. P Lagoda, stressed the need for identifying and phenotyping root traits associated with crop tolerance to nutritional and other abiotic stresses. The Project Officer, Mr J Adu-Gyamfi, gave an overview of the CRP and the need to have outputs that are achievable, quantifiable, and have indicators of success. All the participants presented reports on the characterization of the environment, experimental design and past experiments on the subject area during the first two days. A one-day workshop was organized at the Agency’s Laboratory at Siebersdorf to (i) brief the participants on the laboratory activities related to the use of isotope techniques to quantify nutrient dynamics and nutrient efficiency (ii) discuss data analysis and interpretation of data related to isotopic dilution experiments and (iii) examine sample preparation techniques and analysis related to the CRP.

The following general conclusions were made at the end of the 5-day meeting:

(i) There was general consensus to slightly modify the specific objectives of the CRP to accurately reflect the discussions during the RCM.

(ii) Experimental plans should include the initial collection of at least 200 cereal (maize or upland rice) or grain legume (soybean, cowpea, common bean) genotypes/lines from exotic, landraces and known genotypes, followed by a rapid laboratory screening for root traits (primary root elongation, lateral roots, seminal root branching, root whorls and lateral root branching) conferring P and N acquisition efficiencies. This should be followed by field evaluation of the same number of genotypes in low N/and or low P soils to compare the results of the laboratory screening with that of the field evaluation.
Contrasting genotypes (20-50) could be selected for evaluation using isotopic and nuclear related techniques.

(iii) Agreement was made on common experimental protocols, techniques and standard terminology.

(iv) Participants were encouraged to plant cereals (without fertilization) on the selected sites during the first year to help reduce the N and P content in the soil. Characterization (soil physical and chemical properties) of the selected sites was required to determine the initial total and available concentrations of N and P in soils at the beginning of the experiment.

(v) The issue of low plant available N and P concentrations in soils was discussed. Participants were encouraged to select sites with relatively low plant available N and P after soil testing. The selected site before planting is be designated low N and P to be compared with applied N and P rates. An excel Table requesting participants to provide detail information on the physical and chemical properties of the soil (including the methods used for the extractions) is attached in the Annexes.

The RCM was a marked success, stimulating significant exchanges between plant physiologists, plant breeders, soil scientists and agronomists, and served as a platform for strong collaboration between the SWMCN sub-program and the Plant Breeding and Genetics Section. Expressions of interest were received from the contract holders from MEX and CPR to host the 2\textsuperscript{nd} RCM in 2008.
2. PRESENTATIONS BY PARTICIPANTS

Participants made presentations on their on-going work that could be relevant to CRP. In addition each participant was to outline the workplan for 2007/2008 for discussion. Below are the summary of the presentations made by the participants

2.1. W Horst (University of Hanover, Germany, N use efficiency in crops)

- Defined nitrogen use efficiency (NUE) of a crop as the capacity of a genotype to produce a higher yield (grain, biomass, sugar, protein) than the mean of the population under conditions of limited N supply, and made a graphical illustration of the concept which is presented below. Limited-N supply was not well defined in the illustration.

- The two main components of N efficiency are N uptake efficiency (root growth, morphology and root physiology) and N utilization efficiency (N retranslocation/ N harvest index and photosynthetic nitrogen use).

- There are important genotypic differences in N efficiency in most crops and N uptake efficiency appears to be of major importance compared to N utilization efficiency.

- For N efficiency a delayed senescence of older leaves during the reproductive stage (“stay green”) is important because it allows a higher root activity particularly in the subsoil and thus a better acquisition of nitrate from the subsoil.
Screening for “stay green”/higher photosynthetic activity of N-deficient leaves at the early vegetative stage appears to be possible.

- The maintenance of higher N concentrations in senescing leaves through import rather than delayed export appears to be involved in maintaining their photosynthetic activity.

2.1.2. K Traore (Burkina Faso) Upland Rice

- Work will focus on N uptake by new rice of Africa (NERICA) genotypes from the Genebank from WARDA.
- Two sites (Farako-ba and Banfara) in the South Sudanian (650-1200 mm annual precipitation) zone have been selected and soil characterization done.
- N-15 will be used to estimate NUE.

2.1.3. I. Baggie (Sierra Leone) Upland Rice

- Two sites (Bo and Rokupr) with annual precipitation ranging from 2032 to 5080 mm have been selected and soil characterization done.
- Earlier identified 3 groups: crops that showed (i) poor growth at both low and high fertility conditions (ii) poor growth under low fertility and good growth under high fertility and (iii) efficient or produces high biomass under low fertility.

2.1.4. M de Vries (WARDA) Upland Rice

- NERICA is considered one of the major advances in the field of rice varietal improvement of the past decades (Van Nguyen & Ferrero, 2006)
- 18 released NERICA varieties are suitable for the upland rice ecology of sub-Saharan Africa (SSA)
- Many upland NERICA lines developed, but no systematic evaluation for N and P efficiency
• A large number of promising lines (up to 400) together with their parents and check varieties will be used for evaluation of NUE

2.1.5.  Z Abdul Rahman (Malaysia) Upland Rice

• Several upland rice varieties, such as Lentik, Merah and Liba Pasir showed higher yields even without added fertilizers
• These three varieties will be used for further evaluation and selection on nutrient requirement and their response to N and P fertilizers
• Root development with adequate applications of N and P fertilizers will also be studied using rhizotron boxes

2.1.6.  C The (Cameroon) Maize

• Three approaches for development of germplasm tolerant to nutrient stress are (i) breeding for responsiveness to a range of applied (ii) breeding for N efficiency under low-input and (iii) breeding for both responsiveness and efficiency have been reported
• Breeding for both responsiveness and efficiency is recommended

2.1.7.  J Lynch (Univ. Penn) P and Legumes

Summary of P efficiency traits

• Root architecture is an important component of phosphorus efficiency, by enhancing topsoil foraging and reducing inter-root competition
• Phosphorus efficient genotypes have metabolically cheaper root systems, achieved by greater allocation to cheaper root classes, such as adventitious roots, and reduced maintenance respiration through mechanisms such as aerenchyma
• Bean and soybean genotypes incorporating efficient root architectural traits are being developed and used in Africa, Asia, and Latin America
• The challenge remains to understand agroecological tradeoffs for specific root traits, metabolic costs, synergy/antagonism with other root traits, plasticity, effects on competition/intercropping/nutrient cycling, tradeoffs for contrasting soil resources

Summary of how to find P efficiency traits

• survey wide range of germplasm including landraces, emphasizing regions with poor soils.
• evaluate performance under single, defined stress in an environment that permits expression of root traits
• compare contrasting genotypes for growth under low plant available, but otherwise well adapted, good yield potential, hopefully genetically similar
• understand P stress biology (immobility, mobilization)
phenotype an array of genotypes for known/putative traits, then correlate with performance under low P
controlled environments (ie pots) can be quite useful
seek primary (causal) traits rather than downstream effects (growth and yield are effects, not causes)

2.1.8. J Bayuelo-Jimenez (Mexico) P and maize
- Although there is an ample demonstration of genetic variation of P efficiency in beans and other crops, increasing understanding of genetic variation of root traits that confer P efficiency, and growing recognition of Mexico maize landraces is very innovative
- Most traditional breeding in Mexico has no focus on edaphic stress and the biology of root traits is poorly understood
- Evaluation and characterization of P efficient germplasm among the existing varieties of the Pruchepecha region can serve as a basis for improvement of plant available P use by selection and breeding

2.1.9. J Yang (China) P and maize
- Two locations (Hunan and Beijing provinces) with annual precipitation ranging from 555-600 mm have been selected and soil characterization done.
- On-going work include time-dependent and concentration-dependent kinetics of $^{32}\text{P}$ using maize

2.1.10. C Atkins (Univ. Western Aust.) N and P in legumes
- The main areas of research included (i) root adaptations that could enhance nutrient uptake, (ii) nodulated and cluster (proteoid) roots (ii) translocated ‘signals’ that could influence nutrient uptake and nutrient/assimilate allocation and (iii) use of ion probe imaging to trace nutrient movement at the soil/root interface and in the plant, looking for sites limiting uptake/transfer

2.1.11. F Kumaga (Ghana) N and P in legumes
- Two locations (Accra and Kpeve) with annual precipitation ranging from 850-1200 mm have been selected and soil characterization done.
- Isotope dilution method to be used to estimate nitrogen fixation by soybean genotypes at both low and high P

2.1.12. J J Devron (ENSA, France) P in legumes
- Contrasting lines for phosphate utilization efficiency (PUE) and symbiotic nitrogen fixation for (i) participatory approach at the field level in reference agro-ecosystems (ii) role of soil phosphatase, especially phytase, and the nodulated-root proton efflux in phosphorus bioavailability for legume-cereal cropping systems
2.1.13. M Miguel (Mozambique) P and common bean

- Bean genotypes vary substantially in root hair formation when grown under low soil P availability
- Genotypes with longer, denser hairs can acquire more soil phosphorus and gain more shoot dry weight in low phosphorus soil (again another term!!!).
- Genotypic variation for traits conferring plant PUE (be specific!) opens a possibility of reasonable crop yield increases in low phosphorus availability.


Potential threats to soil fertility management

- Depletion of non-renewable P stocks through export in harvested produce (2-3 kg P ton\(^{-1}\) of cowpea grain; 4-5 kg P ton\(^{-1}\) of soybean grain)
- P use efficiency under low soil P conditions is simply an efficient mining of soil P; it is only a short term solution. P use efficiency under high P conditions (P fertilizer application) is useful
- Long term agricultural sustainability requires soil P replacement and use of efficient and responsive genotypes

2.1.15. A Garcia Altunaga (Cuba) P and maize, common bean

- Outputs such as the identification of common bean genotypes tolerant to soil acidity, a new Cuban phosphate rock-based product, and the positive effect of soil liming on grain yield and the symbiotic nitrogen fixation (SNF) of common bean genotypes and Recombinant Inbred Lines (RILs) of BAT 477 x DOR 364 cross were identified in an earlier CRP.
- The screening of common bean genotypes and RILs of BAT 477 x DOR 364 cross revealed the existence of genotype difference in yield in regard to soil P content.

2.1.16. T Muraoka (Brazil) N and P and crop species

- Twenty species of crops have been classified as efficient, intermediate and inefficient based on \(^{32}\)P isotopic dilution
- Comparison of the ability of different corn hybrids to access poorly plant-available Cerrado soil P using \(^{32}\)P dilution technique has been done

3 Revised Project Document
3.1 Overall objective

To develop integrated crop, soil and nutrient management practices to increase crop production in marginal lands by identifying and promoting the development of food (cereal and legume) crop genotypes with enhanced nutrient (N and P) use efficiency and greater productivity in low fertility soils

3.2 Specific objectives

3.2.1 Develop and validate screening protocols for plant traits that enhance N and P acquisition and utilization in major food cereal and legume crops grown in low fertility soils (no modification)

3.2.2 “Employ validated screening protocols including the use of isotopic tracer techniques and induced mutations to identify genotypes with superior N and P acquisition and/or utilization. This might include mutants identified for novel traits”

3.2.3 “Assess the selected genotypes with traits for enhanced nutrient acquisition and/or utilization in selected cropping systems, including yield and productivity. This assessment could include long-term sustainability of soil fertility”

4 Workplans

The work plan as agreed on by all participants should include

(a) Phenotypic screening: laboratory screening for root characteristics At least 200 genotypes (diverse background including RIL parents) evaluated for root hairs (length and density), root length and density; basal root whorl numbers.

Field screening: Evaluate the same number of genotypes (or at least 50) selected from the laboratory screening for root traits and correlate with results from the laboratory screening

The issue of the low plant available N and P have been well defined in the summary

<table>
<thead>
<tr>
<th>Country</th>
<th>Crop and Nutrient</th>
<th>Trials</th>
<th>Source of Germplasm</th>
<th>Milestones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sierra Leone</td>
<td>Upland Rice, N</td>
<td>(i) Laboratory screening of 200 varieties for root characteristics</td>
<td>155 lines from WARDA, IRRI, 35 from Malaysia and</td>
<td>Potential root traits at seedling stage identified Field performance of selected genotypes correlated with data at</td>
</tr>
<tr>
<td>WARDA</td>
<td>(ii) Field screening at 2 sites of 50 rice genotypes selected from laboratory screening</td>
<td>10 from Sierra Leone seedling stage</td>
<td>Genotypes with contrasting N use efficiency identified</td>
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<tr>
<td>Mozambique</td>
<td>Common bean, P 200+ genotypes from exotic and land races, known genotypes as standards for P acquisition efficiency</td>
<td>At least 200 genotypes from 5 locations in Mozambique CIAT* BILFA NARS Penn State*</td>
<td>Validated screening methods for root traits conferring P efficiency Genotypes with contrasting P use efficiency identified for field evaluation</td>
<td></td>
</tr>
<tr>
<td>Ghana</td>
<td>Soybean, P Laboratory screening for rapid screening in the lab for root traits (root hairs, root angle, root architecture): Rapid field screening - 50 soybean genotypes selected from laboratory to be grown at 2 P levels (0 and 30 kg P ha⁻¹)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Country</td>
<td>Crops and Traits</td>
<td>Evaluation Details</td>
<td>Results</td>
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<tr>
<td>Brazil</td>
<td>Legume, N and P</td>
<td>Lab evaluation of 100 – 200 genotypes. Field evaluation (genotype P acquisition/utilization). 100-200 genotypes (low P and normal P). Lab: ($^{32}$P evaluation) 30 genotypes (low P)</td>
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</tr>
<tr>
<td>Cameroon</td>
<td>Maize and N</td>
<td>Laboratory and field screening of 159 lines</td>
<td>50 S4 Inbred lines, 30 S6 Al tolerant lines, 29 Composites, N susceptible, drought tolerance 29, local and other crosses 50 (Total 159)</td>
<td></td>
</tr>
<tr>
<td>Mexico</td>
<td>Maize and P</td>
<td>Laboratory screening for rapid screening in the lab for root traits (root hairs, root angle, root architecture): Rapid field screening - 50 soybean genotypes selected from laboratory to be grown at 2 P levels (10 and 30 kg P ha$^{-1}$) single row plots with</td>
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<tr>
<td>Country</td>
<td>Crop and P</td>
<td>Description</td>
<td>Germplasm</td>
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</tbody>
</table>
| Cuba        | Common bean and P | Rapid screening of 200 Recombinant Inbred Lines (RILs) and commercially cultivated common bean lines in Cuba.  
Field evaluation of at least 10 RILs and 5 commercially cultivated genotypes with poor root development and P acquisition and 15 lines including 10 RILs with rapid root development and high P acquisition. | RILs of DOR 364 X BAT 477 from CIAT, Columbia |
| China       | Maize and P | Preliminary screening of 150-200 inbred and hybrid varieties under hydroponics conditions. Pot experiment using 10-12 of the inbred lines with different P efficiency selected from the 150-200 lines | Germplasm from China |
| USA (Univ of Penn) | Common beans, maize and P | Develop a manual in English, French, Spanish and Portuguese that describes screening | |
protocols for root traits conferring P efficiency and validation trials in the greenhouse and field

Create a new version of SimRoot with a source code in C++. The model will be used to estimate the potential contribution of root aerenchyma as a trait for enhanced P acquisition in bean and maize.

<table>
<thead>
<tr>
<th>Country</th>
<th>Crop</th>
<th>Activity</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>France (ENSA)</td>
<td>Common bean, N and P</td>
<td>Evaluation of 200 Recombinant Inbred Lines (RILs) and commercially cultivated common bean lines from Cuba for their contrasting PUE and Symbiotic nitrogen fixation (SNF). This will be followed by selection of 3 efficient and 3 inefficient RILs for field evaluation. P mechanistic studies to measure the rhizosphere proton exudation of contrasting RILs and correlate with nitrogenase-linked respiration.</td>
<td>RILs of DOR 364 X BAT 477 from CIAT, Columbia</td>
</tr>
<tr>
<td>Nigeria (IITA)/Kenya (TSBF)</td>
<td>Cowpea and soybean</td>
<td>Laboratory screening of 180 soybean and 300 cowpea</td>
<td>Genotypes from IITA, Nigeria, and</td>
</tr>
<tr>
<td>P</td>
<td>genotypes of diverse origin for variations in root architectural and morphological characteristics. Field screening of the same genotypes under low and high P conditions and identify relationship between root traits (laboratory and field measurements) and field-based P use efficiency measurements</td>
<td>Niger</td>
<td></td>
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</tbody>
</table>
Workshop on data analysis and interpretation related to isotopic dilution experiments at Seibersdorf on 18 October 2006

As part of the activities related to the 1st research coordination meeting of the CRP “Selection and evaluation of food (cereal and legume) crop genotypes tolerant to low nitrogen and phosphorus soils through the use of isotopic and nuclear-related techniques”, a one-day workshop was organized at the Agency’s Laboratory at Seibersdorf with the participation of staff from the SWMCN Section and the Soil Science Unit. The objective of the workshop attended by the 17 participants from 17 countries was to (i) brief the participants on the laboratory activities related to the use of isotope techniques for nutrient dynamics and nutrient efficiency (ii) discuss data analysis and interpretation of data related to isotopic dilution experiments and (iii) examine sample preparation techniques and analysis related to the CRP. During the opening session, a representative of the Director of NAAL, Mr C Schmitzer welcomed the participants and presented an overview of the Agency’s Laboratories research mandate and current activities. Presentations at the workshop included (i) general introduction of isotopes [M-L Nguyen] (ii) isotope techniques in N studies: theory, applications and data interpretation [P M Chalk, G Hardarson and C Atkins] (iii) isotope techniques for studying P dynamics and evaluating agronomic effectiveness of P fertilizers: theory, application and problem set [J Adu-Gyamfi and M-L Nguyen] The participants later visited the analytical laboratory and were briefed on sample preparation and the principles underlying the operation of the mass spectrometer for the analysis of $^{15}$N, $^{13}$C and $^{18}$O in plant, soil and water samples.

Final Discussions

Ten (10) hypothetical traits for rapid screening of maize genotypes for N use efficiency were discussed

1. Primary root elongation
2. Lateral roots
3. Depth of seminal roots
4. Seminal roots whorls
5. Lateral root branching
6. Quantity and depth of adventitious roots (shallow)
7. Root cortical aerenchyma
8. Root hairs
9. Angle and number of nodal roots
10. Depth of nodal roots

The first 5 were considered relatively more important

At least 100-200 genotypes are to be used for the rapid laboratory screening. Participants were encouraged to use a normal distribution curve approach to select efficient and inefficient genotypes for further evaluation in the field.
Participants indicated that there are problems with exchange of germplasm material in all countries except China.

It was agreed that modeling will be useful for addressing output 6 (related to objective 3); relevant but not urgent; we should be focused and hope that a future CRPs will address the third objective.

CONCLUSIONS

(i) There was a general consensus to slightly modify the specific objectives to reflect the discussions during the workshop

(ii) Experimental plans should include the initial collection of at least 200 cereal (maize or upland rice) or grain legume (soybean, cowpea, common bean) genotypes/lines from exotic, landraces and known genotypes, followed by a rapid laboratory screening for root traits (primary root elongation, lateral roots, seminal root branching, root whorls and lateral root branching) conferring P and N acquisition efficiencies. This should be followed by the field evaluation of the same number of genotypes in low N and low P soils to compare the results of the laboratory screening with that of the field evaluation. Contrasting genotypes (20-50) could be selected for evaluation using isotopic and nuclear related techniques.

(iii) Agreement was achieved on common experimental protocols, techniques and standard terminology.

(iv) The issue of low plant available N and P concentrations in soils was discussed. Participants were encouraged to select sites with relatively low plant available N and P after soil testing. The selected site before planting is be designated low N and P to be compared with applied N and P rates. An excel Table requesting participants to provide detail information on the physical and chemical properties of the soil (including the methods used for the extractions) is attached in the Annexes

Annex 1 Program
Annex 2 List of Participants
Annex 3 Abstracts
Annex 1  Program

Monday, 16 October 2006

Opening

09:00-10:30  Opening Session, Chairperson: L. Nguyen

Welcome address: L. Nguyen, Head, SWMCN Section

The IAEA Research Contract Programme: Ms. T. Benson, Head, NACA Section

Integrated plant-soil approaches to increase crop productivity in harsh environments: P.J. Lagoda, Head, Plant Breeding Section

Introduction of participants

Objectives of the meeting: J. Adu-Gyamfi, Scientific Secretary

10:30-11:00  Coffee/tea Break- Group Photo

Session 1:  Presentations from Participants (35 min each + 10 min discussion)
Chairperson: L. Nguyen

11:00-11:45  W. Horst (Univ. Hannover, Germany)
Genotypic differences in nitrogen use-efficiency in crop plants

11:45-12:30  K. Traore (Burkina Faso)
Increasing upland rice productivity through the use of high N acquisition genotypes and improved agronomic practices

12:30-14:00  Lunch Break

Session 2  Presentations from Participants (continued)
Chairperson: P. Moutonnet

14:00-14:45  I. Baggie (Sierra Leone)
Identification of upland rice genotypes tolerant to low N and P soils

14:45-15:30  V.B. Bado (WARDA, Senegal)
Improvement of rice-based system productivities through integrated management of fertilizers, legumes and rice genotypes

15:30-16:00  Coffee/tea Break

16:00-16:45  Z. Abdul Rahman (Malaysia)
Selection and evaluation of upland rice genotypes tolerant to low nitrogen and phosphorus soils in Malaysia
Tuesday, 17 October 2006

Session 3: Presentations from Participants (continued)
Chairperson: Ph. Chalk

08:15-09:00 J. Lynch (Univ. Pennsylvania, USA)
Characterization of root traits contributing to enhanced phosphorus acquisition from low fertility soil

09:00-09:45 J. Bayuelo-Jimenez (Mexico)
Traits associated with improved P-acquisition efficiency in maize landraces grown on an acid Andisol in the Purhepecha Plateau, Michocan, Mexico

09:45-10:30 J. Yang (China)
Selection and evaluation of maize genotypes tolerant to low phosphorus soils

10:30-11:00 Coffee/tea Break

11:00-11:45 C. Atkins (Univ. Western Australia, Australia)
Novel approaches to study factors limiting N and P nutrition in legumes

11:45-12:30 F. Kumaga (Ghana)
Selection of soybean (Glycine max (L.) Merill) genotypes for increased nitrogen and phosphorus use efficiency and seed yield

12:30-13:30 Lunch Break

Session 4: Presentations from Participants (continued)
Chairperson: L. Nguyen

13:30-14:15 J. J. Devron (ENSA, France)
Phosphorus efficiency, phytic acid metabolism and symbiotic nitrogen fixation for sustainable production of common bean, cowpea and chickpea in Mediterranean environments
14:15-15:00  M. Miguel (Mozambique)
Evaluation and selection of common bean (*Phaseolus vulgaris* L.)
genotypes for root hairs - a trait conferring phosphorus (P) acquisition
efficiency in low P soils

15:00-15:45  R. Abaidoo (IITA, Nigeria)
Variations in biological nitrogen fixation and P use efficiency among
cowpea and soybean genotypes in the West African moist savanna

15:45-16:15  Coffee/tea Break

16:15-17:00  A. Garcia Altunaga (Cuba)
Evaluation of common bean and maize genotypes tolerant to low N and
P soils in Cuba

17:00-17:45  T. Muraoka (Brazil)
Phosphorus and nitrogen use efficiency by selected crops in tropical
(Brazilian) soil

17:45-18:30  N. Sanginga (TSBF-CIAT, Kenya)
Evaluation of promiscuous soybean for phosphorus use efficiency and N
balance in low P soils in maize-based cropping systems in the derived
savanna zone in Nigeria

**Wednesday, 18 October 2006**

09:00-17:00  One-day workshop on isotopic and nuclear techniques in soil-plant
studies
IAEA Laboratories, ABL, Soil Science Unit

**Thursday, 19 October 2006**

**Session 6:**  Work planning
*Chairperson: G. Hardarson*

09:00-09:30  Review of project objectives and expected outputs (Scientific Secretary)

09:30-10:00  Coffee/tea Break

10:00-12:30  Review of project logical framework and work plans (Scientific Secretary)

12:30-14:00  Lunch Break
Session 7: Work planning (continued)
14:00-15:30 Constitution of two working groups (one on N and another on P),
election of rapporteurs and development of experimental protocols.
15:30-16:00 Coffee/tea Break
16:00-17:30 Development of experimental protocols (continued)

Friday, 20 October 2006

Session 8: Work planning (continued)
Chairperson: L. Nguyen
09:00-10:30 Presentation and discussion of experimental protocols
10:30-11:00 Coffee/tea Break
11.00-12:30 Presentation of individual work plans
12:30-14:00 Lunch Break
14:00-15:00 Formulation of conclusions and recommendations
15:00-15:30 Coffee/tea Break
15:30-16:00 Presentation of conclusions and recommendations
16:00 Closing remarks
16:00-18:00 Individual discussions
Annex 2    List of Participants

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Annex 3  Abstracts

Genotypic differences in nitrogen use-efficiency in crop plants

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Contract number: GFR/13762

To improve nitrogen (N) efficiency in agriculture, integrated N management strategies that take into consideration improved fertilizer, soil and crop management practices are necessary. Among these, breeding and cultivation of N-efficient cultivars have to play an important role in both low-input and high-input sustainable agricultural production. Agronomic N efficiency is the ability of a genotype to achieve a higher yield under the conditions of an N supply yield-limiting for the mean of the population. Knowledge of secondary plant traits expressing together with N efficiency would facilitate the breeding of N-efficient cultivars.

This paper reports results of field experiments in which grain-maize in Africa and silage-maize and oilseed-rape cultivars in Germany were compared with respect to their agronomic N efficiency (yield at a given N supply), N uptake efficiency (N accumulation at a given N supply), N utilization efficiency (reproductive yield per unit N taken up by the plant), and morphological/physiological plant traits that contribute to N efficiency.

Significant differences among cultivars were found in grain yield. N efficiency was related more closely to N uptake efficiency than utilization efficiency. The experiments showed a significant correlation between N efficiency and leaf senescence during grain filling. The same cultivars were studied for leaf senescence under N deficiency in short-term nutrient solution experiments. Leaf chlorophyll contents as estimated by SPAD values and photosynthesis rates were used as measures for leaf senescence. Cultivars differed both in SPAD values and photosynthesis rates of the older leaves during N deprivation. Significant correlations were found between SPAD values, photosynthesis rates in the nutrient-solution experiment and leaf senescence scores in the field experiments and between photosynthesis rates and grain yield under low-N conditions in the field. Relationships between physiological root parameters, which were investigated in nutrient solution experiments and N uptake or grain yield of the cultivars in the field could not be established. Therefore, a technique was developed which allowed the assessment of root growth and nitrate uptake activities under field conditions.

From our studies is concluded, that the maintenance of a higher photosynthetic capacity of older leaves during N deficiency-induced senescence during grain filling allows the plants to maintain root growth and root activity during this developmental stage thus acquiring soil nitrate particularly from deeper layers more efficiently. The capacity of a genotype to maintain a higher photosynthetic capacity of older leaves during N
deficiency-induced senescence may be assessed at the seedling stage and thus may be suited as a selection parameter for the N efficiency of crops.
Increasing upland rice productivity through high N acquisition genotypes use and agronomic practices

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Contract number: BKF/13753

Crop production in the sahelian countries of Africa is limited by many factors. The most important are low potential yields of local varieties; low inherent soil fertility and low applications of external inputs (organic and mineral fertilizers). In fact the upland soils in the area are deficient in N and P. Although considerable efforts have been devoted to the improvement of soil conditions for crop production through the design and diffusion of best soil-nutrient management practices, the adoption rate of these technologies remained low due to mainly limited incomes of small poor farmers and socio-economic constraints. The design and the use of crop genotypes adapted to these hard environments and the use of organic amendments combined with small quantities of mineral fertilizers are of importance for thousands of smallholders in the Sahel. For example, N fixing legume crops could be integrated in the crop rotation in the objective to design a low-cost management options to improve agricultural productivity.

Rice is a strategic crop in West Africa; it constitutes an important source of food and farm income for many rural households in the region. In most appropriate ecological zone, farmers are more and more involved in producing upland rice. For instance, the area cropped with upland rice was increased by 50% in the south sudanian zone of Burkina Faso during the last 10 years. The current level of upland rice production can be significantly increased by using improved and adapted rice cultivars in combination with low-cost agronomic practices. Burkina National Institute for agriculture researches (INERA) in partnership with West African Rice Network (WARDA) has developed new rice varieties called new rice for Africa (NERICA) which are able to increase nutrients recovery from the soil and fertilizers. But little information is available on which cultivars are the most efficient in N and P recovery under sahelian conditions.

The current project aims to improve upland rice-based system productivity through an integrated management of improved rice genotypes and agronomic practices as a mean to deliver low-cost management options accessible to small farmers.

The execution of the current work will consist of intensive collaboration between WARDA and INERA. The WARDA will supply rice genotypes and technical support for rice selection and characterization. Agronomic field experiments will be carried out by INERA in closed collaboration with scientists from both institutions.
Identification of upland rice genotypes tolerant to low N and P soils through use of isotopic technique

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Contract number: SIL/13754

Soils in the humid and semi-humid of Africa are generally infertile, acidic, low in either nitrogen (N) or phosphorus (P) or both and can rarely sustained yield without high rate of fertilization. Unfortunately, resource-poor farmers can not afford high rate of fertilization. An approach is to provide the farmers with adaptable genotypes to acid-related infertile soils (low N and P) conditions and could efficiently utilized N and P at low rates of fertilization. Upland eco-system is important in rice production in the region and rice is grown in over two-thirds of total land area.

A research is proposed with the objective of evaluating and identifying upland rice genotypes that are tolerant to acid-related infertile soil (low N and P). In the preliminary year, a uniformity trial with rice grown unfertilized prior to the screening will be done. About 30 – 40 upland rice genotypes will be field screened at two rates of fertilization (0 and 60 kg N and P ha$^{-1}$); at two locations using participatory varietal selection (PVS) approach. Data to be collected will include grain yield, shoot and root weights, N, P and Al contents in the shoot and P in the root; and farmers’ selection and criteria. It is expected that at the end of the season, potential efficient and tolerant rice genotypes would have been selected for further evaluation.
Improvement of rice-based system productivities through integrated management of fertilizers, legumes and rice genotypes

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Contract number: BEN/13834

Upland soils of West Africa are characterized by inherently acid, N and P-deficiencies and low organic matter contents. Low potential yields of local upland rice varieties, nutrient deficiencies, soil fertility constraints and low applications of external inputs (organic and mineral fertilizers) are some of the most important factors limiting rice yields. Low inherent soil nutrient status, in particular N and P deficiencies are the most common nutritional stresses in upland soils. Although considerable efforts have been devoted to the improvement of soil conditions for plant growth through the development and use of best soil-nutrient management practices, their adoption has been low because of several socio-economic constraints, mainly due to the low incomes of small poor-resource farmers.

But in most appropriate ecological zone, farmers are more interested by upland rice cultivation. Upland rice yields could be increased with more adapted varieties, coupled with low-cost agronomic practices. In partnership with national research institutes (NRI) Africa, WARDA has developed new promising rice varieties (NERICA) that are able to increase N recoveries from soil and fertilizers as a technological alternative to improve rice-based system productivity through an integrated management of improved rice genotypes and agronomic practices and as a mean to deliver low-cost management options accessible to small farmers. The main goal is to develop integrated genotypes, soil and external inputs and agronomic practices to increase rice-based system productivity. The capacities and adaptation mechanisms of the NERICA varieties for nutrient acquisition (from soil and fertilizers), particularly N and P will be studied. In order to select varieties which improve both soil and fertilizer N acquisition by testing different management options of fertilizers and N\textsubscript{2}-fixing legume crops in rotation with rice. This information can be further utilised to develop integrated crop, soil and external input management practices to increase rice-based system productivity. A modeling approach using decision support tools could be used to identify, test and deliver low-cost management options for farmers.

A networking strategy based on intensive collaboration between WARDA and national agricultural research institutions (NARI) will be used. WARDA will supply rice genotypes and technical support for selection and characterization and the field experiments will be undertaken by scientists of NARI in closed collaboration with WARDA’s scientists.
Selection and Evaluation of Upland Rice Genotypes Tolerant to Low Nitrogen and Phosphorus Soils in Malaysia

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Contract number: MAL/13700

Lowland rice in Malaysia is grown on about 667,000 ha of land and contributed to about 70% of total rice requirement of the country. The other 30% is imported from Thailand, Vietnam, Pakistan and India. Land area planted to upland rice was about 98,000 ha annually, with 75,000 ha in Sarawak, 13,000 ha in Sabah and 10,000 ha in Peninsular Malaysia. The cultivation of upland rice is still an important agricultural activity in Sabah, especially in the rural areas where suitable land for wet rice cultivation is scarce and is grown mainly for home consumption. The current average yield ranged from 0.4 to 1.1 metric tonnes/ha.

To initiate this project, a survey was carried out at nine upland rice growing areas. Most of the farmers were found to still use slash and burn technique, except for one place in Jerantut, Pahang, where the rice was grown every year in the same place. The highest yield recorded during this survey was 1.8 ton/ha of grain which was obtained in Sabah. The majority of the soils showed a textural class of sandy loam, sandy clay loam or clay loam, which were acidic with low CEC, N, P and K contents.
Evaluation, Identification and Development of maize genotype tolerant to low N

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Contract number: CMR/13852

In Africa, soils are generally low in available N and P. These elements are highly required to sustain adequate food production in tropical soils mainly (40%), made of oxisols and ultisols (Keerthisinghe et al. 2001). The application of 60 Kg/ha of P in weathered acid soil of the tropics did not yield significant grain yield increase (The et al. 2006). These authors concluded that it was of no use and costly to applied small quantities of P, especially when acid soil tolerant cultivar was cultivated.

The identification and/or the development of germplasm combining N, P use efficiency as well as Al tolerance, could significantly contribute to sustain maize production on stress environments of the tropical area. The knowledge of the correlated response of plant agronomic characteristics and grain yield on low N, and low P soils, as well as the combining ability and gene action of selected genotypes could help to speed up the development of high yielding stable and adapted cultivars. The potential use of $^{15}$N technique to select for efficient N use genotypes, the studies on rhizosphere mechanisms (root exudates, genotypes x mycorrhizas fungi interaction, genotypes x cultural system) will enhance the attainment of the expected results since research will be conducted in a multi-disciplinary manner.

Activities planned for the next three to five years include:

Evaluation of maize genotypes tolerant to low N, to drought and Al toxicity, for identification of low N donor genes.

The development of cultivars with N and P use efficiency for release to poor resource farmer

The identification of plant agronomic parameters correlated to low N and P

The diffusion of the improved technologies

Studies will be carried out in multi-disciplinary teams.
Characterization of root traits contributing to enhanced phosphorus acquisition from low fertility soil

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Low soil phosphorus availability is a primary constraint to crop production in developing countries. In recent years a number of root traits have been identified which improve phosphorus acquisition in crop plants. These include topsoil foraging via shallower basal roots and greater adventitious rooting, greater rhizosphere exploitation via increased length and density of root hairs, and decreased root metabolic costs through the formation of root cortical aerenchyma and root etiolation. These traits are quite powerful—for example in common bean, genotypic variation in basal root shallowness accounts for a 600% difference in P uptake from soil and is significantly correlated with yield in low P field trials, genotypic variation in root hair length accounts for a 250% difference in P uptake in the field, and genotypic variation in root metabolic costs accounts for a 200% difference in root biomass and soil exploration. Several strong QTL have been identified, accounting for up to 65% of phenotypic variation. The effects of these traits on agroecosystem processes including drought tolerance, nutrient cycling and soil erosion are being researched in field trials in Central America. Phosphorus-efficient bean genotypes identified in this effort yield up to 200-300% more in low P soil than existing cultivars, and are being used as parents in bean breeding programs in Africa and Latin America. Similar genotypic variation exists in maize. In China, selection for root traits in soybean has resulted in the release of 5 new cultivars with superior yield in low fertility soil.

The proposed project will develop an integrated ideotype for efficient phosphorus acquisition in maize and bean, using the geometric simulation model SimRoot. In addition the project will develop standard protocols and methods for the identification of crop genotypes with root characteristics contributing to efficient phosphorus acquisition from low fertility soils. Root traits to be considered include root architecture, root hair length and density, specific root length, root cortical aerenchyma, and production of phosphorus mobilizing root exudates.
Traits associated with improved P-acquisition efficiency in maize landraces grown
on an acid Andisol in the Purhepecha Plateau, Michocan, Mexico

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Contract number: MEX 13800

The Purhepecha Plateau is a high-altitude land located at the centre Michoacan state, Mexico. Because of its geographical location a broad range of different ecological niches, allow the wide distribution of wild relatives and the cultivation of numerous native maize landraces. Landrace diversity comes from age-long cultivation by ethnic population and farmers who use the genetic potential of their crops in their particular agro- and ecological environments. In this region, maize production is carried out predominantly under rain-fed conditions and in small production units where levels of rural poverty are high, often operating on sloping terrain of low fertility. Over 20 percent of total arable land is affected to some degree by soil acidity caused by high phosphate retention. Much of Michoacan’s agriculture takes place on Andisol soils, originated from volcanic ash, which are generally low productive soils for plant growth. In this region, maize production relies more heavily on unique landraces (early white, late white, yellow and blue) since they perform better than improved varieties and hybrids. Although these producers have low yields, maize landraces vary substantially in their ability to grow and yield in poor soils, making it feasible to increase yields in infertile soils through genetic improvement.

Low soil fertility, especially suboptimal phosphorus availability is a principal, pervasive constraint to maize production at the Purhepecha Plateau. Application of lime and P-containing fertilizers is usually the recommended treatment for enhancing soil P availability and stimulating crop yields. However, in this region, few maize producers can afford intensive chemical inputs. Genetic improvement of phosphorus efficiency, defined as yield ability at low phosphorus supply and better response to phosphorus inputs, is therefore an attractive prospect for the productivity of low-input systems. Several genetic traits have been identified with potential in breeding phosphorus efficient crops, including root exudates, root-hair traits, cortical aerenchyma, topsoil foraging through basal or adventitious rooting, and use of multiline mixtures of root phenotypes. This project aims to identify and evaluate local maize germplasm for root traits conferring phosphorus acquisition efficiency, characterize specific traits responsible for P uptake efficiency among local landraces and quantify the root traits’ contribution to the overall phosphorus uptake under low P environments. To achieve these objectives, different approaches will be combined, including phenotypic screening and analysis of root traits, root distribution, arbuscular mycorrizal infection, time course of phosphorus uptake and accumulation using quantitative autoradiography, and genotypes perform on farm. Genotype evaluation of maize landraces for root traits conferring phosphorus efficiency would lead to an improvement of maize production and productivity in these low fertile soils, by
incorporating these traits into superior and desirable genotypes through breeding programs.
Selection and evaluation of maize genotypes tolerant to low phosphorus soils through the use of isotopic and nuclear-related techniques

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Low inherent soil nutrient status, in particular severe phosphorus (P) deficiency is the most common nutritional stresses in many regions of the world, especially in the developing countries. It is estimated that 70% of the cultivated land is phosphorus deficiency in China. Phosphorus deficiency is a major abiotic stress that limits plant growth and crop productivity. Phosphorus fertilizer application is therefore currently the only way to meet the P demand of plant. Applying fertilizers in a large amount not only exhausts limited phosphorus resources but also brings the pollution of the environmental. In fact, total phosphate is quite abundant in many soils, but it is largely unavailable to plants, which results in “Phosphorus deficiency in heredity”. Aiming at these actualities, a complementary approach that has received much less attention is improving the adaptation of crops to these unfavorable soil conditions by selecting, improving and developing crop genotypes with enhanced nutrient use efficiency in soils with low nutrient status, and/or plants that require moderately low external inputs to express their genetic potential for adequate production. This strategy is now considered as a more promising, energy-efficient, ecological-friendly and socio-economic feasible approach than major reclamation processes and related soil management practices.

Maize is an important grain and forage crop in the world, the same as that in China, which faces the same problem of “Phosphorus deficiency in heredity”. To study on the genetic and physiological-biochemical mechanism of plant with high-efficient absorbing, transporting and utilizing soil phosphorus, developing phosphorus efficient cultivars, this proposal will focus on (1) the glancing selection for 5-10 P-tolerance and P-sensitive varieties which employing the dry weight and P deficient symptoms as the indicators, among 100-150 maize inbred lines by hydroponics, (2) the fast selection of maize genotypes tolerant and sensitive to the low phosphorus soils through the use of $^{32}$P tracer technique, (3) the further evaluation of the selected genotypes associated with the root traits, root exudates, relative total biomass, the nutrient uptake of Mg and Ca, the content of chlorophyll, Malonaldehyde (MAD) and phosphatase of the leaves, P uptake and utilization efficiency through the pot experiment, (4) the last determination of long-term effects to identify 5-10 maize genotypes with superior phosphorus acquisition and nutrient use efficiencies.

In conclusion, the proposed project is to exploit the physiological-biochemical mechanism on P-uptake, P-transport and P-reallocation of selected maize genotypes in low-P soils through the use of $^{32}$P tracer technique associated with hydroponic and pot experiment, field trial and long-term experiment, to establish the fast screening protocols of maize to identify genotypes with superior P acquisition genotypes tolerant to P
deficiency soils during 2006 to 2010. The selection and evaluation of N-efficient, P and N-efficient, P and N-inefficient genotypes may be conducted during 2007 to 2010.
Novel approaches to study factors limiting N and P nutrition in legumes

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Contract number: AUL/13699

Some species of lupin develop unique and effective adaptations to low nutrient soil conditions and specifically low available P. The roots of *Lupinus albus* (white lupin) develop ‘cluster roots’ which are proliferations of short determinate lateral roots in dense clumps or clusters. The clusters engage in intense exudation of organic acids, particularly citrate and malate, which alter P availability and are believed to enhance P uptake from the localized microenvironment created around the clusters. Research in the School of Plant Biology at UWA has documented a number of aspects related to the regulation of cluster root development. An important finding is that a shoot-derived regulatory ‘signal’, analogous to the signals that determine autoregulation of *Rhizobium* infection and nodule development, drives the adaptation. Systemic signals are translocated through the plant’s vasculature and in the case of basipetal movement, in phloem. Among the pulses lupin are unique in bleeding freely from incisions in vasculature permitting collection of large amounts of phloem exudate. We have currentwell funded research projects to seek the nature of these systemic ‘signal’ molecules, concentrating at present on small regulatory RNA species, particularly micro RNAs (miRNA). The latter target specific transcripts and regulate gene expression by preventing translation. Already we have cloned around 1000 small RNA species from lupin phloem and identified some 20 as miRNAs, most of which target transcription factor genes. The nodulation response to low soil N and the cluster root response to low soil P are being studied in this respect and we have identified one miRNA in phloem that could potentially regulate expression of P transporter genes. Significantly, the spectrum of miRNAs in phloem is different at different collection sites on the plant; those translocated to roots differ from those translocated upwards. Identification of these regulatory molecules and their mode(s) of action will provide markers for selection of genotypes with greater nutrient use efficiency and could potentially provide a means to express features like cluster roots in many crop legumes.

One of the unique and powerful tools we are using is imaging isotope ratios with ion microprobes. In addition to low resolution probes we can image isotopes at the ultracellular level with a Cameca NanoSIMS® instrument. The nanoSIMS® will resolve isotope abundances for $^{15}$N/$^{14}$N, $^{13}$C/$^{12}$C and $^{31}$P with better than 100nm resolution at the root cell/soil boundary, between cells and among subcellular organelles. Secondary ion mass spectrometry (SIMS) involves atomic layer sputtering of the surface of a sample with a scanning primary ion beam. During the course of the sputtering, secondary ion species are liberated from the sample and sorted by passage through a mass spectrometer to form ion distribution images. We have considerable expertise imaging a wide array of stable isotopes in diverse biological materials including plant roots. These ion image data
will provide new insights into processes that transfer P and N from the soil into roots and will identify sites where accumulation of isotope indicates limitation. The techniques are not limited to P, N and C but can be applied to any stable isotope that ionizes under the conditions of analysis.
Selection of soybean (Glycine max (L.) Merill) genotypes for increased nitrogen and phosphorus use efficiency and seed yield

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Contract number: GHA/13798

Deficiencies in Nitrogen and Phosphorus (N and P) are among the major constraints to productivity in most tropical soils. Although poor and marginal soils may be fertilized to increase crop yields, there is an urgent need to identify and exploit the genetic variability in root and other adaptive plant traits for better adaptation of crops to soils with low N and P.

A series of greenhouse (soil and solution culture) experiments will be conducted to screen 20 – 25 genotypes of early and medium maturing soybean genotypes (currently being used in Research Station trials) for their ability to produce high yields in both nitrogen (N) and phosphorus (P) – deficient and – sufficient conditions. The study will also assess the extent of genotypic variation among the varieties in P – use efficiency and investigate the possible mechanisms for the adaptation of the varieties to low – N and low – P conditions.

To compare the amount of N2 fixed by soybean genotypes at low and high P conditions, the 15N isotope dilution technique will be used to quantify N2 fixation.
Phosphorus efficiency, phytic acid metabolism and symbiotic nitrogen fixation for a sustainable production of common bean, cowpea and chickpea, in the Mediterranean environments

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Contract number: FRA/13816

Grain legumes can contribute to cropping systems through their ability to fix nitrogen from air (N$_2$) in their root-nodules. However, the symbiotic nitrogen fixation process requires additional phosphorus. Thus, the production of grain legume, particularly common bean, is limited by P-deficiency in many soils, mostly in tropical and mediterranean areas. The primary goal of this project is to increase and sustain the production and quality of seeds of common bean, as a model grain legume, and chickpea and cowpea as major sources of proteins for human nutrition, through their efficiency to use P for N$_2$ fixation and through their proton efflux to increase soil P bioavailability.

In this project, legume genotypes already identified for high PUE and SNF, or for low phytic acid, in previous projects, will be assessed in participation with farmers in reference areas for growth, yield and seed quality in relation with their rhizobial and mycorhizal symbiosis. Contrasting genotypes among recombinant inbred lines (F8) from a cross of common bean BAT477 with DOR364 will be selected to elucidate the physiological and molecular bases of the relation on P deficiency to SNF in controlled environment with reference myccorhiza and rhizobia. The later will be compared with rhizobia selected within the reference areas where some may be eventually proposed as inoculants.

For contrasting genotypes already available, the nodule acid phosphatase (AP) activity was found to increase under P deficiency. Among this nodule AP activity, 10% was attributed to phytase which to our knowledge, is the first description of this enzyme activity in mature legume-nodule. Primers of AP with in situ RT-PCR on nodule sections show preferential localization of AP transcripts in vascular traces and the nearby layers of cells surrounding the infected zone, namely the inner cortex. P deficiency increased the amount of the later and the permeability of nodule cortex to oxygen diffusion, a parameter that regulates the nitrogenase-catalysed N$_2$ reduction. Further work is planned to focus on phytase activities in the nodulated-root rhizosphere, with a larger number of segregating lines, in the broader context of an integrated project.

Thus our hypothesis is that a virtuous cycle of N and P fertility is associated with improved efficiency in the use of P for symbiotic N$_2$ fixation. It is expected that this multidisciplinary approach will deliver legume symbioses better adapted to low phosphorus conditions of each reference area. They will be made available for future breeding programmes in order to contribute to the economic feasibility of grain legumes in sustainable farming systems of the targeted countries.
Evaluation and Selection of Common Bean (Phaseolus Vulgaris L.) Genotypes for Root Hairs - a Trait Conferring Phosphorus (P) Acquisition Efficiency in Low-P Soils

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Contract number: MOZ/13803

Edaphic stress, especially low phosphorus (P) availability is one of the main limiting factors to bean production and productivity in Southern and Eastern Africa (CIAT 1992). In these regions, the use of fertilizers is limited because of the associated economic costs, and the majority of farmers do not apply chemical fertilizers. Therefore, alternative means to improve crop performance are needed. Recent studies by Lynch and co-workers reveal that bean genotypes substantially vary in relation to P uptake efficiency when grown under P stressed soils. Some genotypes exhibit root traits that confer P efficiency. These traits include number of root whorls, root architecture, adventitious rooting and root hairs. In the proposed research project we intend to a) collect and evaluate existing Mozambique germplasm of common bean (Phaseolus vulgaris L.) for root hairs; b) evaluate the field performance of the selected bean materials with long and dense root hairs in low phosphorus soils; c) determine the traits’ contribution to the overall phosphorus uptake; and d) develop protocol for use of P$^{32}$ in studies on P uptake of contrasting genotypes. The experiments will be conducted both on-station and on-farm, involving 4 locations in Central Mozambique. The outcome information can be used by the breeders in the selection of genotypes for P efficiency in the breeding programs devoted to the development of nutrient efficient varieties for the region.
Variations in biological nitrogen fixation and P use efficiency among cowpea and soybean genotypes in the West African moist savanna

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Contract number: NIR/13823

Phosphorus and N are the two nutrients that most limit growth in cropping systems of sub-Saharan Africa where soil fertility depletion is a major cause of the declining per capita food production. The soils’ available-P (Bray-1) contents range from 2.5 to 33 mg kg\(^{-1}\), and the total N values range from 0.3 to 1.3 g kg\(^{-1}\) in the West African moist savanna. Therefore, to overcome P and N deficiencies and their effects on crop productivity, the application of soluble inorganic fertilizers has been recommended. However, due to the difficulties associated with the use of inorganic fertilizers in the region and the negative environmental effects of this option, other more sustainable alternatives have been proposed such as the use of genotypes with high efficiency at acquiring P from sparingly soluble soil P pools. The incorporation of N fixing legumes, such as cowpea and soybean, in cereal cropping systems is anticipated to improve the N nutrition of the cereal crop, increase its yield, and reduce erosion. However, biological N fixation (BNF) can be limited if the high requirements for P in the process are not satisfied. Eight genotypes of cowpea and soybean were screened for P use efficiency and enhanced BNF in the Sudan, Guinea, and derived savannas of Nigeria to identify efficiency traits and establish the role of soil-available P in the BNF capability of the promiscuous genotypes studied. The variation among genotypes in P use efficiency traits appeared to be narrow. The proportion of N fixed in association with indigenous rhizobia populations did not significantly increase with increasing rates of P application. All the genotypes produced sufficient grain yields under low-P conditions. Consequently, it has become difficult to develop the screening protocols needed by cowpea and soybean breeders to incorporate P-use efficiency into their breeding programmes. We have begun new activities with 300 genotypes of cowpea and 185 of soybean of diverse genetic background with the objective of identifying extreme N-fixing and P-use efficiency traits and subsequently using these genotypes to study the mechanisms associated with P-use efficiency.
Selection of cereal and legumes for sustainable crop production in Cuban soils

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Contract number: CUB/13844

At present the Cuban agriculture faces a fertilizer deficit with the consequent negative impact upon the quantity and quality of the agricultural production. Soils nutrient deficiencies – nitrogen (N) and phosphorous (P) mainly - in many of the small farmer agricultural areas, soil acidity, the use of plants with low adaptability to these constraint factors and inadequate management practices, among others, limit the agricultural production and at the same time there is not an improvement of the soil fertility and consequently the agricultural production. One solution way to these problems is the use of adaptive genotypes to adverse conditions.

Common bean is cultivated along the Cuban Archipelago, principally by small farmers who use low input quantities and the yields they get are far from the crop potential because of different factors such as: i) use of genotypes of slight adaptability to soil limiting factors as nutrient deficiencies, little water availability, acidity ii) inappropriate agricultural practices iii) economic constraints. In the context of this kind of low inputs agriculture characterized by different agricultural practices, it is necessary the assessment of common bean germplasm in order to choose those with high yield and SNF and adaptive to soil stress conditions.

In this context, some experiments were developed recently under controlled conditions and field where agronomic responses of common bean genotypes and selected Recombinant Inbred Lines (RIL) of BAT 477 x DOR 364 cross were evaluated.

The proposed research will be carried out in laboratory, glasshouse and field using conventional and isotopic techniques. The overall objective is to identify cereal (maize) and legume (common bean) tolerant to nutritional stress in Cuban soils with high yields and high N and P use efficiency.

Specifically, it will be investigated common bean genotypes, including Recombinant Inbred Lines of common bean from BAT 477 x DOR 364 cross obtained at CIAT-Colombia, with high SNF (legumes) and yield and with high N and P use efficiency.

In order to achieve these objectives the work plan includes:
1. Identifying benchmarks areas: Soil sampling and soil characterization
2. Laboratory experiments:
   i) Evaluation of soil P dynamic under different treatments
   ii) Evaluation of soil N dynamic under different treatments
3. Greenhouse experiments: Screening of common bean genotypes and Recombinant Inbred Lines of BAT 477 x DOR 364 cross regarding to
i) Soil nutrient stress
ii) Higher P use efficiency
iii) Higher SNF and N use efficiency

4. Field experiment: Screening of common bean genotypes and Recombinant Inbred Lines of BAT 477 x DOR 364 cross regarding to soil nutrient stress in maize – common bean system.
Phosphorus and nitrogen use efficiency by some crops in tropical (Brazilian) soil

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Contract number: BRA/13779

Phosphorus and nitrogen deficiencies are the most growth limiting factors in tropical and sub-tropical soils of the world, in which includes Brazil.

The high P capacity of these soils, originally low in natural P, in forms largely unavailable to plants, presents serious agronomic and economic constraints, by the needs to apply heavy dose of P for obtaining high crop yield. Estimates of overall efficiency of applied fertilizer have been reported to be around 20% for P and 50% for N. Data obtained with isotopic techniques (\(^{32}\)P and \(^{15}\)N), however, have shown that the efficiency in Brasil rarely surplus 10% for P and very often is lower than 40% for N. This low efficiency for nitrogen is due to significant losses of nutrient by leaching, run-off, gaseous emission and fixation by soil. These are compelling reasons of need to increase P and N nutrient use efficiency (NUE) by the crops.

Increased NUE in plants is vital for better crop yield, reducing input cost and contributing to sustainable agriculture system, improving ecosystem quality. More efficient crop genotypes in nutrient use are important in Brazil for both, improved and resource-poor, farmers as one of the reasons for low fertilizer nutrient recovery of crops is the low NUE of most of cultivars or genotypes utilized by them.

In the former IAEA-CRP (Acid Soil) the isotope dilution technique, using \(^{32}\)P, showed to be a very good tool for screening crop species or genotypes for P use efficiency. For nitrogen, \(^{15}\)N labeled fertilizer permits to evaluate precisely the N use efficiency of crops.

The maize genotypes, rice and bean cultivars, green manure species (including millet) and forage grass crops will be screened for their ability to absorb poorly-available soil P, using isotopic dilution technique (\(^{32}\)P). The crops, except for legume green manure species, will also be evaluated for nitrogen NUE using \(^{15}\)N labeled urea. The selected most efficient cultivars or genotypes will then be grown in field condition for productivity evaluation.
Evaluation of promiscuous soybean for phosphorus use efficiency and N balance in low P soils maize-based cropping systems in the derived savanna zone in Nigeria

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Contract number: KEN/13765

The selection of agriculturally important plant germplasms tolerant to low P could increase crop productivity on P deficient soils in the moist savanna zones of West Africa and therefore reduce P fertilizer requirement. Differences in growth, nodulation and arbuscular mycorrhizal fungi (AMF) root infection among recent promiscuous soybean breeding lines (13 lines) were evaluated at a low and at high phosphate levels (0 and 90 kg P ha\(^{-1}\)) in the pot and field experiments in 1997 in a site within low P level (5.33ug Pg\(^{-1}\) soil Bray\(^1\)) at Fashola in southern Guinea savanna of Nigeria. The three sources of P were (i) soil P, (ii) rock P from Togo and (iii) single superphosphate. Large differences in growth, mycorrhizal infection rate and nodulation were related to the different P sources and soybean lines. Differences between soybean lines in their growth response to P levels occurred at all sources of P (Table 1). The three most interesting groups were: (i) the efficient cultivars or those which produced high biomass with low available P such as V435 (TGM 1251) that did not respond to P application. (ii) the inefficient ones which required high P to grow well such as V391 (TGM 1293) and V398 (TGM 1420) lines had their shoot dry matter increased by 33% and 27 % respectively by SSP application but not with RP. V447 and V459 responded only to RP application and (iii) intermediate plants producing similar biomass at both low and high P. Soybean lines V313 (TGM 1511) was low yielding. Results also showed that P response of some lines was mycorrhizal dependent; P responder lines such as V 398 to SSP and V459 to RP had a lower mycorrhizal infection rate (15%) than the non P responder and high yielding lines such as V435 (23% infection rate). The correlations between shoot dry matter and grain yield were highly significant (P=0.69; P=0.01, n= 190) Results in the pot experiment showed more pronounced differences between lines in their response to the different P sources but the trend was generally the same as for the field data. These results indicate a great potential for selection for growth in P deficient soil. Based on the above results lines such as V435 should be recommended in area with low P soils where cropping systems involving soybean are the target.

In a subsequent year, maize was grown in all the plots in order to assess the residual effect of the previous legumes and P treatments. The major question was to find out if in P and N-limited cropping systems, (1) yield of maize succeeding some high N\(_2\) soybean fixers varieties is equal to that of mucuna and lablab and (2) if residual P from RP is more important than that of SSP. The residual effect of previous P application was significant for shoot and root dry biomass and total N and P accumulation (Table 1). There was no significant difference between SSP and RP. Differences in maize growth and grain yield as affected by the previous soybean lines and the herbaceous legumes are shown in Table
2. Three groups were identified: (i) soybean cultivars TGM 1540, 1251, 944 and TGX1456 and lablab produced the highest maize biomass and grain yield (ii) while lines such as TGM 1360 and 1039 lines had 39% less shoot dry matter and grain yield than lablab and the soybean lines in the same group (iii) other soybean lines produced similar biomass with mucuna and were considered as intermediate. There was a strong correlation between shoot dry biomass and total N and P accumulation. These results refute the common assumption that the N balance after soybean growth is negative. They also indicate a great potential for selection of soybean lines that could be as good as the herbaceous legumes as source of N in maize based cropping systems. Based on the above results lines such as TGM 1540, 1251, 944 and TGX 1456 should be recommended in area with low P soils where cropping systems involving soybean are the target.

Table 1: Effect of previous source of P (soil P, Rock P and SSP) on the growth parameters and grain yield of maize grown in low P soil at Fashola

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot weight (g/plant)</th>
<th>Grain yield (kg/ha)</th>
<th>Shoot N (kg/ha)</th>
<th>Shoot P (kg/ha)</th>
<th>% AMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.27b</td>
<td>2427.1a</td>
<td>11.06b</td>
<td>1.82b</td>
<td>15.80a</td>
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<tr>
<td>RP</td>
<td>36.55a</td>
<td>2579.3a</td>
<td>18.49a</td>
<td>2.98e</td>
<td>17.85b</td>
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<tr>
<td>SSP</td>
<td>37.90a</td>
<td>2594.0a</td>
<td>19.51a</td>
<td>3.19a</td>
<td>24.93e</td>
</tr>
</tbody>
</table>

Means that have same alphabets are not significantly different

Table 2: Effect of previous soybean lines and herbaceous legumes on growth parameters and grain yield of maize grown in low P soil at Fashola

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Shoot DWT (g/plant)</th>
<th>Grain yield (kg/ha)</th>
<th>Shoot N (Kg/ha)</th>
<th>Shoot P (Kg/ha)</th>
<th>AMF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGM 1540</td>
<td>37ab</td>
<td>2797abc</td>
<td>19.73ab</td>
<td>2.38bc</td>
<td>19bcdef</td>
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<tr>
<td>TGM 1251</td>
<td>32abc</td>
<td>2700abc</td>
<td>15.88bc</td>
<td>2.55bc</td>
<td>25a</td>
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<td>TGM 1566</td>
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<td>2480abc</td>
<td>15.27bc</td>
<td>2.70abc</td>
<td>16gh</td>
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<td>TGM 1360</td>
<td>29c</td>
<td>2350bc</td>
<td>15.09c</td>
<td>2.69abc</td>
<td>13h</td>
</tr>
<tr>
<td>TGM 1293</td>
<td>31bc</td>
<td>2349bc</td>
<td>16.56abc</td>
<td>2.73abc</td>
<td>17efg</td>
</tr>
<tr>
<td>TGM 1039</td>
<td>27c</td>
<td>2337bc</td>
<td>14.56c</td>
<td>2.10bc</td>
<td>17defg</td>
</tr>
<tr>
<td>TGX 1456</td>
<td>34abc</td>
<td>2435abc</td>
<td>17.12abc</td>
<td>2.88abc</td>
<td>17efg</td>
</tr>
<tr>
<td>TGM 0944</td>
<td>34abc</td>
<td>2700abc</td>
<td>15.97bc</td>
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<td>21bc</td>
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<td>TGM 1196</td>
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<td>2921ab</td>
<td>15.21bc</td>
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<td>TGM 1511</td>
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<td>TGM 1420</td>
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<td>2183bc</td>
<td>14.54c</td>
<td>2.02c</td>
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<td>TGM 1419</td>
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<td>13.81c</td>
<td>2.43bc</td>
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<td>TGM 1576</td>
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<td>2034c</td>
<td>17.32abc</td>
<td>2.57bc</td>
<td>18bcdef</td>
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<td>Crop</td>
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<td>Value 2</td>
<td>Value 3</td>
<td>Value 4</td>
<td>Value 5</td>
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<tr>
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<tr>
<td>Lablab</td>
<td>39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3180&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.00&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20&lt;sup&gt;bde&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mucuna</td>
<td>31&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2267&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>16.59&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>20&lt;sup&gt;bde&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maize</td>
<td>39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2558&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>20.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means that have same alphabets are not significantly different