# I. Progress Report 2004

# *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 2004, 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) Screening of soybean germplasm materials from the 'core collection' was continued in the field, with more contrasting materials identified for on-going physiological studies and breeding work. Meanwhile, our breeding program continued to produce phosphorus-efficient, high-yielding soybean lines, among which 18 promising lines (9 Spring varieties and 9 Summer varieties) were included in the China National Regional Variety Trials in 2004 with outstanding results. 2) Physiological studies both in China and USA were continued to identify important root traits as well as other physiological parameters contributing to phosphorus efficiency in soybean. 3) Genetic studies were continued for mapping and cloning of genes conferring superior root traits and phosphorus efficiency. A molecular linkage map was constructed with some important QTLs identified, and some candidate genes previously isolated were further analyzed for their possible functions in phosphorus efficiency and/or root development. 4) Agroecological studies were continued both in the field of South China and in controlled environment at PSU to study the ecosystematic impacts of improved soybean germplasm. 5) Data from agroeconomic and social survey were 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 during the CCRP conference in the Netherlands followed by a trip of a PI to Mozambique and Colombia to delineate research strategies in Africa in the second phase of the project.

# *B. Research and Training Progress (by Module)*

## 1. Module 1: Germplasm Screening and Conventional Breeding

In 2004, screening of soybean germplasm materials from the 'core collection' was continued in the field for two more seasons (Spring and Fall) at the Boluo field site. These materials, together with breeding lines resulting from our conventional breeding programs, 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 years continued to prove their superiority over local varieties for P uptake, biomass and grain yield under low P conditions. Promising genotypes were collected in our own germplasm bank which will be used either as future breeding parents and contrasting materials for physiological and genetic studies.

Meanwhile, our conventional breeding programs were continued in the three breeding nurseries located at SCAU, Boluo, and Guangxi using different approaches previously described (i.e., hybrid breeding, recurrent breeding, and mutation breeding. See Annual Reports 2002, 2003). This has resulted in many phosphorus-efficient, high-yielding soybean lines, among which 18 promising lines (9 Spring varieties and 9 Summer varieties) were included in the China National Regional Variety Trials in 2004 (Fig. 1). The results are summarized in Tables 1 and 2. It can be seen from the tables that four of our Spring breeding lines tested were better than or equal to the official “best check” variety, while all of our Summer breeding lines tested were significantly better than the official “best check” variety.

Table 1. Average yield of selected soybean breeding lines in the National Regional Variety Trials (Spring, 2004)

| Line Name | Plot Yield (kg/10m$^2$) | Estimated Yield (kg/ha) | Multiple Comparison p=0.05 | Multiple Comparison p=0.01 | Increase over CK (%) |
|---|---|---|---|---|---|
| Gui0120-1 | 2.70 | 2702 | a | A | 10.47 |
| Yuechun03-4 | 2.59 | 2592 | ab | AB | 5.95 |
| Yuechun03-5 | 2.58 | 2579 | b | AB | 5.41 |
| Yuechun03-3 | 2.50 | 2506 | bc | BC | 2.43 |
| **Liudou1(CK)** | **2.45** | **2446** | **cd** | **BCD** | — |
| Gui0118-1 | 2.39 | 2390 | cd | CD | -2.32 |
| Yuechun03-2 | 2.33 | 2333 | d | DE | -4.65 |
| Gui0112-2 | 2.18 | 2183 | e | EF | -10.79 |
| Yuechun03-1 | 2.07 | 2072 | ef | FG | -15.31 |
| Gui0112-1 | 2.01 | 2008 | f | G | -17.9 |

Note: Values in the table are means of 9 trial plots located in 5 different provinces of South China. Liudou1 is an official “best check” Spring soybean variety for tropical-tropical regions in China.

Table 2. Average yield of selected soybean breeding lines in the National Regional Variety Trials (Summer, 2004)

| Line Name | Plot Yield （kg/10m$^2$） | Estimated Yield (kg/ha) | Multiple Comparison p=0.05 | Multiple Comparison p=0.01 | Increase over CK （%） |
|---|---|---|---|---|---|
| Yuexia03-3 | 3.02 | 3018 | a | A | 36.64 |
| Gui0114-2 | 2.97 | 2971 | ab | AB | 34.5 |
| Gui0114-1 | 2.95 | 2949 | abc | AB | 33.49 |
| Gui0103-1 | 2.86 | 2861 | bcd | ABC | 29.51 |
| Gui0104-1 | 2.75 | 2746 | de | CDE | 24.33 |
| Yuexia03-5 | 2.71 | 2716 | def | CDE | 22.95 |
| Yuexia03-1 | 2.64 | 2645 | ef | DE | 19.75 |
| Yuexia03-4 | 2.58 | 2579 | f | EF | 16.76 |
| Yuexia03-2 | 2.43 | 2431 | g | FG | 10.04 |
| **Guixia1（CK）** | **2.21** | **2209** | **h** | **H** | — |

Note: Values in the table are means of 9 trial plots located in 5 different provinces of South China. Guixia1 is an official "best check" summer soybean variety for tropical-tropical regions in China.

## 2. Module 2: Physiological Mechanisms and Root Trait Identification

At SCAU, we continued to characterize root traits of contrasting genotypes both in the field and in the lab using the paper pouch growth system, sand culture system and the phytotron-rhizotron system, with focus on phosphorus-induced changes in root morphology (root hairs) and root architecture and their relationship to soybean's ability in response to altered P nutrition. Studies were also 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 began a wide germplasm survey for genetic variations of root hair traits in soybean using a soybean collection from USDA. She has identified one particular set of parents (Essex and Forrest) that are contrasting in root hair length and density under the growth conditions used (Fig 2). These materials, together with their recombinant inbred line progenies, will be used for further physiological and genetic studies.

Meanwhile, research was conducted to investigate physiological parameters other than root traits that may contribute to phosphorus efficiency in soybean. Among this, PSU graduate student Eric Nord conducted two field trials to understand the effect of differing maturity times on P efficiency in soybean varieties. The first trial was carried out at the Boluo field site in Guangdong province, China, in collaboration with the Root Biology Center at SCAU. The second trial was carried out at the Rock Springs Experimental Farm at PSU. Preliminary results indicate that increased time to maturity is correlated with increased seed yield under both low and high P conditions (Fig. 3).

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

### 1) Gene Mapping

At SCAU, a soybean genetic map was constructed for tagging of QTLs associated with P efficiency, using a $F_2$ population with 110 individuals derived from a cross between BD2 and BX10. Totally 442 SSR primers distributed over the 20 chromosomes of soybean (Soybase, 2003) were screened for polymorphism between BX10 and BD2, and 252 markers generated polymorphic band patterns between parents. Among them 161 SSR markers (36.4%) were co-dominant and were used in map construction. The map consists of 21 linkage groups, which include 146 SSR markers (15 markers unlinked) and 1 morphological marker—flower color, and covers 1698.1 cM, with an average genetic distance of 11.5 cM. For mapping traits associated with agronomic traits under low P stress, 120 F2 seedlings were grown in a low-P (0.2 uM) nutrient solution for two weeks. Three traits, i.e., root dry weight (RDW), shoot P content (SPC), and stem node number (SNN) were evaluated, and QTLs conferring these traits were detected and shown on Fig. 4.

At PSU, a genetic map of Essex × Forrest based on 181 RAPD and RFLP markers plus 240 microsatellite markers was employed by graduate student Catalina Posada for identification of QTL related to root hair traits in soybean. RILs of Essex × Forrest were obtained from Dr. D.A. Lightfoot of the Southern Illinois University at Carbondale. These 100 RILs and the parents were screened in cigar roll growth system in high and low P nutrient media using buffered Al-P as a source of phosphorus. Selected RILs contrasting in root hair and density are being evaluated in a greenhouse experiment using growing media containing red soil in order to determine whether the differences observed in the cigar roll growth system reflects root hair growth in a more realistic medium. Phenotypic data obtained will be used to identify QTL associated with different traits.

### 2) Gene Cloning

At SCAU, more candidate genes putatively related to root traits and/or P efficiency were identified using the suppression subtraction hybridization (SSH) technique. 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. The cDNA clones previously identified are being characterized for their spatial and temporal expression patterns (Fig. 5)

At PSU, two trainees from SCAU, Mr. Yingxiang Wang and Dr. Chuxiong Zhuang, conducted functional analysis of a candidate gene (*GmMAPK*1) under the supervision of Prof. Hong Ma at PSU. They employed *in situ* hybridization and other techniques to study the possible function of this gene in relation to root growth and development (Fig. 6).

## 4. Module 4: Agroecological Analysis

### 1) Phosphorus-efficient Soybean Genotypes in Different Cropping Systems

In 2004, a third-year field experiment was continued at 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 in a soybean-maize intercropping/rotation system. Our results of the third year continued to provide evidence for the beneficial effects of P-efficient genotypes on plant growth and soil fertility maintenance. The effect of soybean intercropping and/or rotation on maize yield is shown on Fig. 7.

Meanwhile, we continued to conduct greenhouse experiments at SCAU using both root growth container (RGC) and minirhizotrons to study the root dynamics of different soybean genotypes (Fig. 8). The possible effects of plant interactions with mycorrhizal and rhizobium symbioses were also investigated.

### 2) Phosphorus Cycling

In 2004, early and late season field experiments were continued at the Boluo field site using run-off and leachate collection facilities installed in field plots 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, etc) were analyzed to estimate phosphorus budgets in the field. This year's result continued to indicate that the P-efficient genotype tend to reduce P losses from both run-off water and leaching into the subsoil (Table 3)

Table 3. Soluble P content (mg/L) in the run-off water and leachate in the field planted with two contrasting soybean genotypes

| Soybean Variety | Low P Treatment | | High P Treatment | |
|---|---|---|---|---|
| | Runoff | Leachate | Runoff | Leachate |
| BX 10 (P efficient) | 0.74±0.07 a | 0.37±0.08 a | 1.51±0.24 a | 0.29±0.05 a |
| BD 2 (P inefficient) | 1.00±0.06 b | 0.33±0.08 a | 2.16±0.28 b | 0.67±0.08 b |

Note: Values in the tables are means of 4 replicates with standard errors. Values with the same letter in the same column are not significantly different (p=0.05).

Meanwhile at PSU, graduate student Amelia Henry conducted a rainfall simulation on soil plots with bean genotypes DOR364 (deep-rooted) and G19833 (shallow-rooted) and soybean genotypes 224 (deep) and Brazil 10 (shallow) plus unplanted control plots using the protocol established by the National Phosphorus Research Project to collect runoff (Figure 9). Significant differences in erosion between genotypes with different root structure were not observed. This might be due to the young age of the plants at the time of rainfall simulation or the lack of steep slope in the plot. Therefore, computer model RUSLE is being employed to examine the effects of slope on erosion.

Also at PSU, graduate student Raul Jaramillo continued his efforts in the assessment of crop growth and phosphorus cycling employing simulation models. Several changes have been made to our geometric model SimRoot in order to accommodate the construction and maintenance carbon costs of the root. At this moment this model creates a root in response to the parameters passed by the user, and considering the limitation of assimilates at different moments. An important improvement could be attained if a mechanistic representation of carbon acquisition and allocation could be coupled with SimRoot. For this, we have selected the CROPGRO model (Boote et al 1998), which is part of the Decision Support System for Agrotechnology Transfer (DSSAT, Jones et al 2003). A deficiency in this model is the over-simplification of root growth and distribution. The combination of SimRoot with CROPGRO is a potential solution to this limitation, and as mentioned this will provide us with a realistic estimation of assimilates allocated in the root inside SimRoot simulations. Several contacts were made with the group of developers working with CROPGRO and DSSAT, who kindly facilitated the use of a beta-version of DSSAT v4 in which numerous simulations were made to estimate the flexibility of the model in the response to changes in root allocation (Figure 10).

## 5. Module 5: Agroeconomic Studies and Impact Assessment

At PSU, efforts were continued by Dr. R. Weaver and his Ph.D. student Lin Zhang to analyze the farm survey data obtained previously from South China to assess the agroeconomic impacts of phosphorus efficient soybean varieties. A SCAU faculty member in agroeconomics, Ms Huixia Cui, went to PSU to assist Dr. Weaver and his student for these activities. Costs, returns, and profitability of soybeans, sugarcane, rice, and other minor crops were estimated based on 2002 farm level survey. This work clarified the substantial relative unprofitability of current varieties of soybeans relative to other crops (Table 4). These results suggest that household nutritional preferences drive the current growing of soybeans. It further motivates the market level studies to be conducted in 2005.

Table 4. Farm budget estimated from data of farm level survey in 2002

| Production system | Average Yield (kg/mu) | Non-labor cost (Yuan/mu) | Hired labor cost (Yuan/mu) | Total expenses (Yuan/mu) | Profits (based on mkt price 2.49 Yuan/kg) |
|---|---|---|---|---|---|
| Without oxen and tractor | 179.6 | 428.18 | 53.78 | 481.96 | -50.38 |
| Use oxen | 175.3 | 444.26 | 74.41 | 518.67 | -90.09 |
| Use tractor | 161.46 | 557.74 | 53.78 | 611.52 | -182.84 |
| Average | 172.12 | | | | -108.81 |

In addition, domestic resource costs analysis was conducted using the previous farm level survey data to determine the profitability from a social perspective of soybean production in South China. The results indicate that not only are current practices and varieties unprofitable from a private perspective, but they are also not economically rational to produce from a societal perspective. This analysis considers the cost of inputs and the value of the output at the farm compared to use of imported inputs and purchase of imported soybeans. The results suggest that substantial costs are currently associated with bringing imported inputs and soybeans to the farm. This suggests that substantial costs are also associated with current marketing systems that would be used to collect and distribute soybeans from the farm to consumers. This motivates the need for the market level studies to be conducted in 2005.

In conclusion, the major findings from the agroeconomic analyses are: 1) Profit will determine whether rural households adopt new varieties; 2) Current varieties and production systems are not viable on a monetized basis. Households produce only for their own needs; 3) Potential of doubling or tripling of yields will produce no economic value unless supported by development of markets; 4) Market sale of soybean supply will only occur when rural households view soybeans as providing returns to labor that exceed returns available from other activities.

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

### 1) Joint training

Three trainees from SCAU went to PSU for collaborative research in 2004. Mr. Yingxiang Wang, a graduate student supervised by Dr. Xiaolong Yan at SCAU, visited Dr. Hong Ma's lab at PSU in September 2004 for a 6-month training in molecular studies on root growth and development in soybean. During his stay at PSU, he conducted functional analysis of a candidate gene (*GmMAPK1*) under the supervision of Dr. Hong Ma at PSU. Another trainee from SCAU, Dr. Chuxiong Zhuang, later went to PSU in October to participate in the same research project. Ms Huixia Cui, a newly-recruited project member in the agroeconomics module at SCAU, visited Dr. R. Weaver's lab at PSU to assist him and his graduate student Lin Zhang to analyze the farm survey and data obtained previously from South China. As those trainees previously sent, Zhuang and Cui returned to SCAU immediately after the training period was over and Wang will come back in March 2005 as scheduled.

Reciprocally, Eric Nord, a graduate student supervised by Dr. Jonathan Lynch of PSU, visited SCAU for one month in May 2004 to conduct agroecological studies on soybean P nutrition in the field. He carried out a field trial at the Boluo field site in Guangdong province, PRC, in collaboration with the Root Biology Center at SCAU.

## 2) Communications and Management

As usual, the two PIs representing SCAU and PSU partnerships, Drs. Xiaolong Yan and Jonathan Lynch, have been in regular contact through email and telephone calls during the past year to discuss scientific and management issues related to the project. At both SCAU and PSU, the sub-committee met periodically, sometimes on module basis, to report project progress and discuss problems and constraints encountered. We successfully constructed a web site (http://www1.scau.edu.cn/zyhjxy/mcknight/index.htm) specifically devoted to this project to facilitate communications among the participants in this project. This website is linked to the McKnight Foundation's CCRP website for better information exchanges among participants from other CCRP projects as well as scientists from all over the world.

In November 2004, seven members of the China-Soybean project, including Drs. Xiaolong Yan, Yaoguang Liu, Mantong Mei and Hai Nian from SCAU and Drs. Jonathan Lynch, Rob Weaver and Miss Amelia Henry from PSU, attended the 2004 Biennial CCRP Grantee Conference held in Vaals, the Netherlands. In addition to the scheduled program by CCRP, the project team had several discussions concerning the strategy for the next phase of the project that will be focus on research and extension of legume production technologies to African countries beyond the current project phase. The result of our strategy planning activities at Vaals is shown in Figure 11.

After the Vaals conference, Dr. Jonathan Lynch (PI-PSU) traveled to Mozambique and Colombia to discuss the second phase of the China-soybean CCRP project with team members and collaborators. In Mozambique he held discussions with national program scientists, university administrators, and USAID staff. In Colombia he held discussions with CIAT scientists working with bean genetics and breeding. He has drawn following conclusions from these meetings: 1) improved legume productivity, especially for common bean, in Mozambique and Malawi would make an important contribution to food security and economic development, 2) human capacity building, modest research infrastructure investments, and operational support are priority needs in Mozambique, 3) the overall project strategy and team composition was supported by all involved. A detailed proposal for the second phase of the project is being prepared.

# *C. List of Publications*

## 1. Peer-reviewed papers

1) Zhao J., Fu J., Liao H. et al., 2004. Characterization of root architecture in an applied core collection for phosphorus efficiency of soybean germplasm. Chinese Science Bulletin, 49(15): 1611-1620.
2) Wang L., Liao H., Yan X. et al., 2004, Genetic variability in root hairs as regulated to phosphorus status in soybean. Plant and Soil. 261 (1-2): 77-84.
3) Dong D., Peng X., Yan X. 2004. Organic acid exudation induced by phosphorus deficiency and/or aluminum toxicity in two contrasting soybean genotypes. *Physiologia Plantarum* 122:190-199

## 2. Theses

1) Zhao J. 2004. Adaptive Responses of Root Architecture to Phosphorus Deficiency and Cloning of Related Genes in Soybean. Ph.D. Thesis, South China Agricultural University, Guangzhou, China, 104 pp
2) Tian J. 2004. APase Activity in Soybean and Common Bean as Related to P Availability. Ph.D. Thesis, South China Agricultural University, Guangzhou, China, 118 pp
3) Wang LD. 2004. Genetic Variability for Root Hair Traits in Soybean as Affected by Phosphorus Availability. M.S. Thesis, South China Agricultural University, Guangzhou, China, 51 pp
4) Kuang RB. 2004. Nitrogen and Phosphorus Interactions as Related to Root Morphological-Architectural and Nodular Traits in Soybean. M.S. Thesis, South China Agricultural University, Guangzhou, China, 44 pp
5) Sun ZJ. M.S. 2004. Construction of A Soybean Genetic Linkage Map with SSR Markers and QTL Mapping. M.S. Thesis, South China Agricultural University, Guangzhou, China, 68 pp
6) Zhu LX. 2004. Effects of Different Soybean Genotypes on Phosphorous Efficiency and Microbial Biodiversity in the Soil-Plant Ecosystem. M.S. Thesis, South China Agricultural University, Guangzhou, China, 77 pp.

# *D. Emails from PIs*

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

----- Original Message -----
From: Jonathan Lynch
To: Xiaolong Yan
Sent: Saturday, January 29, 2005 5:27 AM
Subject: 2004 annual report

Dear Xiaolong,

this message is to confirm that I have read the annual report for the McKnight project in 2004, and endorse it.

Thanks

Jonathan

Jonathan Lynch
Professor
Dept. Horticulture
Penn State University
University Park, PA, 16802
814-863-2256, fax 3-6139
http://www.personal.psu.edu/faculty/j/p/jpl4/
"Science is the belief in the ignorance of experts"
Richard Feynman

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

----- Original Message -----
From: <ygliu@scau.edu.cn>
To: <xlyan@scau.edu.cn>
Sent: Saturday, January 29, 2005 12:34 PM
Subject: Re: 2004 annual report

Xiaolong:

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

Yaoguang

> Yaoguang Liu
> Professor of Molecular Biology
> Laboratory of Genetic Engineering
> South China Agricultural University
> Guangzhou 510642, China
> Tel. 86-20-85281908, Fax. 86-20-85280200

## 3. An Email From Dr. Hong Ma (coPI-PSU)

----- Original Message -----
From: Hong Ma
To: xlyan
Sent: Saturday, January 29, 2005 11:57 AM
Subject: Re: 2004 annual report

Dear Xiaolong:

I have no changes in the report. I agree with the report.

Thank you very much.

Hong

Hong Ma
Professor of Biology
Department of Biology
405D Life Sciences Building
The Pennsylvania State University
University Park, PA 16802