I. Progress Report 2003

A. Cover Page

Project Title:

Adaptation of Soybean to Low Phosphorus Soils of Tropical and Subtropical South China: A Radical Approach

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Executive Summary

In 2003, we continued to make excellent progress towards the research and training objectives of the project. The following progress and results were achieved during the past year: 1) About 50 elite soybean germplasm materials identified from last years' 'core collection' screening were further verified for P efficiency in two separate field sites for the second year to provide future breeding materials and to elucidate the relationship between important root traits and P efficiency. Meanwhile, our breeding program has resulted in 8 P-efficient, high-yielding soybean lines that will be included in the next year's official Chinese regional variety trials. 2) Physiological studies in the labs of both China and USA continued to provide evidence that important root traits including root morphology, root architecture and root organic acid exudation play important roles in P uptake efficiency and adaptation to adverse soil conditions. 3) Genetic systems were established for mapping and cloning of genes conferring superior root traits and P efficiency. A number of genetic populations were developed, a genetic map was constructed, and some candidate genes for P efficiency/root development were cloned. 4) Agroecological studies were continued in the field and information on the biotic environment for soybean production in South China was gathered to facilitate modeling of ecosystematic impacts of improved soybean germplasm. 5) Data from agroeconomic and social survey were being analyzed to assess likely economic impacts of improved soybean germplasm in South China. 6) Joint-training programs continued with reciprocal visits of students between PSU and SCAU. A meeting between the PIs of both partners was held to discuss issues concerning joint efforts in research extension to African countries beyond the current project phase.

B. Research and Training Progress (by Module)

1. Module 1: Germplasm Screening and Conventional Breeding

In 2003, about 50 elite soybean germplasm materials identified from the previous years' 'core collection' screening was further verified for P efficiency for two more seasons (Spring and Fall) in the Boluo and Yingde field sites. These materials were compared with several local conventional varieties under both low and high P conditions in terms of biomass and grain yield. Most of the P-efficient genotypes identified in the previous year continued to prove their superiority over local varieties for P uptake, biomass and grain yield under low P conditions (Fig. 1). These genotypes were selected as future breeding parents and contrasting materials for physiological and genetic studies.

Simultaneously, conventional breeding programs were continued in 2003 in the three breeding nurseries located at SCAU, Boluo, and Guangxi using different approaches including:

- Hybrid breeding: In the Spring of 2003, more than 2000 lines were obtained from our on-going soybean breeding program. These lines were tested in both normal and low-P soils for two seasons. About 30 desirable lines were selected and tested in Hainan Island (in the tropics) during the winter season. Some well-adapted lines with high-yielding characters were selected to participate in the Chinese regional variety trials for tropical and subtropical regions in 2004 (Fig.2). Meanwhile, we propagated about 200 populations derived from crosses made over the last several years and obtained seeds for the F_1, F_2, F_3, F_4, and F_5, respectively. Many desirable lines from F_4 and F_5 populations were identified and will be tested over various environments next year.

- Recurrent breeding: Recurrent breeding was continued in 2003 from the diallel crossing program conducted last year. The recurrent selection populations are being propagated and selection of promising populations/lines will be made from beginning in 2004.

- Mutation breeding: Mutagenesis with ^{60}Co radiation did not result in significant variations for traits of interest in previous work. In 2003 we tried a double mutagenic method by using ^{60}Co radiation followed by ethyl methane sulfonate (EMS) treatment. The M_0 generations showed significant variation for both shoot and root morphology. Seeds of M_1 have been obtained and the plants will be further screened and selected in the field in 2004.

2. Module 2: Physiological Mechanisms and Root Trait Identification

1) Root Morphology and Architecture

At SCAU, root traits of contrasting genotypes from the field screening were further characterized in the lab with the paper pouch growth system, sand culture system and the recently established phytotron-rhizotron system (Fig 3). Additional evidence is being accumulated that supports the hypothesis that changes in root morphology and root architecture are induced by P treatments, and such changes are related to soybean's adaptation to low P availbility. Further studies are being conducted to characterize the physiological and molecular mechanisms of P-induced root exudation and its significance in phosphorus efficiency.

At PSU, graduate student Catalina Posada has initiated a survey of a wide range of material from the USDA soybean germplasm collection for genetic variation in root hair traits. Selected genotypes were characterized for root hair and other traits important for P acquisition in soybean. Digital images of root hairs were collected for analysis of root hair density, elongation, and distribution. Meanwhile, Chinese graduate student Ruibin Kuang from SCAU visited PSU for short-term training under the supervision of Dr. Jonathan Lynch, where she completed a study assessing the role of root architectural patterns in regulating nitrogen fixation in soybean under low P conditions.

2) Root Exudation

Experiments were continued at SCAU to investigate the role of root exudates in mobilizing sparsely available soil P. We focused on exudation of organic acids induced by phosphorus deficiency (-P) and/or aluminum toxicity (+Al) in contrasting soybean genotypes as related to internal organic acid concentration and related enzyme activities. Our data showed that soybean genotypes differ in the type and quantity of organic acids excreted from the roots under P stress, which contributes to greater P uptake in P-efficient genotypes . Further studies demonstrated that organic acid exudation is differentially induced by -P and +Al in soybean plants, with specific induction of oxalate and malate by -P and citrate by +Al, indicating their respective functions in P efficiency and Al resistance (Fig. 4).

Our physiological studies on P efficiency as related to important root traits in soybean have resulted in many new scientific findings that are being submitted to both Chinese and international journals for publication.

3. Module 3: Molecular Biology of Root Traits and Development of Genetic Systems for Improving Root Traits and P Efficiency

1) Gene Mapping

More RILs and other genetic populations of soybean were developed in 2003. We also began construction of a soybean genetic map for identification of QTLs associating with P efficiency. First, a specific mapping population was developed using the seeds from one F_1 plant from the cross between two parents contrasting in P efficiency and root traits from previous field and greenhouse experiments. The F_2 segregating population with 110 individuals was used for map construction. About 360 SSR (Simple Sequence Repeat) markers distributing over all 20 chromosomes of soybean were screened for polymorphism between the parents, among which 117 markers were detected with SSR length polymorphism, with a polymorphic rate of 36.6%. Segregation analysis was conducted for alleles at each polymorphic SSR locus in the above F_2 population (Fig. 5), and the map is being constructed with MAPMAKER program.

2) Gene Cloning

At SCAU, suppression subtraction hybridization (SSH) techniques were employed to identify cDNAs closely related to root traits and P efficiency (Fig. 6). More than 100 candidate genes were cloned and sequenced, among which several genes were confirmed by Northern Blot to be specifically expressed in the root, with enhanced expression under low P availability. cDNA clones previously identified are being characterized for their possible physiological functions.

At PSU, several mutations in the model plant *Arabidopis thaliana* affecting root hair development have been obtained and characterized. We investigated the effects of these mutations on phosphorus signaling and response. After growth in high and low P media, images of roots and root hairs were taken and were analyzed using the METAMORPH program. An interesting response to P starvation was found in three of the mutants. In the mutants *glabra 2(gl2)*, *transparent testa glabra* (*ttg*) and *werewolf* (*wer*), root hairs develop both from cells which normally produce root hairs (H cells) and from the epidermal cells which do not normally produce root hairs (N cells). Under P deficiency, only the root hairs forming from H cells have the typical elongation response to P deficiency. Based on this finding we decided to use one of these mutants as a background for mutagenesis in order to find mutants that are impaired in the response to P starvation but not in root hair development. On the basis of information from this approach, candidate genes controlling root hair development are being identified in soybean.

4. Module 4: Agroecological Analysis

1) Phosphorus-efficient Soybean Genotypes in Different Cropping Systems

A second-year field experiment was conducted in the Boluo field site with previously identified soybean genotypes contrasting in P efficiency to study the effects of P-efficient genotypes on the crop yield of soybean and maize in a soybean-maize intercropping/rotation system. More data are being accumulated on the beneficial effects of P-efficient genotypes, which seemed to have greater overlapping of the root systems between soybean and maize.

Meanwhile, greenhouse experiments were conducted at SCAU employing both root growth container (RGC) and minirhizotrons to study the root dynamics of different soybean genotypes and possible effects of root overlapping on maize growth (Fig 7). Other aspects of root interactions including mycorrhizal and rhizobium symbioses were also investigated.

2) Phosphorus Cycling

In 2003, early and late season field experiments were conducted in the Boluo field site to study the impacts of adopting P efficient genotypes on nutrient cycling in low-P red soils. Four soybean genotypes (2 P-efficient and 2 P-inefficient) were planted in the field with both low and high P treatments. The P input (P from seeds, irrigation water, fertilizers, rain etc.) and P output (P from run-off, percolation, harvest etc) were analyzed to estimate phosphorus budgets in the field. Run-off and percolation collection facilities were installed in field plots to collect water and analyze the P content of collected water (Fig). Genotypic effects on soil P fractions (Ca-P, Fe-P, A1-P, O-P and organic P) are also being characterized.

In October 2003, PSU graduate student Raul Jaramillo visited SCAU to study ecosystem impacts of improved soybean germplasm. Meteorological information available at SCAU was collected and entered in the DSSAT model with the help of WEATHERMAN, a utility that assists the input and analysis of weather information. Descriptive statistics were obtained and compared to the summary information available from FAOCLIM 2.01 and to the averages of the Boluo field site. Besides, soil information was obtained from several sources describing the typical soils of South China. Soil samples were taken from the field site at Boluo and analyzed for texture and bulk density at the facilities of SCAU. Combining the information from secondary sources and the data obtained from Boluo, a general soil file was created and entered on DSSAT with the characteristics of the "Deep Latosol" sub-group. With the weather and soil information collected, the DSSAT model is being tested to assess ecosystem impacts of improved soybean germplasm.

5. Module 5: Agroeconomic Studies and Impact Assessment

Based on the background information obtained previously, a farm survey was designed, pretested and administered in Chinese within the project area to establish base line estimates of the costs of production, input requirements (for labor, material, and machinery), and identify key constraints on current productivity and efficiency of key crops (rice, sugarcane, soybean). Students from SCAU were trained to conduct the farm surveys and the survey was administered to over 100 farm operators. Responses were translated to English and entered into data set. Preliminary statistical analysis was completed for soybean. As expected, substantial variations exist across observations; however, some clear conclusions can be drawn. First, soybean germination rates and yields per land area are extremely low by world standards. Second, reliance on hand seeding, weeding, cultivation, and harvesting is typical. Third, producers view soybean as a food crop for home consumption rather than market commercialization. The data are being analyzed by Dr. R. Weaver and his Ph.D. student at PSU to 1) examine heterogeneity across operators, 2) establish simple operating budget for each crop and for each unique production configuration, 3) evaluate the profitability of production of existing crops based on nearby international border prices for the crop outputs and inputs to gauge relatively comparative advantage based on alternative cultivars, and price structure. A new faculty member at SCAU with expertise in economics was recently recruited to assist Dr. Weaver and his student for these activities.

6. Module 6: Joint-Training, Communication and Management

1) Joint training

Ruibin Kuang, a graduate student supervised by Dr. Xiaolong Yan at SCAU, visited Dr. Jonathan Lynch's lab at the Pennsylvania State University (PSU) from July to December 2003 for a 6-month training in physiological studies on phosphorus and nitrogen interactions in soybean. During her stay at PSU, Ms Kuang conducted physiological studies on phosphorus and nitrogen interactions in soybean with significant progress made. Two more trainees were selected to conduct collaborative research at PSU in 2003. Unfortunately, they did not realize the trips to the US due to visa problems from the US consulate.

Reciprocally, Raul Jaramillo, a graduate student supervised by Dr. Jonathan Lynch of PSU, visited SCAU for one month in October 2003 to conduct agroecological studies on soybean P nutrition in the field.

At PSU, 3 new students were recruited for this project: Lin Zhang (Chinese citizen) in Economics, Eric Nord in Ecology, and Amelia Henry in Ecological and Molecular Plant Physiology. All three have outstanding qualifications for this project and have begun their research in the Fall of 2003.

2) Communications and Management

During the past year, the two PIs representing SCAU and PSU partnerships, Drs. Xiaolong Yan and Jonathan Lynch, have been in regular contact through email and telephone calls to discuss scientific and management issues related to the project. Dr. Xiaolong Yan visited PSU from September to November, during which he closely interacted with his PSU partners to further strengthen the partnership. During his stay at PSU, he summarized research progress and delineated future plans for both sides. He also had a telephone conference with Dr. Robert Shaffert, the PI of the Brazil-Soil project, to discuss issues concerning inter-project research collaborations and joint efforts in research extension to African countries. Before returning to China, Drs. Yan and Lynch visited Dr. Rebecca Nelsen, the CCRP program leader, at Cornell University to provide project updates and discuss future research and extension of legume production technologies to African countries beyond the current project phase.

At both SCAU and PSU, the sub-committee met periodically, sometimes on module basis, to report project progress and discuss problems and constraints encountered. An annual report meeting was held at the Root Biology Center of SCAU on December 30, 2003. More than 20 project participants at SCAU (including both staff members and students) attended the meeting, during which the major participants of each research module (i.e., conventional breeding, physiology, molecular biology, agro-ecology, and agro-economics) reported research progress in the past year and proposed plan for the next year.

C. Problems and Constraints

In general, this project is progressing very well and many preliminary results are promising and encouraging. Previous constraints mentioned in the last year's annual report (i.e. severe drought problem in one of the field sites and vacancy of the Chinese agroeconomist) were solved by having successfully selected a new field site and recruited a new economics faculty member at SCAU. We did not encounter major difficulties last year and do not foresee major problems in 2004, except that we may still have visa problems for the trainees to visit PSU. Two of the trainees were blocked for their entry visas to the US for some unknown reasons by the US consulate in Guangzhou. We hope things will be better in the year of 2004.

D. Summary of the Status of the IP Plan

As soon as the project was funded in 2001, the partnership of this project began preparation of the Intellectual Property Management Plan (IP Plan) required for CCRP grantees with the expectations and guidelines provided by the McKnight Foundation. During the Mexico conference in March 2002, an outline of the IP plan was presented for comments and suggestions from the partners as well as from IP professionals. Later in July 2002, a draft of the IP plan was sent to the Central Advisory Service (CAS) selected by the McKnight Foundation. With the assistance of professionals from CAS, an IP plan is being completed. Communications between the PIs of the project and CAS continued in 2003 to improve the IP plan.

E. Emails from PIs

1. An Email From Dr. Jonathan Lynch (PI-PSU)

----- Original Message -----
From: Jonathan Lynch
To: Xiaolong Yan
Sent: Tuesday, January 27, 2004 9:56 PM
Subject: Re: 2003 annual report

Dear Xiaolong,

I have read the final version of the 2003 annual report for the McKnight project, and I approve and endorse it completely.

Thank you for your collaboration in 2003- I look forward to working together in 2004.

Warm Regards,

Jonathan

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2. An Email From Dr. Yaoguang Liu (coPI-SCAU)

----- Original Message -----
From: <ygliu@scau.edu.cn>
To: <xlyan@scau.edu.cn>
Sent: Monday, January 26, 2004 5:34 PM
Subject: Re: 2003 annual report

Xiaolong:

Thank you for the report. The report was prepared well and I agree with the contents.

Yaoguang

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> Yaoguang Liu
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3. An Email From Dr. Hong Ma (coPI-PSU)

----- Original Message -----
From: Hong Ma
To: xlyan ; Jonathan Lynch ; Liu Yaoguang
Sent: Monday, January 26, 2004 2:27 AM
Subject: Re: 2003 annual report

Dear Xiaolong:

Thank you very much for working with Jonathan on writing an excellent report. I agree with the content of the report. In addition, I have made some editorial revisions, using track change function so you can see them easily. Please feel free to accept or reject them.

I attach the files for revised parts I and III.

Thank you and best wishes.

Hong

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