Final Report

รายงานวิจัยฉบับสมบูรณ์

Varietal Improvement of Peanut and Jerusalem Atichoke for Increasing Product Value and Functional Food Quality Project

โครงการปรับปรุงพันธุ์ถั่วลิสงและแก่นตะวัน เพื่อเพิ่มมูลค่าและคุณค่าเชิงอาหารสุขภาพ

By

Professor Dr. Aran Patanothai

Supported by

the Thailand Research Fund
and the Office of Higher Education Commission

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สัญญาเลขที่ **RTA5080001**

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Project Leader

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Acknowledgment

This project received financial support from the Thailand Research Fund (TRF) and the Office of Higher Education (CHE). The supports from the TRF were provided both directly to this project and through the Royal Golden Jubilee (RGJ) Ph.D. Program for the parts of thesis research of the RGJ Ph.D. students and the expenditures for their oversea advisors who have given valuable suggestions and have helped in editing the manuscripts for journal publication. In additions, the Department of Plant Science and Agricultural Resources of the Faculty of Agriculture and the Department of Biology of the Faculty of Science, Khon Kaen University provided supports for field, greenhouse and laboratory facilities for the implementation of project research. Thanks are extended to all persons who have given valuable advices, suggestions and assistances, which have helped the project to be able to accomplish the planned mission.

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Varietal Improvement of Peanut and Jerusalem Atichoke for Increasing Product Value and Functional Food Quality

Project Leader: Prof. Dr. Aran Patanothai

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Abstract

Peanut is an important food legume of Thailand. Its improvement in functional food quality will add more value to the crop. Jerusalem artichoke or “kaentawan” is a new crop to Thailand which has a great potential. The crop has inulin, a chemical compound that is beneficial to health. This project aimed to provide basic knowledge to support varietal improvement for increasing functional food quality of the two crops.

This project is essentially Phase III of the TRF Senior Research Scholar Project of the project leader. During Phases I and II, for peanut, basic research has been undertaken in four selected topics, i.e., peanut bud necrosis disease, utilization of peanut residues for soil improvement, the use of a crop simulation model, CROPGRO-Peanut, in assisting peanut variety evaluation, and various aspects of drought resistance of peanut genotypes. The latter three topics were continued in this project. A new area of research to support peanut breeding for high oleic to lenoleic ratio (O/L ratio) was also under taken to improve the functional food quality of peanut. For kaentawan, research to support varietal improvement for high inulin content was conducted.

The project covered the period from 1 September 2007 – 31 August 2010. The research team included 14 researchers, 4 research assistants, 13 Ph.D. students and 8 M.S. students. Research was organized into six sub-projects: (1) Multi-environment evaluation of advanced peanut lines, (2) Basic research to support breeding of peanut for drought resistance, (3) Basic research to support breeding of peanut for high oleic to linoleic acid (O/L) ratio, (4) Application of crop simulation model in crop breeding, (5) Utilization of peanut stover and other crop residues to improve crop productivity, and (6) Varietal improvement of kaentawan (jerusalem artichoke) for high inulin. The numbers of studies conducted were 3, 13, 4, 5, 3 and 4 for Sub-projects 1, 2, 3, 4, 5 and 6, respectively.

The project has strengthened the research capability of the 14 researchers in the team; all have gained their experiences in conducting quality research and supervising M.S and Ph.D. thesis research, and can seek their own research funds. For the 13 Ph.D. students, ten have finished their program. Seven of the eight M.S. students have graduated. As of 2 September 2010, 27 papers have been published or accepted for publication in accredited international journals, and one more has been submitted. Three annual seminars had been held during the period of the project. Linkages have also been established with 8 foreign institutes and 5 private enterprises

Research findings of the project have been utilized in the peanut and kaentawan breeding programs of Khon Kaen University. As a result, one new larges-seeded early-maturing peanut cultivar is about to release and three cultivars of kaentawan have been released to farmers.

สัญญาเลขที่ RTA5080001

โครงการปรับปรุงพันธุ์ถั่วลิสงและแก่นตะวันเพื่อเพิ่มมูลค่าและคุณค่าเชิงอาหารสุขภาพ

หัวหน้าโครงการ : ศ. ดร. อารันต์ พัฒโนทัย
ภาควิชาพืชศาสตร์และทรัพยากรการเกษตร คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น

เรื่องย่อ

ถั่วลิสงเป็นพืชตระกูลถั่วที่สำคัญชนิดหนึ่งของประเทศไทย การปรับปรุงคุณค่าเชิงอาหารสุขภาพจะเป็นการเพิ่มมูลค่าให้แก่พืชนี้ แก่นตะวันเป็นพืชใหม่สำหรับประเทศไทยแต่มีศักยภาพสูง พืชนี้มีสารอินนูลินซึ่งเป็นประโยชน์ต่อสุขภาพ โครงการนี้มุ่งที่จะสร้างองค์ความรู้พื้นฐานที่จะสนับสนุนการปรับปรุงพันธุ์พืชทั้งสองให้มีคุณค่าเชิงอาหารสุขภาพสูงขึ้น

โครงการนี้เป็นโครงการเมธีวิจัยอาวุโส สกว. ระยะที่ 3 ของหัวหน้าโครงการ ในช่วงระยะที่ 1 และระยะที่ 2 งานวิจัยพื้นฐานของถั่วลิสงได้ดำเนินการในสี่หัวข้อ ได้แก่ ต่อโรคใบไหม้ การใช้ซากถั่วลิสงในการปรับปรุงบำรุงดิน การใช้แบบจำลองการเจริญเติบโตของพืชในงานปรับปรุงพันธุ์ถั่วลิสง และประเด็นต่าง ๆ เกี่ยวกับการทนแล้งของถั่วลิสง สามหัวข้อหลังได้ดำเนินการต่อเนื่องมาถึงโครงการนี้ด้วย ประเด็นใหม่คืองานวิจัยที่จะสนับสนุนการปรับปรุงพันธุ์ถั่วลิสงให้มีสัดส่วนของกรดโอเลอิค/กรดลิโนเลอิคสูงเพื่อเพิ่มคุณค่าเชิงอาหารสุขภาพให้แก่ถั่วลิสง สำหรับแก่นตะวัน ได้ดำเนินงานวิจัยที่จะสนับสนุนการปรับปรุงพันธุ์เพื่อให้มีสารอินนูลินสูงขึ้น

โครงการมีช่วงเวลาตั้งแต่ 1 กันยายน 2550 ถึง 31 สิงหาคม 2553 ทีมวิจัยประกอบด้วยนักวิจัย 14 คน ผู้ช่วยวิจัย 4 คน นักศึกษาปริญญาเอก 13 คน และ นักศึกษาปริญญาโท 8 คน งานวิจัยแบ่งออกเป็นโครงการย่อย 6 โครงการคือ (1)การทดสอบพันธุ์ถั่วลิสงในหลายสภาพแวดล้อม (2) งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์ถั่วลิสงให้ทนแล้ง (3) งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์ถั่วลิสงให้มีสัดส่วนของกรดโอเลอิค/กรดลิโนเลอิคสูง (4) การใช้แบบจำลองการเจริญเติบโตของพืชในการปรับปรุงพันธุ์ถั่วลิสง (5) การใช้ซากถั่วลิสงและซากพืชอื่น ๆ เพื่อปรับปรุงประสิทธิภาพการผลิตพืช และ (6) งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์แก่นตะวันให้มีสารอินนูลินสูง จำนวนหัวข้อที่ศึกษาในแต่ละโครงการย่อย คือ 3, 13, 4, 5, 3 และ 7 สำหรับโครงการย่อยที่ 1, 2, 3 และ 4 ตามลำดับ

ในการสร้างนักวิจัย โครงการฯได้เพิ่มขีดความสามารถของนักวิจัยในทีมทั้ง 14 คน กล่าวคือ นักวิจัยมีประสบการณ์ในการทำวิจัยที่มีคุณภาพ และในการให้คำปรึกษาและควบคุมการทำวิจัยที่เป็นวิทยานิพนธ์ของนักศึกษาปริญญาโทและเอก และทุกคนมีความสามารถในการหาทุนวิจัยได้เอง ในบรรดานักศึกษาปริญญาเอก 13 คน 10 คนสำเร็จการศึกษาแล้ว ส่วนนักศึกษาปริญญาโท 7 ใน 8 คนสำเร็จการศึกษาแล้ว จนถึง 2 กันยายน 2553 โครงการฯมีรายงานวิจัยที่ได้รับการตีพิมพ์หรือได้รับการตอบรับให้ตีพิมพ์ในวารสารวิชาการนานาชาติที่เชื่อถือได้ 27 เรื่อง และมีรายงานที่ส่งไปตีพิมพ์อีก 1 เรื่อง ได้มีการจัดสัมมนาวิชาการประจำปีไปแล้ว 3 ครั้ง และได้เชื่อมโยงกับสถาบันในต่างประเทศ 8 สถาบัน และกับบริษัทเอกชน 5 บริษัท

ผลงานของโครงการฯ ได้นำไปใช้ในโครงการปรับปรุงพันธุ์ถั่วลิสงและโครงการปรับปรุงพันธุ์แก่นตะวันของมหาวิทยาลัยขอนแก่น เป็นผลให้มีพันธุ์ถั่วลิสงเมล็ดโตอายุสั้นพันธุ์ใหม่ที่กำลังจะเผยแพร่ออกสู่เกษตรกร และมีพันธุ์แก่นตะวันที่ได้เผยแพร่ออกสู่เกษตรกรแล่ว 3 พันธุ์

Project Code: RTA508001

Project Title : Varietal Improvement of Peanut and Jerusalem Atichoke for Increasing Product Value and Functional Food Quality

ชื่อโครงการ : การปรับปรุงพันธุ์ถั่วลิสงและแก่นตะวันเพื่อเพิ่มมูลค่าและคุณค่าเชิงอาหารสุขภาพ

Executive Summary

Project leader:

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Type of funding support: Senior Research Scholar

Research area: Agriculture

Duration: 1 September 2007 – 31 August 2010

Research team:

Researcher – 14
Research assistants - 4
Ph.D. students – 13
M.S. students – 8

Overall objectives:

1. To evaluate advanced peanut lines in multi-environment trials.
2. To generate basic knowledge for supporting varietal improvement of peanut and jerusalem artichoke.
3. To generate published papers in accredited international journals.
4. To improve the capability of researchers and train new researchers to produce high quality research.
5. To strengthen collaboration in peanut and jerusalem artichoke research between KKU and other institutes both within the country and overseas.

Expected outputs:

The project aimed to produce the following outputs:

1. New cultivars of peanut: 2
2. New cultivars of kaentawan: 2
3. Number of published papers in accredited journals: 15

4. Number of researchers with improved capability: 11
5. Number of M.S. and Ph.D. graduates produced: 15
6. Annual meeting held: 3
7. Number of foreign institutes that linkage established: 5
8. Number of private companies that linkage established: 2.

Background and scope:

Rapid changes in socioeconomic, environmental and cultural conditions have called for the adoption of sufficiency economy philosophy in agricultural development. This means that agricultural production must generate sufficient income to support decent livelihood of farmers and minimize all the risks. Production of diversified, high value products with functional food quality will both generate good income and reduce the risk from price fluctuation.

Peanut fits well with the above direction. The crop provides a significant source of supplementary income to a large number of rural people, and thus being a safeguard for the risk of failure or low price of the main crop. It also plays an important role in sustaining soil productivity through its nitrogen fixing ability. Current demand for peanut is high, particularly for processed products for both internal and export markets, but production is insufficient. Peanut products also have great potential for export if their quality meets the international standard. Improvement of product quality and production efficiency is, thus, essential for peanut production in Thailand. These require a strong support in research.

This project is essentially Phase III of the TRF Senior Research Scholar Project of the project leader. During Phases I and II, basic research has been undertaken in four selected topics, i.e., peanut bud necrosis disease, utilization of peanut residues for soil improvement, the use of a crop simulation model, CROPGRO-Peanut, in assisting peanut variety evaluation, and various aspects of drought resistance of peanut genotypes. The latter three topics were continued in this project. A new area of research to support peanut breeding for high oleic to lenoleic ratio (O/L ratio) was also under taken to improve the functional food quality of peanut.

To provide a new alternative to farmers and diversify the products, we have selected jerusalem artichoke (*Helianthus tuberosus* L.) to work on as it high has functional food quality which can command high price. We have named the crop in Thai as “kaentawan”. The crop produces tubers which contain a high amount of inulin which can be used in various ways and is high in economic value. The crop is currently grown commercially in many countries, but it is a new crop to Thailand. Initial testing showed a great potential of the crop and various promotion activities have been undertaken by KKU. The crop is becoming widely known, and is being grown commercially. The key component for the success of kaentawan production in Thailand is crop variety. Therefore, varietal improvement for high inulin content is of high priority, and supportive research was conducted in this project.

Research in the project is organized into six sub-projects:

Sub-project 1. Multi-environment evaluation of advanced peanut lines.

Sub-project 2. Basic research to support breeding of peanut for drought resistance.

Sub-project 3. Basic research to support breeding of peanut for high oleic to linoleic acid (O/L) ratio.

Sub-project 4. Application of crop simulation model in crop breeding.

Sub-project 5. Utilization of peanut stover and other crop residues to improve crop productivity.

Sub-project 6. Varietal improvement of kaentawan (jerusalem artichoke) for high inulin.

Highlights of research results:

Highlights of results for the individual subprojects are as follows:

Sub-project 1. Multi-environment evaluation of advanced peanut lines

1.1 Multi-environment trials (METs) of advanced large-seeded peanut lines selected for earliness have identified two lines that gave yield and seed size equivalent to the released large-seeded cultivars but were more than 10 days earlier in maturity.

1.2 METs of advanced peanut lines selected for peanut bud necrosis virus (PBNV) resistance have identified two high yielding PBNV resistant lines, one large-seeded and one small-seeded.

1.3 METs of advanced peanut lines selected for drought tolerance have identified three lines with higher yields than the standard checks, one large seeded and two small-seeded.

Sub-project 2. Basic research to support breeding of peanut for drought resistance

2.1 Chlorophyll content and density were found to be stable across environments. Their strong correlations with total dry matter and transpiration efficiency pointed out that they are useful traits conferring drought tolerance in peanut. Moreover, SPAD chlorophyll meter reading (SCMR) could be used as a rapid and cost effective tool for assessment of relative chlorophyll status in peanut leaves, and could be applied for indirect selection of drought tolerance in peanut.

2.2 Three peanut lines were identified as having drought avoidance mechanism based on root length density (RLD) and root distribution. The higher RLD at lower soil depths enhanced drought tolerance in these genotypes, enabling them to stabilize pod yield and HI under water stress conditions. Breeding for deeper rooting may facilitate the development of improved peanut cultivars in specific water limited-environments where water is available in deep soil.

2.3 The study on the effect of drought stress on nitrogenase activity, nodule number and nodule dry weight of 11 peanut genotypes with different degrees of drought resistance revealed that severe drought stress reduced all the three traits about two times greater than did mild drought stress. Nodule dry weight was closely related with nitrogenase activity under drought conditions. High nitrogenase activity under mild drought condition was related to its high potential and to a low rate of its reduction in response to stress. The contribution of the potential was lower under more severe drought conditions. Selection for high nitrogenase activity as a surrogate trait to improve nitrogen fixation under drought conditions should be more effective under mild than severe stress.

2.4 A study in four peanut crosses showed high heritability of HI, SLA and SCMR, indicating that breeding progress should be possible for these traits. HI, SCMR and

SLA observations can be recorded at both stressed and non-stressed conditions. This gives peanut breeders a large flexibility to record these observations in a large number of segregating populations and breeding lines in the field, making it easy to incorporate these drought tolerance related traits in breeding and selection schemes. SCMR should be particularly useful as a selection criterion for drought tolerance in peanut because of high heritability, and the simplicity in gathering.

2.5 The investigation on response to drought for reproductive characters of drought resistant peanut genotypes showed that high numbers of flowers and pegs are advantageous but not necessary for high yield under drought conditions. Early peak of flowering is important for the formation of mature pods, and number of mature pods is the most important character determining pod yield under both non-stressed and stressed conditions. Seed size is also important for pod yield under drought but in lesser extent. Our findings showed that high pod yield under non-stressed conditions are important for high pod yield under drought conditions in some genotypes, whereas low reduction in pod yield under drought conditions is more important in others. This information should help breeders to formulate more effective and efficient breeding strategies for improving drought resistance in peanut.

2.6 Chlorophyll density and SPAD chlorophyll meter reading (SCMR) were found to vary among water regimes, times of sampling and genotypes, but water regime x genotype interactions were not significant for these two traits. The correlation coefficients between chlorophyll density and SCMR were positive and significant across irrigation treatments and each water regime, plant age and leaf position. The results indicated that evaluation of chlorophyll density by SCMR can be carried out at any water regime conditions in the second or third-fully expanded leaves after 40 days of crop growth.

2.7 The investigation on the relationship between drought tolerance traits and aflatoxin contamination in a range of peanut genotypes showed that a combination of traits related to drought tolerance including SLA, RLD and kernel colonization could be potentially used as selection traits for resistance to aflatoxin contamination of peanut. Combinations of traits tended to have higher correlations with aflatoxin contamination than single traits, except for SLA, which had a high correlation, especially under drought conditions.

2.8 Water use efficiency (WUE) of peanut genotypes was found to be consistent across seasons and water regimes. The genotypes with larger root systems and low SLA could maintain relatively high WUE under water stress conditions. Our studies demonstrated that root dry weight and SLA are important traits related to WUE under moderate (2/3 AW) and severe (1/3 AW) drought, respectively. Root dry weight and SLA should be useful selection criteria for high WUE under moderate (2/3 AW) and severe (1/3 AW) drought conditions, respectively.

2.9 Water deficit at early season drought and subsequent recovery could increase pod yield of peanut. Peanut genotypes differed significantly in ability to recover from early season drought, but did not show large differences in harvest index under early season drought treatments. Thus, biomass production might be the cause of differences in pod yield, and high yield under early season drought was attributed to maintenance of green leaf area and high capacity of photosynthesis and biomass production. These findings could be useful for improving genotypic performance of peanut for early season drought.

2.10 An investigation on the relationships between root dry weight (RDW) and transpiration efficiency (TE) under early season drought showed that peanut genotypes were fairly consistent in TE under early season drought. The genotypes with larger root systems could maintain relatively high TE under water stress conditions. RDW showed a strong relationship with TE and should be useful as selection criteria for high TE under early season drought conditions.

2.11 A study on the physiological basis for genotypic variation in tolerance to and recovery from pre-flowering drought in peanut indicated that pod yield differences among genotypes under pre-flowering drought were associated with variation in N_2 fixation, LA and RGR. Strong correlations between glasshouse and field conditions for N_2 fixation and LA were also found, suggesting that initial screening and selection for N_2 fixation and LA could be conducted in a glasshouse environment and promising material then evaluated for pod yield responses under field conditions.

2.12 Root characteristics of peanut genotypes grown in hydroponics culture were positively correlated with those of peanut genotypes grown in soil medium. Thus, hydroponics evaluation of genotypes for possible drought tolerance could replace evaluations in pot experiment if facilities are available.

2.13 The relationships of increased drought tolerance and reduced aflatoxin production were confirmed. Drought tolerance traits have the potential to serve as indirect selection tools for lower preharvest aflatoxin contamination. Drought tolerant of biomass, specific leaf area (SLA), chlorophyll density, relative water content, and drought stress rating can be used as efficient tools for selection of peanut genotypes with terminal drought tolerance and low levels of aflatoxin contamination.

Sub-project 3. Basic research to support breeding of peanut for high oleic to linoleic acid (O/L) ratio

3.1 The study on genotypic variability and genotype x environment interactions in oil and fatty acids in high, intermediate and low oleic acid peanut genotypes showed that genotypic variation was the main source of variation for fatty acid composition and significant genotype × environment (G × E) interactions for oleic acid were found mostly among peanut genotypes in low and intermediate groups. However, non-significant G × E interactions were found in the high group indicating that the high group is less affected by environmental changes and, therefore, selection for high oleic acid will be effective.

3.2 Additive gene action was found to be more important than nonadditive gene action for oleic content. Therefore, it is possible to improve oleic content in peanut and selection can be conducted effectively in early generation. SunOleic 97R and Georgia–02C were identified as good donor parents for high oleic acid breeding program.

3.3 Estimation of heritability by parent-offspring regression for high-oleic acid in three peanut crosses gave intermediate and high heritability values for oleic, linoleic acids and O/L ratio, suggesting a high possibility to improve these characters in the study peanut populations. The negative association between oleic acid and linoleic acid indicated that selection for higher oleic acid will result in lower linoleic acid.

3.4 Near-infrared reflectance spectroscopy (NIR) method was found to give oleic acid values of segregating peanut populations that correlated well with the corresponding values obtained from the gas liquid chromatography (GLC) method. As measurement with NIR is non-destructive and inexpensive, the method is very useful for screening segregating populations of peanut for high oleic acid content.

Sub-project 4. Application of crop simulation model in crop breeding

4.1 The cultivar coefficients of peanut lines that were derived from the reduced field data set consisting of observed dates of two critical developmental stages and plant growth analysis data on three dates can accurately predict yield ranking and stability of the lines in independent multi-environment trials. The above procedure for determining the cultivar coefficients, thus, could be used in assisting with multi-environment evaluation of breeding lines at the early stage of yield evaluation when the number of lines is large and available seeds are limited.

4.2 It is feasible to estimate the cultivar coefficients for new peanut lines from typical data of routine performance trials by using GENCALC. The procedure should also be applicable to other crops for which crop simulation models are available.

4.3 The CSM-CROPGRO-Peanut can be used as a tool for determining the most efficient test sites for evaluation of peanut breeding lines in Thailand. The procedure could be extended to other areas and to other crops, particularly for developing countries where the number of METs is normally limited.

4.4 Model sensitivity analysis can be used as a breeding tool to study the causes of G x E interaction. The plant traits that affect both the differences in yield between peanut genotypes and the G x L interaction for pod yield are LFMAX, XFRT, SDPM, SFDUR, and PODUR. Model sensitivity analysis can be used to hypothesize yield improvement likelihoods of a given peanut genotype for a peanut production environment based on improving single or multiple traits.

4.5 Model sensitivity analysis also showed that it is not only one environmental factor that causes G x L interaction between locations, but it is a combination of several environmental factors. Extractable soil water was the major factor influencing both yield and G x L interaction, followed by temperature and amount of rainfall. These findings are useful in determining appropriate breeding strategies, especially for selection of test sites for the multi-environment trials.

4.6 The CSM-CROPGRO-Peanut model could be used for determining the effects of different traits on performance of the peanut genotype and in designing peanut ideotypes for a target environment. The characteristics of the designed peanut ideotypes are early flowering, long time between first flower and end of leaf expansion, large leaf size and high SLA, high leaf photosynthesis rate, determinate growth habit and high partitioning of assimilates to pods and seeds, rapid pod adding period, and moderately long seed filling durations. The designed ideotypes gave pod yield 15 and 27 % higher than the original long and short duration lines, respectively. Among all these traits, adjusting LFMAX, XFRT and SFDUR appeared to give the highest impact on yield improvement.

Sub-project 5. Utilization of peanut stover and other crop residues to improve crop productivity

5.1 The study on the effects of tropical legume residues on N dynamics, CO_2 evolution, sugarcane growth and soil nutrient contents showed that application of peanut residue at the rate equivalent to 12.50 Mg ha^{-1} gave higher mineral N after 53 days from incorporation to the soil and higher final sugarcane biomass than those of other treatments including chemical N fertilizer. Application of peanut residues resulted in the highest levels of N, K and Mg in the sugarcane plant. However, CO_2 emission was significantly higher with added legume residues than with mineral fertilizer N.

5.2 A change from burning to sugarcane residues retention led to alterations in N cycling and improved soil organic matter. However, it did not significantly affect N_2 fixation due to the uniform action of ploughing and the extended time gap between sugarcane residue incorporation and planting of the following the legume crop.

5.3 The millable cane and sugar yield were positively affected by sugarcane residue mulching and incorporation compared to burning. Residual effects of legumes increased sugarcane tillering and yield compared to the fallow treatment without N fertilizer. Soybean residues of higher C:N ratio and lignin content compared to groundnut residues decomposed slower and improved N synchrony with cane N demand. This led to a better conservation of residue N in the system. Recycled legume residues were able to substitute basal fertilizer N application but not topdressing after 6 months.

5.4 During the pre-rice lag phase, adding groundnut residues increased mineral N initially, while added rice straw led to initial microbial N immobilization. Soil microbial N and apparent efficiency were higher, while microbial C were often lowest in groundnut and mixed treatments. Microbial C:N ratio increased with increasing proportion of added rice straw. CH_4 emissions were largest in mixed treatments, but observed gaseous losses were greater than predicted from a purely additive effect. It appears possible to regulate N dynamics by mixing rice straw with groundnut residues; however, at a trade-off of increased CH_4 emissions.

5.5 Mixing groundnut residues and rice straw could delay N release during the pre-rice lag phase leading to an improved synchrony in N demand/supply and increased growth and yield of the succeeding rice and reduced N losses from the soil–plant system.

Sub-project 6. Varietal improvement of kaentawan (jerusalem artichoke) for high inulin

6.1 The test of 15 Kaentawan genotypes over nine environments showed that environment effects contributed to the largest portion of variations in fresh tuber yield, tuber number and tuber size, followed by genotypic effects, while genotype x environment interactions were significant but were much smaller than genotypic effects in their contribution to the variations of all characters. JA 89 was the most promising cultivar for wide adaptation and high tuber yield, whereas HEL 65 was the most promising cultivar for its bigger tubers and acceptable yield.

6.2 The 87 Jerusalem artichoke germplasm accessions at KKU had high variation in maturity, fresh tuber yield, biomass and inulin content, indicating that selection of superior genotypes should be possible among these accessions. Selection for early maturity and high yield should also be possible because of their weak correlations. There was no relationship between fresh tuber yield and inulin content, suggesting that these two traits are segregated independently making possible to select for both traits simultaneously. CN52867 and JA37 were suitable to be parental lines for crossing to generate breeding populations as they were high in yield and inulin content and also in their genetic dissimilarity.

6.3 Genetic analysis with ISSR and RAPD markers indicated considerable genetic diversity among the 147 accessions of Jerusalem artichoke germplasm accessions at KKU that obtained from the three sources. These accessions were groups into four clusters by each marker.

6.4 A simple spectrometric method for determining inulin content in Jerusalem artichoke tuber was developed. The proposed method was found to give more or less the same results as the chromatographic method for the determination of plant inulin after its hydrolysis.

Project outputs

1) Capacity building of researchers

Research team:

Number of researchers: 14
Number of Ph.D. students: 13
Number of M.S. students: 8
Number of research assistants: 3

Improved research capacity of researchers

Researchers in the team have gained their experiences in conducting quality research and in supervising thesis research of graduate students both at the M.S. and Ph.D. levels.

All of the researchers in the team have the capability of seeking their own research funds, and have their own projects in which they are the project leaders.

Training of graduate students

Thirteen Ph.D. and 8 M.S. students are trained under the project. Ten of the Ph.D. students have finished their program, and three are still on-going. Seven of the M.S. students have graduated and one is expected to finish by the end of 2010.

2) Published papers in accredited journals (as of 2 September 2010):

Number of published or accepted papers: 27.
Number of first-submitted papers: 1.

3) Annual technical seminars

Three annual seminars had been held during the period of the project. All of these had been organized together with the Senior Research Scholar projects of Prof. Dr. Peerasak Srinives of Kasetsart University and Prof. Dr. Benjawan Rerkasem of Chiang Mai University.

4) Linkages with foreign institutes

Linkages have been established and collaborative research on peanut has been undertaken with the following foreign institutes:

4.1 USDA-ARS, Coastal Plain Experiment Station
Tifton, Georgia, USA

4.2 Queensland Department of Primary Industries
Kingaroy, Australia

4.3 Department of Biological and Agricultural Engineering,
University of Georgia,
Griffin, Georgia, USA.

4.4 Department of Agronomy, University of Florida,
Gainesville, Florida, USA

4.5 International Crop Research Institute for the Semi-arid Tropic (ICRISAT)
Patancheru, Andar Pradesh, India.

4.6 Institute of Plant Production and Agroecology, University of Hohenheim
Stuttgard, Germany

4.7 Agricultural Science Center at Clovis,
New Mexico University
Clovis, New Mexico

4.8 Department of Agricultural and Biosystems Engineering,
Iowa State University
Ames, Iowa, USA

Project Code: RTA5080001

Project Title: Varietal Improvement of Peanut and Jerusalem Atichoke for Increasing Product Value and Functional Food Quality

ชื่อโครงการ: การปรับปรุงพันธุ์ถั่วลิสงและแก่นตะวันเพื่อเพิ่มมูลค่าและคุณค่าเชิงอาหารสุขภาพ

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Type of funding support: Senior Research Scholar

Research area: Agriculture

Duration: 1 September 2007 – 31 August 2010

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8. Miss Dolrat Sudlah

Overall objectives:

1. To evaluate advanced peanut lines in multi-environment trials.
2. To generate basic knowledge for supporting varietal improvement of peanut and jerusalem artichoke.
3. To generate published papers in accredited international journals.
4. To improve the capability of researchers and train new researchers to produce high quality research.
5. To strengthen collaboration in peanut and jerusalem artichoke research between KKU and other institutes both within the country and overseas.

Expected outputs:

The project aimed to produce the following outputs:

1. New cultivars of peanut: 2
2. New cultivars of kaentawan: 2
3. Number of published papers in accredited journals: 15
4. Number of researchers with improved capability: 11
5. Number of M.S. and Ph.D. graduates produced: 15
6. Annual meeting held: 3
7. Number of foreign institutes that linkage established: 5
8. Number of private companies that linkage established: 2.

Background:

Degradation of natural resources, risks in price fluctuations and loss of agricultural products from pests, diseases, drought and flood, strong market competition, and changing consumer demands have called for the adoption of sufficiency economy philosophy in agricultural development. This means the development will take “the middle path” and “self-immunity” needs to be devised for sufficient protection from impact arising from extensive and rapid socioeconomic, environmental and cultural changes in the world. Applying these principles to agricultural development means that agricultural production must generate sufficient income to support decent livelihood of farmers, and all the risks should be minimized. Diversity is a powerful self-immunity. Production of diversified, high value products will both generate good income and reduce the risk from price fluctuation. Improving the quality as functional food will be a response to market demand and the products will command higher price. Good agricultural practices need to be employed, and emphasis should be given to the maintenance and/or improvement of natural resources both in quantity and quality.

Peanut fits well with the above direction. The crop is grown as a secondary crop by small farmers in all parts of the country, providing a significant source of supplementary income to a large number of rural people, and thus being a safeguard for the risk of failure or low price of the main crop. The peanut crop also plays an important role in soil improvement through its nitrogen fixing ability, thus, help sustaining soil productivity. Almost all of the nuts produced in Thailand are used domestically. Lately, there has been increasing uses of peanut in food processing, both at home and industry levels. The demand for these products is high in both internal and overseas markets. Currently, production of peanut in the country is insufficient for the demand. As a consequence, importation of peanut has increased substantially in the recent years. There

is also a great export potential of peanut products if their quality meet the international standard, especially on the level aflatoxin contamination. Improvement of product quality and production efficiency is, thus, essential for peanut production in Thailand. These require a strong support in research.

This project is essentially Phase III of the TRF Senior Research Scholar Project of the project leader (For Phases I and II, the project title was “Basic Research for Supporting Peanut Production”). During Phase I, basic research has been undertaken to support the improvement of production efficiency and quality of peanut and peanut products. The main research topics included peanut bud necrosis which is a new peanut disease in Thailand, the utilization of peanut residues for soil improvement, and the use of a crop simulation model, CROPGRO-Peanut, in assisting peanut variety evaluation. In Phase II, work on peanut bud necrosis was continued in some aspects. Work on the utilization of crop residues was expanded to cover the entire cropping systems. The use of crop simulation model was also expanded to include studies on genotype x environment interactions and identification of desirable physiological traits for peanut genotypes. The project has also embarked upon a new area of research on drought resistance in a holistic approach.

The findings from the above basic research have been utilized in the peanut breeding work that has been carried on in parallel under a separate project. Large-seeded peanut lines that are early in maturity, resistant to peanut bud necrosis disease, and drought resistant are now in the advanced stage of yield evaluation. In this project, these lines will be evaluated in multi-environment yield trials, and superior lines will be selected for possible release to farmers. A part of the work on drought resistance will be conducted under a separate project (also funded by the TRF). However, a number of studies which have been initiated in Phase II will be conducted under this project. Studies on the applications of the CSM-CROPGRO-Peanut model in peanut breeding will continued on those that have not yet finished in Phase II, with only a new study on the simulation of root responses to drought stress. Work on the utilization of crop residue will be only the unfinished studies from Phase II. A new area is the research to support peanut breeding for high oleic to lenoleic ratio (O/L ratio) which is a new direction of peanut breeding to improve the functional food quality of peanut so that the product will be of higher value.

To develop a new crop for Thailand to provide a new alternative to farmers and diversify the products, we have looked for the crop which is of high value and possesses quality as functional food to be in line with the trend in market demand. We have selected jerusalem artichoke (***Helianthus tuberosus*** L.) as it fits the above criteria, and we have named the crop in Thai as “kaentawan”. The crop produces tubers which contain a high amount of inulin. This compound is used in various ways and is high in economic value. The tuber can be consumed directly as functional food which is good for health. The use of inulin to replace antibiotics in animal feed are quite important to the animal industry, not only in reducing the bad smell problem of animal farm but more importantly in helping the industry meeting the food safety requirement for free antibiotic in meat. As a carbohydrate-producing crop, kaentawan is also used as a raw material for renewable energy. Kaentawan fields can be a tourist attraction and can benefit the bee keeping industry.

The crop is currently grown commercially in many countries including the United States, Canada, France, Italy, Germany, Russia, China, India and Australia. However, it is a new crop to Thailand, but initial testing showed great production potential in the country. To establish kaentawan as multi-purpose crop in Thailand, various promotion activities have been carried out at Khon Kaen University. Currently, kaentawan is well known in Khon Kaen and nearby provinces as a vegetable for good health. A private company, Kaentawan Biotech Company Limited, has now been established to do business on kaentawan products in Thailand. Information on kaentawan is now available in more than 1,000 local websites.

The key component for the success of kaentawan production in Thailand is crop variety. Several varieties have been introduced and tested for productivity. Although some of them gave good yield, they still have disadvantages such as susceptible to stem rot disease, long crop duration, slow growth rate at early growth stages, and most importantly low inulin content. Therefore, varietal improvement for high inulin content is of high priority. The development of high inulin varieties requires broad genetic base of germplasm. More genetic materials should be introduced and evaluated for adaptation and for inulin content to identify the best parents for developing segregating populations for further selection of superior genotypes. Molecular markers associated with high inulin content should be sought to facilitate the effective selection for high inulin among kantawan breeding lines.

Scope of research:

The project consisted of 6 sub-projects:

Sub-project 1. Multi-environment evaluation of advanced peanut lines.

Sub-project 2. Basic research to support breeding of peanut for drought resistance.

Sub-project 3. Basic research to support breeding of peanut for high oleic to linoleic acid (O/L) ratio.

Sub-project 4. Application of crop simulation model in crop breeding.

Sub-project 5. Utilization of peanut stover and other crop residues to improve crop productivity.

Sub-project 6. Varietal improvement of kaentawan (jerusalem artichoke) for high inulin.

Results

Sub-Project 1

Multi-environment Evaluation of Advanced Peanut Lines

Associate Prof. Dr. Sanun Jogloy

Sub-project Leader

The peanut breeding program at Khon Kaen University has been focusing on breeding for earliness of the large-seeded type peanut, breeding for resistance to peanut bud necrosis virus and breeding for drought tolerance. During the first and second phases of this project, a number of peanut breeding lines had been generated for these breeding objectives. These peanut lines had also been evaluated sequentially for their yield performances in the preliminary and the standard yield trials, and superior lines had been selected. However, these selected lines needed to be further evaluated in the more advanced stages of yield evaluation. At the beginning of this project, there were three groups of advanced peanut lines that were selected for the different breeding objectives, i.e., early-maturing large-seeded lines, peanut bud necrosis virus resistant lines and drought tolerant lines. These lines were tested in three series of multi-environment trials under this sub-project.

1.1 Multi-environment trials of early maturing large-seeded peanut lines

Wilawan Tula, Thanwan Kesmala and Sanun Jogloy

There were two sets of early maturing large-seeded peanut lines that were evaluated. Set 1 consisted of five breeding lines and three standard checks. These lines were tested in a regional yield trial that was conducted at Khon Kaen and Kalasin in the dry and rainy seasons of 2008 and at Khon Kaen in the dry and rainy seasons of 2009, totaling six environments. Set 2 consisted of three breeding lines and three standard checks. They were tested in a farm trial that was conducted at Khon Kaen, Kalasin and Mahasarakarm in the dry season of 2008 and at Khon Kaen field 1, Khon Kaen field 2 and Kalasin in the rainy season of 2008, and at Khon Kaen in the dry and rainy seasons of 2009. Both trials were grown under rainfed conditions in the rainy season and under irrigation in the dry season. Data were recorded for days to maturity, pod yield, seed yield, seed size and shelling percentage.

The results of Set 1 trials are shown in Table 1. KK 6 gave the highest average pod yield (1,179 kg/ha) and seed yield (1,194 kg/ha) over the six environments. This line showed the most stable yield as it could maintain a favorable pod yield of 1,471 kg/ha even in the poorest trial in the rainy season 2008 at Kalasin. It also gave the largest seed size, with an average 100-seed weight across six environments of 65.8 g. The varieties or lines that gave pod yields comparable to that of KK 6 were (Luhua11 x Singburi)F8-12 (1,473 kg/ha), KKU 60 (1,357 kg/ha) and (Luhua11 x Singburi)F8-14 (1,284 kg/ha). Among them, (Luhua11 x Singburi)F8-12 gave the highest pod and seed yields under the most favorable environment, and its average yield was not significantly lower than that of the best yielding variety KK 6. It also was 15-20 days earlier in maturity than KK 6 (data not shown), and had a comparable seed size to KK 6.

Table 1. Means for pod yield, seed yield, 100-seed weight and shelling percentage of large-seeded early maturing peanut breeding lines evaluated in the regional yield trial for six environments in the dry and rainy seasons of 2008 and 2009.

Line/Variety	Pod yield (kg/ha)	Seed yield (kg/ha)	100-seed wt. (g)	Shelling (%)
(Luhua11xSingburi)F8-8	1,188b	758b	59.5ab	61.4ab
(Luhua11xSingburi)F8-11	1,194b	711b	61.4ab	57.1ab
(Luhua11xSingburi)F8-12	1,473ab	886ab	59.6ab	56.5b
(Luhua11xSingburi)F8-14	1,284ab	773b	59.2ab	58.1ab
Luhua11 x KK 60-3)F8-6	1,113b	684b	58.3ab	59.6ab
KK 6	1,779a	1,194a	65.8a	63.5ab
KKU 60	1,357ab	858b	60.7ab	61.9ab
KK 5	1,106b	723b	51.7b	65.2a
Mean	1,314	823	59.5	60.4
F-Test	**	**	**	**
CV (%)	27.5	27.6	10.8	8.4

** Significant at P $\leq$ 0.01.
Numbers in the same column with the same letter are not significantly different by DMRT.

The results of the farm trials of Set II lines are presented in Table 2. The three tested lines gave average pod yields that were not statistically different from that of the standard checks KK 6. However, under the most favorable environment in the dry season in 2008 at Khon Kaen, (Luhua11 x China 97-2)F5-7 and (Luhua11 x China 97-2)F6-11-4 gave the highest pod yields of 3,142 and 3,092 kg/ha, respectively. Seed sizes of these two lines were not statistically different from KK 6, but (Luhua11 x China 97-2)F6-11-4 was 10.5 days earlier than KK 6.

Table 2. Means for pod yield, 100-seed weight, shelling percentage and days to maturity of advanced large-seeded early maturing peanut lines evaluated in a farm trial at eight environments in the dry and rainy seasons of 2008 and 2009.

Line/variety	Pod yield (kg/ha)	100-seed wt. (g)	Shelling (%)	Days to maturity
(Luhua11 x China 97-2)F6-11-4	1,569ab	60.2a	61.1	109.5
[(Nc.17090xB1)-25 x KK 60-3)] F6-11-5	1,513ab	58.0ab	62.2	120.0
(Luhua11 x China 97-2)F5-7	1,487ab	60.0a	62.6	110.3
KK6	1,719a	61.0a	62.5	120.7
KKU 60	1,491ab	59.0ab	60.7	109.4
KK5	1,334c	52.7b	61.0	112.8
Mean	1,519	58.5	61.7	113.8
F-Test	*	**	ns	
CV.(%)	35.1	17.6	13.0	

ns, *, ** not significant and significant at P $\leq$ 0.05 and 0.01, respectively.
Numbers in the same column with the same letter are not significantly different by DMRT.

1.2 Multi-environment trials of peanut bud necrosis resistant lines

Wilawan Tula, Thanwan Kesmala and Sanun Jogloy

Eleven advanced large-seeded peanut breeding lines selected for peanut bud necrosis resistance together with two standards checks and one PBNV resistant check were evaluated in a standard yield trial at Khon Kaen in the dry season of 2008, the dry and rainy seasons of 2009 and the dry season of 2010, totaling four environments. Data were recorded on pod yield, seed yield, seed size, shelling percentage and peanut bud necrosis incidence. However, only pod yield was recorded in dry season of 2010.

High incidence of PBNV occurred only in the rainy season of 2009. In this season, KKU 6 showed a high disease incidence of 31.3 %, while the resistant check IC 10 had only 4.8 % PBNV incidence (Table 3). The line (Luhua11x ICGV 86031) showed a low PBNV incidence closed to the resistant check (5.0 %). Three other lines, i.e., (KKU72-2 x ICGV 86388)-2, (Luhua 11 x IC34)-10 and (KKU72-2 x ICGV 86388)-1, also showed a good level of PBNV resistance with the incidences of 8.4, 9.6 and 9.9 %, respectively. It was noted that KKU 60 also showed a moderate level of PBNV resistance with an incidence of 13.2 %.

Table 3. Means for pod yield, seed yield, 100-seeded weight, and PBNV incidence of advanced peanut breeding lines selected for peanut bud necrosis virus resistance evaluated in a standard yield trial at KKU in the dry and rainy seasons during 2008-2010 (four environments).

Line/variety	Pod yield (kg/ha)	Seed yield (kg/ha)	100-seed wt. (g)	PBNV incidence (%)†
(KKU72-2xICGV 86388)-1	1,682a-d	976a-d	38.6e-g	9.9
(KKU72-2xICGV 86388)-2	1,848a-d	1,119a-d	46.9c-e	8.4
(KKU72-2xIC 10)	1,499cd	1,001a-d	32.9fg	16.3
(Luhua11x ICGV 86031)	2,142a-c	1,338a-c	51.8cd	5.0
(KK 60-3x ICGV 86031)	1,373d	829d	44.4c-e	22.4
(KKU 72-1x IC 10)-6	1,673a-d	1,030a-d	42.0d-f	12.0
(KKU 72-1xICGV86031)-8	1,916a-d	1,084a-d	42.6d-f	13.9
(KKU 72-2x ICGV 86031)-8	2,032a-d	1,209a-d	42.5d-f	10.8
(KKU 72-2x ICGV 86031)-11	1,624b-d	916b-d	48.8c-e	20.7
(Luhua 11 x IC 10)-1	2,225ab	1,428a	54.4bc	13.2
(Luhua 11x IC34)-10	2,201ab	1,403ab	43.6c-f	9.6
KK 6	2,041a-d	1,404ab	63.9ab	31.3
KKU 60	2,369a	1,468a	66.9a	13.2
IC 10	-	-	-	4.8
Mean	1,894	1,169	47.6	13.7
F-test	**	**	**	ns
CV (%)	26.9	30.9	17.5	79.3

† Data from the 2009 rainy season only.

ns, ** non-significant and significant at $P \leq 0.01$.

Numbers in the same column with the same letter are not significantly different by DMRT.

KKU 60 showed the highest average pod and seed yields (2,369 and 1,468 kg/ha, respectively) over the four test environments. The PBNV resistant line (Luhua11x ICGV 86031) had average pod and seed yields (2,142 and 1,338 kg/ha, respectively) not significantly differed from those of KKU 60. This line, however, had a slightly smaller seed size than KKU 60, with a 100-seed weight of 51.8 g compared to 63.9 g of KKU 60. Another PBNV resistant line (Luhua 11x IC34)-10 also had average pod and seed yields (2,201 and 1,403 kg/ha, respectively) not significantly differed from those of KKU 60, but its seed size (43.6 g/100 seeds) was much smaller than KKU 60. Other two PBNV resistant lines had average yields somewhat lower than KKU 60 (Table 3).

1.3 Multi-environment trials of drought tolerant peanut lines

Wilawan Tula, Thanwan Kesmala and Sanun Jogloy

There were 15 advanced breeding peanut lines in this category. These lines and seven standard checks were evaluated in a standard yield trial at Khon Kaen in the rainy and dry seasons of 2008 and 2009, totaling four environments. The trials in the rainy season were conducted under rainfed conditions while the trials in the dry season were irrigated. The data were recorded for pod yield, seed yield, seed size and shelling percentage.

Three peanut lines gave average pod and seed yields over four environments higher than those of the standard checks. These included [ICGV 98324 x KK60-3]F4-4, [ICGV 98324 x KK60-3]F4-26 and [ICGV 98308 x TN 9]F4-35. Their pod yields were 2,013, 1,996 and 1,993 kg/ha and their seed yields were 1,337, 1,338 and 1,381 kg/ha, respectively, compared to pod and seed yields of the best standard check KK 6 which were 1,836 and 1,259 kg/ha, respectively (Table 4). These lines also had higher average pod and seed yield than the drought tolerant check ICGV 98324. The line [ICGV 98308 x TN 9]F4-35 had a large seed size with a 100-seed weight of 76.8 g, comparable to the large-seeded check KK 6 which had a 100-seed weight of 76.3 g. The other two lines were small-seeded type having seed size comparable to that of the most popular small-seeded peanut cultivar Tainan 9.

Table 4. Means for pod yield, seed yield, 100-seed weight of 15 advanced drought tolerant peanut breeding lines and seven standard checks evaluated in a standard yield trial at KKU in the dry season and rainy seasons of 2008 and 2009 (four environments).

Line/variety	Pod yield (kg/ha)	Seed yield (kg/ha)	100-seed wt. (g)	Shelling (%)
[ICGV 98308 x KK60-3]F4-12	1,552a-c	1,024a-d	55c-f	67.5a
[ICGV 98308 x KK60-3]F4-56	1,164bc	774cd	52.8d-f	65.4ab
[ICGV 98308 x KK60-3]F4-120	1,439a-c	947a-d	54.7c-f	65.9ab
[ICGV 98308 x TN 9]F4-22	1,587a-c	1,121a-d	70.0ab	69.8a
[ICGV 98308 x TN 9]F4-35	1,993a	1,381a	76.8a	69.5a
[ICGV 98308 x TN 9]F4-52	1,140bc	738cd	60.1b-e	63.6ab
[ICGV 98308 x TN 9]F4-119	1,312a-c	863b-d	57.7b-f	64.4ab
[ICGV 98324 x KK60-3]F4-4	2,013a	1,337ab	54.3d-f	65.8ab
[ICGV 98324 x KK60-3]F4-26	1,996a	1,338ab	53.4d-f	66.8ab
[ICGV 98324 x KK60-3]F4-68	1,316a-c	872a-d	53.1d-f	65.7ab
[ICGV 98324 x TN 9]F4-6	1,344a-c	914a-d	65.3a-d	67.5a
[ICGV 98324 x TN 9]F4-44	1,170bc	769cd	57.1b-f	64.7ab
[ICGV 98324 x TN 9]F4-64	1,247bc	838b-d	59.7b-e	67.1ab
[ICGV 98324 x TN 9]F4-148	1,142bc	743cd	56.7b-f	65.3ab
[ICGV 98324 x TN 9]F4-154	1,395a-c	942a-d	58.6b-f	66.1ab
KKU 60	1,804a-c	1,153a-c	68.8a-c	64.1ab
KK 5	1,269bc	861b-d	57.3b-f	69.0a
KK 6	1,836ab	1,259a-c	76.3a	68.3a
KKU 1	1,145bc	767cd	57.0b-f	67.6a
Tainan 9	1,231bc	880a-d	53.8d-f	73.8a
ICGV 98324	1,467a-c	960a-d	50.8ef	66.5ab
Kalasin 2	1,101c	621d	44.4f	56.3b
Mean	1,439	959	58.8	66.4
F-Test	**	**	**	**
CV.(%)	29.2	31.6	14.5	9.9

** significant at $P \leq 0.01$.

Numbers in the same column with the same letter are not significantly different by DMRT.

Sub-Project 2

Basic Research to Support Breeding for Drought Resistance

Associate Prof. Dr. Sanun Jogloy

Sub-Project Leader

More than 80% of world's peanut production comes from rainfed agriculture. Drought is a major abiotic stress affecting yield and quality of rainfed peanut worldwide. Yield losses due to drought are variable in nature depending on timing, intensity, and duration, coupled with other location specific environmental factors such as irradiance and temperature (Nageswara Rao and Nigam, 2001). In Thailand, more than 60 % of peanut production is under rainfed, and certainly drought is one of the major constraints to production (Patanothai *et al.*, 1987).

Drought effects on peanut are manifested in several ways both on quantity and quality. Water deficits depending on the timing of occurrence can cause significant reduction in yield by affecting physiological processes i.e. nitrogen fixation (Ludwing and Matthew, 1993; Ramos *et al.*, 1999; Serraj *et al.*, 1999), photosynthesis (Williams and Boote, 1995), and calcium uptake by developing pods (Rajendrudu and Williams, 1987). Crop responses to drought can be morphological and physiological, including reduction in leaf area and size of new leaves, leaf senescence, changing leaf angle, increasing stomata resistance, reducing leaf water potential, and increasing root length and density (Wright and Nageswara Rao, 1994). Ramos *et al.* (1999) found that increasing level of drought stress resulted in greater reduction in stover dry weight, total biomass, root dry weight, nodule dry weight, nodule number and nitrogenase activity. Effect of drought in reducing nitrogen fixing ability of peanut by reducing nitrogenase activity was also reported by Nambiar and Dart (1983) and Venkateswarlu *et al.* (1990). A decline in the rate of N-fixation was positively correlated with a decline in leaf water potential (Venkateswarlu *et al.*, 1990). Response to drought, however, varies among peanut genotypes, and yield reduction from drought stress could be up to 56-85% (del Rosario and Fajardo, 1988). The unpredictability of drought events causes enormous variability in production and hence problems for continuity of supply, which directly affects domestic and export-market requirements. Reduction in N-fixation efficiency by drought stress also lowers the value of peanut in soil improvement, affecting the contribution of the crop to long-term sustainability of land productivity.

Drought also affects peanut quality, particularly the contamination of aflatoxin produced by *Aspergillus flavus* and *A. parasiticus*. It is well documented that end-of season drought can predispose the crop to aflatoxin contamination which can severely impact the economic value of the crop (Davidson *et al.* 1983; Mehan, 1989). The level of aflatoxin contamination was found to increase with the length of the drought predisposal period (Sander *et al.*, 1985). Thus, the problem of drought contributes significantly to the problem of aflatoxin contamination.

Plants have evolved complex, integrated adaptive mechanisms against drought stresses. Plant utilize more photo-assimilates to promote root growth at the expense of shoot growth in order to explore more volumes of soil as drought becomes progressively severe. Changes in leaf size, leaf thickness, stomatal density, and cuticle thickness are morphological adaptation of plant leaves under water stress. These adaptive mechanisms

allow plants to maximize water uptake while minimize water loss during drought stress (Kramer, 1980; Wright and Nageswara Rao, 1994; Xiong and Zhu, 2002). Under drought conditions, plant cells loose water resulting in a decrease in turgor pressure. This would trigger signaling cascades leading to expression of water-stress-inducible genes. These gene products are classifies into two main groups. The first group includes functional proteins that are indirectly responsible for regulating osmotic adjustment and involved in protecting cellular processes under drought conditions (Bray, 1997). Those in the second group are regular proteins or transcription factors involved in further regulation of signal transduction and gene expression (Shinozaki and Yamaguchi-Shinozaki, 1997).

Some responses to water stress are similar in tolerant species (Bray, 1997). Drought stress induces expression of functional proteins and activates regular proteins or transcription factors. The latter group of proteins will trigger the induction of more genes encoding functional proteins (Shinozaki and Yamaguchi-Shinozaki, 1997). Genes induced under stress condition are thought to function in stress tolerance. This has been proved by the fact that engineered over-expression of drought-inducible genes in transgenic plants confers some tolerance to water stress (Bohnert and Jensen, 1996; Bray, 1997; Holmberg and Bulow, 1998). Variations in drought-stress-induced gene expression among plant species can be used to study cellular responses to water deficits. During stress conditions, profile of gene products or protein will be different among plant species and can be analyzed to identify plants with varying capacity to tolerate drought. Protein profiles can be studied using SDS-PAGE (Laemmli, 1970). Protein patterns can be generated and unique between plant organs (Saha *et al.*, 1997) or even among leaves within the same plant (Masclaux *et al.*, 2000). Although morphological and physiological responses of peanut cultivars to drought stresses have been reported (Collino *et al.*, 2001; Nautiyal *et al.*, 2002; Wright and Nageswara Rao, 1994), cellular mechanisms responsible for drought tolerance are largely unknown and warrant further investigation.

A number of methods have been used in evaluating peanut genotypes in their responses to drought stress in terms of changes in morphological and physiological characters and in final pod yield and quality. These include application of different levels of water by line source sprinkler (Nageswara Rao *et al.*, 1988), drip irrigation to induce drought stress during pod and seed development stages (Collin *et al.*, 2001), and rain out shelter in field evaluation (Nautiyal *et al.*, 2002). Nautiya *et al.* (2002) created water-deficit conditions at 40-80 and 60-100 days after emergence in field evaluation and 60 days after sowing in greenhouse evaluation. However, the appropriate time to impose drought stress should depend on the nature of drought occurring in local cropping systems. The stages that peanut is susceptible to drought are flowering and pod and seed development. At these stages plant will show water deficit at ½ field capacity of soil moisture (Del Rosario and Fajardo, 1988). The normal procedure for evaluation is to omit watering for a given period and record morphological and physiological characters such as root length and density, water use efficiency, partitioning of dry matter into pods, and ability to recover from drought stress. The most frequent measured characters are water use efficiency and specific leaf area (SLA). The latter character was found to be highly correlated with water use efficiency (Wright *et al.*, 1994).

Different peanut genotypes differ in their yielding ability under drought stress depending on the time of occurrence. Del Rosario and Fajardo (1988) reported that the peanut cultivar 33-1 gave 13-19 % yield increase when drought stress occurred before flowering, but showed 56-83 % yield reduction when drought stress occurred during flowering and pod and seed development stages. They also found that the peanut cultivars

Acc 847, 55-437 and GNP 1157 had potential to be resistant to drought morphologically, physiologically, and in yielding ability. Collino *et al.* (2001) observed that response mechanisms to drought stress at R2-R7 stage of two peanut cultivars were different. The cultivar Manfredi 393 INTA had the ability to extract more water while the cultivar Florman INTA developed faster and produced more number of pods.

Genetic improvement for water-use efficiency has been a major research thrust in most of arable crops including peanut to cope with drought constraint. At ICRISAT, considerable efforts have been spent in screening peanut germplasm for resistance to drought, and several drought resistance lines have been identified. These lines have been used in the peanut breeding program of the institute and also supplied to national programs (Reddy *et al.*, 1994). Several of these lines also showed resistance to drought in the test in China (Liang *et al.*, 1996).

The peanut breeding program at Khon Kaen University has embarked upon breeding for drought resistance (or for improving water-use efficiency). Although the problem of aflatoxin contamination is of prime importance, breeding for aflatoxin resistance in peanut has not been successful anywhere. Eventhough some lines have been identified as having resistance to the causal fungus in dry-seed inoculation, they failed to show resistance to aflatoxin contamination in field evaluation. In Thailand, a lot of effort had also been spent in breeding for aflatoxin resistance without a success; the resistant lines identified lost their resistance in later generations (Patanothai *et al.*, 1993). The aim of the effort in breeding for drought resistance, thus, is not only to alleviate the problem of yield reduction from drought alone but also to reduce the problem of aflatoxin contamination. Experiences in breeding for drought resistance at ICRIAT were considered as a lesson learned. At ICRISAT, an empirical approach was first followed for selection among segregating populations and evaluation of advanced breeding lines for their sensitivity to mid-season and end-season droughts, based on pod and seed yields. While the empirical approach was partly successful, it was concluded that a more efficient breeding approach requires the selection of traits associated with drought resistance. There has been significant improvement in physiological understanding of the genotypic response to drought in peanut, suggesting scope for selecting genotypes with traits contributing to superior performance under water limited conditions (Nageswara Rao and Nigam, 2001). Selection for physiological traits associated with drought resistance has been the approach taken by the KKU peanut breeding program.

Most of the physiological traits associated with drought resistance are quantitative in nature. Using molecular markers, quantitative trait loci (QTL) can be detected in an appropriate population of plants. A locus for any quantitative trait can be mapped as long as polymorphism is observed in segregating populations under analysis and phenotypic information is available for lines in the population. However, for traits as complex as drought tolerance, the success of the QTL approach is conditioned by the effectiveness of the phenotypic procedure in detecting repeatable, highly heritable differences among recombinant lines, that permit the identification of robust QTLs. Therefore, a special effort is needed for the conceptualization, design and management of phenotyping programs for drought tolerance, to maximize the chances of identifying QTL that will be useful in the future improvement of tolerance in the target crop in the target environment. Compared to other crops, cultivated peanut with currently available DNA markers shows limited polymorphic variation, which had made it difficult to construct a genetic map for cultivated peanut. However, polymorphic variation in DNA has been recently detected in selected germplasm of cultivated peanut, using RFLP and AFLP methods (He and

Prakash, 1997; Subramanian *et al.*, 2000). On the other hand, there is still limited information on the biochemical and molecular basis for variation among genotypes for drought resistance (Nageswara Rao and Nigam, 2001). Further research is necessary to develop linkages between the drought resistance traits and the molecular markers so that marker-assisted selection tools could be applied in drought resistance breeding.

This sub-project has undertaken basic research to generate information that will be useful to the work on breeding for drought resistance. A comprehensive approach is adopted, i.e. examining from the field level down to the crop, cell and molecular levels. The lengths and frequency of drought stress occurring in major peanut production areas will be characterized to establish the conditions of drought stress for subsequent studies. Responses to drought stress are being examined in terms of yield and yield components, morphological and physiological characters, and biochemical processes. Studies on techniques to evaluate peanut genotypes for their resistance to drought are underway, including identification of molecular markers. Studies will also be conducted on the inheritance of drought resistance and of the associated trait (SLA), and on the relationship of drought resistance to aflatoxin contamination and N-fixing efficiency.

Thirteen studies were conducted under this sub-project. These include:

2.1 Chlorophyll stability is an indicator of drought tolerance in peanut.
2.2 Root distribution of drought resistant peanut genotypes in response to drought.
2.3 Effect of drought stress on traits related to N_2 fixation in eleven peanut genotypes differing in degrees of resistance to drought.
2.4 Heritability of drought–resistance traits and genotypic and phenotypic correlation of drought–resistance and agronomic traits in peanut.
2.5 Response of reproductive characters of drought resistant peanut genotypes to drought.
2.6 Stability of relationship between chlorophyll density and soil plant analysis development chlorophyll meter readings across different drought stress conditions in peanut.
2.7 Association between aflatoxin contamination and drought tolerance traits in peanut.
2.8 Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water.
2.9 Variability in yield responses of peanut (*Arachis hypogaea* L.) genotypes under early season drought.
2.10 Association of root dry weight and transpiration efficiency of peanut genotypes under early season drought.
2.11 Physiological basis for genotypic variation in tolerance to and recovery from pre-flowering drought in peanut.
2.12 Relationship between root characteristics of peanut (*Arachis hypogaea* L.) in hydroponics and pot studies.
2.13 Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought

These studies are reported in this section.

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2.1 Chlorophyll stability is an indicator of drought tolerance in peanut

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Two thirds of the global peanut production occurs in rain-fed regions of the semi-arid tropics which are characterized by unpredictable droughts (Wright and Nageswara Rao, 1994). Bringing rain-fed land into irrigation is not feasible and hence, genetic improvement for drought resistance is a cost-effective approach to improve peanut productivity under rainfed conditions.

The conventional approach of breeding for drought resistance has been based primarily on pod yield, a complex trait which integrates a number of local adaptation factors. Although such an approach has generally been successful for a given location, it has not been successful across locations due to large genotype x environment interactions for yield (Araus et al., 2002). An alternative approach is to identify and use surrogate traits which are positively associated with yield under drought conditions and less affected by environmental variations in breeding for drought resistance. Identification of such surrogate traits is, thus, needed.

Transpiration efficiency (TE, defined as the biomass produced per unit of water transpired) has been widely perceived as a useful trait for drought resistance (Richards, 1997). Significant relationships between TE, specific leaf area (SLA), chlorophyll and the photosynthetic enzyme ribulose 1-5 di-phosphate carboxylase (Rubisco) content in peanut suggested that photosynthetic capacity is the main cause of variation for TE in peanut (Nageswara Rao et al., 1995). The rubisco enzyme constitutes the major portion of chlorophyll in the leaf and hence chlorophyll density and stability are believed to be important factors contributing to drought resistance in peanut.

Recent research has shown that the leaf chlorophyll content can be rapidly assessed using the SPAD chlorophyll meter (Akkasaeng et al., 2003). Furthermore, close relationships between SPAD chlorophyll meter readings (SCMR) and TE (Sheshshayee et al., 2006) in peanut suggest that SCMR could be used as a rapid and in situ tool for assessing leaf chlorophyll in peanut. However, there is little information on the genotype x drought interactions on chlorophyll content and whether stability of leaf chlorophyll contributes to drought resistance in peanut. The objectives of the current study were to (a) estimate genotype x drought interactions for chlorophyll status in peanut leaves (b) assess if chlorophyll stability under drought can be used as a potential selection criterion for drought tolerance in peanut and (c) assess the potential of using SCMR as a selection tool for assessing chlorophyll status in peanut genotypes.

Materials and Methods

Two field experiments were conducted during the 2003/2004 and 2004/2005 dry seasons in a split plot design with four replicates. Main-plot treatments were three water

regimes (field capacity (FC), 2/3 available water (AW) and 1/3 AW) and sub-plot treatments were twelve peanut genotypes. These genotypes were selected on their reputed superior drought resistance characteristics. Eight genotypes (i.e. ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348 and ICGV 98353) were drought resistant germplasm introduced from the International Crops Research Institute for Semi-Arid Tropics (ICRISAT). Tifton-8 is a drought resistant line introduced from the US. Two cultivars (KK 60-3 and Tainan 9) are commercial varieties grown in Thailand, and are known to be sensitive to drought. A non-nodulating line (yellow-green leaf color) was used as a control for low chlorophyll genotype.

Total dry matter (TDM), chlorophyll density, chlorophyll content per plant and SCMR were recorded at 30, 60 and 90 day after emergence. Data obtained were used to compute transpiration (T) and transpiration efficiency (TE).

Results

There were significant differences among genotypes for TE and chlorophyll parameters. The genotype x drought interaction effects for chlorophyll characters (content and density) were not significant suggesting a strong genetic effect (Table 1). Drought stress significantly reduced TDM, T and chlorophyll content across genotypes but significantly increased TE and chlorophyll density in peanut (data not showed).

The correlation coefficients between TDM and chlorophyll content (r = 0.51** to 0.91**) (Table 2) and between TE and chlorophyll density (r = 0.46* to 0.77**) (Table 3) were positive and significant. These findings suggest that chlorophyll parameters are strongly linked with drought tolerance in peanut. There were highly significant and positive relationships between chlorophyll density and SCMR (r = 0.67**to 0.93**) (Fig. 1) and between SCMR and TE (r = 0.41*to 0.80**) (Table 3) suggesting that SCMR could be used as a tool for rapid assessment of relative chlorophyll status in peanut genotypes as well as for the indirect selection of drought tolerance in peanut.

Table 1. Pooled analysis of variance over two years of total dry matter, transpiration, transpiration efficiency, chlorophyll density and chlorophyll content.

Source of variance	df	Mean squares									
		Total dry matter at final harvest (g plant^{-1})		Transpiration (mm m^{-2})		Transpiration efficiency (g mm^{-1})		Chlorophyll density at 90 DAE (µg cm^{-2})		Chlorophyll content at 90 DAE (mg plant^{-1})	
Season (s)	1	11.8		146855.0	**	5.0	*	4.9		2554.1	**
Reps. within year	6	1059.4		8689.0		4.2		126.5		424.4	
Water regimes (w)	2	6903.5	**	1664308.0	**	3.8	**	51.4	*	687.8	**
S x w	2	689.3	**	69783.0	**	0.4		46.4	*	4.4	
Error A	12	190.4		7193.0		0.7		47.7		70.4	
Genotypes (g)	11	5810.7	**	15346.0	**	15.3	**	624.4	**	1755.7	**
S x g	11	349.1	*	17148.0	**	1.5	**	53.2		182.8	
W x g	22	1158.6	**	4084.0	**	1.8	*	74.5		247.4	
S x w x g	22	625.3	**	5823.0	**	1.7	*	67.2		216.5	
Error B	198	2976.4		14061.0		9.2		608.2		1812.5	

*' ** Significant at the 0.05 and 0.01 probability levels, respectively. DAE = day after emergence

Table 2. Correlation coefficients (r) (n = 36) between total dry matter (g plant^{-1}) at three sampling dates and at harvest with chlorophyll content (mg plant^{-1}) at three sampling dates in 2003/04 and 2004/05.

Chlorophyll content (mg plant^{-1})	Total dry matter (g plant^{-1})							
	2003/04				2004/05			
	30 DAE	60 DAE	90 DAE	harvest	30 DAE	60 DAE	90 DAE	harvest
30 DAE	0.88**			0..51**	0.79**			0.63**
60 DAE		0.91**		0.70**		0.76**		0.74**
90 DAE			0.85**	0.78**			0.83**	0.84**

** Significant at the 0.01 probability levels. DAE = day after emergence.

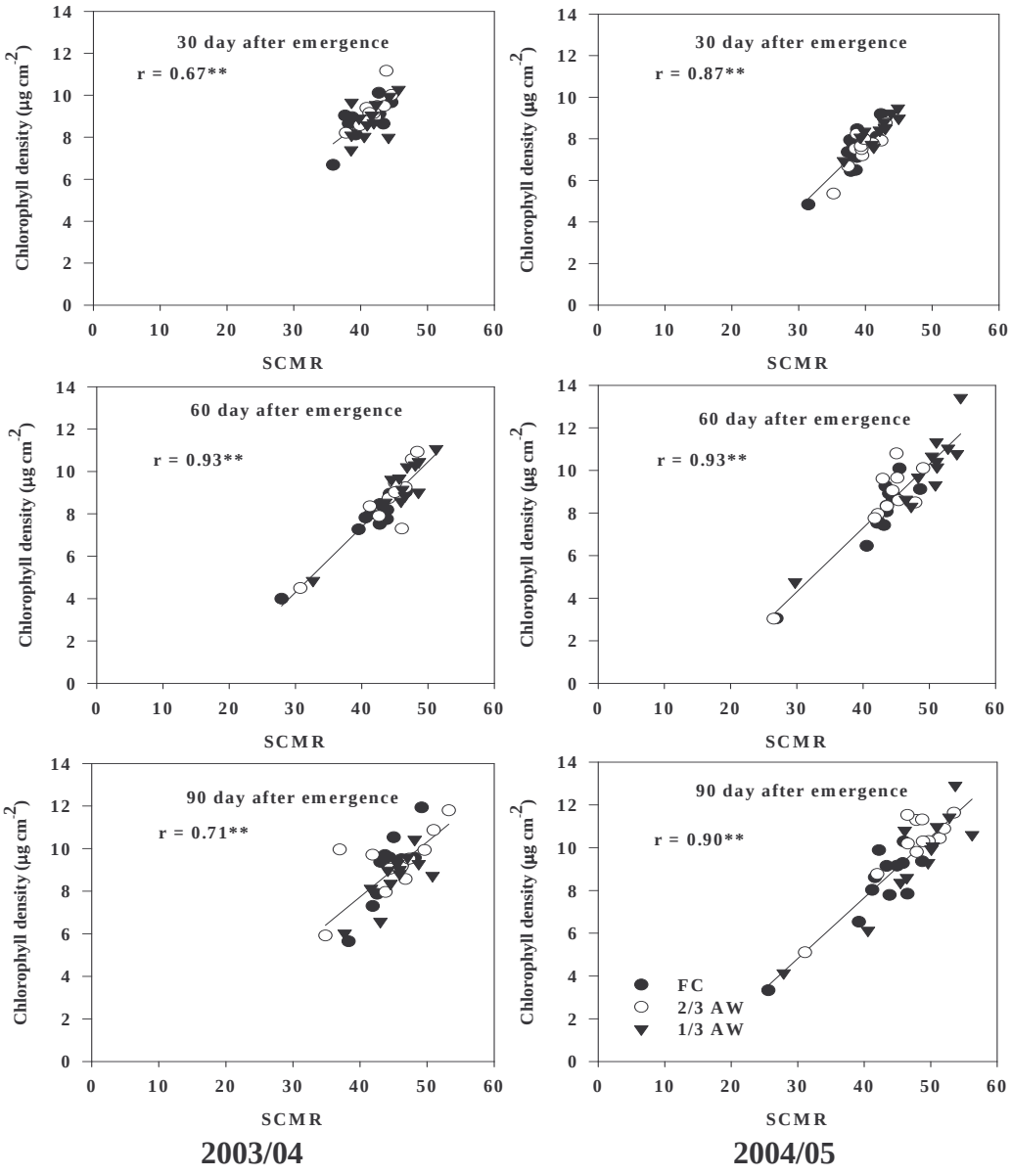

Fig. 1. Relationship between chlorophyll density and SPAD chlorophyll meter reading (SCMR) in 2003/04 and 2004/05; r = correlation coefficients (n=36), *' ** Significant at the 0.05 and 0.01 probability levels, respectively. FC, 2/3 AW and 1/3 AW = field capacity, 2/3 available water and 1/3 available water, respectively.

Table 3. Relationship between transpiration efficiency (TE) and chlorophyll density, and between TE and SCMR in 2003/04 and 2004/05.

	2003/04	2004/05
	r (n=36)	r (n=36)
TE & chlorophyll density		
30 DAE	0.65 **	0.71 **
60 DAE	0.77 **	0.70 **
90 DAE	0.46 **	0.61 **
TE & SCMR		
30 DAE	0.58 **	0.74 **
60 DAE	0.80 **	0.79 **
90 DAE	0.41 *	0.76 **

*' ** Significant at the 0.05 and 0.01 probability levels, respectively. r = correlation coefficient. DAE = day after emergence.

Conclusion

Non-significant G x E interaction for chlorophyll content and density indicated that they were more stable than TDM, T and TE. High correlations between TDM and chlorophyll content and between TE and chlorophyll density across water regimes pointed out that chlorophyll content and density are useful traits conferring drought tolerance in peanut. The traits should be useful as selection criteria for drought tolerance. Moreover, SCMR could be used as a rapid and cost effective tool for assessment of relative chlorophyll status in peanut leaves, and could be applied for indirect selection of drought tolerance in peanut.

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2.2 Root distribution of drought resistant peanut genotypes in response to drought

P. Songsri, S. Jogloy, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook

Rainfed regions occupy over two-thirds of the global peanut production area. In these regions, drought is a major production constraint (Wright and Nageswara Rao, 1994; Reddy et al., 2003). The ability of a plant to modify its root distribution to exploit deeper stored soil water may be an important mechanism to avoid drought. In peanut, selection for drought resistance in the past has primarily been based on biomass production and pod yield under drought conditions. The mechanisms by which the resistant peanut genotypes achieve high yield under drought are not well understood. Information on the ability of these drought resistant peanut genotypes to alter root distribution contributing to high yield under water stress might reveal the avoidance mechanism and could result in the development of improved breeding strategies for drought resistance in peanut.

We have received eight drought resistant peanut genotypes from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). They were selected based on high biomass and pod yield under drought conditions. We raised the following questions: (1) Do these drought resistant genotypes differ in root distribution? (2) What are the behaviors of these genotypes for root characters in response to drought? and (3) What are the relationships between root characters in response to drought and yield?

The objectives of this study were to assess root distributions, variations in root length density (RLD) and percentage of root distribution, and the relevance of root traits for yield of drought resistant peanut genotypes under different available soil water levels.

Materials and methods

The experiment was conducted in the dry seasons of 2003/04 and 2004/05. Eleven peanut genotypes were used, of which eight (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348 and ICGV 98353) are elite drought resistant lines from ICRISAT, one (Tifton-8) is a drought resistant line from USDA and two (KK 60-3 and Tainan 9) are released cultivars in Thailand. A split-plot in a randomized complete block design with four replicates was used. Main-plot treatments were three soil moisture levels [field capacity (FC), 2/3 available soil water (2/3 AW) and 1/3 available soil water (1/3 AW)], and sub-plot treatments were 11 peanut lines.

Subsurface drip-irrigation system (Super Typhoon®, Netafim Irrigation equipment & Drip systems, Israel) at a distance of 20 cm between emitters was installed with a spacing of 50 cm between driplines at 10 cm below the soil surface mid-way between peanut rows to supply water to the crop. Soil water level was maintained uniformly at field capacity at 0-60 cm from planting to 21 DAS. Afterward, soil moistures for the stress treatments were allowed to gradually decline until reaching the predetermined

levels of 2/3 AW and 1/3 AW at 0-60 cm at 28 DAS and 35 DAS, respectively, then were held more or less constant until harvest.

Roots were sampled by a core sampler at 37, 67 and 97 days after sowing (DAS). Root length was determined by a scanner and the WINRHIZO Pro 2004a software. RLD was calculated as the ratio of root length (cm) and soil volume (cm^3). Graphical illustration of root distribution was constructed by merging RLD in the first and second soil layers (0-40 cm) as upper roots and pooling RLD at the third, fourth and fifth layers (40-100 cm) as lower roots. Pod yield, biomass and harvest index (HI) were recorded at harvest. A drought tolerance index (DTI) was calculated for each parameter as the ratio of the parameter under stress treatment to that under well-watered conditions.

Results

Variations in RLD in 40 to 100 cm layer ($RLD_{40\ to\ 100\ cm}$) were found under well-watered conditions, and the peanut genotypes could be readily identified as high, intermediate and low for this trait. Changes in RLD in the 40 to 100 cm soil layer were found at 2/3 AW and were more evident at 1/3 AW. ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308 and KK 60-3 were classified as drought responsive as they increased RLD in the deeper subsoil level in response to drought (Fig. 1).

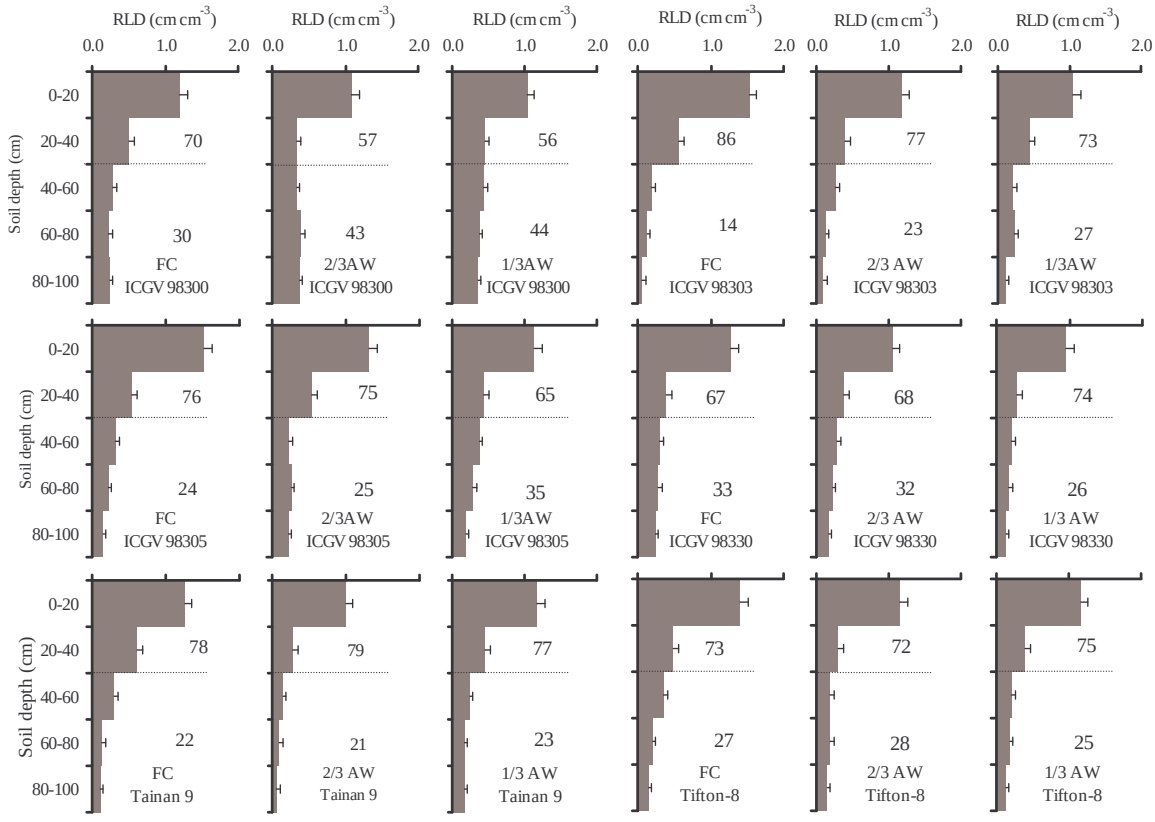

Fig. 1. Root length density distribution with depth for selected peanut genotypes grown under 3 available soil water regimes at 97 DAS for the year 2003/04 and 2004/05. Numeric values above and below dotted line indicate percent of total root length in 0-40 and 40-100 cm soil layers.

In general, RLD under drought conditions was not related to biomass production. The ability to maintain percentage of RLD (DTI for %RLD) was related to pod yield, DTI for pod yield and DTI for HI. In this study we demonstrated drought avoidance mechanisms of peanut genotypes in obtaining higher pod yield under drought conditions. Under drought conditions, ICGV 98300 ICGV 98303 and ICGV 98305 had high pod yield was due to the lower reduction in pod yield and harvest index. Smaller reductions in pod yield and harvest index were closely related with the increased root distribution into deeper soil profile especially under 1/3 AW (Fig. 2). We finally identified the peanut genotypes in the latter group as putatively drought avoidant.

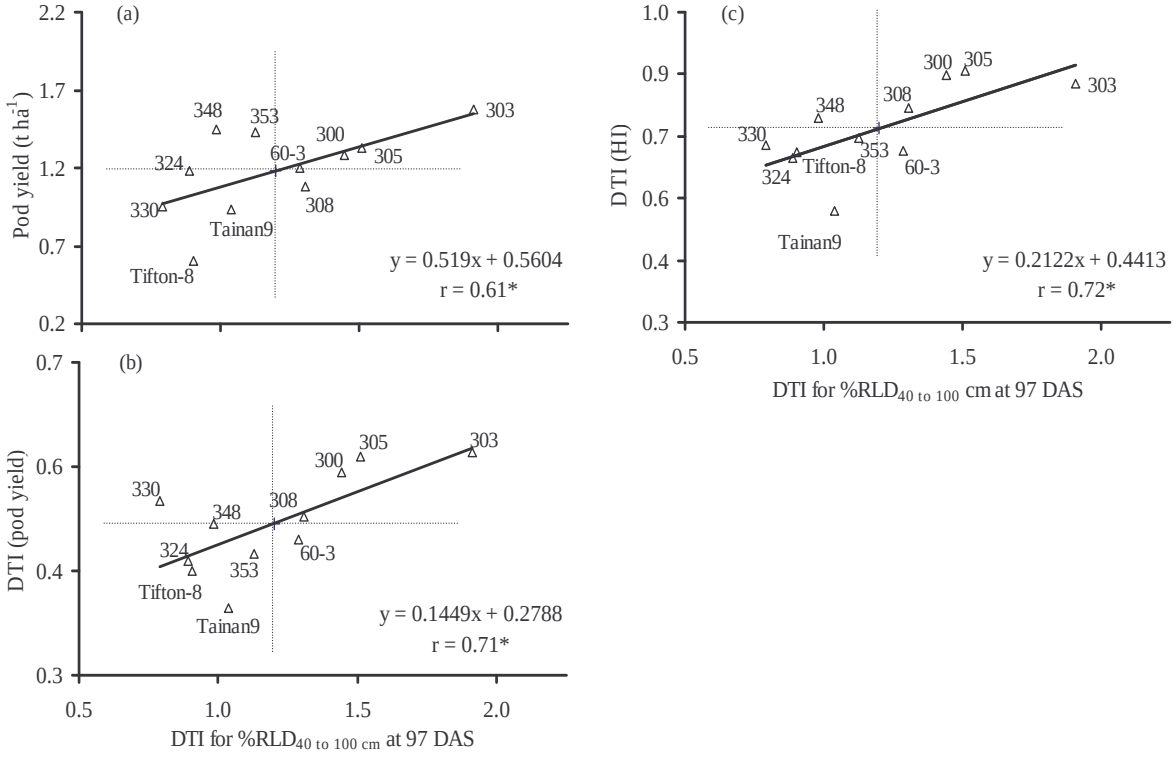

Fig. 2. DTI for % $RLD_{40 \text{ to } 100 \text{ cm}}$ (40-100 cm) at 97 DAS related to pod yield (a), DTI for pod yield (b), and DTI for HI (c) under 1/3 AW condition of 11 peanut genotypes in 2003/04 and 2004/05.

Conclusion

Based on RLD and root distribution characters, ICGV 98300, ICGV 98303 and ICGV 98305 were identified as the genotypes having drought avoidance mechanism. The higher RLD at lower soil depths enhanced drought tolerance in these genotypes, enabling them to stabilize pod yield and HI under water stress conditions. Plants might possess one or several mechanisms of drought resistance or avoidance in order to achieve high yields under water stress. Drought avoidance by changing root distribution into deep soil is one of the mechanisms that helps peanut to obtain high pod yield and HI under drought conditions. Breeding for stabilizing yield under water-limited conditions by deeper rooting may facilitate the development of improved peanut cultivars in specific water limited-environments where water is available in deep soil.

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Publication

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2.3 Effect of drought stress on traits related to N_2 fixation in eleven peanut genotypes differing in degrees of resistance to drought

S. Pimratch, S. Jogloy, N. Vorasoot, B. Toomsan, T. Kesmala,
A. Patanothai, and C.C. Holbrook

Drought is a recurring problem limiting peanut yield in rain-fed areas of the semi-arid tropics (Wright et al., 1991) and it also reduces N_2 fixation (Sinclair et al., 1995; Serraj et al., 1999). As access to irrigation in these areas is limited, utilization of drought resistant genotypes is a viable alternative to alleviate the problem. Improvement of genotypes that can fix more nitrogen under drought stress conditions is important to enhance productivity of the crop.

The objective of this study was to determine the effect of drought stress on nitrogenase activity, nodule number and nodule dry weight of eleven peanut genotypes with different degrees of drought resistance.

Materials and Methods

Eleven peanut genotypes were tested in a split-plot design with four replications under field conditions with rhizobium inoculation but without nitrogen fertilizer in the dry seasons of 2003/2004 and 2004/2005. Main-plot treatments were three water regimes [field capacity (FC), 2/3 available soil water (AW) and 1/3 AW], and sub-plot treatments were 11 peanut lines. Eight (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348 and ICGV 98353) are drought resistant lines from ICRISAT, one (Tifton-8) is drought resistant line from USDA and two (KK 60-3 and Tainan 9) are released cultivars in Thailand. Data were collected on nodule number, nodule dry weight, and nitrogenase activity (acetylene reduction assay) at 30, 60 and 90 days after emergence (DAE).

The analyses of variance were done using MSTAT-C package. Simple correlation was used to determine the relationship between nitrogenase activity, with nodule number and nodule dry weight and between nodule number and nodule dry weight. Multiple-linear regression was used to determine the relative contribution of nitrogenase activity under non-stressed condition and reduction in nitrogenase activity under water stress condition to nitrogenase activity under each stress condition.

Results

The genotypes were significantly different for most evaluations except for nitrogenase activity at 1/3 AW at 60 and 90 DAE (Table 1). Drought stress reduced nitrogenase activity, nodule number and nodule dry weight, but the reductions in these traits differed among traits and between levels of drought stress. The results indicated that levels of stress and times of evaluations had significant effects on the responses of peanut genotypes for these traits. The variation in nitrogenase activity was not high and significant differences among genotypes were found at FC and 2/3 AW only. This could be due to high reduction in nitrogenase activity under severe drought conditions. Tifton-8 and KK 60-3 were the best genotypes, showing consistently higher nitrogenase activity than other genotypes. The reductions in nitrogenase activity of Tifton-8 and KK 60-3 were also higher than those of the others. The drought resistant lines from ICRISAT did not differ greatly for nitrogenase activity and nodule traits. Tifton-8 had a moderate number of nodules at FC, but relatively high numbers under drought conditions especially at 90 DAE. This might be due to low reductions at both stress levels. KK 60-3 had a high number of nodules at FC and it also performed well under drought conditions at both sampling dates. Low reduction in nodule numbers was also the cause. KK 60-3 showed consistently high nodule dry weight across water levels and sampling dates due to its high performance at FC and relatively low reduction. Nodule dry weight for Tifton-8 was not as high as that of KK 60-3 at 60 DAE under well-watered conditions, but similar at 90 DAE. Under drought conditions, Tifton-8 has lower nodule dry weight than did KK 60-3 at 60 DAE, but its nodule number was similar to that of KK 60-3 at 90 DAE. Tifton-8 also had relatively low reduction in nodule dry weight in response to water stress.

Significant correlation coefficients were not found among nitrogenase activity, nodule number and nodule dry weight under FC, but significant correlation coefficients were found between the nodule dry weight and the nitrogenase activity at 2/3 AW at both sampling dates ($r = 0.68^*$ and 0.65^* for 60 and 90 DAE, respectively) and at 1/3 AW at 60 DAE ($r = 0.63^*$) (Table 2). The results indicated that nodule dry weight was more important than nodule number for nitrogen fixation under drought conditions and also implied that nitrogen derived from the air is necessary for the crop as nitrogen uptake is limited by drought.

Regression analysis showed that at 2/3 AW the joint contributions of nitrogenase activity under well-watered conditions and the amount of reduction under stress were high, accounting for 96.2 and 98.8% of total contributions at 60 and 90 DAE, respectively (Table 3). The reductions (41.1% for 60 DAE and 46.5% for 90 DAE) were slightly lower than those of nitrogenase activity under well-watered conditions (55.1% for 60 DAE and 52.4% for 90 DAE). The results indicated that both potential and reduction are equally important for the performance under mild drought. Under severe drought, the relative contributions of these factors were also high, with total contributions of 94.6 and 98.2% for 60 and 90 DAE (Table 3), respectively. However, the contributions of the reduction, accounting for 94.5 and 79.1% of total contributions at 60 and 90 DAE, respectively, were much higher than those of nitrogenase activity under well-watered conditions (0.10% for 60 DAE and 19.1% for 90 DAE). These results indicated that under severe drought the performance of peanut genotypes for nitrogenase activity was dependent largely on the reduction.

Conclusion

Severe drought stress reduced nitrogenase activity, nodule number and nodule dry weight about two times greater than did mild drought stress, causing uniform performance of peanut genotypes for nitrogenase activity under severe drought conditions. However, differences among peanut genotypes in nodule traits were found at all water levels. Tifton-8 and KK 60-3 in general performed better than the drought resistant lines from ICRISAT for all traits. Nodule dry weight was closely related with nitrogenase activity under drought conditions. High nitrogenase activity under mild drought conditions was related to high nitrogenase activity under well watered conditions (potential) and to a low rate of reduction in nitrogenase activity in response to stress. The contribution of the potential was lower under more severe drought conditions. Selection for high nitrogenase activity as a surrogate trait to improve nitrogen fixation under drought conditions should be more effective under mild than severe stress.

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Table 1. Nitrogenase activity (Acetylene reduction assay; ARA), nodule number, nodule dry weight and the reductions in these traits of 11 peanut genotypes under different water regimes at 60 and 90 day after emergence (DAE).

Genotypes	ARA					Nodule number					Nodule dry weight				
	(µmole plant $^{-1}$ hr^{-1})			Reduction (%)		(nodules plant $^{-1}$)			Reduction (%)		(g plant $^{-1}$)			Reduction (%)	
	FC	2/3 AW	1/3 AW	2/3 AW	1/3 AW	FC	2/3 AW	1/3 AW	2/3 AW	1/3 AW	FC	2/3 AW	1/3 AW	2/3 AW	1/3 AW
							60 DAE								
Tifton-8	15.7a	12.3a	7.5	21.4ab	52.1a	327cd	284abc	155b	12.2c	53.0cd	0.150cd	0.146b	0.080bc	1.6d	46.6abc
KK 60-3	15.6a	10.8ab	6.9	30.7a	55.7a	348b	307a	181a	11.7c	46.0d	0.208a	0.164a	0.110a	21.0abc	46.4abc
ICGV 98300	11.7b	9.6b	7.2	16.8abc	36.5bc	278f	230d	138bcd	16.2c	49.5d	0.174bc	0.132bcd	0.107a	18.5bcd	31.4c
ICGV 98348	11.0b	9.5b	6.8	12.6bc	37.3bc	296ef	184e	88g	34.1a	70.4a	0.192ab	0.110ef	0.084bc	38.1a	49.5ab
ICGV 98353	10.7b	10.5ab	6.9	4.0cd	34.9bc	286f	184e	100fg	30.3a	63.7b	0.150cd	0.080g	0.091abc	39.9a	34.1bc
ICGV 98324	11.3b	9.9b	7.5	12.0bc	32.6bc	373a	302a	148bc	20.7bc	61.1b	0.161cd	0.120de	0.073bc	24.2abc	52.3a
ICGV 98330	10.4b	10.2b	7.4	1.1d	28.2c	328cd	288ab	135cd	12.5c	59.2bc	0.143d	0.124cde	0.072c	8.3cd	46.7abc
ICGV 98308	11.2b	10.5ab	7.0	3.2cd	35.6bc	342bc	265c	129de	20.78bc	61.0b	0.159cd	0.119de	0.081bc	22.1abc	43.9abc
ICGV 98305	10.9b	9.9b	7.5	8.0bcd	30.4c	314de	267bc	113ef	15.34c	64.1ab	0.156cd	0.139bc	0.090abc	8.7cd	40.2abc
ICGV 98303	10.8b	10.2b	7.2	5.1cd	32.8bc	322d	264c	155b	16.8c	50.8cd	0.155cd	0.118de	0.095ab	18.6bcd	31.6c
Tainan 9	12.3ab	10.1b	7.1	16.7abc	41.0b	342bc	234d	110f	28.0ab	65.2ab	0.151cd	0.099f	0.074bc	29.9ab	45.8abc
Mean	12.0	10.4	7.2	12.0	37.9	295	255	132	19.9	58.5	0.164	0.123	0.087	21.0	42.6
							90 DAE								
Tifton-8	12.6a	8.3a	4.9	34.4a	60.4a	345b	266a	165ab	19.7d	46.7e	0.227b	0.199a	0.118b	13.1e	50.4cde
KK 60-3	11.8ab	8.0ab	4.6	31.4ab	59.4a	379a	271a	175a	25.6cd	49.3e	0.237ab	0.183a	0.143a	22.1de	39.6e
ICGV 98300	10.3bc	7.5ab	4.3	27.4abc	57.5ab	285cd	157de	105e	44.6ab	62.0abc	0.242ab	0.143bc	0.085def	37.8bcd	61.9abc
ICGV 98348	9.5c	7.2b	4.4	24.1bcd	52.5abc	264d	130e	104e	48.9a	58.3bcd	0.229b	0.135bc	0.112bc	39.3bcd	46.8de
ICGV 98353	9.5c	7.4ab	4.4	22.6bcd	52.2abc	285cd	156de	83f	39.3abc	68.4a	0.183cd	0.073d	0.0578g	57.2a	65.8a
ICGV 98324	9.8c	7.7ab	4.3	21.6bcd	54.9abc	369ab	233b	122d	32.4bcd	62.0abc	0.201c	0.143bc	0.080ef	25.8cde	57.9a-d
ICGV 98330	9.2c	7.1b	4.7	23.7bcd	47.4c	367ab	220bc	132d	39.8abc	64.0ab	0.183cd	0.141bc	0.071fg	32.4bcd	62.5ab
ICGV 98308	9.3c	7.7ab	4.6	16.6d	48.5bc	291cd	174d	134cd	34.1bc	47.4e	0.204c	0.106cd	0.086def	47.6ab	57.3a-d
ICGV 98305	9.4c	7.8ab	4.6	16.7d	49.5bc	369ab	201c	150bc	43.9ab	54.1cde	0.252a	0.149b	0.098cd	40.6bc	60.5abc
ICGV 98303	9.6c	7.7ab	4.2	19.4cd	55.6abc	312c	172d	139cd	42.2ab	52.2de	0.182cd	0.136bc	0.092de	26.2cde	51.0b-e
Tainan 9	10.6bc	7.4ab	4.3	31.1ab	59.1a	271d	171d	92ef	34.7abc	63.9ab	0.162d	0.106cd	0.058g	33.8bcd	63.8a
Mean	10.1	7.6	4.5	24.4	54.3	322	196	127	36.8	57.1	0.209	0.138	0.091	34.2	56.1

Different letters in each column show significant at 99% level of probability; FC = field capacity, AW = available soil water.

*,** = Significant at 95% and 99% probability levels, respectively.

Table 2. Correlation between nitrogenase activity (Acetylene reduction assay, ARA), with nodule number and nodule dry weight different water regimes.

Parameters	Nitrogenase activity		
	FC	2/3 AW	1/3 AW
60 DAE			
Nodule number	0.28	0.37	0.30
Nodule dry weight	0.24	0.68*	0.63*
90 DAE			
Nodule number	0.39	0.35	-0.37
Nodule dry weight	0.28	0.65*	0.42
		Nodule dry weight	
Nodule number			
60 DAE	-0.03	0.72*	0.35
90 DAE	0.29	0.74**	0.77**

*, ** = Significant at 95% and 99% probability levels, respectively.
FC = field capacity, AW = available soil water.

Table 3. Contribution of potential of nitrogenase activity at field capacity and reduction in nitrogenase activity fixed under drought stress at 60 and 90 days after emergence (DAE).

Category	Explained by regression (%)	
	60 DAE	90 DAE
At 2/3 AW		
Regression	96.2**	98.8**
Potential of nitrogenase activity at FC	55.1**	52.4**
Reduction in nitrogenase activity at 2/3 AW	41.1**	46.5**
At 1/3 AW		
Regression	94.6**	98.2**
Potential of nitrogenase activity at FC	0.10**	19.1**
Reduction in nitrogenase activity at 1/3 AW	94.5**	79.1**

** = Significant at the 0.01 probability level.
FC = field capacity, AW = available soil water.

2.4 Heritability of drought–resistance traits and genotypic and phenotypic correlation of drought–resistance and agronomic traits in peanut

P. Songsri, S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, A. Patanothai, and C.C. Holbrook

Drought is the major abiotic constraint affecting peanut productivity and quality worldwide. Improving water access and management are practically difficult since water is a scarce resource. Therefore breeding for drought resistance is an important strategy in alleviating the problem and offers the best long term solution. While direct selection for yield under stressed conditions can be effective, the limitations of this approach are high resource investment and poor repeatability of the results due to the large G x E (genotype x environment) interaction that results in slow breeding progress (Wright et al., 1996).

More rapid progress may be achieved by using physiological traits such as harvest index (HI) or water use efficiency (WUE), specific leaf area (SLA), and SPAD chlorophyll meter reading (SCMR) (Nigam et al., 2005). WUE, however, is not an easy trait to measure, and therefore is not practical for use in large-scale breeding programs for improving drought tolerance. SLA and SCMR have also been used as surrogate traits for WUE (Sheshshayee et al., 2006; Nigam et al., 2005).

Information on the inheritance of HI, SLA and SCMR and the genetic correlations among these traits will be useful for planning a suitable breeding strategy for improving drought tolerance. Drought can alter the heritability estimates of these traits, therefore, genetic gain through conventional selection might be different under drought and well-watered conditions. Genetic correlations between drought resistance traits and agronomic traits have to be studied in details under drought and well-watered conditions to evaluate correlated responses to selection of drought resistance traits on agronomic traits.

The objectives of this study were to estimate the (i) heritabilities of drought resistance traits, (ii) genotypic and phenotypic correlations between drought resistance traits and agronomic traits in peanut under different water levels, and (iii) relationship between drought resistance traits under stressed and non-stressed condition.

Materials and methods

Four peanut F_1 hybrids (ICGV 98308 x KK60-3, ICGV 98324 x KK60-3, ICGV 98308 x Tainan 9, and ICGV 98324 x Tainan 9) were generated from the hybridization of 2 drought resistant lines (ICGV 98308 and ICGV 98324) selected for low yield reduction under drought stress with two high yielding cultivars KK60-3 and Tainan 9. ICGV 98324 and KK 60-3 are know to have high SCMR and low SLA, ICGV 98308 had moderate SLA and moderate SCMR, and Tainan 9 had high SLA and low SCMR under both stressed and non-stressed conditions.

The F_1 seeds were planted and their seeds harvested in bulk for each cross. In F_2 and F_3 generations, two pods were kept for each plant and bulked for each cross. Line separation was carried out in the F_4 generation. A total of 140 lines (35 lines for each cross) were randomly selected and multiplied in the F_5 and F_6 generation.

The 140 families from 4 crosses were evaluated in the $F_{4:7}$ and $F_{4:8}$ generations (F_4–derived lines in the F_7 and F_8 generations, respectively) under two soil moisture levels (field capacity (FC) and 2/3 available soil water (2/3 AW)) for two years in the dry seasons of 2005/06 and 2006/07 at Khon Kaen University. A split plot design with four replications was used. Two soil moisture levels [FC (11.0 %) and 2/3 AW (8.8 %) in 0-60 cm depth] were assigned as main plots, and peanut lines were laid out in subplots.

Data were recorded for specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR), and for biomass, pod yield, harvest index, number of mature pods per plant and seed per pod and seed size. Drought tolerance index (DTI) was calculated for biomass (DTI (BIO)), and pod yield (DTI (PY)) as the ratio of each parameter under stressed treatments (2/3 AW) to that under well-watered (FC) condition.

Estimates of broad-sense heritability for the 4 crosses were calculated by partitioning variance components of family mean squares to pooled environmental variance (δ^2_E) and genotypic variance (δ^2_G), and then broad-sense heritability estimates (h^2_b) were calculated following Holland et al. (2003). Phenotypic and genotypic correlations between drought resistance traits and agronomic traits were calculated following the methods of Falconer and Mackay (1996). Simple correlation was used to determine the relationship between biomass, pod yield and drought resistance traits under well-watered and drought conditions to understand whether the performance of peanut genotypes was consistent across environments.

Results

The h^2 for biomass (BIO), pod yield (PY), DTI (Drought tolerance index) (PY), DTI (BIO), HI, SLA and SCMR were high for all tested crosses (0.54 to 0.98) (Table 1). Most characters had similar heritability estimates when compared between different water levels. This should make selection for drought tolerance easier. However, DTI is still useful in explaining how some genotypes had higher pod yield under drought.

Table 1. Estimates of heritability with standard error for biomass (BIO), pod yield (PY), drought tolerance index for biomass (DTI† (BIO)) and pod yield (DTI (PY)) and harvest index (HI) at harvest and specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) at 67 day after sowing of 4 crosses of peanut under stressed and non-stressed conditions.

	Heritability						
Cross	BIO	PY	DTI (BIO)	DTI (PY)	HI	SLA	SCMR
Stressed							
ICGV 98308 x KK60-3	0.94±0.06	0.93±0.07	0.93±0.07	0.86±0.11	0.94±0.05	0.93±0.07	0.89±0.10
ICGV 98308 x Tainan9	0.81±0.16	0.95±0.05	0.54±0.25	0.92±0.07	0.89±0.08	0.81±0.15	0.96±0.03
ICGV 98324 x KK60-3	0.73±0.20	0.93±0.07	0.67±0.21	0.87±0.11	0.95±0.04	0.91±0.08	0.92±0.08
ICGV 98324 x Tainan9	0.96±0.04	0.97±0.03	0.86±0.12	0.96±0.03	0.89±0.08	0.95±0.05	0.96±0.04
Non stressed							
ICGV 98308 x KK60-3	0.89±0.12	0.91±0.08	—	—	0.94±0.04	0.83±0.15	0.89±0.11
ICGV 98308 x Tainan9	0.98±0.02	0.98±0.02	—	—	0.97±0.02	0.91±0.09	0.97±0.02
ICGV 98324 x KK60-3	0.93±0.07	0.93±0.06	—	—	0.92±0.06	0.91±0.09	0.90±0.08
ICGV 98324 x Tainan9	0.98±0.02	0.98±0.01	—	—	0.96±0.03	0.95±0.05	0.96±0.04

† DTI were calculated by the ratio of stressed (2/3 available water (AW)) / non-stressed (field capacity (FC)) conditions.

The r_G (-0.61 and -0.66) and r_P (-0.61 and -0.66) between SLA and SCMR were strong and negative under 2/3 AW and FC (Table 2). Our finding showed that genotypic and phenotypic correlations between SLA and SCMR were consistent under both FC and 2/3 AW conditions. The results showed consistency of SLA and SCMR in a wide range of soil water levels and drought conditions.

SCMR was positively correlated with PY (0.21) and seed size (0.43) under 2/3 AW conditions. SCMR had higher r_G and r_P with PY, BIO and other agronomics traits than did SLA (Table 3). DTI (PY) had strong and positive genotypic correlation with DTI (BIO) (0.69; $P \leq 0.01$). HI was quite low correlated with DTI (PY) under stressed condition (0.37; $P \leq 0.01$) and also was correlated with SCMR both under drought and well-watered conditions (0.13; $P \leq 0.01$and 0.33; $P \leq 0.01$, respectively). Nonetheless, the integration of physiological traits (or their surrogates) in the selection scheme would be advantageous in selecting genotypes which are more efficient water utilisers (SCMR) or partitioners of photosynthates into economic yield (HI). The SPAD chlorophyll meter provides an easy opportunity to integrate a surrogate measure of water-use-efficiency with pod yield, in the selection scheme of a drought resistance breeding program in peanut.

Significant correlations between FC and 2/3 AW conditions were found for PY, BIO, SCMR and SLA, indicating that these traits could be selected under FC or 2/3 AW conditions (Table 4). SCMR which is easy to measure is potentially useful as a selection trait for drought resistance because of high h^2 and positive correlation with PY and agronomic traits. As heritability estimates were high under both well-watered and stress conditions and the traits under different water regimes were correlated well, it is advisable to first select peanut genotypes under well-watered conditions in large segregating populations because drought simulation is much more difficult, and later the selections can be refined under both drought and non-stress conditions in advanced generations.

Table 2. Genotypic (r_G) correlation estimates among drought resistance traits for all 4 peanut cross of 140 genotypes in the dry seasons of 2005/06 and 2006/07 in Khon Kaen, Thailand (degree of freedom = 556).

	Stressed				Non stressed		
	DTI† (PY)	SCMR	SLA	HI	SCMR	SLA	HI
DTI (BIO)	0.69**	-0.34**	0.05	0.06	-	-	-
DTI (PY)		-0.28**	0.06	0.37**	-	-	-
SCMR			-0.61**	0.13**		-0.66**	0.33**
SLA				0.11*			-0.10*

* and ** significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.

† DTI were calculated by the ratio of stressed (2/3 AW)/non-stressed (FC) conditions.

Table 3. Genotypic (r_G) correlation estimates between drought resistance traits and agronomic traits for all 4 peanut cross of 140 genotypes in the dry seasons of 2005/06 and 2006/07 in Khon Kaen, Thailand (degree of freedom = 556).

Drought resistance traits	Agronomic traits				
	BIO	PY	Seed size	No. mature pods/plant	Seed/pod
Stressed					
DTI† (BIO)	0.47**	0.34**	0.01	0.34**	0.29**
DTI (PY)	0.52**	0.57**	0.25**	0.45**	0.14**
SCMR	0.18**	0.21**	0.43**	-0.20**	-0.04
SLA	0.07	0.07	0.06	0.04	0.10*
HI	0.19**	0.76**	0.50**	0.62**	0.16**
Non stressed					
SCMR	0.41**	0.51**	0.48**	0.02	0.24**
SLA	0.01	-0.09*	-0.12**	0.02	0.06
HI	0.01	0.79**	0.47**	0.49**	0.26**

* and ** significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.
† DTI were calculated by the ratio of stressed (2/3 AW)/non-stressed (FC) conditions.

Table 4. Correlation coefficients of biomass (BIO), pod yield (PY), harvest index (HI), specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) of 4 peanut crosses under stressed (d) and well-watered (w) conditions during 2005/06 and 2006/07 in Khon Kaen, Thailand (degree of freedom = 33).

Correlation	Peanut cross			
	ICGV 98308 x KK60-3	ICGV 98308 x Tainan9	ICGV 98324 x KK60-3	ICGV 98324 x Tainan9
BIO_w *vs* BIO_d	0.48**	0.79**	0.62**	0.84**
PY_w *vs* PY_w	0.35*	0.73**	0.61**	0.71**
HI_w *vs* HI_d	0.75**	0.62**	0.58**	0.46**
$SCMR_w$ *vs* $SCMR_d$	0.73**	0.84**	0.53**	0.86**
SLA_w *vs* SLA_d	0.59**	0.35*	0.52**	0.86**

* and ** significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.

Conclusion

Most traits measured in these 4 peanut crosses had high heritability indicating that breeding progress should be possible. HI, SCMR and SLA observations can be recorded at both stressed and non-stressed conditions. This gives peanut breeders a large flexibility to record these observations in a large number of segregating populations and breeding lines in the field, thus making it easy to incorporate these physiological traits associated with drought tolerance in breeding and selection schemes in peanut. SCMR should be particularly useful as a selection criterion for drought tolerance in peanut because of high heritability, and the simplicity in gathering.

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2.5 Response of reproductive characters of drought resistant peanut genotypes to drought

P. Songsri, S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook

Drought is a major cause of yield reduction for peanut production worldwide (Reddy et al. 2003; Wright and Nageswara Rao 1994; Vorasoot et al. 2003). Information on the effects of water stress on reproductive characters of peanut genotypes varying in degrees of drought resistance would provide a better understanding of the differences in yield performance under drought. Pod yield can be considered as the sequential processes of flower production, peg initiation, conversion of peg to pods, and pod filling. However, the contributions of reproductive characters such as number of flowers, pegs and mature pods to pod yield stability under drought have not been well researched.

The objectives of this study were to evaluate genetic variations in yield and reproductive developmental characters among peanut genotypes in response to drought and relate these responses to pod yield under different available soil water levels. This information should provide a better understanding on how genotypes could achieve high yield under drought, and could have important implications on breeding for drought resistance in peanut.

Materials and methods

Eleven peanut genotypes varying in degrees of drought tolerance were tested under three soil moisture levels [field capacity (FC), 2/3 available soil water (AW) and 1/3AW] in field experiments at Khon Kaen University in the dry seasons of 2003-2004 and 2004-2005. Eight were drought tolerance lines from ICRISAT, one was a drought tolerant line from the US and the remaining two were released cultivars in Thailand. In maintaining the specified soil moisture levels, water was added to the respective plots by subsurface drip-irrigation based on crop water requirement and surface evaporation which were calculated following the methods described by Songsri et al. (2008). Data were recorded for number of flowers, pegs, immature pods and mature pods per plant, seed per pod, 100-seed weight, and pod yield at harvest. A drought tolerance index (DTI) for pod yield was calculated as the ratio of pod yield under stress treatment to that under well-watered conditions.

Results

Soil water status showed reasonable management of soil moistures. A clear distinction among soil moisture levels was noted at 30 cm of soil depth except in the year 2003-04, when rainfall of 71 mm at 73-75 DAS, which caused high soil moistures in the drought treatments. Relative water content (RWC) and leaf water potential (LWP) were significantly lower in the plants experiencing soil-moisture-deficit stress than their respective controls (Fig. 1). The highest LWP and RWC were observed for soil moisture at FC followed by 2/3 AW and 1/3 AW, respectively. Observations found visual wilting in 2/3 AW and more severe wilting in 1/3 AW in the afternoon. RWC and LWP of the plants in the 1/3 AW treatment were extremely low at 97 DAS.

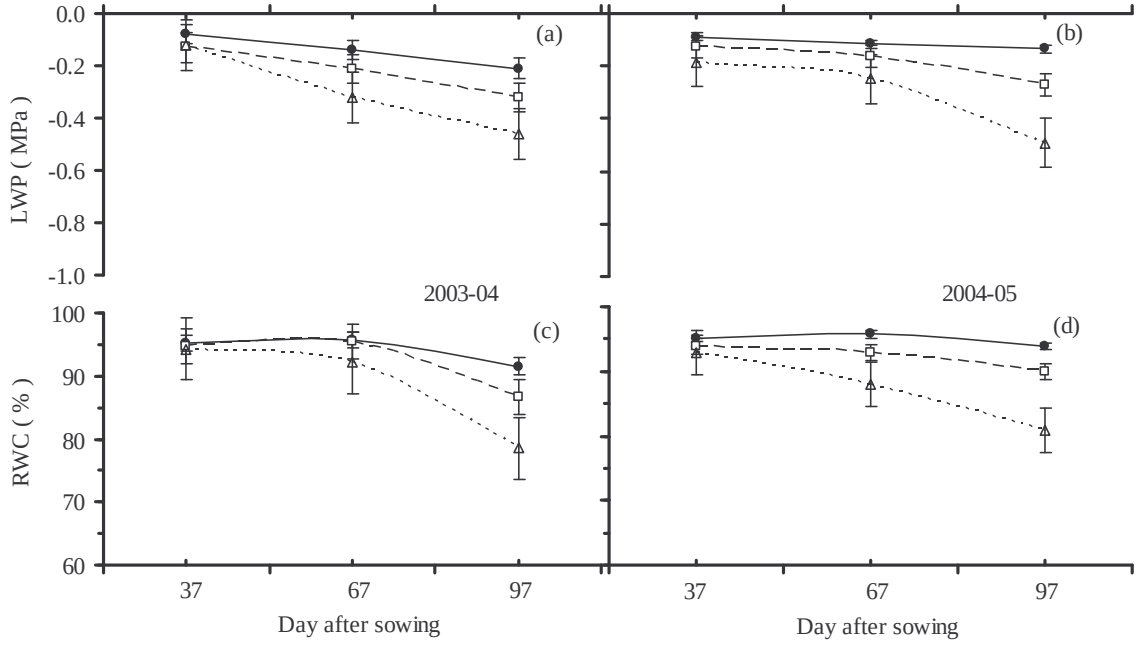

Fig.1. Leaf water potential (LWP) (a, b) and relative water content (RWC) (c, d) in three available soil water regimes (FC, ●; 2/3 AW, □ and 1/3 AW, Δ) at 37, 67 and 97 DAS during the 2003-04 and 2004-05 dry seasons.

The differences among water regimes were significant for pod yield, number of pegs, immature pods and mature pods per plant, seed per pod and 100 seed weight, and differences among genotypes were significant for all traits. Drought reduced pod yield, number of pegs, pods and mature pods per plant. Early peak of flowering is important for the formation of mature pods under drought conditions.

Two different strategies were used in maintaining high pod yield under drought (Fig. 2). High yield potential was important for ICGV 98348 and ICGV 98353, whereas low pod yield reduction was important for ICGV 98305, ICGV 98303 and ICGV 98300 (Table 1). Tifton 8 showed the lowest pod yield and poor seed filling. High number of pegs and well-filled mature pods were the most important traits contributing to high pod yield in drought resistant genotypes (Table 2).

Conclusion

High numbers of flowers and pegs are advantageous but not necessary for high yield under drought conditions. Early peak of flowering is important for the formation of mature pods, and number of mature pods is the most important character determining pod yield under both non-stressed and stressed conditions. Seed size is also important for pod yield under drought but in lesser extent. Our findings showed that high pod yield under non-stressed conditions are important for high pod yield under drought conditions in some genotypes, whereas low reduction in pod yield under drought conditions is more important in others. This information should help breeders to better understand the factors that lead to higher pod yield under drought and may help breeders to formulate more effective and efficient breeding strategies for improving drought resistance in peanut.

Table 1. Pod yield (PY, t ha^{-1}) and drought tolerance index (DTI) for pod yield of 11 peanut genotypes grown under different water regimes at harvest in dry seasons of 2003-04 and 2004-05.

Genotype	2003-04					2004-05				
	PY FC	PY 2/3 AW	PY 1/3 AW	DTI (2/3 AW)	DTI (1/3 AW)	PY FC	PY 2/3 AW	PY 1/3 AW	DTI (2/3 AW)	DTI (1/3 AW)
ICGV 98353	3.19 a	2.56 a	1.81 a	0.80	0.57	3.14 ab	2.38 ab	0.99 a-d	0.76	0.32
ICGV 98348	3.00 ab	2.52 ab	1.79 a	0.84	0.60	3.16 ab	2.48 a	1.00 a-d	0.78	0.32
Tainan 9	2.45 bc	2.22 abc	1.13 bc	0.91	0.46	2.57 bcd	1.34 de	0.74 d	0.52	0.29
ICGV 98303	2.35 c	2.20 a-d	1.84 a	0.94	0.78	3.19 ab	1.83 b-e	1.23 a	0.57	0.39
ICGV 98324	2.41 bc	2.17 a-d	1.14 bc	0.90	0.47	3.42 a	1.75 cde	1.21 ab	0.51	0.35
ICGV 98305	2.27 c	2.05 a-d	1.67 a	0.90	0.74	2.17 de	2.17 abc	0.96 bcd	1.00	0.44
ICGV 98300	2.31 c	2.03 a-d	1.42 ab	0.88	0.61	2.40 d	2.11 abc	1.22 a	0.88	0.51
ICGV 98308	2.18 c	1.98 bcd	1.09 bc	0.91	0.50	2.45 cd	2.34 ab	1.01 abc	0.96	0.41
ICGV 98330	2.09 c	1.80 cd	1.09 bc	0.86	0.52	2.14 de	1.79 b-e	0.84 cd	0.84	0.39
KK 60-3	2.29 c	1.62 d	1.41 ab	0.71	0.62	3.07 abc	1.85 bcd	1.00 a-d	0.60	0.33
Tifton -8	1.23 d	0.91 e	0.70 c	0.74	0.57	1.75 e	1.23 e	0.42 e	0.70	0.24
Mean	*2.34*	*2.01*	*1.37*	*0.85*	*0.59*	*2.68*	*1.93*	*0.97*	*0.74*	*0.36*

Mean in the same column with the same letters are not significantly different by DMR at $p \leq 0.05$.

DTI for a genotype were calculated by the ratio of stressed (2/3 AW or 1/3 AW)/non stressed (FC) conditions.

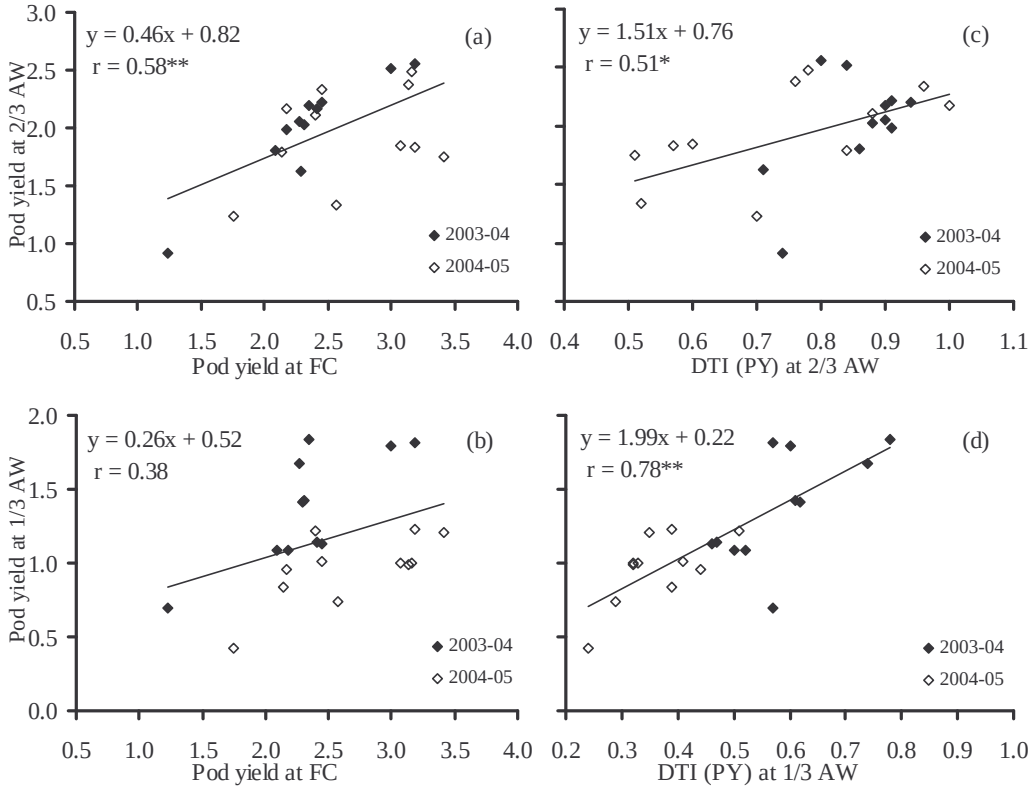

Fig. 2. Relationship between pod yield (t ha^{-1}) under FC and 2/3 AW (a) and 1/3 AW (b) and DTI for pod yield (DTI (PY)) under 2/3 AW (c) and 1/3 AW (d) of 11 peanut genotypes in 2003-04 and 2004-05.

Table 2. Correlation coefficients among the pod yield and drought tolerance index for pod yield (DTI (PY)) and number of flowers, pegs, pods, mature pods per plant, seed per pod and 100 seed weight of 11 peanut genotypes under three water regimes grown in the field during 2003-04 and 2004-05.

Trait	Flower No. $plant^{-1}$	Pegs No. $plant^{-1}$	Pods No. $plant^{-1}$	Mature pods No. $plant^{-1}$	Seed No. pod^{-1}	100 seed weight
Pod yield at FC	0.06	0.59**	0.67**	0.29	0.28	-0.30
Pod yield at 2/3 AW	0.39	0.53*	0.61**	0.63**	0.26	-0.43*
DTI (PY) at 2/3 AW	0.42	0.01	-0.02	0.44*	-0.14	-0.25
Pod yield at 1/3 AW	0.51*	0.28	0.41	0.87**	0.47*	0.38
DTI (PY) at 1/3 AW	0.51*	-0.10	-0.02	0.76**	0.45*	0.57**

* and ** significant at $p \leq 0.05$ and significant at $p \leq 0.01$, respectively.

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2.6 Stability of relationship between chlorophyll density and soil plant analysis development chlorophyll meter readings across different drought stress conditions in peanut

A. Arunyanark, S. Jogloy, C. Akkasaeng, N. Vorasoot, T. Kesmala and A. Patanothai

Chlorophyll content per unit leaf area (chlorophyll density) has been used as an index of photosynthetic capacity and growth of plants (Bowyer and Leegood, 1997). The ability to maintain high chlorophyll density under water deficit conditions has been suggested as a drought tolerance mechanism of many crop plants (This et al., 2000; van der Mescht et al., 1999) including peanut (Arunyanark et al., 2008). Although chlorophyll density is a drought tolerance trait, this measurement process is laborious, time consuming, costly, destructive and inconvenient for a large number of samples. These limitations preclude its use for plant breeding programs in which large numbers of samples are involved. Indirect methods which are easy and rapid to use, economical, effective and reliable to assess chlorophyll density are required.

Minolta SPAD-502 meter measures green color intensity in leaves *in vivo,* and is, therefore, an ideal instrument for obtaining the data without destructive sampling of a large number of chlorophyll data in the field, and the reading is performed in very short time. Close associations between SPAD chlorophyll meter reading (SCMR) value and chlorophyll density had been reported in peanut (Arunyanark et al., 2008). Thus, SCMR could be used indirectly for evaluation of drought tolerance in peanut genotypes.

However, breeding and selection scheme for drought tolerance was limited by other important problems. Such breeding programs have to be conducted under dry season only or in glasshouse where drought conditions can be simulated. In field evaluation, although the number of genotypes evaluated is not limited, it can be grown

only one crop a year. In contrast to field evaluation, although the experiment in greenhouse can simulate drought, it has a limited space to grow a large number of peanut genotypes. Moreover, in large-scale breeding programs, it is difficult to complete SCMR observations within a specified time and plant stage, or could not take sample at same leaf position for all.

Surrogate traits for drought tolerance such as chlorophyll density and SCMR will help to increase potential of breeding scheme if they are able to identify drought tolerance genotypes with chlorophyll density and SCMR stability across water supply conditions. Thus, the evaluation can be carried out for all seasons. Moreover, SCMR would be helpful to breeders if these measurements could be observed in several times or several leaf positions. Therefore, information on the effects of different water regimes, plant ages and leaf positions on chlorophyll density and SCMR relationships is required. The objectives of this study were to (i) examine the stability of chlorophyll density and SCMR, and (ii) evaluate the relationships between chlorophyll density and SCMR in different leaf positions at different times under different drought stress conditions.

Materials and Methods

The field experiment was conducted at the Field Crop Research Station, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, in Thailand, during the dry seasons (November 2003 – May 2004). A split plot design with four replications was used. Three different water regimes (field capacity; FC, 2/3 available water (AW) and 1/3 AW) were assigned in main plots, and twelve peanut cultivars in sub-plots. Twelve peanut cultivars were chosen based on their differences in drought resistance and leaf color. Eight of these lines (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98300, ICGV 98348 and ICGV 98353) were drought resistant germplasm introduced from the International Crops Research Institute for Semi-Arid Tropics (ICRISAT). Tifton-8 is a drought resistant line introduced from the United State Department of Agriculture (USDA). Two cultivars (KK 60-3 and Tainan 9) were commercial varieties grown in Thailand. A non-nodulating line (yellow- green leaf color) was used as a control for low chlorophyll genotype. The crop was grown in eleven-row plots, 6 m long, with a spacing of 0.5 m between rows and 0.2 m between plants within row. A subsoil-drip-irrigation system installed at 10 cm below the soil surface and fitted with a pressure valve and water meter ensured the uniform supply of measured amount of water across each plot.

Data were recorded for SCMR, chlorophyll density (chlorophyll content per unit leaf area) at three times as 20, 40 and 60 DAE. One healthy peanut plant in each plot was randomly sampled, and the first, second and third fully-expanded leaves from the top of the main stems were used for SCMR assessment at 9.00-10.00 a.m., SCMR were recorded by a Minolta SPAD-502 meter (Tokyo, Japan) on the four leaflets of each individual leaf. The average of all leaflets within a leaf was recorded as SCMR value of the individual leaf. Terminal leaflet of each sample leaf were removed and keep chilling until use. The sample leaves were bored using disc borer with the size of 1 cm^2. This disc leaf was soaked in 5 ml of *N,N*-dimethylformamide and kept for 24 hour in dark chamber. Then, chlorophyll extraction was measured the light absorption with spectrophotometer and chlorophyll contents were done following the procedures described by Moran (1981).

Results

Although water regimes and peanut genotypes were significantly different, their interaction effects were not significant for both chlorophyll density and SCMR (Table 1). This pattern was found in all leaf positions. The findings indicate the stability of SCMR and chlorophyll density in peanut across water regimes and peanut genotypes. Both chlorophyll density and SCMR gave similar information on the effects of drought stress in peanut. The result indicates that more stress higher is the density of chlorophyll (Table 2). Moreover, the peanut genotypes showed significant differences in chlorophyll density and SCMR with similar rankings at all sampling times. Differences in chlorophyll density and SCMR among leaf positions were found as early as 20 DAE and were likely to rank in the order of first < second < third, respectively.

The relationships between chlorophyll density and SCMR were high, positive and significant (Table 3), suggesting that SCMR is a useful tool for assessing chlorophyll density in peanut. Plant ages had a small effect on the relationships between chlorophyll density and SCMR, whereas water stress and leaf positions had no significant effect on the relationship (Table 3). It is clear that using SCMR for evaluation of chlorophyll density can be recorded at any water regime conditions in the second or third-fully expanded leaves after 40 days of crop growth. This gives a large flexibility to breeders who have to record observations in a large number of segregating and breeding populations in the field. Drought simulation is not necessary because selection under drought condition gives the results similar to those under well watered condition.

Conclusion

Chlorophyll density and SCMR varied depending on water regimes, times of sampling and genotypes, but water regime x genotype interactions were not significant for chlorophyll density and SCMR. Chlorophyll density and SCMR gave similar information on the differences among water regimes, peanut genotypes and leaf positions. The correlation coefficients between chlorophyll density and SCMR were positive and significant across irrigation treatments and each water regime, plant age and leaf position. The result concluded that evaluation of chlorophyll density by SCMR can be carried out at any water regime conditions in the second or third-fully expanded leaves after 40 days of crop growth.

Table 1. Pooled analysis of variance for measurements over time in split-plot design of chlorophyll density and SPAD chlorophyll meter reading (SCMR).

Source of variation	df	Mean squares					
		Chlorophyll density			SCMR		
		L1	L2	L3	L1	L2	L3
Replication	3	4.5	14.4	18.8	6.9	11.4	5.7
Water regimes (W)	2	9.6	14.5	16.0	192.6*	138.8*	107.8
Error (a)	6	4.5	8.5	10.1	30.4	21.9	30.8
Genotypes (G)	11	43.0**	41.9**	37.8**	467.9**	381.4**	318.2**
W x G	22	1.1	2.0	2.5	20.9	13.8	10.2
Error (b)	99	2.0	2.0	2.4	28.3	12.1	12.1
Time of sampling (T)	2	92.0**	58.5**	59.1**	1037.2**	386.6**	119.8**
W x T	4	9.3**	4.5	11.4**	202.9**	25.7	25.5
G x T	22	3.9**	5.7**	4.1**	39.7*	26.1**	26.1**
W x G x T	44	2.8	2.9*	2.0	24.3	15.9	15.2
Error (c)	216	2.0	1.9	2.1	24.6	13.1	12.6

Significant at *P ≤ 0.05 and **P ≤ 0.01 levels.
L1, L2 and L3 = first, second and third leaf positions.

Table 2. Chlorophyll density and SPAD chlorophyll meter reading (SCMR) of peanut at 20, 40 and 60 day after emergence (DAE).

Treatment	Chlorophyll density (μ*g* cm^{-2})			SCMR		
	20 DAE	40 DAE	60 DAE	20 DAE	40 DAE	60 DAE
Water regimes						
Field capacity	7.9	8.4	8.7 b	39.4	41.3	41.2 b
2/3 available water	7.6	8.4	8.8 b	39.6	42.0	42.3 b
1/3 available water	8.2	8.3	9.9 a	40.0	42.2	45.6 a
LSD (0.05)	ns	ns	0.8	ns	ns	1.4
Peanut cultivars						
ICGV 98300	7.0 ef	8.5 c	9.6 a-d	37.8 de	42.3 cde	44.1 b
ICGV 98303	7.8 cd	7.9 de	9.1 cde	39.5 cd	40.9 ef	43.3 b
ICGV 98305	8.0 cd	8.5 cd	9.0 de	40.4 bc	42.9 bcd	44.2 b
ICGV 98308	7.5 de	7.9 de	9.4 bcd	38.7 cde	40.3 f	42.9 b
ICGV 98324	8.6 ab	9.2 ab	10.2 a	43.0 a	45.2 a	47.5 a
ICGV 98330	8.8 a	9.4 a	10.3 a	43.0 a	45.1 a	47.5 a
ICGV 98348	8.4 abc	8.7 bc	9.9 ab	37.7 e	41.7 def	43.1 b
ICGV 98353	8.1 bcd	8.8 bc	9.8 abc	39.0 cde	41.9 def	43.0 b
Tainan 9	8.0 cd	7.7 e	8.4 e	37.8 de	40.3 f	40.3 c
KK 60-3	8.4 abc	9.4 a	10.1 ab	42.1 ab	43.7 abc	46.7 a
Tifton-8	7.9 cd	8.8 bc	9.3 bcd	41.6 ab	44.0 ab	43.4 b
non-nod	6.4 f	5.4 f	4.6 f	34.9 f	33.6 g	30.4 d
LSD (0.05)	0.6	0.6	0.7	1.7	1.7	2.2
Leaf positions						
First	7.2 c	7.1 c	8.5 b	34.5 c	37.3 c	39.9 b
Second	8.1 b	8.6 b	9.3 a	41.0 b	43.3 b	44.1 a
Third	8.4 a	9.4 a	9.6 a	43.4 a	44.9 a	45.1 a
LSD (0.05)	0.3	0.3	0.4	0.9	0.8	1.1

Mean in the same column within the same factors with the same letters are not significantly different by least significant difference (LSD) at $P \leq 0.05$.

Table 3. Correlation coefficients between chlorophyll density and SPAD chlorophyll meter reading.

Water regimes	Leaf positions	Correlation coefficients (r)		
		20 DAE	40 DAE	60 DAE
Field capacity (n=12)	First	0.67 *	0.99 **	0.94 **
	Second	0.31	0.94 **	0.97 **
	Third	0.75 **	0.77 **	0.93 **
	Overall (n=36)	0.65 **	0.93 **	0.92 **
2/3 available water (n=12)	First	0.75 **	0.92 **	0.91 **
	Second	0.75 **	0.95 **	0.87 **
	Third	0.80 **	0.96 **	0.92 **
	Overall (n=36)	0.76 **	0.94 **	0.88 **
1/3 available water (n=12)	First	0.88 **	0.92 **	0.90 **
	Second	0.78 **	0.79 **	0.94 **
	Third	0.91 **	0.77 **	0.88 **
	Overall (n=36)	0.88 **	0.91 **	0.91 **

*, **Significant at the 0.05 and 0.01 probabily level. DAE = day after emergence

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2.7 Association between aflatoxin contamination and drought tolerance traits in peanut

A. Arunyanark, S. Jogloy, S. Wongkaew, C. Akkasaeng, N. Vorasoot, G.C. Wright, Rao C.N. Rachaputi and A. Patanothai

Alleviation of aflatoxin contamination through genetic manipulation has been a long–term goal of crop scientists. However, large and unexplained G x E interaction and high analytical costs of aflatoxin (Anderson et al., 1995), have limited the progress of genetic improvement for resistance to aflatoxin contamination in peanut. Therefore, there is a need to explore alternative breeding strategies including the potential to identify traits that could be used as indirect selection criteria for resistance to aflatoxin contamination.

Drought tolerance traits in peanut may have the potential to be used as indirect selection criteria for resistance to pre-harvest aflatoxin contamination, as many drought tolerant lines showed lower levels of pre-harvest aflatoxin contamination (Holbrook et al., 2000). These observations suggest that drought tolerant genotypes may possess some degree of tolerance to aflatoxin contamination. Thus, the physiological traits involving drought tolerance could potentially be applied as a potential selection criterion for improving resistance to aflatoxin production in peanut.

Transpiration efficiency (TE) has been identified as an important drought tolerance trait in a number of crop species (Richards, 1997). Further research in peanut has shown that TE is strongly correlated with the easily measurable trait i.e., specific leaf area (SLA) (leaf thickness) (Nageswara Rao and Wright 1994). Another important trait that has been found to correlate with drought tolerance is rooting efficiency, the ability of a plant to modify its root distribution to exploit deeper stored soil water (Songsri et al., 2008). More efficient development of aflatoxin resistant genotypes might be feasible, if the drought tolerance traits that contribute to aflatoxin resistance can be identified. The objective of the current investigation was to investigate the relationship between drought tolerance traits and aflatoxin contamination in a range of peanut genotypes.

Materials and Methods

Two field experiments were conducted at Khon Kaen University during November 2003 to March 2004, and October 2004 to February 2005. A split–split plot design with four replications was used, with three water regimes (field capacity, 2/3 available water and 1/3 available water) as main plots and eleven peanut genotypes as sub–plots and two levels of *A. flavus* inoculation (uninoculated and inoculated) as sub–sub plots. The water regimes are referred throughout the text as irrigated (Irr), medium drought (MD) and severe drought (SD), respectively. Eight genotypes (ICGVs 98300, 98303, 98305, 98308, 98324, 98330, 98348 and 98353) identified as drought tolerance types were obtained from ICRISAT. Tifton–8 was an introduction from the US with known resistance to drought and aflatoxin contamination. Two cultivars, KK 60–3 and Tainan 9, were released cultivars in Thailand. The population of *A. flavus* in the soil was monitored at intervals ranging from before inoculation to harvest. The effects of environment factors (temperature and moisture in soil) and *A. flavus* population in soil on kernel infection and aflatoxin contamination were studied, and related with drought tolerant traits (chlorophyll density; Chl.D, specific leaf area; SLA and root length density; RLD).

Results

The results showed that drought causes elevated soil temperatures (>22 ^{o}C) and reduced soil moisture contents, causing favorable conditions for aflatoxin production (data not shown). Although sampling times differed slightly between seasons, it was clear that inoculated soils in both seasons had consistently higher CFU of *A. flavus* compared to the uninoculated control (Fig. 1). CFU of *A. flavus* were generally higher in drought treatments compared to irrigated soils. It was clear that inoculation of *A. flavus* resulted in an increase of the *A. flavus* population, while drought also promoted the growth and persistence of the *A. flavus* population.

Analysis of variance (Table 1) showed significant effects of water regimes (W), genotypes (G) and soil inoculation (Inoc) for kernel colonization and aflatoxin contamination for both seasons. G x E interactions were pronounced for both aflatoxin contamination and kernel colonization. The irrigated treatment had lower kernel colonization (2–37 %) and aflatoxin contamination (1–19 ppb) compared to up to 53 % kernel colonization and up to 62 ppb aflatoxin contamination in severe drought conditions in uninoculated plots (data not shown). Drought consistently increased kernel colonization by *A. flavus* and aflatoxin contamination in both years. Further, incorporation of *A. flavus* into the soil had a significant effect on kernel colonization and aflatoxin contamination, with inoculated soils having much higher kernel colonization and aflatoxin contamination compared to the uninoculated control (data not shown).

The combination traits of drought tolerance as SLA, chlorophyll density and RLD are strongly related with aflatoxin contamination (Table 2). Most trait combinations had higher correlation with aflatoxin contamination than single traits except for SLA which had high correlation especially under severe drought. These results indicated that drought tolerance traits (SLA and RLD) could be contributing to resistance to aflatoxin contamination suggesting that a combination of SLA, RLD and kernel colonization could be used as selection criteria in selecting parents for aflatoxin resistance. The genotypes with higher levels of traits such as SLA and RLD might be able to maintain photosynthetic capacity and water status and thus functional plant metabolism during drought stress, which is necessary to maintain immune responses and avoid susceptibility to aflatoxin contamination.

The combination traits of SLA and kernel infection, and single trait of SLA could be the best choices in terms of economic and simplicity because of high correlation with aflatoxin contamination. The most appropriate selection condition should be under drought stress.

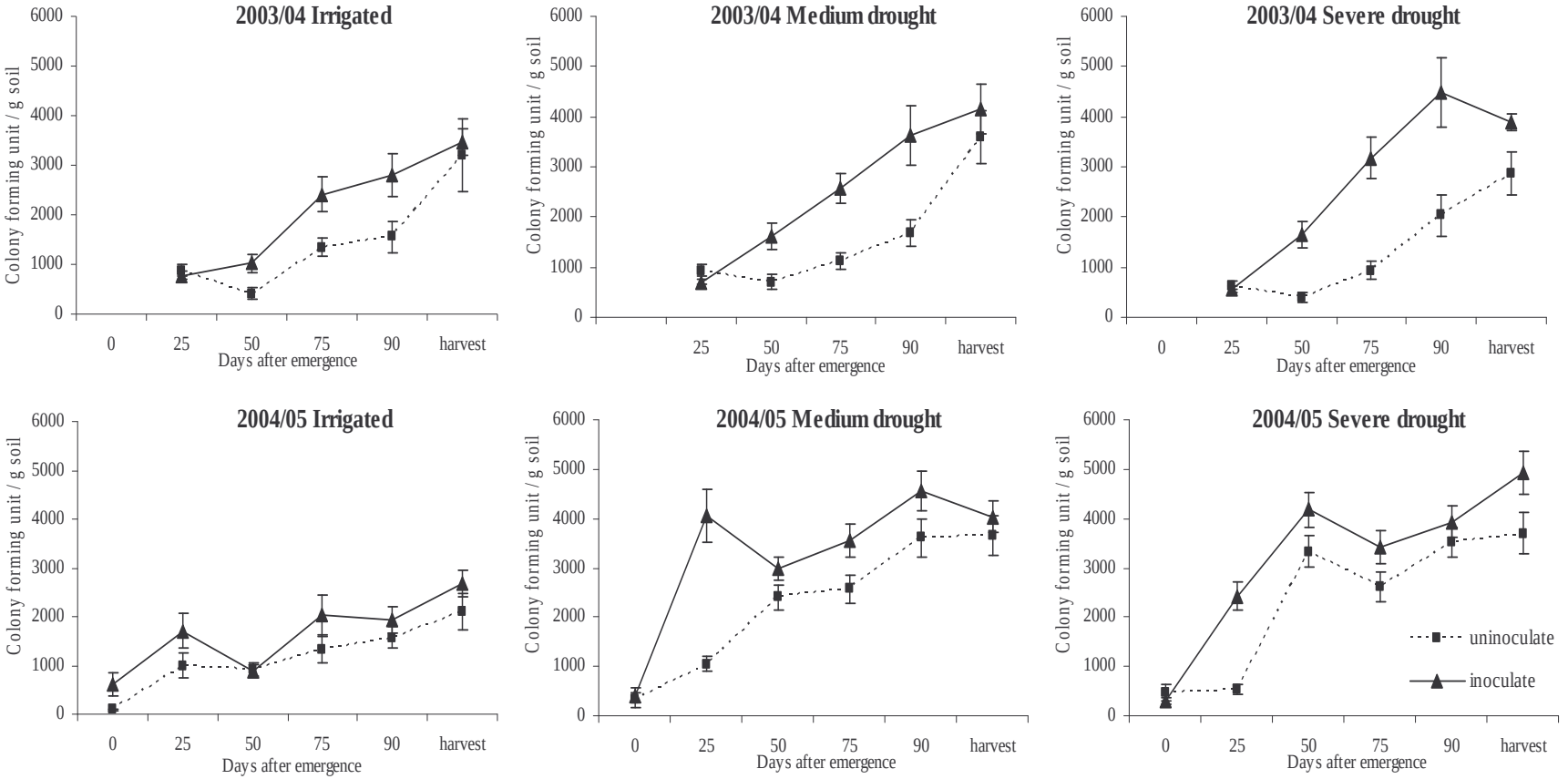

Fig. 1. Soil populations of *A. flavus* in uninoculated and inoculated areas under irrigated, medium drought and severe drought treatments in 2003/04 and 2004/05 seasons. Error bars represent ± SE.

Table 1. Mean squares from analysis of variance in split–split–plot design of kernel colonization by *A. flavus* and aflatoxin B_1 contamination.

Source of variation	df	Mean squares			
		2003/04		2004/05	
		Kernel colonization	Aflatoxin	Kernel colonization	Aflatoxin
Replication	3	0.27	0.21	0.18	0.24
Water regimes (W)	2	1.13 *	3.65 **	2.46 **	6.29 **
Error (a)	6	0.19	0.17	0.05	0.23
Genotypes (G)	10	0.47 **	0.32 **	0.31 **	0.47 **
W x G	20	0.05	0.13 **	0.03	0.17 **
Error (b)	90	0.05	0.05	0.02	0.06
Soil inoculation (Inoc)	1	0.45 **	5.84 **	2.13 **	0.67 **
W x Inoc	2	0.10	0.17 *	0.60 **	0.23 *
G x Inoc	10	0.08	0.06	0.05 *	0.23 **
W x G x Inoc	20	0.04	0.05	0.05 **	0.10 *
Error (c)	99	0.05	0.04	0.02	0.05

Data were log–transformed (Y = log(x+1.5))
*, significant for $P < 0.05$; **, significant for $P < 0.01$.

Table 2. Simple and multiple correlations (r) between drought tolerance traits and aflatoxin contamination.

Trait	2003/04 (n=11)		2004/05 (n=11)		Pooled seasons (n=22)	
	Irr	SD	Irr	SD	Irr	SD
SLA	0.01 ns	0.72 *	0.11 ns	0.68 *	–0.07 ns	0.58 **
RLD	–0.46 ns	0.49 ns	0.24 ns	0.20 ns	0.29 ns	0.42 *
colonization	–0.44 ns	0.59 ns	0.03 ns	0.48 ns	0.56 **	0.59 **
SLA & RLD	0.55 ns	0.75 **	0.31 ns	0.68 *	0.32 ns	0.59 **
SLA & colonization	0.45 ns	0.72 *	0.14 ns	0.73 *	0.56 **	0.65 **
RLD & colonization	0.63 *	0.71 *	0.25 ns	0.53 ns	0.56 **	0.61 **
SLA & RLD & colonization	0.67 *	0.76 **	0.32 ns	0.73 *	0.56 **	0.66 **

Irr, irrigated; SD, severe drought; SLA, specific leaf area; RLD, root length density.
ns,*,** non-significant and significant at $P < 0.05$ and $P < 0.01$, respectively.

Conclusion

The results support that drought conditions and inoculation of *A. flavus* affected the persistence of *A. flavus* populations in soil, and thus, increased kernel colonization by *A. flavus* and subsequent aflatoxin contamination. This is the first report showing evidence that a combination of traits related to drought tolerance including SLA, RLD and kernel colonization could be potentially used as selection traits for resistance to aflatoxin contamination of peanut. Combinations of traits tended to have higher correlations with aflatoxin contamination than single traits, except for SLA, which had a high correlation, especially under drought conditions.

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2.8 Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water

P. Songsri, S. Jogloy, C.C. Holbrook, T. Kesmala, N. Vorasoot, C. Akkasaeng, and A. Patanothai

Drought is the major abiotic constraint affecting peanut productivity and quality worldwide (Reddy et al. 2003; Wright and Nageswara Rao 1994). There is a pressing need to improve the water use efficiency (WUE) of rain-fed peanut production. Breeding varieties with higher water use efficiency is seen as providing part of the solution. Although some agronomic interventions to conserve soil moisture and enhance water use efficiency (WUE) are available, developing peanut varieties tolerant to drought and efficient in water use offers the best long term and cost effective solution to the

uncertainty of availability of water. So far, the approach to breeding cultivars with superior yield performance under water limited conditions has remained empirical, *via* selection for yield under stress conditions. More rapid progress may be achieved by a prior knowledge of the physiological basis of trait performance such as the ability of root systems to capture water for transpiration, specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR), and partitioning efficiency or harvest index (HI) under drought conditions.

In this study, peanut genotypes differing in drought resistance were evaluated to address the following questions: (i) Do these drought resistant genotypes differ in WUE, harvest index (HI), root growth, SLA and SCMR? (ii) What are the behaviors of these genotypes for WUE and root growth in response to drought stress? and (iii) What are the relationships between WUE, root growth, SLA and SCMR in response to drought stress? The objectives were to (i) evaluate genetic variations in WUE, HI, root dry weight, SLA and SCMR among peanut genotypes in response to different available soil water levels; and (ii) assess the relevance of root dry weight, SLA and SCMR to WUE in peanut under receding soil moisture.

Materials and methods

Pot experiments were conducted under greenhouse conditions at Khon Kaen University during December 2002 to May 2003 (GH1), and repeated during June to November 2003 (GH2). Each treatment consisted of two pots in a replicate. The 11 peanut genotypes (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348, ICGV 98353, Tainan 9, KK 60-3 and Tifton-8) and three soil moisture levels [field capacity (FC), 2/3 available soil water (AW) and 1/3 AW] were laid out in a factorial randomized complete block design (RCBD) with six replications.

Prior to planting, uniform water was applied to all the pots to bring them to FC (17.81%) for uniform germination. The water for additional applications was divided into four fractions, and the first fraction was given to the soil surface and the remaining fractions though plastic tubes into three holes at 15, 30 and 45 cm below the soil surface. The soil moisture for all pots was maintained at FC until 21 DAS. After 21 DAS, the irrigation treatments were initiated. Soil water levels were maintained uniformly at field capacity from planting until harvest in the well-watered treatment, and allowed to gradually reduce until they reached predetermined levels for the 2/3 AW (14.14%) and 1/3 AW (10.47%) stress treatments at 28 and 35 DAS, respectively. For each treatment, moisture content was maintained uniformly with no more than 1% moisture change of the predetermined levels until harvest

At 37, 67, and 97 day after sowing (DAS), data were recorded for SLA and SCMR. Root dry weight, harvest index (HI) and WUE were recorded at harvest.

Results

Drought reduced WUE, root dry weight and HI in both seasons. Tifton-8 and ICGV 98300 had high WUE and also had large root systems under drought conditions. ICGV 98324 and Tifton-8 had low SLA and high SCMR under stressed and non-stressed condition. Under drought conditions, ICGV 98324 had high HI and Tifton-8 had low HI.

Root dry weight had a greater contribution to WUE under well-watered and mild drought (2/3 AW). Under severe drought (1/3 AW), SLA showed a more important contribution to WUE than the other traits (Table 1).

Table 1. Contributions of root dry weight (RDW), specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) to water use efficiency (WUE) under FC, 2/3 AW and 1/3 AW conditions.

	Explained by regression (%)		
	FC	2/3 AW	1/3 AW
Regression	*59.39*	*96.50***	*72.31**
RDW	54.70**	85.37**	18.35*
SLA	4.48*	10.24*	52.65*
SCMR	0.21	0.89	1.30*

* significant at $p < 0.05$.
** significant at $p < 0.01$.

Among water regimes, the highest positive correlation was found between WUE and root dry weight ($r = 0.93$; $p \leq 0.01$) under 2/3 AW. The correlation coefficient between WUE and SLA was highest under severe stress conditions ($r = -0.75$; $p \leq 0.01$). These were in agreement with the results from regression analyses (Fig. 1).

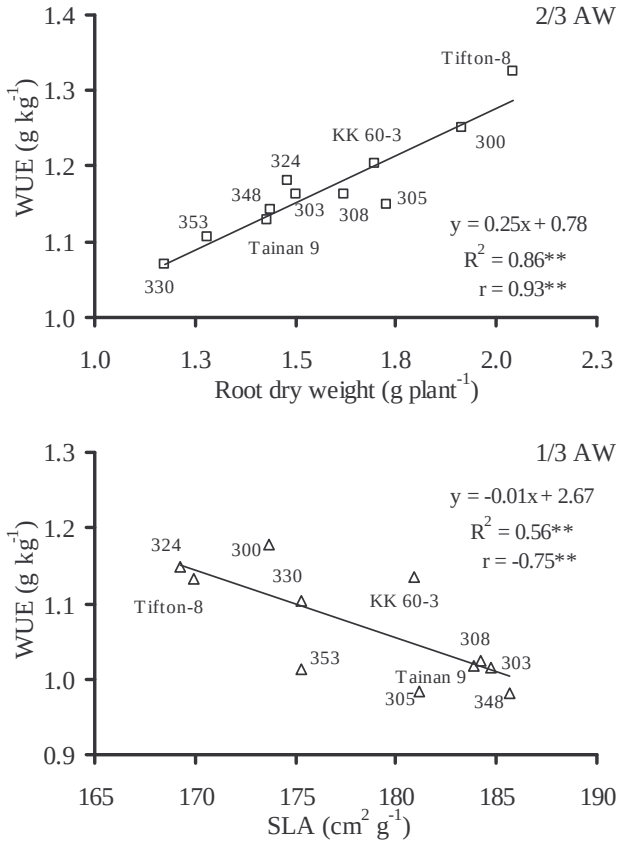

Fig.1. Relationship between water use efficiency (WUE) (g kg^{-1}) and root dry weight (g $plant^{-1}$) under 2/3 AW and specific leaf area (SLA) (cm^2 g^{-1}) under 1/3 AW of 11 peanut genotypes.

Under mild drought stress when available soil water was 66.7% and enough for plant uptake, the genotypes with large root systems could mine more water than the genotypes with small root systems. Under severe drought stress when available soil water

was only 33.3% and not enough for plant uptake, photosynthesis could play a more important role for water use efficiency. The closer association between SLA and SCMR and WUE at 1/3 AW indicated that plants may need more chlorophyll or photosynthetic capacity to produce an equivalent amount of dry matter compared to the situation under non-stressed and mild drought stress conditions. The variation in WUE is associated with variation in photosynthetic capacity per unit leaf area because thicker leaves usually have a higher density of chlorophyll per unit leaf area and hence have a greater photosynthetic capacity compared with thinner leaves. It could be hypothesized that peanut genotypes with high SCMR and low SLA have more photosynthetic machinery per unit leaf area and hence have potential for greater assimilation under drought stress.

Conclusion

WUE was consistent across season and water regime. The genotypes with larger root systems and low SLA could maintain relatively high WUE under water stress conditions. Our studies demonstrated that root dry weight and SLA are important traits related to WUE under moderate (2/3 AW) and severe (1/3 AW) drought, respectively. Root dry weight and SLA should be useful selection criteria for high WUE under moderate (2/3 AW) and severe (1/3 AW) drought conditions, respectively.

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2.9 Variability in yield responses of peanut (*Arachis hypogaea* L.) genotypes under early season drought

D. Puangbut, S. Jogloy, N. Vorasoot, C. Akkasaeng, T. Kesmala, and A. Patanothai.

Drought stress at the vegetative phase or pre-flowering stage had no detrimental effect on pod yield, whilst, in many cases the yield was increased. Several physio-morphological characters have been reported as associated traits for increasing pod yield under pre-flowering drought stress (Awal and Ikeda 2002). The recovery knowledge of physio-morphological traits underlying the increase pod yield of peanut grown under pre-flowering drought are important for both irrigation management and breeding if peanut genotypes are different in yield responses. However, the accumulative knowledge on the recovery of these traits in peanut so far has been limited to a few reports on a variety, while, the extent of useful genetic variability remains unknown. The objectives of this study were to investigate the variability in yield response of peanut genotypes subjected to early season drought and to evaluate physio-morphological characters associated with yield.

Materials and methods

Field experiments were conducted in the rainy season of and the dry season of 2005-2006 at Khon Kaen University. Eleven peanut genotypes and two water regimes (i.e. fully-irrigated control and 1/3 available soil water from emergence to 40 days after emergence followed by adequate water supply) were laid out in a split-plot design with four replications. Water regimes were assigned in main-plots and 11 peanut genotypes were laid out in sub-plots. Measurements of leaf area index (LAI), SLA, and SCMR were made at 40 and 60 days after emergence. At harvest, biomass and pod yield were recorded and then harvest index was calculated. In addition, root growth was measured at harvest in another experiment conducted in the glasshouse.

Combined analyses of variance of two-season data were first performed. Because of season x genotype and water regime x genotype interactions were significant, data of each season and each water regime were analyzed separately.

Results

Imposition of early season drought followed by re-watering resulted in an increase in pod yield compared to the irrigated treatment (Fig 1). The current study supports the earlier findings that imposition of pre-flowering drought can result in higher yield compared to irrigated conditions (Nageswara Rao et al., 1988). Significant genotypic differences in yield response in relation to early season drought were observed. The highest pod yields were found in ICGV 98303 and Tainan 9 in the rainy season, whereas, in the dry season, ICGV 98303 was still highest for pod yield followed by ICGV 98300. After re-watering, SCMR (Table 1), LAI (Table 2) and biomass productions (data not presented) were increased. Thus, increase in yield was associated with high biomass production after recovery combined with great green leaf area and concentration of leaf chlorophyll.

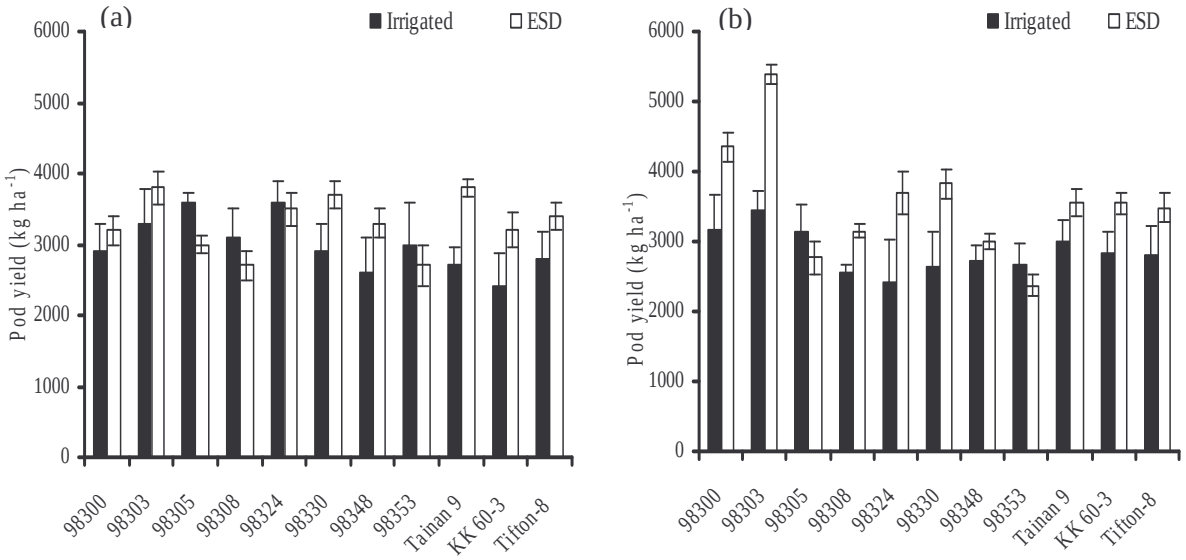

Fig. 1. Pod yield of 11 peanut genotypes under irrigated and early season drought (ESD) treatments in the rainy season (2005) (a) and in the dry season (2005/06) (b), vertical bars shown standard error difference of means.

Table 1. SPAD chlorophyll meter reading (SCMR) of 11 peanut genotypes under irrigated and early season drought (ESD) treatments during water stress (40 DAE) and after re-watering (60 DAE) in the dry season (2005/06).

Genotypes	SCMR			
	Drought		Recovery	
	Irrigated	ESD	Irrigated	ESD
ICGV 98300	40.2 e	48.0 b	43.9 cd	48.7 c
ICGV 98303	41.2 cde	50.5 a	42.6 d	49.0 c
ICGV 98305	42.1 bcd	46.8 c	44.5 bc	46.5 c
ICGV 98308	40.3 e	45.6 d	42.7 d	45.9 d
ICGV 98324	44.7 a	50.9 a	45.2 bc	50.0 b
ICGV 98330	44.6 a	51.8 a	47.9 a	51.5 ab
ICGV 98348	43.3 ab	46.5 d	40.3 e	44.4 de
ICGV 98353	40.9 de	45.1 d	44.8 bc	45.2 de
Tainan 9	41.8 b-e	45.7 d	42.3 d	45.1 e
KK 60-3	42.7 bc	51.0 a	47.4 a	50.7 b
Tifton-8	44.7 a	50.6 a	45.6 b	52.4 a
Mean	42.4	48.4	44.3	48.1

Mean in the same column with the same letters are not significantly different by DMRT at $P < 0.05$, DAE = days after emergence.

Table 2. Leaf area index (LAI) of 11 peanut genotypes under irrigated and early season drought (ESD) treatments during water stress (40 DAE) and after re-watering (60 DAE) in the dry season (2005/06).

Genotypes	LAI			
	Drought		Recovery	
	Irrigated	ESD	Irrigated	ESD
ICGV 98300	1.6 cd	1.1	3.1 a	3.4 b
ICGV 98303	1.4 d	1.1	3.1 a	3.1 c
ICGV 98305	2.0 a	1.3	2.8 b	2.3 e
ICGV 98308	1.6 bcd	1.1	2.8 b	3.0 d
ICGV 98324	1.3 d	0.9	3.0 ab	2.9 d
ICGV 98330	1.6 cd	1.1	2.7 b	3.1 c
ICGV 98348	1.8 abc	1.1	3.0 a	3.3 c
ICGV 98353	1.8 abc	1.1	2.5 c	2.0 e
Tainan 9	2.0 abc	1.1	3.0 ab	4.0 a
KK 60-3	1.5 cd	1.4	3.3 a	3.8 b
Tifton-8	1.4 d	1.4	3.3 a	4.1 a
Mean	1.6	1.2	3.0	3.2

Mean in the same column with the same letters are not significantly different by DMRT at $P < 0.05$**,** DAE = days after emergence

Conclusion

Water deficit at early season drought and subsequent recovery could obviously increase pod yield. Peanut genotypes differed significantly in ability to recover from early season drought, but did not show large differences in harvest index under early season drought treatments. Thus, biomass production might be the cause of differences in pod yield. High yield under early season drought was attributed to maintenance of green leaf area and high capacity of photosynthesis and biomass production. These findings could be useful for improving genotypic performance of peanut for early season drought.

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2.10 Association of root dry weight and transpiration efficiency of peanut genotypes under early season drought

D. Puangbut, S. Jogloy, N. Vorasoot, C. Akkasaeng, T. Kesmala,
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Because peanut is usually grown in rainfed conditions, it has been hypothesized that improving water use efficiency (WUE) would be the best strategy to cope with episodes of intermittent drought. Selection for traits such as transpiration efficiency (TE) and WUE offers the best long term and cost effective solution for growing peanut under unpredictable or limiting soil water available conditions (Krishnamurthy et al., 2007). An understanding of the traits associated with TE such as the ability of roots to increase water uptake and maintain high photosynthetic capacity should be useful in improving TE under drought stress conditions.

Studies on the surrogate traits for TE in peanut have been focused on specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) (Songsri et al., 2009). Very limited information is available on the relationships between TE and root systems under early season drought conditions (ESD). Selection of superior peanut genotypes for their ability to maintain high root system during water stress and recovery may help breeders to identify peanut genotypes with drought tolerance. The objective of this study was to investigate the relationships between root dry weight and TE under early season drought.

Materials and Methods

Two greenhouse experiments were conducted during February to May 2004 and November 2004 to March 2005 at Khon Kaen University. A randomized complete block design (RCBD) with four replications was used. The treatments were factorial combinations of two factors. Factor A consisted of two water regimes, i.e., irrigated control (FC) and 1/3 available soil water (AW) from emergence to 40 days after emergence (DAE) followed by adequate water supply, and factor B comprised of 11 peanut genotypes. Data were recorded on SLA and SCMR at 40 and 60 DAE, and on TE and root dry weight (RDW) at harvest.

The data were subjected to standard analyses of variances and comparisons among means. Multiple-linear regression was used to determine the relative contribution of RDW, SLA and SCMR to TE under FC and early season drought treatments.

Results

Early season drought increased SCMR, TE and RDW but reduced SLA. Strong and more consistent variation for TE was observed among 11 peanut genotypes across seasons (Table 1). ICGV 98300, KK 60-3 and Tifton-8 had high TE and large root systems under drought conditions. KK 60-3 and Tifton-8 had low SLA and high SCMR under early season drought conditions. RWD had a contribution to TE under well-watered and especially under drought condition (Table 2). SCMR and SLA had smaller contributions to TE under well-watered and early season drought conditions.

Most of the characters studied were higher under early season drought than under well-watered conditions, except for SLA that was clearly lower. Interestingly, there was an increase in RDW in all peanut genotypes under early season drought conditions. The strong relationship between RWD and TE (Fig. 1) suggested that RDW is one of the

characters contributing to TE under early season drought, especially in genotypes with high RDW. The large root system can support accelerated plant growth under drought conditions**.**

Conclusion

Overall, these 11 peanut genotypes showed fairly consistence in TE under early season drought treatment. The genotypes with larger root systems could maintain relatively high TE under water stress conditions. Tifton-8 and KK 60-3 had high RWD and low SLA. Based on large RDW, ICGV 98300, Tifton-8 and KK 60-3 was identified as the genotypes having drought avoidance mechanisms. Our studies demonstrated that RDW is important trait related to TE under early season drought. RDW should be useful as selection criteria for high TE under early season drought conditions.

Table 1. Transpiration efficiency (TE), root dry weight (RDW) at harvest and specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR) at 40 day after emergence (DAE) of 11 peanut genotypes under irrigated and early season drought (ESD).

Genotypes	TE ($g\ kg^{-1}$)		RDW ($mg\ plant^{-1}$)		SLA ($cm^2\ g^{-1}$)		SCMR	
	Irrigated	ESD	Irrigated	ESD	Irrigated	ESD	Irrigated	ESD
ICGV 98300	2.1 ab	2.8 a	1.24 bc	1.29 bc	230 ab	186 bc	45.6	49.9 b
ICGV 98303	1.9 b	2.3 b	0.88 e	0.91 d	223 b	199 a	45.4	45.0 c
ICGV 98305	1.8 b	2.3 b	1.05 cde	1.08 c	234 ab	201 a	43.3	46.9 b
ICGV 98308	2.0 ab	2.4 ab	1.03 cde	1.08 c	234 ab	186 bc	43.9	47.5 bc
ICGV 98324	2.1 ab	2.5 ab	1.16 bcd	1.20 bc	228 b	187 bc	45.8	51.4 a
ICGV 98330	2.1 ab	2.5 ab	0.90 de	1.04 c	231 ab	193 b	45.2	50.5 ab
ICGV 98348	1.7 b	2.1 c	0.86 e	0.96 d	255 a	201 a	43.9	45.1 c
ICGV 98353	1.5 bc	2.3 b	0.96 de	1.09 c	228 b	196 ab	44.8	46.9 b
Tainan 9	2.4 a	2.5 ab	1.19 bc	1.33 b	222 bc	195 ab	45.8	46.4 b
KK 60-3	2.4 a	2.8 a	1.66 a	1.76 a	227 b	186 bc	45.6	50.0 ab
Tifton-8	2.3 a	2.9 a	1.71 a	1.85 a	223 b	185 bc	45.6	51.0 a
Mean	2.0	2.5	1.10	1.20	230	192	45.0	48.2

Different letters in each column show significance at $P < 0.01$ by Duncan's multiple range test.

Table 2. Contributions of root dry weight (RDW), specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) to transpiration efficiency (TE) under irrigated and early season drought (ESD) treatments.

Trait	Explained by regression (%)	
	Irrigated	ESD
Regression	63.00 *	87.75 **
RDW	51.06 *	74.41 **
SLA	0.02	11.56 **
SCMR	11.92 *	1.78 **

* and ** significant at $p < 0.05$ and significant at $p < 0.01$, respectively.

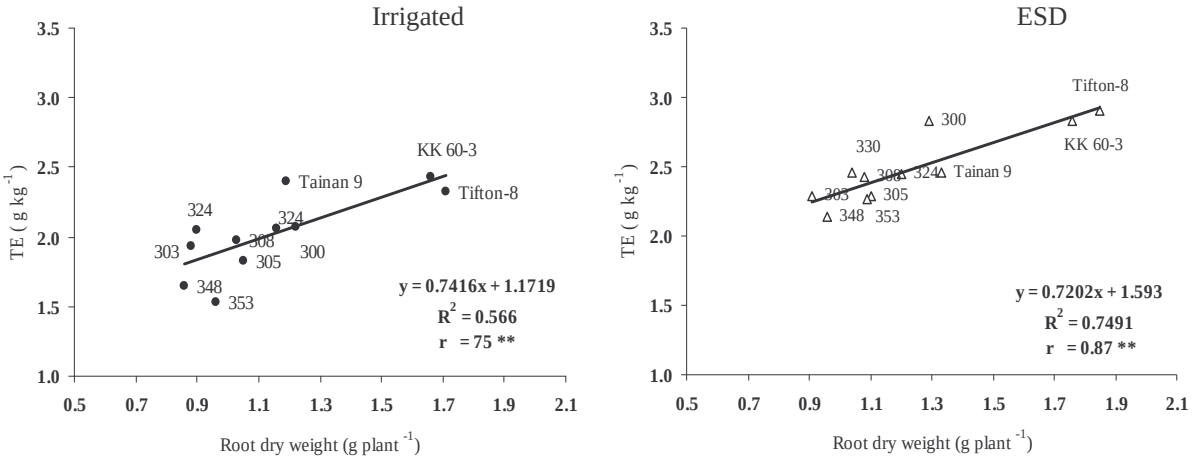

Fig.1. Relationship between root dry weight and transpiration efficiency (TE) of 11 peanut genotypes under irrigated and early season drought (ESD) treatments.

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2.11 Physiological basis for genotypic variation in tolerance to and recovery from pre-flowering drought in peanut

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Although yield benefits of pre-flowering drought have been established in a number of independent studies, these studies involved a limited number of genotypes and the physiological mechanisms underlying these yield responses are not fully understood (Nautiyal et al. 1999; Awal and Ikeda 2002). In a more recent study, a positive correlation was found between N_2 fixation and biomass production under drought (Pimratch et al. 2008), suggesting that maintenance of N_2 fixation under drought could be an important adaptive mechanism in sustaining yields under drought. However, the effect of pre-flowering drought on N_2 fixation and root growth in peanut is not well understood. Furthermore, there is limited information on genotypic variation for these important physiological traits during pre-flowering drought and recovery. A better understanding of mechanisms contributing to yield benefits of pre-flowering drought in peanut is important for both irrigation management and crop improvement. The objectives of this study were to determine the effects of moisture stress during the pre-flowering phase on pod yield and to understand some of the physiological responses underlying genotypic variation in response to, and recovery from, pre-flowering drought.

Materials and Methods

A glasshouse and field experiments were conducted at Khon Kaen University, Thailand. The glasshouse experiment was a factorial in a randomized complete block experiment consisting of two watering regimes i.e. fully-irrigated control and 1/3 available soil water from emergence to 40 days after emergence followed by adequate water supply, and 12 peanut genotypes. The field experiment was a split-plot design with two watering regimes as main-plots, and 12 peanut genotypes as sub-plots. Measurements of N_2 fixation and leaf area were made in both experiments at 40 days after emergence and at harvest. In addition, root growth was measured in the glasshouse experiment. At harvest, pod yield was recorded.

Data for each watering regime were analyzed separately as the genotypes x water (G x W) interaction was significant. Duncan's multiple range test was used to compare means. Combined analysis of variance for glasshouse and field experiments was conducted for N_2 fixation and leaf area for each watering regime following a randomized complete block design.

Simple and multiple correlations were computed to determine the relationship between pod yield and physiological traits and the relationship between N_2 fixation and nodule traits.

Results

Imposition of pre-flowering drought followed by recovery resulted in an average increase in yield of 24% (range from 10% to 57%) and 12% (range from 2% to 51%) in the field and glasshouse experiments, respectively (Table 1). Significant genotypic variations for N_2 fixation, leaf area and root growth were also observed after recovery (data not presented). The study revealed that recovery growth following release of pre-flowering drought had a stronger influence on final yield than tolerance to water deficits

during the pre-flowering drought. A combination of N_2 fixation, leaf area and root growth accounted for a major portion of the genotypic variation in yield (r = 0.68 to 0.93) (Table 2) suggesting that these traits could be used as selection criteria for identifying genotypes with rapid recovery from pre-flowering drought. A combined analysis of glasshouse and field experiments showed that leaf area and N_2 fixation during the recovery had low genotype x environment interaction (Table 3), indicating potential for using these traits for selecting genotypes in peanut improvement programs.

Table 1. Pod yield of 11 peanut genotypes under irrigated and pre-flowering drought (PFD) conditions in glasshouse and field experiments.

Genotypes	Pod yield			
	Glasshouse (g plant^{-1})		Field (kg ha^{-1})	
	Irrigated	ESD	Irrigated	ESD
ICGV 98300	8.1 a	8.5 bc	3170 b	4350 b
ICGV 98303	7.6 b	8.1 c	3450 a	5400 a
ICGV 98305	5.8 c	6.8 d	3150 b	2770 d
ICGV 98308	8.3 a	8.5 bc	2550 c	3150 cd
ICGV 98324	7.5 b	7.5 c	2420 cd	3700 c
ICGV 98330	7.2 b	8.9 bc	2650 c	3820 c
ICGV 98348	7.9 ab	7.7 c	2720 c	3000 b
ICGV 98353	7.1 b	7.9 c	2670 c	2370 d
Tainan 9	7.1 b	8.4 bc	3000 ab	3550 bc
KK 60-3	7.3 b	7.9 c	2837 c	3551 bc
Tifton-8	7.7 ab	11.6 a	2804 c	3477 d
Mean	7.4	8.3	2856	3558

Means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2. Simple and multiple correlation (r) between pod yield and physiological traits (n = 11) under glasshouse and field conditions.

Trait	Glasshouse			Field	
	Irrigated	PFD		Irrigated	PFD
RGR	0.53	0.93 **	LA	0.34	0.61*
N_2 fixed & LA	0.24	0.68 *	N_2 fixed & LA	0.46	0.71 *
N_2 fixed & RGR	0.60 *	0.93 **			
RGR & LA	0.63 *	0.93 **			

*, ** significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.
RGR = root growth rate, LA = leaf area

Table 3. Mean squares from the combined ANOVA for N_2 fixation and leaf area (LA) in glasshouse and field experiments measured on the last day of drought (40 DAE) and after 40 DAE (following recovery) under PFD treatment.

Source of Variation	df	N_2 fixation		LA	
		PFD	Recovery	PFD	Recovery
Environment (E)	1	27853**	45916	691240**	216400**
Rep. within E	6	136	11376	2665	68292
Genotypes	10	406**	35921**	5671**	490427**
G x E	10	252 *	8065	3526	12334
Pooled error	60	49	5446	2815	2232

*, ** significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Conclusion

This study demonstrated significant genotypic variation in yield and physiological responses under PFD and subsequent recovery. Pod yield differences were associated with variation in N_2 fixation, LA and RGR. The strong correlations between glasshouse and field conditions for N_2 fixation and LA suggest that initial screening and selection for N_2 fixation and LA could be conducted in a glasshouse environment and promising material then evaluated for pod yield responses under field conditions. It is expected that selection of genotypes under a PFD pattern would increase breeding efficiency.

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2.12 Relationship between root characteristics of peanut in hydroponics and pot studies

T. Girdthai, S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew,
C.C. Holbrook and A. Patanothai

Drought is a major constraint limiting productivity and quality of peanut. Breeding for drought tolerance can increase long-term productivity in drought prone environments. In addition, breeding approaches utilizing physiological and morphological traits have been proposed to improve selection efficiency for superior drought tolerant genotypes. Root responses when soil moisture dries out are important mechanisms for drought avoidance (Ketring, 1984), and the ability to extract soil water has been related to improved drought resistance in peanut. A large root system can be an important character for drought tolerance (Meisner and Karnok. 1992). However, measuring root characteristics in soil medium is tedious, time consuming, and labor intensive. Hydroponics culture has been used in peanut and other crops, and could be useful in screening large numbers of germplasm lines or segregating populations in a breeding program to reject entries with poor root traits (Mian et al. 1993; Ogbonnaya et al., 2003). This method would be valuable if it provides information similar to that observed in soil medium. The objective of this study was to determine the association between root characteristics of peanut grown in hydroponics and in pot studies.

Materials and Methods

Two parallel experiments were conducted at Khon Kaen University. Twelve peanut genotypes (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348, ICGV 98353, Tainan 9, KK 60-3, Tifton-8, and non-nod) were planted in a randomized complete block design with 4 replications in a hydroponics study and a pot experiment in two years during 2004-2005. Shoot dry weight, root dry weight, root to shoot ratio, root length, root surface, average diameter of roots, and root volume were measured.

Results

In the hydroponics study, the largest root production of peanut genotypes was the Virginia-type followed by the Spanish-type. Tifton-8 and KK 60-3 had consistently higher values for all root characteristics compared to the other genotypes. ICGV 98300, ICGV 98324, ICGV98330, ICGV 98348, and non-nod performed poorly for all characteristics (Table 1). Average shoot dry weight and root to shoot ratio in the hydroponics were 21.31 g plant^{-1}and 0.132, respectively. Shoot dry weight ranged from 18.80 to 26.39 g plant^{-1} and root to shoot ratio ranged from 0.092 to 0.172. KK 60-3 and Tifton-8 had the highest value for shoot dry weight and ICGV 98305 had the highest value for root to shoot ratio. ICGV 98300 had the lowest value for shoot dry weight and root to shoot ratio. Among root characteristics evaluated, diameter of roots had the lowest variation, whereas root dry weight, root length, root surface, and root volume could be well-differentiated among the peanut genotypes.

Table 1. Mean of shoot dry weight, root dry weight, root to shoot ratio, root length, root surface, average diameter of roots, and root volume of 12 peanut genotypes grown in hydroponics culture at 80 days after transplanting in 2004/05 and 2005.

Genotypes	Shoot dry Weight g plant^{-1}	Root dry Weight (g plant^{-1})	Root to shoot ratio	Root length (cm plant^{-1})	Root surface (cm^2 plant^{-1})	Average diameter of roots (mm plant^{-1})	Root volume (mm^3 plant^{-1})
ICGV 98300(s§)	18.80 c †	1.64 f	0.092 d ‡	6953 fg	1137 d	0.586 bcde	12.5 e
ICGV 98303(s)	21.48 c	2.88 bc	0.149 ab	12133 bc	1991 ab	0.593 bcd	21.3 b
ICGV 98305(s)	19.63 c	2.94 b	0.172 a	12402 bc	1996 ab	0.606 ab	22.9 ab
ICGV 98308(s)	21.93 bc	2.77 bc	0.151 abc	11901 c	1823 b	0.560 defg	20.6 b
ICGV 98324(s)	20.26 c	2.13 e	0.121 cd	8524 e	1492 c	0.567 cdef	15.6 cd
ICGV 98330(s)	21.31 c	2.24 de	0.114 bcd	7809 ef	1395 c	0.599 bc	16.2 cd
ICGV 98348(s)	19.33 c	2.09 e	0.123 cd	8816 e	1357 c	0.539 fg	13.9 de
ICGV 98353(s)	20.41 c	2.58 cd	0.151 abc	12495 bc	1929 ab	0.581 bcde	21.4 b
Tainan9(s)	21.28 c	2.28 de	0.132 cd	10198 d	1530 c	0.552 efg	16.9 c
KK 60-3(v)	26.39 a	3.09 ab	0.133 abcd	13851 a	2096 a	0.640 a	24.8 a
Tifton-8(v)	25.45 ab	3.35 a	0.153 abc	13350 ab	2094 a	0.640 a	25.7 a
Non-nod(s)	19.37 c	1.72 f	0.095 d	6305 g	1041 d	0.523 g	11.5 e
Mean	21.31	2.48	0.132	10395	1657	0.582	18.6
SE	1.41	0.13	0.03	478	74.29	0.02	1.12

† For each genotype, along the column, means followed by the same letter are not significantly different (P<0.05) by LSD.

‡ Mean of each genotype in this column is original mean and the means separation test was performed on the transformed data.

§s, Spanish type; v, Virginias type.

The pot experiment was carried out in a greenhouse and peanut genotypes were maintained under well-watered conditions. Average shoot dry weight and root to shoot ratio were 12.35 g plant^{-1} and 0.095, respectively (Table 2). Shoot dry weight ranged from 9.59 to 15.98 g plant^{-1} and root to shoot ratio ranged from 0.081 to 0.117. Root dry weight for all genotypes ranged from 0.93 to 1.43 g plant^{-1}. Root length ranged from 4363 to 6597 cm plant^{-1}. Root surface ranged from 517 to 864 cm^2 plant^{-1} while average diameter of roots ranged from 0.445 to 0.606 mm plant^{-1} and root volume ranged from 5.0 to 8.8 cm^3 plant^{-1}. KK 60-3 and Tifton-8 were the highest in root characteristics, whereas non-nod was the lowest.

In general, the performance of peanut genotypes for root characteristics in pot conditions was quite similar to that of peanut genotypes grown in hydroponics culture. Close relationships between peanut grown in hydroponics and in pots for root characteristics were observed (Fig. 1). Root dry weight, root length, root surface, average diameter of roots, and root volume of peanut in hydroponics were positively correlated with root characteristics in pot study (r = 0.74**- 0.93**). Means of root characteristics in the pot experiment were lower than those root characteristics of peanut grown in hydroponics conditions.

Table 2. Mean of shoot dry weight, root dry weight, root to shoot ratio, root length, root surface, average diameter of roots, and root volume of 12 peanut genotypes grown in pot experiment at 100 days after planting in 2004/05 and 2005.

Genotypes	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Root to shoot ratio	Root length cm plant^{-1}	Root surface cm^2 plant^{-1}	Average diameter of roots mm plant^{-1}	Root volume mm^3 plant^{-1}
ICGV 98300(s§)	13.11 cd †	1.06 bcde	0.082 d ‡	5285 de	667 cde	0.526 bc	6.7 bcd
ICGV 98303(s)	11.98 def	1.13 bcd	0.096 bcd	6177 ab	702 bcd	0.542 b	6.3 bcde
ICGV 98305(s)	11.41 ef	1.20 b	0.106 ab	6017 abc	813 ab	0.533 bc	7.6 ab
ICGV 98308(s)	10.59 fg	1.10 bcd	0.105 abc	5757 bcd	706 bcd	0.486 bcd	6.4 bcde
ICGV 98324(s)	12.78 cde	1.13 bc	0.090 bcd	5929 abcd	779 abc	0.525 bc	7.2 abc
ICGV 98330(s)	14.35 bc	1.16 bc	0.081 cd	5839 bcd	738 abcd	0.535 bc	7.3 abc
ICGV 98348(s)	11.53 def	0.93 e	0.081 cd	5384 cde	612 def	0.491 bcd	5.4 de
ICGV 98353(s)	10.54 fg	1.18 bc	0.117 a	5891 abcd	707 bcd	0.477 cd	6.6 bcd
Tainan9(s)	11.29 ef	1.03 cde	0.092 bcd	4744 ef	551 ef	0.460 d	6.0 cde
KK 60-3(v)	15.98 a	1.37 a	0.087 bcd	6408 ab	824 ab	0.542 b	7.9 ab
Tifton-8(v)	15.06 ab	1.43 a	0.095 bcd	6597 a	864 a	0.606 a	8.8 a
Non-nod(s)	9.59 g	0.98 de	0.102 bcd	4363 f	517 f	0.445 d	5.0 e
Mean	12.35	1.14	0.095	5699	707	0.514	6.8
SE	0.61	0.05	0.02	256.	46.96	0.02	0.62

† For each genotype, along the column, means followed by the same letter are not significantly different ($P<0.05$) according to Least significant difference (LSD) test.

‡ Mean of each genotype in this column is original mean and the means separation test was performed on the transformed data.

§s, Spanish type; v, Virginias type.

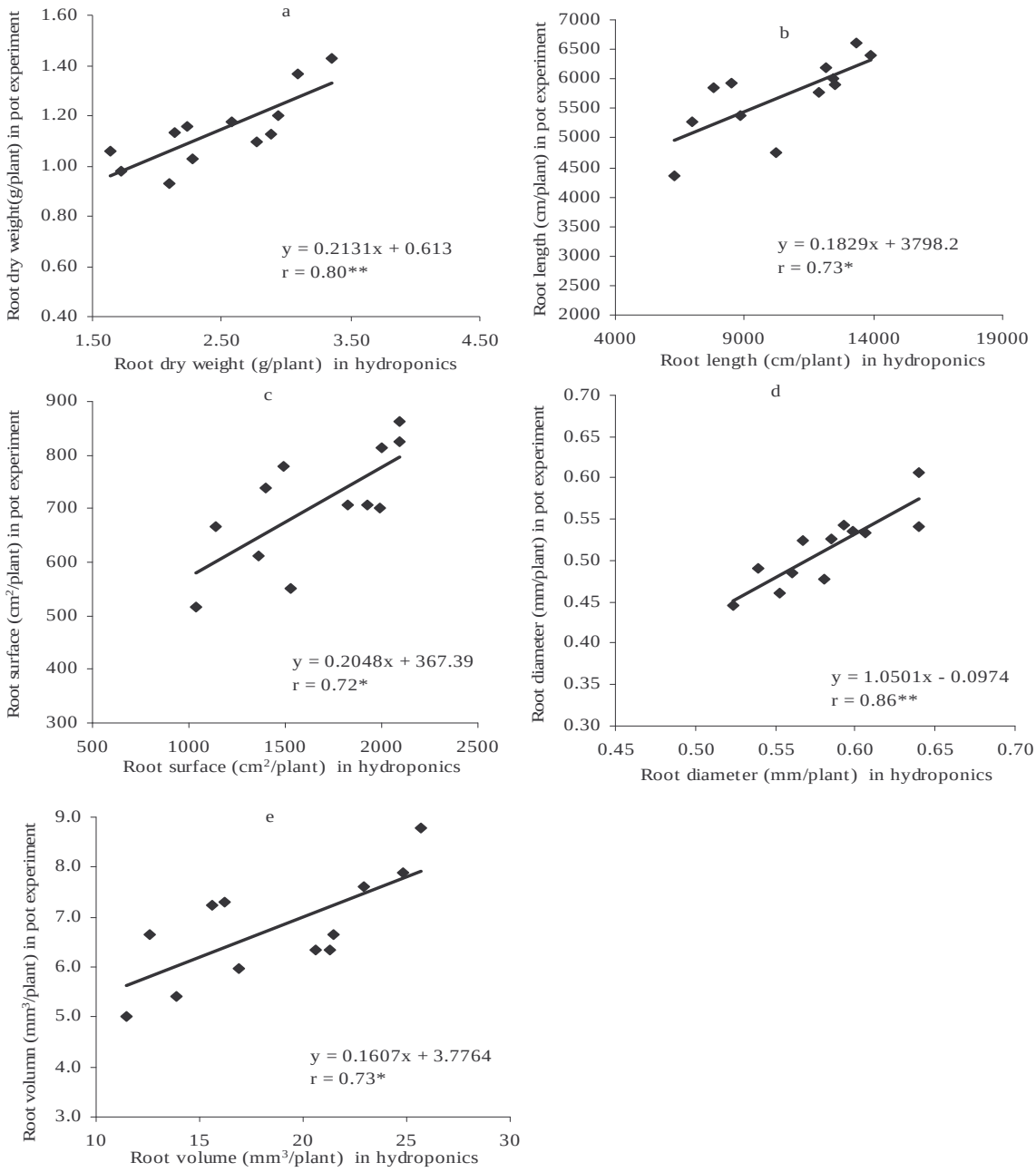

Fig. 1. Relationship between root dry weight (a), root length (b), root surface (c), average diameter of roots (d), and root volume (e) of peanut grown in hydroponics and pot experiment.

Conclusion

Root characteristics of peanut genotypes grown in hydroponics culture were positively correlated with those of peanut genotypes grown in soil medium. Hydroponics could also replace evaluations in pot experiment if facilities are available. These results imply that hydroponics systems could be used to select peanut with different root characteristics for possible drought tolerance instead of assessment in soil medium conditions.

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2.13 Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought

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Aflatoxin contamination in peanuts is a serious human health concern. Severe drought during the latter part of the growing season induces preharvest invasion of *Aspergillus flavus* and subsequent preharvest aflatoxin contamination (PAC). Some drought resistance genotypes have low *Aspergillus* infection and aflatoxin contamination (Holbrook et al., 2008). Therefore, drought resistance traits are promising as indirect selection tools for resistance to preharvest aflatoxin contamination. Developing a drought-tolerant cultivar appears to be a promising method for reducing aflatoxin contamination of peanut (Dorner et al., 1989; Wotton and Strange, 1985). However, success in this effort has been slow due to poor understanding of the mechanism of drought/aflatoxin tolerance to assist in the selection of drought-tolerant peanut genotypes. The objectives of this study were to determine the effects of terminal drought stress on *Aspergillus* invasion and aflatoxin contamination, and investigate the association between surrogate traits of drought tolerance and aflatoxin contamination.

Materials and Methods

Two field experiments were conducted in the dry seasons 2004/05 and 2005/06. A split-plot design with four replications was used. Two water regimes (field capacity (FC) and 1/3 available soil water (AW)) were assigned to main plots, and eleven peanut genotypes (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348, ICGV 98353, Tainan 9, KK 60-3, and Tifton-8) were assigned to subplots. Water was withheld to reach the desired moisture of 1/3 AW at 80 days after planting (DAP) until harvest. Rainout shelters were used to maintain stress during the growing season. At 30 DAP, all plots were inoculated with *A. flavus*. Data were recorded for physiological traits, total biomass, pod yield, drought tolerance index (DTI), percentage of *A. flavus* infection and aflatoxin contamination.

Results

Aflatoxin accumulation were increased when peanut were exposed to terminal drought. Drought resistance genotypes were resistant to aflatoxin contamination. The genotypes ICGV 98330, ICGV 98348, ICGV 98353, and Tifton- 8 which are elite drought-resistant lines were observed to have low aflatoxin contamination. Physiological traits for drought tolerance are consistently related to aflatoxin contamination in peanut under terminal drought (Table 1). Correlations between DTI of biomass and *A. flavus* colonization and PAC were significant (ranged from $r = -0.59$ to $r = -0.67$, $P < 0.05$). These results revealed that peanut genotypes with an ability to maintain high biomass production under terminal drought also had relatively low aflatoxin production. Positive correlations between specific leaf area (SLA), drought stress rating (DSR), and canopy temperature and *A. flavus* colonization and PAC were significant (ranged from $r = 0.58$, $P < 0.05$ to $r = 0.77$, $P < 0.01$). Negative and significant correlations between chlorophyll density (CHID), and relative water content (RWC) and *A. flavus* colonization and PAC (ranged from $r = -0.57$, $P < 0.05$ to $r = -0.75$, $P < 0.01$) were also observed.

Table 1. Correlation between *A. flavus* colonization (%) and aflatoxin contamination (ppb) and surrogate traits for drought tolerance of 11 peanut genotypes under terminal drought in 2004/05 and 2005/06 dry seasons.

Surrogate trait for drought tolerance	2004/05 (n=11)		2005/06 (n=11)	
	A. flavus	Aflatoxin	*A. flavus*	Aflatoxin
DTI of biomass	-0.67 *	-0.59 *	-0.52	-0.59 *
SLA	0.60 *	0.68 *	0.67 *	0.77 **
SCMR	-0.53	-0.35	-0.36	-0.53
ChlD	-0.67 *	-0.57 *	-0.60 *	-0.61 *
RWC	-0.71 *	-0.58 *	-0.75 **	-0.63 *
DSR	0.58 *	0.59 *	0.71 **	0.56
Canopy temperature	0.56	0.50	0.64 *	0.66 *

DTI, drought tolerance index; SLA, specific leaf area; ChlD, chlorophyll density; SCMR, SPAD chlorophyll meter reading; RWC, relative water content; DSR, drought stress rating.

*, ** are significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Multiple regressions showed that the contribution of surrogate traits for drought tolerance to *A. flavus* colonization and aflatoxin contamination were not consistent across years (Table 2). RWC and ChlD contributed 50.9 % and 21.3 %, respectively, to *A. flavus*

colonization in 2004/05 but these traits did not contribute to *A. flavus* colonization in 2005/06. In 2005/06, the contributions of surrogate traits for drought tolerance to *A. flavus* colonization were mainly from drought stress rating (51.2 %). RWC contributed 34.0 % to PAC in 2004/05 and SLA contributed 58.9 % to PAC in 2005/06.

Table 2. Contribution of surrogate traits for drought tolerance to *A. flavus* colonization and aflatoxin contamination of 11 peanut genotypes under terminal drought in 2004/05 and 2005/06 dry seasons.

Surrogate trait for drought tolerance	Explained by regression (%) in 2004/05		Explained by regression (%) in 2005/06	
	A. flavus	Aflatoxin	*A. flavus*	Aflatoxin
Regression	82.6 **	58.1 *	74.3 *	72.8 *
SLA	7.0	0.6	20.5	58.9 *
ChlD	21.3 *	5.5	0.1	11.3
RWC	50.9 **	34.0 *	2.5	0.9
DSR	3.4	18.1	51.2 *	1.7

SLA, specific leaf area; ChlD, chlorophyll density; RWC, relative water content; DSR, drought stress rating.
*, ** are significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Conclusion

This study demonstrated that some drought resistant genotypes had lower *A. flavus* colonization and preharvest aflatoxin contamination. The relationships of increased drought tolerance and reduced aflatoxin production were confirmed by this study. Drought tolerance traits have the potential to serve as indirect selection tools for lower preharvest aflatoxin contamination. DTI of biomass, SLA, ChlD, RWC, and DSR can be used as efficient tools for selection of peanut genotypes with terminal drought tolerance and low levels of aflatoxin contamination.

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Sub-Project 3:

Basic research for supporting peanut varietal improvement for high O/L (oleic acid/linoleic acid) ratio

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Sub-Project Leader

Peanut is a grain legume as well as an oil crop. Oil accounts for 44-45% of peanut kernel (Savage and Keenan, 1994), and high oil concentration causes quality problems in peanut products. Oleic and linoleic acids constitute about 80% of peanut oil. Linoleic acid is less saturated double bonds of carbon skeleton of the molecule structure, and less stable than oleic acid. Low O/L ratio causes rapid rancidity and poor flavor in the oil and peanut products (Mercer et al., 1990). The rancidity in the oil causes low quality and low shelf-life of peanut products especially processed products such as roasted peanut, canned and salted peanut and other products that use peanut as an ingredient. High O/L ratio is high quality oil and can increase the quality of peanut products. Furthermore, saturated oil is good for health and can reduce cholesterol level in blood that can clog blood vessels and increase the risk of heart disease.

Most peanut varieties in Thailand are low in O/L ratios, e.g., 1.5/1 for Tainan 9 (Chaola et al., 1987). Recently, peanut varieties with high O/L ratio such as SunOleic (Gorbet, 2003), Georgia-02C (Branch, 2003), Olin (Simpson et al., 2003), F435 (Norden et al., 1987), Tamran OL 01 and Tamran OL 02 (Texas Foundation Seed Service, 2004) have been released commercially in the United States with O/L ratios ranging from 22/1 to 40/1. Improving O/L ratio will increase peanut quality which will benefit peanut growers, industrial users as well as consumers. The peanut breeding program of Khon Kaen University is aiming at breeding for high O/L ratio as a next step in varietal improvement of peanut. The first step that needs to be done is to assess the genetic variability for this character in the germplasm currently available. The inheritance of high O/L ratio is also not well under stood. This information is needed to guide the implementation in breeding for high O/L ratio in peanut.

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3.1 Genotypic variability and genotype by environment interactions in oil and fatty acids in high, intermediate and low oleic acid peanut genotypes

N. Singkham, S. Jogloy, T. Kesmala, P. Swatsitang, P. Jaisil and N. Puppala

Peanut is an important oil crop, containing 50 % oil in its kernels. Oleic acid and linoleic acid account for 80 % of total fatty acids. Low seed quality is generally associated with rancidity and low shelf-life of the kernels. High oleic acid increase quality and shelf-life of peanut. Therefore, oleic acid can be used to determine oil stability and longer shelf-life of peanut. The variations in genotype, year, season, location, drought stress, and soil temperature could affect percent of fatty acid compositions, and genotype × environment (G × E) interactions were observed for oleic, linoleic and O/L ratio (Andersen and Gorbet, 2002, Dwivedi et al., 1993). Variability of genotype and G × E interactions for fatty acids are important to develop high-oleic types in peanut varietal improvement programs. The objective of this study was to determine the variation in fatty acids and G × E interactions of oil and fatty acids in three groups of genotypes with high, intermediate and low oleic acid. This information will be useful for peanut breeding aiming at improving seed quality and shelf-life through increased oleic acid.

Materials and Methods

Twenty-one genotypes, having with high, intermediate and low oleic acid, were tested at Khon Kaen University for two rainy seasons (2006 and 2007) and one dry season (2006/07). The experiments were conducted in a randomized complete block design with two replications. Measurements were made on shoot dry weight and pod dry weight at final harvest of the individual genotypes.

Fifty mature kernels for each genotype were used for the determination of oil content and fatty acid compositions. Oleic and linoleic acid contents were determined by gas liquid chromatography (GLC) method, and then the ratio of oleic to linoleic acids (O/L ratio) was determined.

A combined analysis of variance was first conducted. Then, the sum of squares attributed to genotypes was further partitioned into orthogonal comparisons among three oleic acid groups and among genotypes within each group. The genotype × season interaction sum of squares was also partitioned into the interactions of peanut groups with seasons. The pooled error was used to test significance of genotype × season interaction.

Results

Significant seasonal differences were shown for most traits except for % oil, whereas the genotypic differences and the genotype × environment (G × E) interactions were significant for all traits (Table 1). Oleic acid was highest and linoleic was lowest in the rainy seasons 2007, whereas dry season favored the higher production of linoleic acid than oleic acid (Table 2). The seasonal differences for these characters were not unexpected because fatty acid compositions are affected by environmental factors. In this study, however, percent oil was not significantly affected by seasonal variation and was more stable than the other characters.

For the three groups of genotypes with different levels of fatty acid compositions, high variations in oleic, linoleic acids, % oil and O/L ratio were found among three groups and within the intermediate and the low groups, whereas the high group had low variations for oleic and % oil. The interaction among groups was the main source of G × E interactions for oleic, linoleic acids and O/L ratio, accounting for more than half of G × E total sum squares. G × E interactions for oleic acid among peanut groups were observed between intermediate and low oleic acid groups only, while the G × E interactions for linoleic acid, % oil were observed in all groups.

Considerable variations due to G × E interactions were observed for pod yield and harvest index (HI), accounting for 18 and 15% of total variation, respectively (Table 1). The lower G × E interaction for oleic acid is possibly due to the simple genetic control of the trait compared to the more complex inheritance for biomass, pod yield and HI.

Table 1. Contribution of sources of variation[†] for oleic, linoleic acids, % oil, O/L ratio, pod yield, and harvest index (HI) evaluated in three environments (seasons).

Source of variation	df	Oleic acid	Linoleic acid	% oil	O/L ratio	Pod yield	HI
Environment (E)	2	0.05**	0.07**	0.19	0.01*	0.45**	0.51**
Rainy season vs. dry season	1	0.04**	0.06**	0.03	0.01**	0.13**	0.03**
Between Rainy seasons 2006 and 2007	1	0.01**	0.01**	0.16	0.00	0.32**	0.49**
Rep within season	3	0.00	0.00	0.09	0.00	0.00	0.00
Genotypes (G)	20	0.92**	0.90**	0.32**	0.94**	0.36**	0.30**
Among genotype groups	2	0.87**	0.86**	0.04**	0.92**	0.18**	0.11**
Among high oleic acid genotypes	2	0.00	0.0002**	0.01	0.01**	0.01**	0.01**
Among intermediate oleic acid genotypes	9	0.04**	0.03**	0.13**	0.01**	0.09**	0.14**
Among low oleic acid genotypes	7	0.02**	0.01**	0.15**	0.00	0.08**	0.05**
Genotypes × Environment (G × E)	40	0.03**	0.03**	0.26**	0.04**	0.18**	0.15**
Among genotype groups × E	4	0.02**	0.02**	0.00	0.01**	0.01**	0.01**
Among high oleic acid genotypes × E	4	0.00	0.001**	0.03*	0.03**	0.01**	0.01**
Among intermediate oleic acid genotypes × E	18	0.01**	0.01**	0.15**	0.00	0.10**	0.08**
Among low oleic acid genotypes × E	14	0.002**	0.003**	0.07*	0.00	0.05**	0.05**
Pooled error	60	0.00	0.00	0.15	0.01	0.01	0.03
Total		1.00	1.00	1.00	1.00	1.00	1.00

*, ** significant at $p \leq 0.05$ and $p \leq 0.01$ probability levels, respectively

[†]Proportion of sum of squares to total sum of squares

Table 2. Percentage of oleic, linoleic acids, % oil and O/L ratio averaged over 21 peanut genotypes.

Season	Oleic acid	Linoleic acid	% oil	O/L ratio
	% of total fatty acid			
Rainy season 2006	59.49b	20.30b	44.19	6.06a
Rainy season 2007	63.06a	17.34c	46.87	6.32a
Dry season 2006/07	55.69c	24.60a	46.51	4.77b
F-test	**	**	NS	*

*, ** and NS significant at 0.05, 0.01and non significant probability levels, respectively
Means in the same column followed by the same letter (s) are not significantly different (at $p<0.05$) by DMRT

Correlations between oleic acid and pod yield (r = 0.08), between oleic acid and biomass (r = –0.05) and between oleic acid and harvest index (HI) (r = 0.14) were not significant. The correlation between oleic acid and pod yield was not significant, indicating that these traits are independently segregated and improvement of individual traits is possible. Oleic acid was inversely correlated with linoleic acid (r = –0.99, $p \leq 0.01$) and positively correlated with % oil (r = 0.22, $p \leq 0.05$) (data not shown).

Conclusion

Genotypic variation was the main source of variation for fatty acid composition and significant genotype × environment (G × E) interactions for oleic acid were found mostly among peanut genotypes in low and intermediate groups. However, non-significant G × E interactions were found in the high group indicating that the high group is less affected by environmental changes and, therefore, selection for high oleic acid will be effective.

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3.2 Combining ability for oleic acid in peanut

N. Singkham, S. Jogloy, P. Swatsitang, P. Jaisil, N. Puppala and A. Patanothai

Peanut quality is a major problem of peanut products worldwide. Low quality and short storability are generally associated with rancidity of the kernels. High-oleic acid peanut has much greater flavor stability and longer shelf life than normal-oleic peanut. High-oleic in oil is of health benefit and associated with lowered blood serum cholesterol in human. Genetic control of oleic acid studied so far has not been conclusive. Many authors found that the inheritance of this character is inherited qualitatively (Isleib et al., 1996; López et al., 2001). If that is the case, breeding for high-oleic acid would be very simple. However, quantitative inheritance of this character has also been reported by Upadhyaya and Nigam (1999), which means breeding for this character would be complex and difficult. A better understanding on the inheritance of oleic acid is important in breeding of peanut for high-oleic acid. The objectives of this study were to determine general combining ability (GCA) and specific combining ability (SCA) for oleic acid in peanut, and to identify parental lines for use in breeding for high-oleic peanut.

Materials and methods

Five peanut genotypes differing in oleic content were crossed in a full diallel matting design. They included SunOleic 97R, Georgia-02C both with high-oleic acid, $[(NC17090 \times B1)\text{-}9\text{-}1 \times KK60\text{-}3]_{F6\text{-}8\text{-}3}$ with intermediate-oleic acid, KKU 1 and KK 5 both with low-oleic acid. The 20 F_1 hybrids and five parental lines were planted in the rainy season of 2008 at Khon Kaen University (KKU) using a randomized complete block design with four replications. Plants were harvested at maturity. The seeds of parental lines were bulked for each plot, while the F_2 seeds from each plant in the plot were bulked and divided into two sets. The first set was analyzed for fatty acids and another set was used for planting in the F_3 generation.

The remnant F_2 seeds of 20 crosses and seeds of parental lines were used in the experiment. The seeds were planted in the dry season of 2008/09 at KKU using a randomized complete block design with four replications was. Ten plants in each plot were harvested at maturity, and seeds from each plot were bulked for fatty acid analysis.

Fifty mature kernels for each sample in the F_2 and F_3 generations were bulked for the determination of oil content and fatty acid compositions. Oleic and linoleic acid contents were determined by gas liquid chromatography (GLC) method, and then the ratio of oleic to linoleic acids (O/L ratio) was determined. Estimates of combining ability in the F_2 and F_3 generations were computed by using Method 1 Model 2 of Griffing (1956). The relative importance of GCA and SCA effects was calculated as GCA/SCA means squares. Tests for significance of GCA and SCA effects were done using the t-test.

Results

General combining ability (GCA) and specific combining ability (SCA) effects were significant for oleic, linoleic acids, % oil and O/L ratio in both the F_2 and F_3 generations (Table 1). The results suggested that additive and nonadditive gene actions contributed to the variations in these characters. However, GCA effects contributed greater than did SCA effects for these characters. High ratios of GCA and SCA mean square were observed for oleic and linoleic acids suggesting that the additive gene action is more important than nonadditive gene action for these characters. However, the

magnitude of GCA variance for percent oil was smaller than SCA in F_2 generation indicating the preponderance of nonadditive gene action for this character.

Moreover, the reciprocal effects were also detected for oleic, linoleic acids, % oil, O/L ratio and IV in both the F_2 and F_3 generations (Table 1), suggesting that cytoplasmic factors were also important for these characters. Similar results were also reported by Mercer et al. (1990).

SunOleic 97R and Georgia–02C are the best parents for high-oleic acid and O/L ratio because they had high GCA effects for these characters (Table 2). Therefore, the progenies of the crosses with SunOleic 97R or Georgia–02C as parent should deserve more attention for selection of high-oleic acid. In addition, the best cross combination was SunOleic 97R × Georgia–02C, which exhibited greater positive SCA effect for oleic acid and O/L ratio.

Conclusion

Additive gene action was more important than nonadditive gene action for oleic content. Therefore, it is possible to improve oleic content in this peanut population and selection can be conducted effectively in early generation. SunOleic 97R and Georgia–02C were found to be good donor parents for high oleic acid breeding program.

Table 1. Mean squares for oleic, linoleic acids, % oil, the ratio of oleic to linoleic acids (O/L ratio) in the F_2 and F_3 generations.

Source	df	Oleic acid	Linoleic acid	% oil	O/L ratio
			F_2 generation		
GCA[1/]	4	609.50**	393.69**	1.15**	219.78**
SCA[2]/	10	5.75**	4.23**	7.22**	37.86**
Reciprocals	10	8.58**	5.60**	4.93**	0.62**
GCA/SCA[3/]		105.98	93.1	0.16	5.8
Error	48	0.89	0.58	1.17	1.05
			F_3 generation		
GCA	4	723.20**	434.55**	11.48**	217.39**
SCA	10	17.10**	19.44**	6.14**	41.63**
Reciprocals	10	22.53**	17.71**	3.32**	7.33**
GCA/SCA		42.28	22.35	1.87	5.22
Error	48	6.19	3.59	1.54	1.54

* and ** significant at $p \leq 0.05$ and $p \leq 0.01$ probability levels, respectively.

[1/] General combining ability, [2/] Specific combining ability.

[3/] The ratio of general combining ability mean squares and specific combining ability mean squares.

Table 2. General combining ability effects for oleic, linoleic acids, % oil, the ratio of oleic to linoleic acids (O/L ratio) correlation between fatty acid compositions of parental lines and their progenies in the F_2 and F_3 generations.

Parental line	Oleic acid	Linoleic acid	% oil	O/L ratio
		F_2 generation		
SunOleic 97R	6.41**	–5.01**	0.14	3.85**
Georgia–02C	7.20**	–5.97**	–0.28	5.94**
F6-8-3[1/]	3.16**	–2.46**	0.01	–1.31**
KK 5	–8.57**	7.14**	–0.36	–4.30**
KKU 1	–8.21**	6.30**	0.48	–4.19**
r[2/]	0.59**	0.58**	–0.09	0.47**
		F_3 generation		
SunOleic 97R	8.02**	–5.75**	0.41	5.58**
Georgia–02C	7.72**	–6.33**	1.30**	4.41**
F6-8-3[1/]	2.16**	–1.85**	0.52	–1.93**
KK 5	-9.48**	7.28**	–1.11**	–3.86**
KKU 1	-8.42**	6.65**	–1.12**	–4.21**
r[2/]	0.56**	0.54**	0.16	0.45**

* and ** significantly different from zero at $p \leq 0.05$ and 0.01 probability levels, respectively.

[1/] the variety[(NC17090 × B1)-9-1 × KK60-3]$_{F6-8-3.}$

[2/] Correlation coefficient between fatty acid contents of parents and their progenies.

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3.3 Estimation of heritability by parent-offspring regression for high-oleic acid in peanut

N. Singkham, S. Jogloy, T. Kesmala, P. Swatsitang, P. Jaisil, N. Puppala and A. Patanothai

Peanut is an economically important oil crop. The major fatty acids in peanut oil are oleic and linoleic acids accounting for 80% of total fatty acid. A high-oleic acid and a high ratio of oleic to linoleic acid (O/L ratio) are associated with longer shelf-life and lower rancidity. Eating high oleic peanut diet can reduce low density lipoprotein (LDL) in human (O'Byrne et al., 1997). As effective selection for characters under improvement depends on sufficient additive genetic variation of the characters that are expressed as heritability, information on the heritability of oleic acid and other fatty acid compositions and the phenotypic correlations among these characters is needed for planning suitable breeding strategies for improving high-oleic acid in peanut seeds (Songsri et al., 2008). Therefore, the objectives of this study were to determine the heritability of oleic acid and other fatty acid compositions in peanut and to estimate the relationship among oil characters.

Materials and Methods

Two peanut genotypes with high-oleic acid (SunOleic 97R and Georgia–02C) and one genotype having low-oleic acid (KKU 1) were used as parents to generate three F_1 crosses including Georgia–02C × KKU 1, SunOleic 97R × KKU 1 and SunOleic 97R× Georgia–02C. The F_1 hybrids were further grown in small plots for producing F_2 seeds for evaluation and generation advance. The experiment was conducted at Khon Kaen University (KKU) in the rainy season of 2008.

The F_2 seeds of individual F_1 plants of each cross were divided into two groups. The first group of the F_2 seeds was evaluated for fatty acids and another group of the F_2 seeds was set aside for evaluation in the F3 generation. There were 35 F_2 progenies, 18 F_2 progenies and 32 F_2 progenies for Georgia–02C × KKU 1, SunOleic 97R × KKU 1 and SunOleic 97R × Georgia–02C, respectively, available for evaluation in the F_3 experiments.

In the F_3 generation, three experiments were conducted separately for each cross the dry season of 2008/09 at Khon Kaen University, using a randomized complete block design with two replications. Ten seeds of each family were planted in one-row plot with 1 m long and 20 cm between plants within row. Ten plants of each plot were harvested at maturity. The seeds of each family of three crosses were bulked and prepare for fatty acid analysis. Fifty mature kernels for each sample in the F_2 and F_3 generations were bulked for the determination of oil content and fatty acid compositions. Oleic and linoleic acid contents were determined by gas liquid chromatography (GLC) method, and then the ratio of oleic to linoleic acids (O/L ratio) was determined.

Heritability estimates in narrow sense (h^2) were calculated by parent offspring method using the data of F_3 on F_2 families (Smith and Kinman, 1965). Simple correlation coefficients were calculated to determine the relationship between the fatty acid compositions of each cross in the F_2 and F_3 generations.

Results

The estimates of narrow sense heritability for oleic acid varied from 0.63 to 0.72 and linoleic acid varied from 0.57 to 0.72 (Table 1). The heritability estimates were intermediate to high for oleic, linoleic acids and O/L ratio. The results indicated that the progress in selection program could be achieved for these characters in early generation because of sufficient additive gene effects. The results were agreed with those of Mercer et al. (1990) and Upadhyaya and Nigam (1999) in which additive and additive × additive gene effects were found to be involved in the inheritance of oleic acid.

Table 1. Heritability by parent-offspring regression and standard errors of three crosses for fatty acid compositions, % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV) and the ratio of unsaturated to saturated fatty acid (U/S ratio).

Fatty acid composition	Narrow sense heritability		
	SunOleic 97R × Georgia–02C	SunOleic 97R × KKU 1	Georgia–02C × KKU 1
Palmitic acid	0.31 ± 0.00	0.61 ± 0.00	0.82 ± 0.00
Stearic acid	0.03 ± 0.00	0.34 ± 0.00	0.66 ± 0.00
Oleic acid	0.68 ± 0.00	0.63 ± 0.00	0.72 ± 0.00
Linoleic acid	0.70 ± 0.00	0.57 ± 0.00	0.72 ± 0.00
Arachidic acid	0.01 ± 0.00	0.57 ± 0.00	0.01 ± 0.00
Eicosenoic acid	0.17 ± 0.01	0.45 ± 0.00	0.67 ± 0.00
Behenic acid	0.02 ± 0.00	0.43 ± 0.00	0.00
Lignoceric acid	0.00	0.28 ± 0.00	0.18 ± 0.00
% oil	0.16 ± 0.00	0.00	0.05 ± 0.00
O/L ratio	0.27 ± 0.00	0.81 ± 0.00	0.61 ± 0.00
IV	0.27 ± 0.00	0.45 ± 0.00	0.49 ± 0.00
U/S ratio	0.12 ± 0.00	0.79 ± 0.00	0.60 ± 0.00

Heritability estimates for palmitic acid, O/L ratio and U/S ratio were high in two crosses between high-oleic and low-oleic acid peanut genotypes (SunOleic 97R × KKU 1 and Georgia–02C × KKU 1) ranging from 0.60 to 0.81, whereas in peanut cross between high-oleic acid peanut genotypes (SunOleic 97R × Georgia–02C) were low ranging from 0.12 to 0.27. Heritability estimates for % oil were low for all peanut crosses, indicating that improvement of % oil in these populations is very difficult.

The correlation coefficients between oleic acid and linoleic acid in both the F_2, F_3 generations were significant and negative (–0.98, $p \leq 0.01$) (Data not shown). The O/L ratio had positive and significant correlations with oleic, eicosenoic acids and U/S ratio, but it had negative and significant correlations with palmitic, stearic, linoleic acids and IV in both the F_2, F3 generations. Percentage of oil was positively correlated with oleic acid ($r = 0.30$, $p \leq 0.01$) in the F3 generation, whereas its relationship with linoleic acid was not significant. The results indicated that selection for higher oleic acid and higher % oil at the same time may be difficult.

Conclusion

Intermediate and high heritability estimates for oleic, linoleic acids and O/L ratio suggested the high possibility to improve these characters in this peanut population. The negative association between oleic acid and linoleic acid indicated that selection for higher oleic acid will result in lower linoleic acid.

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Publication

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3.4 Oleic acid contents determined by gas liquid chromatography and near-infrared reflectance spectroscopy methods in segregating populations of peanut

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Oleic acid is a major fatty acid in peanut oil, comprising about 40–80% of total fatty acid. High-oleic acid peanut seed has much greater flavor stability and shelf life than normal and low oleic peanut during storage. Furthermore, the consumption of high oleic peanut could reduce blood serum cholesterol and low density lipoproteins in humans. Most of breeding program for oil crops usually measured the fatty acids with gas liquid chromatography (GLC). This method has a number of disadvantages, including destruction of samples, expensive, labor-intensive and time-consuming (Kim et al., 2009). There is a need for non-destructive and inexpensive method for screening of segregating populations in peanut breeding programs (Pérez–Vich et al., 1998). Near-infrared reflectance spectroscopy (NIR) method appears to fit well with the need (Tillman et al., 2006), but its accuracy for this purpose has never been validated. The objective of this study was to compare oleic acid contents determined by GLC method with those predicted by NIR method in segregating populations of peanut.

Materials and methods

Comparisons of GLC and NIR methods for determination of oleic acid in segregating populations of peanuts were done in two sets of peanut lines. The lines in set 1 consisted of F_2 seeds of 10 plants from each of the 20 F_1 hybrids from crosses of five peanut lines varying in oleic acid content and other fatty acids that were obtained from Study 3-2. Set 2 consisted of F_1, F_2, BC_{11} and BC_{12} seeds for each of the three crosses of SunOleic 97R, Georgia–02C and KKU 1 that were obtained from Study 3-3. Seeds of each line were divided into two parts, one was analyzed by GLC method and the other was analyzed by NIR method. The results from the two methods were compared graphically, and simple correlation was used to determine their relationship.

Results

For all pairs of comparison between the two methods, oleic acid contents predicted by NIR method were significantly higher than those determined by GLC method (Figure 1 a, b). The results were rather similar for the two segregating populations of peanut, confirming that the predicted values of oleic acid were over-estimated and needs to be calibrated for more accurate estimation of the values.

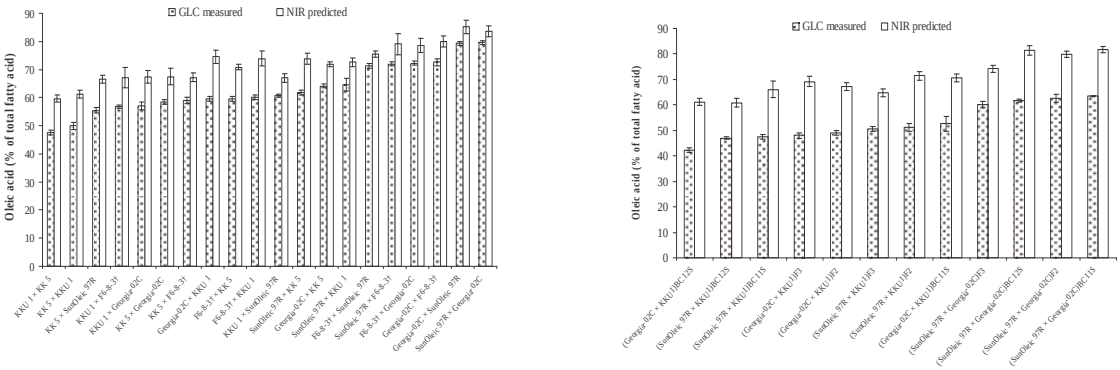
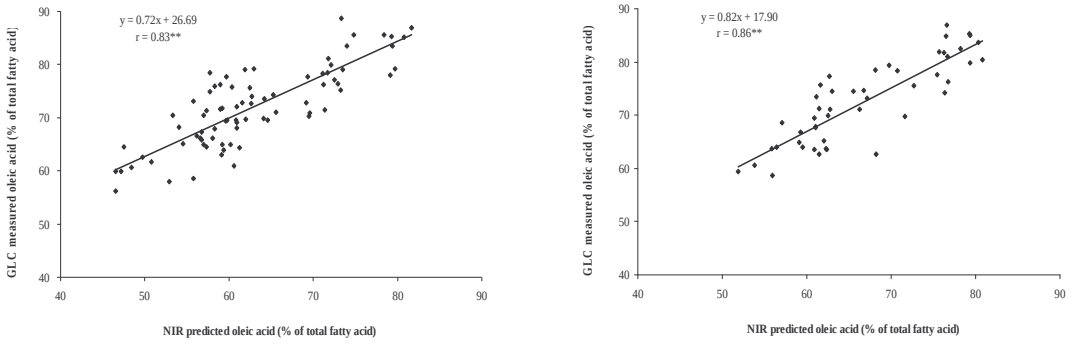


Fig. 1. Mean of oleic acid content for gas liquid chromatography (GLC) and near-infrared reflectance spectroscopy (NIR) methods in the F_2 generation for 20 peanut crosses (a) and in the F_2, F_3 and first backcross generation to parent lines self ($BC_{11}S$ and $BC_{12}S$) in three peanut combinations (b).

The correlation coefficients between oleic acid contents determined by the two methods were high for both experiments with the values of 0.83 ($p \leq 0.01$) in the F_2 generation and 0.86 ($p \leq 0.01$) in different generations (Figure 2 a, b). The results indicated that NIR method could be used in screening peanut genotypes for high oleic acid.



(a) (b)

Fig. 2. Correlation between % oleic acid of gas liquid chromatography (GLC) and near-infrared reflectance spectroscopy (NIR) methods of peanut combinations in the F_2 generation (a) and the F_2, F_3 and first backcross generation to parental lines self ($BC_{11}S$ and $BC_{12}S$) generations in three peanut combinations (b).

Conclusion

The results of this study indicated that measured values of oleic acid of segregating peanut populations by the NIR method, though over-estimated, were highly correlated with the corresponding values obtained from the GLC method. For screening peanut lines in which relative values are of concern, the NIR method could be as accurate as the GLC method. As NIR measurement is non-destructive and inexpensive, the method is suitable for use in screening a large number of breeding lines of peanut for high oleic acid content.

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Sub-Project 4

Application of Crop Simulation Model in Crop Breeding

Professor Dr. Aran Patanothai

Sub-Project Leader

Physiologically-based crop simulation models have been developed as a multipurpose tool for application in agricultural research (Hoogenboom et al., 1992; Penning de Vries et al., 1993; Jones et al., 2003). For peanut, the process-oriented Cropping System Model (CSM)-CROPGRO-Peanut has also been developed and included as a part of the Decision Support System for Agrotechnology Transfer (DSSAT) (Tsuji et al., 1994; Hoogenboom et al., 1999, 2004; Jone et al., 2003). The ability of these models to simulate growth and yields of crop cultivars under various managements and environmental conditions makes them an attractive tool for crop improvement. Suggestions have also been made on their potential contributions to crop improvement process (Aggarwal *et al*., 1995; White, 1998; Matthews and Stephen, 2002). These include assisting with multi-location evaluation of crop breeding lines (Aggarwal et al., 1995), understanding the nature of genotype by environment (G x E) interactions (Hammer et al., 1996; Chapman et al., 2002), identification and evaluation of desirable traits or combination of traits leading to the design of a crop ideotype for a specific environment (Boote and Tollenaar, 1994; Aggarwal et al., 1995; Bastiaans et al., 1997; Boote et al., 2001). However, actual investigations on the applications of crop simulation models in plant breeding have been very limited. During the previous two phases of the project, some of these model applications have been investigated for peanut breeding using the CSM-CROPGRO-Peanut model.

The model application that is of great interest to us and has been our main emphasis since the beginning is the use of a crop simulation model in assisting the multi-environment evaluation of peanut breeding lines. This interest came from the fact that crop genotypes normally respond differently to different environmental conditions. Consequently, breeders are forced to evaluate new breeding lines in multi-environment trials (METs) over a set of sites and years to assess their yield performance and stability. METs, thus, have become a major activity in all crop breeding programs. This process is laborious, time consuming and expensive. Furthermore, it is not possible to evaluate promising breeding lines for the entire range of environments that correspond to local farmers' conditions in different production areas. If a crop simulation model could be used for this purpose, the efficiency of breeding lines evaluation would be greatly improved.

The first study examined the possibility of using the CSM-CROPGRO-Peanut model to evaluate yield performance and stability of 12 large-seeded Virginia type peanut lines across 10 environments and of 14 small-seeded Spanish type peanut lines across 3 environments. The results showed that the CROPGRO-Peanut model could predict the relative performances of the test peanut lines reasonably well (Banterng et al., 2006). A follow up study was also conducted to confirm their finding with a set of 13 diverse peanut breeding lines at the early stage of yield testing and four check cultivars (Suriharn, 2008). Yield performance and stability of these peanut lines over 11 environments were evaluated based on observed and simulated data. The results

confirmed the previous study that the CSM-CROPGRO-Peanut model could be used in assisting with yield performance and stability evaluation of peanut breeding lines.

The use of a crop simulation model for the above purpose would be more useful if it could be done during the early stages of line evaluation, because at this stage actual yield testing is normally done in only a few environments. Model simulation could extend the range of the test environments, making line selection more accurate and effective. A major difficulty, however, is on the estimation of the GCs of new breeding lines which required massive data from field experiments conducted over several planting dates or multiple locations (Hoogenboom et al., 1999). This recommended procedure is not very practical and reduction in data collection is needed.

In this attempt, we first established that two seasons of such data collection were sufficient for the estimation of cultivar coefficients of peanut lines for breeding applications. This was indicated by the study of Banterng et al. (2004) and subsequently confirmed by the study of Suriharn et al. (2007). However, the methodology was still too intensive to be applicable to breeding lines at the early testing stages because the number of lines is large and seed supply is limited. Another followed up study indicated that it is possible to reduce the intensity of data collection required for the determination of GCs, and the minimum data set was suggested (Anothai et al., 2007).

We have also evaluated the application of crop simulation model in studying genotype x environment (G x E) interaction. As effective allocation of resources for testing of genotypes across locations and years is based on the relative importance of genotype x location (G x L), genotype x year (G x Y), a study was conducted to investigate the dynamic patterns of these two interactions for pod yield of peanut using the CSM-CROPGRO-Peanut model. It involved analyses of simulated pod yields of 17 diverse peanut lines at 112 locations covering all peanut production areas in Thailand over 3 seasons (early-rainy, mid-rainy and dry seasons) and 30 years (1972-2002) (Phakamas et al., 2007). The results of this study raise a question on the effectiveness of the strategy for using locations to replace years in varietal testing normally employed by breeders.

Currently, there is a growing interest in breeding crop cultivars for specific adaptation. We have also examined whether all peanut production areas in Thailand are sufficiently diverse to justify a sub-division into mega-environments for breeding for specific adaptation using simulated data generated by the CSM-CROPGRO-Peanut model. The results indicated that there should be only one mega-environment for peanut breeding in Thailand (Putto et al., 2008).

In this phase, seven studies were planned under this sub-project. These include:

Study 4-1. Utilization of the CROPGRO-Peanut model in assisting multi-location evaluation of peanut lines with the GCs derived from reduced data set.

Study 4-2. Determination of cultivar coefficients of peanut lines from yield trial data.

Study 4-3. Determination of effective test sites for breeding lines evaluation using the CROPGRO-Peanut model.

Study 4-4. Evaluation of crop traits and environmental factors responsible for G x L interaction using the CROPGRO-Peanut model.

Study 4-5. Designing peanut ideotype for Thailand using the CROPGRO-Peanut model.

Study 4-6. Characterization of drought stress in different peanut production areas in Thailand.

Study 4-7. Prediction of responses to drought stress on root development of peanut lines using the CSM-CROPGRO-Peanut model.

The first five studies were completed. However, Study 4-6 was omitted because there was no student to undertake this work. Also, Study 4-7 has not yet been done because it requires observed root data from another study and the estimation of the cultivar coefficients of the concerned peanut lines conducted in another study, and these were not yet available at the termination of this project. Therefore, only the first five studies are reported.

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4.1 Utilization of the CSM-CROPGRO-Peanut model in assisting multi-environment evaluation of peanut lines with the GCs derived from reduced data set

J. Anothai , A. Patanothai , K. Pannangpetch, S. Jogloy,
K. J. Boote, and G. Hoogenboom

A major limitation of the application of a crop simulation model is the determination of cultivar coefficients, as the recommended procedure requires extensive data sampling throughout the growing season in field experiments conducted over several planting dates or across multiple locations (Hoogenboom et al., 1999). Such a procedure is laborious and time-consuming, and is very impractical especially if it involves a large number of new genotypes/cultivars as in breeding applications. A recent study by Anothai et al. (2008) demonstrated that the minimum data required for cultivar coefficient determination of the CSM-CROPGRO-Peanut model could be reduced to observing the dates of two critical developmental stages and obtaining plant growth analysis data on three dates, with no need for measured leaf area index (LAI) and specific leaf area (SLA). However, the cultivar coefficients that were derived by this procedure have not been evaluated in actual application. The objectives of this study were to determine the cultivar coefficients of two sets of peanut lines from reduced field data collection and to evaluate the derived cultivar coefficients in assisting multi-environment evaluation of peanut lines with the CSM-CROPGRO-Peanut model.

Materials and methods

Two sets of large-seeded peanut breeding lines were used. Set I consisted of seven breeding lines and two check cultivars under testing in a regional yield trial and Set II consisted of nine breeding lines and a check cultivar under testing in a standard yield trial. These lines were grown in two field experiments during the rainy season of 2004, the dry season of 2005 and the rainy season of 2005 at Khon Kaen University, using a randomized complete block design with four replications.

Data were collected on plant growth and development, soil characteristics, weather and crop management as required for determining the cultivar coefficients of a new peanut cultivar, following the procedures described in IBSNAT (1988) and Hoogenboom et al. (1999). For the 2004 rainy season experiment, growth analysis data were collected at 20, 33, 43, 55, 67 and 86 days after planting and at harvest maturity. However, for the 2005 dry and rainy seasons, these growth data were collected on just three dates following our optimum protocol (Anothai et al., 2008). The data collected from the experiments during the dry and rainy seasons of 2005 were used for model calibration, while the data collected during the 2004 rainy season were used for initial evaluation of the model.

To obtain actual multi-location trials data, the two sets of peanut lines were tested in the regional (Set I) and standard (Set II) yield trials conducted in farmers' fields and research stations located in the northeast and northern Thailand from 2003 to 2005, totaling 10 environments for Set I and 8 environments for the Set II. Pod yield for the individual peanut lines in the two sets was simulated for the same environments in which they were actually tested, using the CSM-CROPGRO-Peanut model. Evaluation of the derived cultivar coefficients from reduced data sets for model

application was performed by comparing yield ranking and yield stability over the test environments of the individual peanut lines in each set based on observed and simulated pod yields.

Results

Model calibration showed good agreement between simulated and observed values of phenology and growth characteristics of the peanut lines. The initial model evaluation with data collected in the 2004 rainy season confirmed that model prediction was good for independent data.

The results also showed that the ranks of observed and simulated means for pod yield for the 10 and 8 actual test environments of Set I and Set II of the individual peanut lines were well correlated, with the coefficient for rank correlation being 0.73 ($P < 0.01$) and 0.81 ($P < 0.01$) for Set I and Set II, respectively. Among the top five highest yielding lines based on mean observed pod yield for the two sets (upper 56% for Set I and 50% for Set II), four lines were identified by model simulation in both sets (Table 1). Furthermore, both model simulation and actual experimentation identified the same top yielding lines in the two sets. These results indicate a reasonably good performance of the model in predicting the yield ranking of the peanut lines with the cultivar coefficients that were derived from a reduced data set.

The results of the stability analyses based on observed and simulated of pod performed by the GGE biplot method (Yan et al., 2001) showed that for Set I Entries 2, 1 and 9 were the most stable genotypes based on both observed and simulated pod yields (Fig. 1a and b). For Set II, Entries 4 and 6 were the most stable genotypes based on observed pod yield, followed by Entries 3, 5 and 7, while Entries 4 and 5 were the most stable genotypes based on the simulated pod yield, followed by Entries 3 and 6. These results indicate that, with the cultivar coefficients that were derived from a reduced data set, the CSM-CROPGRO-Peanut model can predict yield stability of the test peanut lines over multiple environments quite well.

Table 1. Mean over environments and ranking of individuals peanut lines for observed and simulated pod yield of the peanut lines in the Set I and Set II yield trials.

Entry No.[a]	Mean pod yield			
	Observed (t ha^{-1})[b]	Simulated (t ha^{-1})[b]	Observed ranking	Simulated ranking
Set I (regional) yield trial (10 environments)				
2	2.97 a	4.02 a	1	1
9	2.77 a	3.60 b	2	2
1	2.66 a	3.55 b	3	3
7	2.15 b	3.03 d	4	9
5	2.11 bc	3.36 c	5	4
6	2.08 bc	3.29 c	6	5
8	2.05 bc	3.09 d	7	7
4	1.99 bc	3.10 d	8	6
3	1.82 c	3.06 d	9	8
Mean	2.29	3.34		
LSD.05	0.318	0.157		

Table 1. Continued.

Entry No.[a]	Mean pod yield			
	Observed (t ha^{-1})[b]	Simulated (t ha^{-1})[b]	Observed ranking	Simulated ranking
Set II (standard) yield trial (8 environments)				
4	2.25 a	2.79 a	1	1
6	2.09 a	2.71 b	2	3
3	2.08 a	2.67 b	3	4
5	2.08 a	2.78 a	4	2
7	1.78 b	2.50 d	5	7
1	1.75 b	2.59 c	6	5
8	1.75 b	2.44 de	7	9
2	1.68 b	2.42 e	8	10
10	1.57 b	2.50 d	9	6
9	1.50 b	2.47 de	10	8
Mean	1.85	2.59		
LSD.05	0.309	0.077		

[a] See Table 1 for entry description.

[b] Numbers in the same column within each set followed by the same letter are not significantly different at P ≤ 0.05 by LSD (DF = 72).

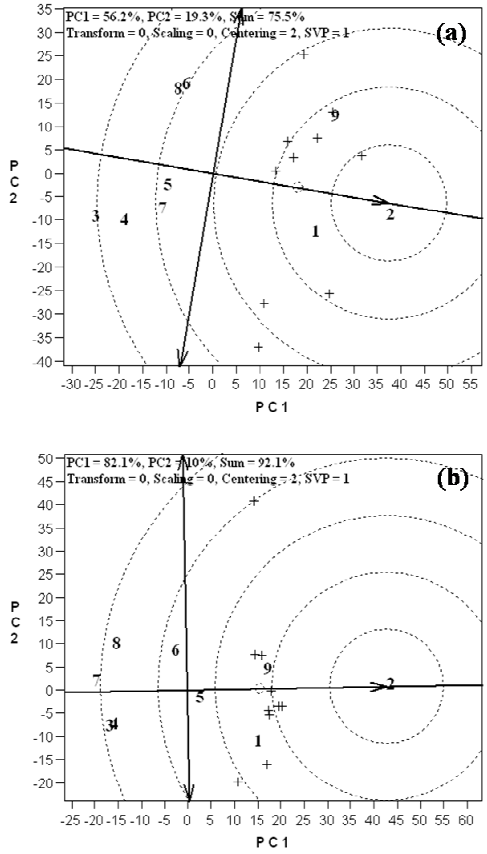

Fig 1. GGE-biplot for mean pod yield of nine peanut lines (number) in the Set I (regional) yield trial across the 10 test environments (+): (a) observed pod yield and (b) simulated pod yield.

Conclusion

The reduced field data collection consisting of observing the dates of two critical developmental stages and obtaining plant growth analysis data on three dates, with no need for measured LAI and SLA, can be used to estimate the cultivar coefficients of peanut lines that accurately predict yield ranking and stability of the lines in independent multi-environment trials. The procedure, thus, could be used in assisting with multi-environment evaluation of breeding lines at the early stage of yield evaluation when the number of lines is large and available seeds are limited.

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4.2 Determination of cultivar coefficients of peanut lines from yield trial data

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The normal procedure for estimating the cultivar coefficients for model simulation is laborious and time consuming. In the previous study, it was found that the minimum data required for the determination of cultivar coefficients by the CSM-CROPGRO-Peanut model could be reduced to dates of two critical developmental stages along with plant growth analysis data on three dates (Anothai et al., 2008). Nevertheless, this “reduced sampling” methodology is still impractical for several situations. Another approach is to estimate the coefficients with typical “end-of-season” data collected in yield trials, as shown by Mavromatis et al. (2001, 2002) for soybean lines. Furthermore, the procedures for the optimization needed for the estimation of the cultivar coefficients were difficult. Therefore, a new procedure for the optimization using the Genotype Coefficient Calculator (GENCALC), a software package that facilitates the calculation of cultivar coefficients (Hunt et al., 1993), is

proposed here. The objective of this study was to determine the feasibility of estimating the cultivar coefficients for new peanut lines using typical data from yield performance trials with the optimization procedures of GENCALC.

Materials and methods

The data used in present study were obtained from previous studies (Suriharn et al., 2007; Suriharn et al., 2008). The entries included thirteen peanut lines and four check cultivars. Two types of data were available; the first set included data from performance trials obtained from Suriharn et al. (2008) and those data were used to estimate the cultivar coefficients; the second set included data from the growth analysis experiments obtained from Suriharn et al. (2007) and was used for model evaluation. The performance trials were conducted in farmers' fields and research stations in northeast and northern regions of Thailand during 2002-2004, both during the rainy and dry seasons, totaling eight environments.

At each of the performance trials, data were recorded for the dates of planting, emergence (VE), plants with first flower (R1) and harvest maturity (R8), final pod yield, final seed yield, final biomass, final seed size, pod harvest index, seed harvest index and shelling percentage. For the growth analysis experiments, the observed development data for the rainy season of 2002 and the dry season of 2003 included emergence (VE), plants with four nodes present on the main stem (V4), plants with first flower (R1), plants with first peg (R2), plants with the first pod beginning to swell (R3), plants with a fully-expanded pod (R4), plants with the first seed beginning to develop (R5), plants with one pod having full-sized seeds (R6), physiological maturity (R7; plants with one matured pod) and harvest maturity (R8; plants with 70-80 % matured pods) (Boote, 1982). However, only R1 and R8 were observed for the dry season of 2004.

The GENCALC program of the Decision Support System for Agrotechnology Transfer (DSSAT Version 4.5) was used to calibrate the cultivar coefficients of the peanut lines/cultivars. Fifteen cultivar coefficients are required for running the CSM-CROPGRO-Peanut model. In this study, only seven candidate coefficients were selected and calculated by running GENCALC.

The derived cultivar coefficients of the peanut lines were evaluated against independent data sets obtained from the experiment conducted in the 2002 rainy, 2003 dry and 2004 dry seasons.

Results

The model calibration with GENCALC resulted in cultivar coefficients that produced simulated values for the development and growth characteristics that were close to their corresponding observed values (Figure 1).

For the model evaluation with independent data sets, the simulated values for R1 and R8 of the 17 peanut lines were in good agreement with the corresponding observed values for all experiments (2002 rainy, 2003 dry and 2004 dry seasons). The simulation of final pod yield and final biomass also showed a reasonably good agreement with the observed values, as indicated by low RMSE and moderate values for mean r^2. An example is shown in Figure 2 for peanut Entry 2.

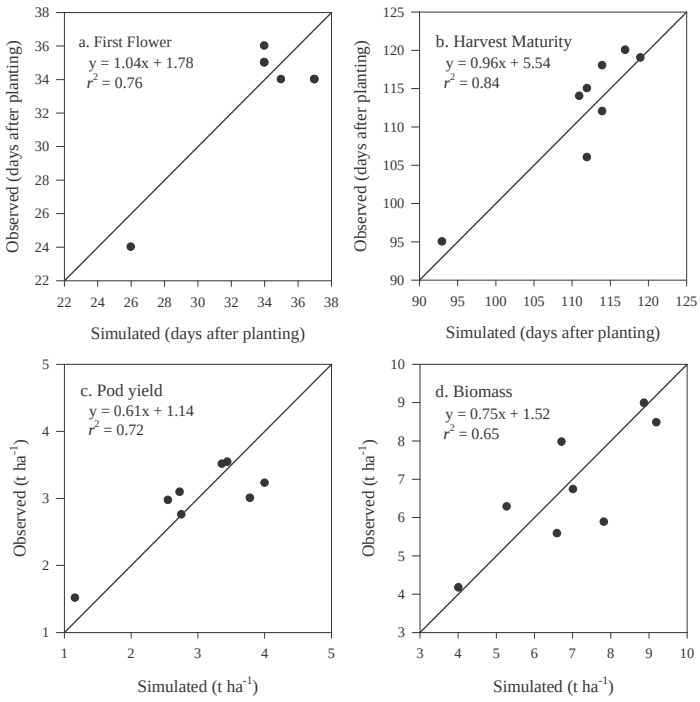

Figure 1. Simulated versus observed values for model calibration of days from planting to first flower (R1) (a) and to harvest maturity (R8) (b), final pod yield (c), and final biomass (d) of Entry 2 grown in performance trials during 2002-2004.

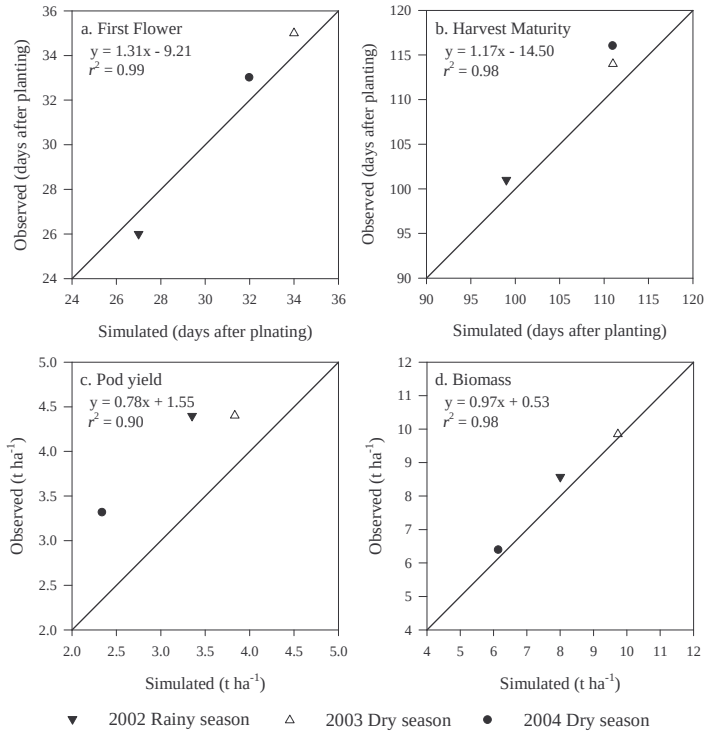

Figure 2. Simulated versus observed values for model evaluation of days from planting to first flower (R1) (a) and to harvest maturity (R8) (b), final pod yield (c), and final biomass (d) of Entry 2 grown in the evaluation experiments during the 2002 rainy, 2003 dry and 2004 dry seasons.

The comparisons between the simulated values of time-series data and the corresponding observed values for total biomass, stem, leaf, pod yield and seed yield of the tested peanut lines in model evaluation also showed good agreement. An example is shown in Figure 2. Overall, the results of model evaluation with independent data indicated that the derived cultivar coefficients could accurately simulate growth, development and final pod and biomass yields of the peanut breeding lines.

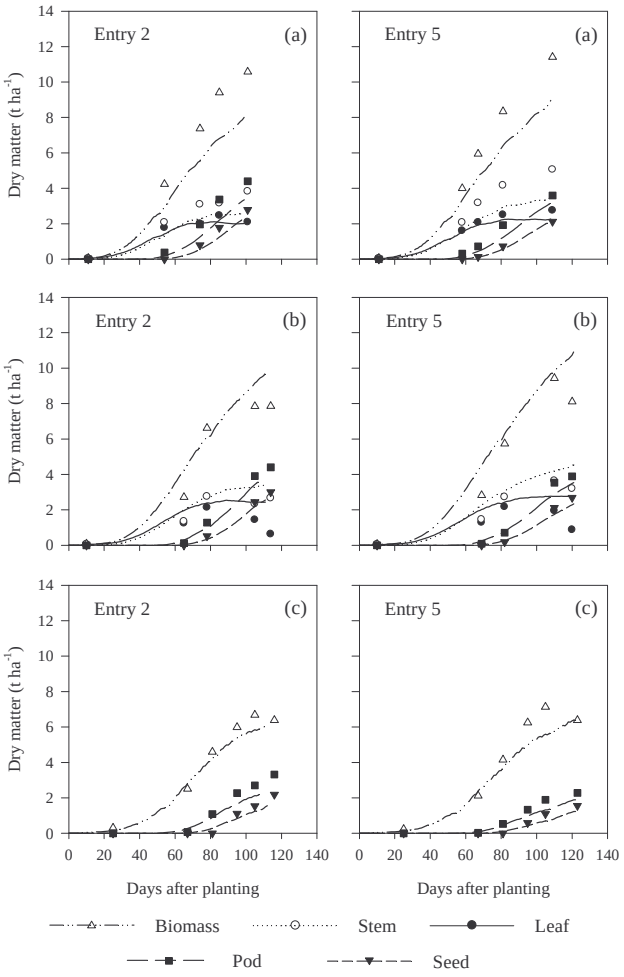

Figure 3. Simulated (lines) versus observed (symbols) values for biomass, stem, leaf, pod and seed at different growth stages for Entries 2 and 5 (see Table 1 for line descriptions) in the evaluation experiments during the 2002 rainy (a), 2003 dry (b) and 2004 dry seasons (c) where the cultivar coefficients were derived from performance trials.

Conclusion

It is feasible to estimate the cultivar coefficients for new peanut lines from typical data of routine performance trials by using GENCALC. The procedure should also be applicable to other crops for which crop simulation models are available.

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Published Paper

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4.3 Determination of effective test sites for breeding lines evaluation using the CROPGRO-Peanut model

C. Putto[a], A. Patanothai, S. Jogloy, K. Pannangpetch,
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Crop breeding lines are generally evaluated in multi-environment trials (METs) over a set of sites and years as the performance ranking might change for different environments due to genotype x environment interaction (Fehr, 1987; Kang, 1990). It has been well recognized that only the crossover type of G x E interaction is associated with significant genotypic rank change (Baker, 1988). If two or more test sites exhibit no or non-crossover G x E interaction, performance ranking of the test genotypes would be the same and testing would only be needed at one of these sites (Fehr, 1987; Annicchiarico, 2002). An efficient testing of crop breeding lines, therefore, requires a set of complementary test sites that adequately sample the environments of interest with minimal duplication (Hambling et al., 1980).

Efforts have been made to determine appropriate test sites for breeding line evaluation. The most widely used approach is to first group the sites based on their similarity in genotypic responses, and then select a representative site from each group. Such approach has been conducted by various grouping methods with actual MET data. However, these have often failed to provide an adequate coverage of the target population of environments, because there is a practical limit in both the number of sites and years for which METs can actually be conducted. Presently, crop simulation

model can provide the required data for a legitimate classification of target environments for determination of appropriate test sites for a crop breeding program. The objective of this study was to determine the efficient test sites for METs of peanut breeding lines in Thailand using the CSM-CROPGRO-Peanut model.

Materials and Methods

The main procedure used in this study consisted of simulating pod yield of a set of peanut lines for all peanut production areas in Thailand for 30 years. The simulated yield data were then used to subdivide the identified locations in each of the three growing seasons, i.e., the early-rainy, mid-rainy and dry season, into groups based on their similarity in genotypic responses. Sets of test sites were determined for the individual seasons based on different criteria. These sets of test sites were evaluated and compared for their efficiency in performance evaluation of peanut breeding lines.

Determination of all peanut production locations in Thailand were done based on the unique combinations of weather station and soil type for the individual villages that have a considerable planted area of peanut. All together, 162 unique locations were identified: 76 for the early-rainy season, 39 for the mid-rainy season and 47 for the dry season. Seventeen peanut lines that are diverse in yield level, plant type and maturity were used in this study. The CSM-CROPGRO-Peanut Model was used to simulate pod yield for each peanut genotype for all 162 locations. For each location, pod yield of each line was simulated for three seasons in which the crop is normally grown (early-rainy, mid-rainy and dry seasons) over 30 years (1972–2002).

The environmental grouping, and hence the determination of test sites, was done separately for each of the three seasons. Two methods were used in location grouping, i.e., cluster analysis (Collaku et al., 2002) and the GGE biplot method (Yan et al., 2000). Both methods were applied to simulate pod yield for the 17 peanut lines at the identified locations in each season for 30 years. Cluster analysis was conducted using the Ward's minimum variance method (Ward, 1963) in hierarchical cluster analysis, which was truncated at four groups when most (> 85 %) of the total G x L interaction could be accounted for by the interaction between genotype and location groups. Location grouping with the GGE biplot method was performed using the GGE biplot software (Yan et al., 2000).

Six sets of test sites were determined based on different scenarios for site selection. Each set consisted of four sites in each of the three seasons, i.e., the early-rainy, mid-rainy and dry seasons, corresponding to the number of groups as determined by the cluster analysis. Set 1 test sites were selected based on geographic distribution. Set 2, 3 and 4 test sites were selected based on location grouping by cluster analysis combined with geographical distribution. For all three sets, the test sites were randomly selected from locations within the individual location-groups that were derived from cluster analysis, with the provision that they were distributed in all regions of the country (Set 2), or only in the northern (Set 3) or northeastern region (Set 4). Set 5 test sites were those that are currently being used for conducting METs by the peanut improvement program of Khon Kaen University. The sites in this set are located in both the northern and the northeastern regions. Set 6 test sites were selected based on location grouping by the GGE biplot, for which a site was randomly selected from locations within the individual location-groups for each season.

For each set of test sites, combined analysis of variance for simulated pod yield of the 17 peanut lines across the years and locations was conducted for each season.

The efficiency of the six sets of test sites were evaluated by comparing the magnitude of the G x L interaction component in the combined analysis of variance and the ranking of the test genotypes based on the average simulated pod yield.

Results

The 162 locations in the present study encompassed a considerable range of climatic conditions and soil types. The 17 peanut genotypes differed in maturity, seed size, and yield level. Thus, the basic requirements for an effective determination of test sites, i.e., diverse genotypes and wide coverage of environments, were fulfilled in the present study.

Grouping of locations in each season by cluster analysis was truncated at four groups, with R^2 values among groups of 0.94, 0.92 and 0.85 for the early-rainy, mid-rainy and dry seasons, respectively. Within a season, the locations for each group were distributed in different geographical regions of the country. There was no pattern of association between locations within a geographical region, neither was a pattern of association found between locations within a geographical region, nor was there a clear relationship between groups and soil and weather conditions.

The results of location grouping for the individual seasons with the GGE biplot method provided the same result in location grouping as those obtained from cluster analysis for many locations, however, the two methods resulted in different outcomes for several locations. Similar to cluster analysis, location grouping with the GGE biplot method showed no correlation with geographic distribution or soil type.

The efficiency in breeding line evaluation for different sets of test sites was first evaluated by their ability to capture the G x L interaction in the analysis of variance. The set that captures more G x L interaction, and thus wider environmental coverage, would be preferred. The results showed that Set 6 gave the greatest contribution of G x L interaction for all three seasons, followed by Set 2 (Table 1). The test sites of Set 6, thus, were considered to best capture the G x L interaction in breeding line evaluation of peanut in Thailand, followed by the Set 2 test sites.

The magnitude of the G x L interaction alone may not be a good indicator of the efficiency of the test sites, because only the crossover type of G x L interaction is important in test site determination. Performance ranking would be more important in breeding line evaluation. The results showed that the different test-site sets gave almost the same ranking of the test peanut genotypes (Table 2). The results from all sets of test sites were highly correlated, with a correlation coefficient that ranged from 0.996 to 1.00 for mean simulated pod yield and from 0.987 to 1.00 for ranking of genotypes. The same results were also obtained for the individual seasons. Apparently all sets of the test sites gave almost the same results for evaluation of the breeding lines. They were, thus, considered equivalent in their testing ability.

Table 1. Relative contributions of different sources of variation in the combined analysis of variance for simulated pod yield of 17 peanut lines over 30 years (1972 to 2001) for each set of test sites**.**

Source	df	SS (% of total SS)					
		Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
Early-rainy season							
Year (Y)	29	16.76**	18.14**	21.96**	28.31**	22.49**	9.30**
Location (L)	3	40.70**	54.72**	41.58**	32.30**	33.83**	40.09**
Y x L	87	26.71**	13.53**	22.17**	23.07**	26.49**	27.46**
Genotype (G)	16	11.81**	9.80**	10.47**	12.06**	12.88**	18.75**
G x Y	464	0.94**	0.99**	1.17**	1.47**	1.43**	0.83**
G x L	48	1.26**	1.57**	1.29**	1.12**	1.08**	1.60**
G x Y x L	1392	1.82	1.25	1.35	1.67	1.80	1.97
Total	2039	100.00	100.00	100.00	100.00	100.00	100.00
Mid rainy season							
Y	29	11.64**	13.29**	21.54**	17.22**	11.73**	8.84**
L	3	23.11**	34.53**	19.19**	4.03**	32.66**	47.45**
Y x L	87	25.77**	25.52**	27.67**	24.16**	14.98**	14.31**
G	16	35.34**	21.69**	26.09**	48.61**	35.99**	24.46**
G x Y	464	0.93**	1.12**	1.59**	2.56**	1.54**	0.79**
G x L	48	1.04**	1.65**	1.23**	0.37**	1.22**	2.30**
G x Y x L	1392	2.18	2.19	2.69	3.06	1.90	1.84
Total	2039	100.00	100.00	100.00	100.00	100.00	100.00
Dry season							
Y	29	2.44**	2.63**	7.40**	5.55**	5.44**	4.68**
L	3	56.69**	43.42**	12.42**	43.62**	42.31**	27.26**
Y x L	87	1.81**	2.13**	1.42**	1.66**	2.73**	4.58**
G	16	35.84**	47.61**	75.21**	45.15**	45.71**	58.16**
G x Y	464	0.31**	0.30**	0.91**	0.77**	0.67**	0.62**
G x L	48	2.44**	3.48**	2.01**	2.64**	2.32**	3.67**
G x Y x L	1392	0.46	0.44	0.62	0.62	0.82	1.02
Total	2039	100.00	100.00	100.00	100.00	100.00	100.00

****** Significance at $P \leq 0.05$.

Set 1: Sites selected to have geographical distribution in all regions.

Set 2: Sites derived from location grouping by cluster analysis with geographical distribution in all regions.

Set 3: Sites derived from location grouping by cluster analysis but only in the north.

Set 4: Sites derived from location grouping by cluster analysis but only in the northeast.

Set 5: Sites currently used, located in the north and northeast.

Set 6: Sites derived from location grouping by the GGE biplot method.

Table 2. Mean and rank for simulated pod yield of peanut genotypes for all test sites of the different sets over 30 years.

Entry No.[a]	Set 1		Set 2		Set 3		Set 4		Set 5		Set 6	
	Mean (t ha^{-1})	Rank	Mean (t ha^{-1})	Rank	Mean (t ha^{-1})	Rank	Mean (t ha^{-1})	Rank	Mean (t ha^{-1})	Rank	Mean (t ha^{-1})	Rank
7	2.47	1	2.31	1	2.46	1	2.34	1	2.30	1	2.41	1
12	2.41	2	2.22	2	2.33	3	2.23	2	2.21	2	2.36	2
9	2.39	3	2.22	3	2.35	2	2.22	3	2.20	3	2.35	3
6	2.35	4	2.18	4	2.28	4	2.20	4	2.16	4	2.31	4
10	2.14	5	1.99	5	2.09	5	1.99	6	1.96	6	2.10	5
5	2.13	6	1.98	6	2.08	6	2.02	5	1.99	5	2.09	6
8	2.12	7	1.96	7	2.06	7	1.96	7	1.93	7	2.09	7
11	2.11	8	1.95	8	2.04	8	1.93	8	1.92	8	2.06	8
1	2.03	9	1.86	9	1.93	9	1.90	9	1.85	9	1.98	9
4	1.92	10	1.77	10	1.84	10	1.77	10	1.73	10	1.89	10
3	1.82	11	1.69	11	1.78	11	1.70	11	1.65	11	1.79	11
2	1.74	12	1.60	12	1.69	12	1.64	12	1.59	12	1.69	12
14	1.64	13	1.50	13	1.56	13	1.50	13	1.47	13	1.60	13
17	1.55	14	1.40	14	1.48	14	1.45	14	1.40	14	1.48	15
13	1.52	15	1.40	15	1.47	15	1.41	15	1.37	15	1.49	14
15	1.20	16	1.08	16	1.13	16	1.11	16	1.07	16	1.14	16
16	1.16	17	1.04	17	1.08	17	1.08	17	1.04	17	1.11	17
Mean	1.92		1.77		1.86		1.79		1.76		1.88	

[a] See Table 1 for entry description.

With the testing ability being equal, the choice of test sites should then be determined based on the convenience, and consequently the cost, in conducting the trials. For our case, the Set 4 test sites, which were all in the northeast region, would be preferred. However, instead of randomly selected, the representative sites could be selected from the individual location-groups based on the convenience in conducting the trial. This would make the METs of breeding lines more cost-effective.

Conclusion

The present study clearly demonstrated that, in the case of Thailand where the test sites for METs of peanut breeding lines do not provide an adequate coverage of the target population of environments, the CSM-CROPGRO-Peanut can be used as a tool for determining the most efficient test sites for breeding line evaluation. The procedure could be extended to other areas and to other crops, particularly for developing countries where the number of METs is normally limited due to resource limitations, providing that a simulation model and the required data for model simulation are available.

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4.4 Evaluation of crop traits and environmental factors responsible for G x L interaction using the CROPGRO-Peanut model

C. Putto, A. Patanothai, S. Jogloy, K. Pannangpetch,
K.J. Boote and G. Hoogenboom

Genotype x environment (G x E) interaction complicates the identification of superior genotypes as the ranking for yield performance of the test genotypes may change for different environments (Cooper and Hammer, 1996; Kang, 2002). Knowledge of the physiological basis for the differential responses of genotypes to specific environments should improve the efficiency with which a breeder can characterize material for its G and G x E interaction, and hence increase the speed at which superior genotypes can be identified (Wright et al., 1996). A good understanding of the environmental factors causing the G x E interaction would also be useful in determining appropriate breeding strategies to cope with or utilize G x E interaction.

Several studies have been conducted to elucidate the causes of G x E interaction for crop yield based on analyzing yield trial data with various statistical methods. These statistical models, however, can neither elucidate the direct effect of each plant trait or each combination of traits on the G x E interaction for crop yield, nor can reveal all the environmental variables that could potentially cause G x E interaction over the concerned target population of environments (TPE).

Crop models have now been developed which can simulate yields of crop cultivars under different environmental and management conditions. In peanut, the Cropping System Model (CSM)-CROPGRO-Peanut is also available (Boote et al., 1998; Jones et al., 2003; Hoogenboom et al. 2004a). In model simulation, the value of the genotype-specific coefficients or the environmental parameters can be changed at will. The model, thus, can be used as a tool in studying the causes of G x E interaction. The genotype x location (G x L) interaction is of special interest as it is related to local adaptation of crop genotypes (Annicchiarico, 2002). The objective of this study was to determine the plant traits and environmental factors that cause the G x L interaction for yield in peanut using the CSM-CROPGRO-Peanut model.

Materials and methods

The CSM-CROPGRO-Peanut Model was used to simulate pod yield for 17 peanut genotypes for 14 locations representative of all peanut production areas in Thailand using 30 years of historical weather data. The response of the peanut genotypes to different locations was determined with a conventional linear regression model (Eberhart and Russell, 1966). The yield response to locations of each peanut genotype was determined by regressing mean yield of the genotype for the individual locations against the location mean yield. The regression coefficient (b) value was used as an index to indicate the response of a genotype to different locations. Pairs of genotypes with different patterns of G x L interaction were identified by comparing the means and the b values of the individual genotypes.

In determining plant traits that cause G x L interaction, six pairs of peanut genotypes showing the three patterns of G x L interaction, i.e., no interaction, crossover interaction and non-crossover interaction, were selected for sensitivity analysis. Sensitivity analysis was performed for each pair by sequentially replacing the values of

each cultivar coefficient and combinations of coefficients of the lower-yielding genotype with the values of the corresponding coefficients of the higher-yielding genotype. Pod yield of the synthetic genotypes with modified cultivar coefficients were simulated for 14 locations, 3 seasons and 30 years, and their mean yield and yield response to locations (b-values) were determined. The change in cultivar coefficients continued until the regression line of the synthetic genotype was more or less the same as that of the higher-yielding genotype. The minimum cultivar coefficients that made the regression lines of the synthetic and high-yielding genotypes move closest together were considered the causal traits for G x L interaction for this pair of genotypes. The process was repeated for all selected pairs of genotypes.

In determining environmental factors that cause G x L interaction, the patterns of G x L interaction for the individual pairs of locations were determined by regressing mean yields of each location for the individual genotypes against the genotypic mean yields. The resulting mean yields and b-values of the individual locations were used in classifying pairs of locations into different patterns of G x L interaction, i.e., no interaction, non-crossover interaction and crossover interaction. Pairs of locations were selected as representatives for the individual patterns of G x L interaction. In each pattern, the procedure to identify the environmental factors causing G x E interaction was initiated by creating combinations of environmental factors, both climatic and soil, into sets of modified environments. They were then used in the crop model sensitivity analyses to determine their effects on mean yield and b-value. For each pair of locations, the values of each environmental factor and combinations of environmental factors of the lower-yielding location were sequentially replaced with the values of the corresponding factors of the higher-yielding location, and the effects of the replacements on mean yield and b-value were determined. The environmental factor or combination of environmental factors that caused G x L interaction was determined with a minimum number of environmental factors that made the regression lines of the low-yielding and high-yielding locations move more closely together. The process was repeated for all selected pairs of locations.

Results

Differential cultivar responses to locations were found for the individual genotypes, and all the responses were essentially linear. The value of the regression coefficient (b) against site mean yield for the individual peanut genotypes varied from 0.69 to 1.25. All three patterns of G x L interaction, i.e., no-interaction, non-crossover and crossover interactions, were found among pairs of peanut genotypes. For all possible pairs of 17 genotypes in the present study, 28 pairs show no G x L interaction, 93 pairs display non-crossover G x L interaction, and 15 pairs exhibit crossover G x L interaction.

Model sensitivity analysis showed that the cultivar coefficients that showed major effects were the duration from first seed to physiological maturity (SDPM), maximum leaf photosynthesis rate (LFMAX), the maximum fraction of daily growth that is partitioned to seed and shell (XFRT), single seed filling duration (SFDUR) and the duration of pod addition (PODUR), and those having minor effects were the duration from emergence to first flower (EMFL), maximum leaf size (SIZLF) and maximum seed weight (WTPSD). The plant characters that caused the differences in both mean yield and yield response to locations between peanut genotypes in different pairs included LFMAX, XFRT, SDPM, SFDUR and PODUR (Figure 1). These traits

are related to the production of photosynthate (LFMAX), the partitioning of assimilates to pods (XFRT), the phonological duration of pod and seed development (SDPM), single seed growth duration (SFDUR), and the rate of pod duration (1/PODUR), respectively. Changing the values of the above traits will affect these processes, and consequently affect crop yield. In addition, they have an interactive effect in determining the yield potential and yield response to locations for a given peanut genotype. Changing the degree of genotypic response to environments is possible through selection for a combination of some of these traits (Figure 2).

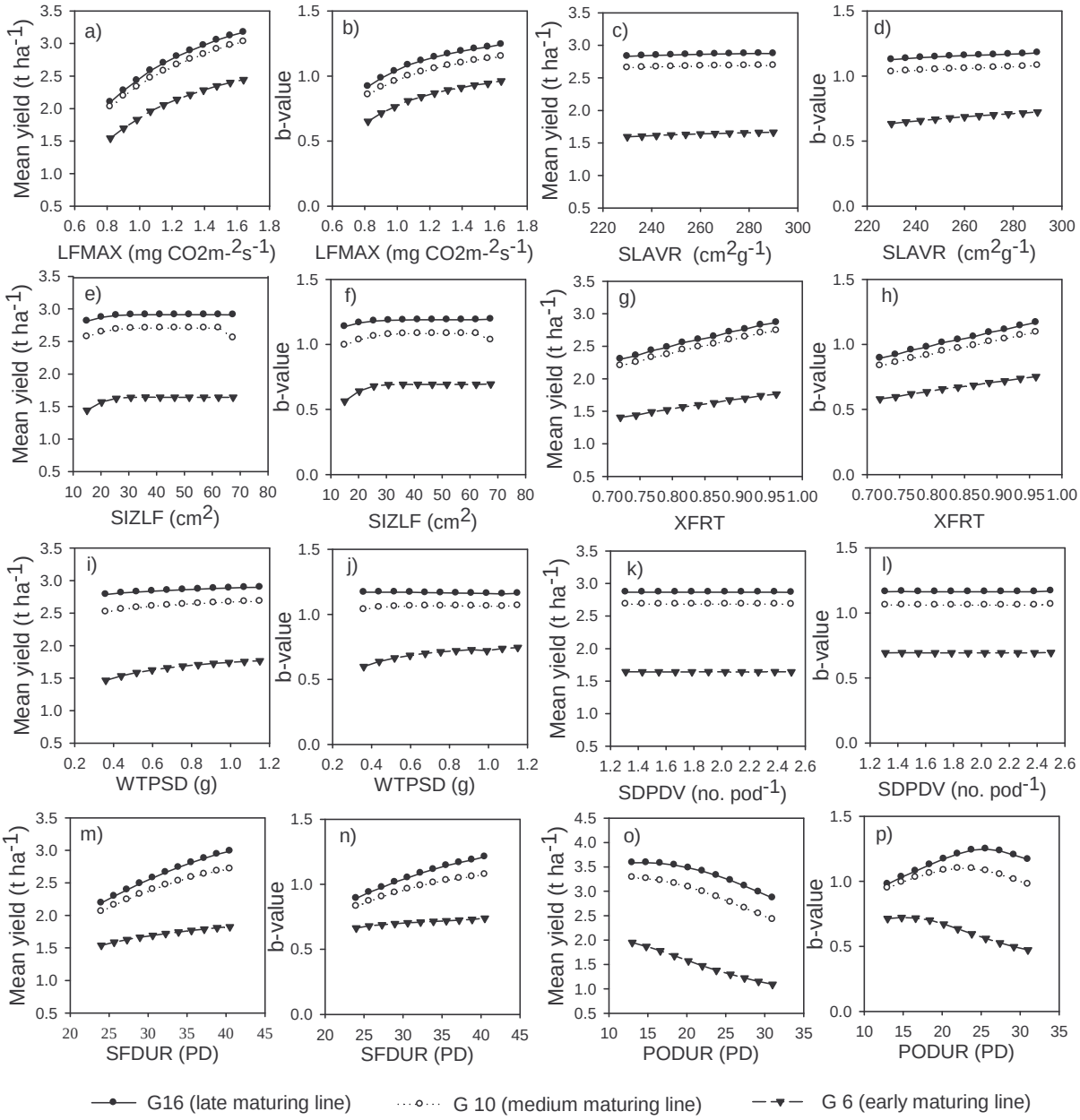

Figure 1. Effects of changing the value of each individual growth parameter on mean yield and yield response to location (b-value) for the early maturing (G 6), the medium maturing (G 10) and the late maturing (G 16) peanut lines.

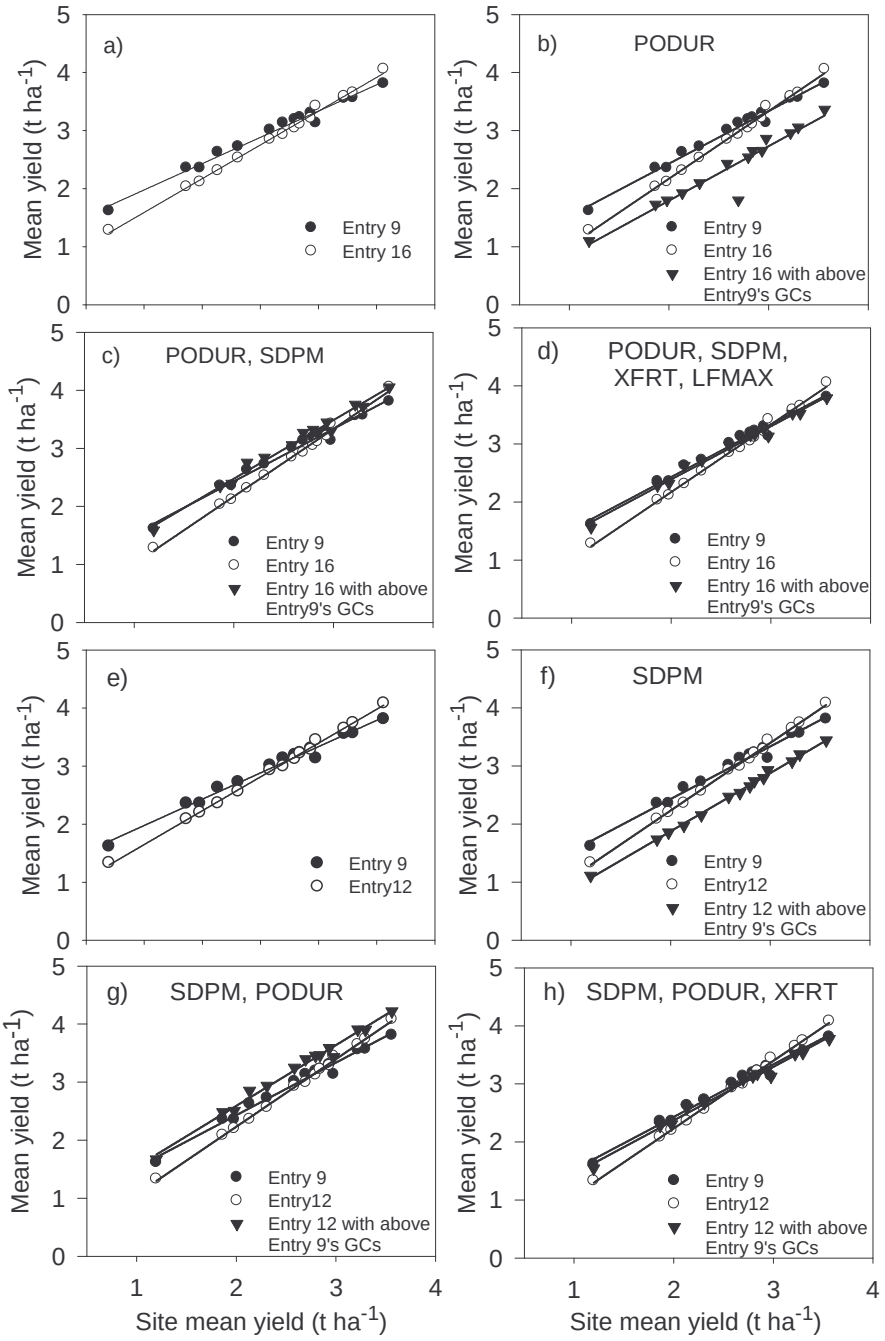

Figure 2. The crossover G x L interaction and the results after the specified cultivar coefficients of the low yielding line in each pair were adjusted to those of the corresponding high yielding line.

Model sensitivity analysis to determine the effect of environmental factors indicated that an increasing value for extractable soil water (ESW) and solar radiation increased both mean yield and yield response of a location across a range of genotypic performances (b-value), while increasing rainfall increased only mean yield, but did not affect the b-value. On the contrary, increasing temperature resulted in a decline of both mean yield and b-value (Figure 3). The changes of both mean yield and b-value for the increments of extractable soil water (ESW) and temperature were relatively large,

while the changes for the varying values of rainfall and solar radiation were rather small. The combinations of environmental factors that affected yield of location across peanut genotypes were different among pairs of locations, e.g., being a combination of soil, temperature and solar radiation for one pair, but being a combination of soil, rainfall and temperature for another pair, and being a combination of soil and temperature for yet another pair. Similarly, the combinations of environmental factors accounting for G x L interaction were different in individual pairs of locations (Figure 4). Our analyses showed that the environmental factors that contributed to yield differences across locations and to G x L interaction depended on the gap of individual environmental factors between locations. Information gained in this study could be useful in selecting locations for use in METs.

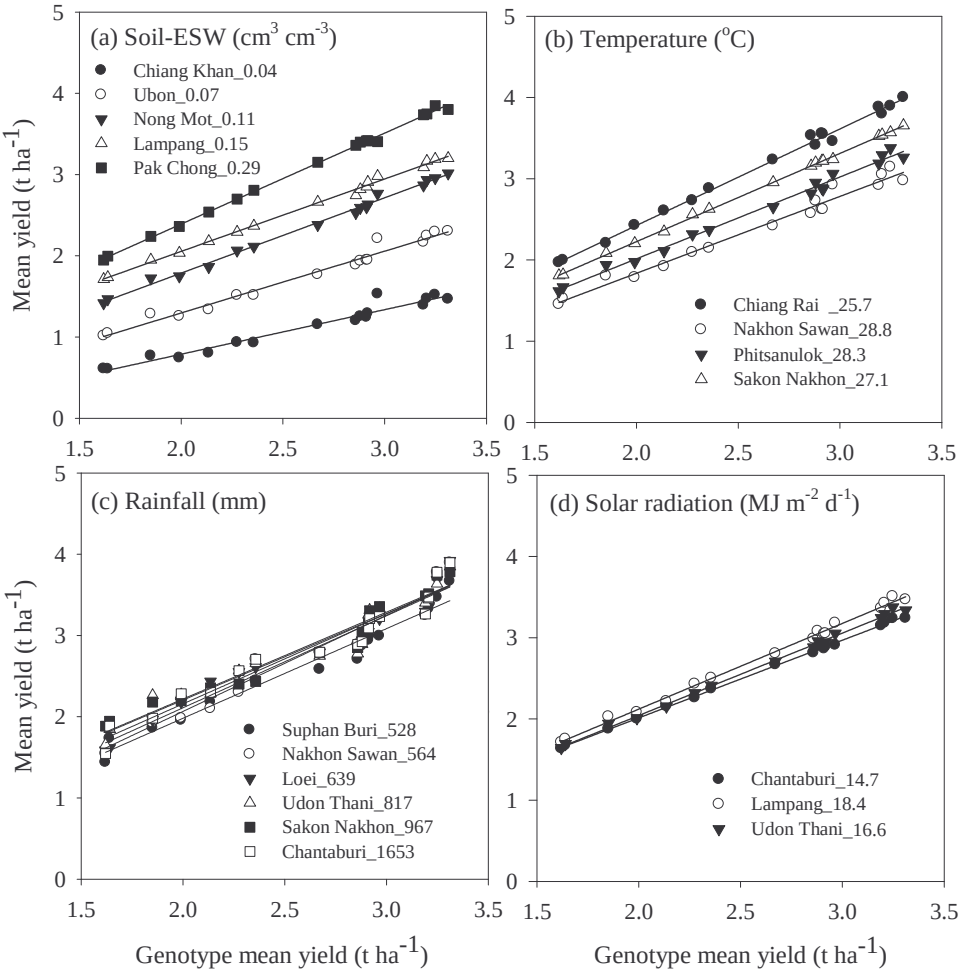

Figure 3. Effect of soil (a), temperature (b), rainfall (c) and solar radiation (d) on yield response of location to varying genotypic performances, shown for the Khon Kaen location except where an environmental factor varied. Each line indicates mean yields over three seasons and 30 years of the 17 peanut genotypes at a particular set of environmental factors. X-axis represents mean yields of the individual peanut genotypes over all 14 locations, three seasons and 30 years (genotype mean yield).

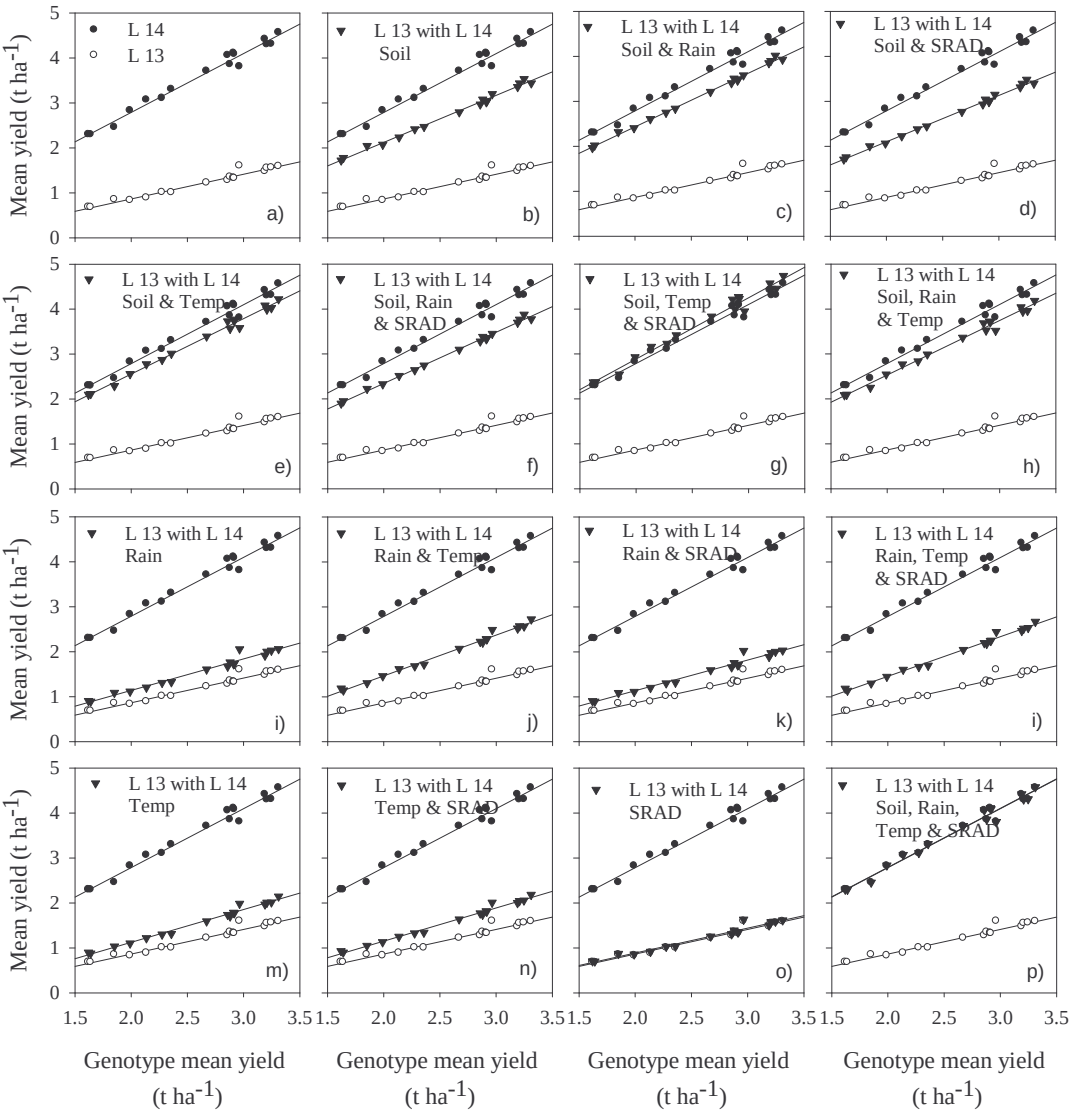

Figure 4. Changes in yield response to different genotypic performances of Location 13 (L 13) with changing environmental factors to Location 14 (L 14) (where there was initially non-crossover G x L interaction).

Conclusions

This study demonstrated that model sensitivity analysis can be used as a breeding tool to study the causes of G x E interaction. The plant traits that affect both the differences in yield between peanut genotypes and the G x L interaction for pod yield are LFMAX, XFRT, SDPM, SFDUR, and PODUR. Model sensitivity analysis can be used to hypothesize yield improvement likelihoods of a given peanut genotype for a peanut production environment such as Thailand based on improving single or multiple combinations of plant traits. The results from this study also showed that it is not only one environmental factor that causes G x L interaction between locations, but it is a combination of several environmental factors. Extractable soil water was the major factor influencing both yield and G x L interaction, followed by temperature and amount of rainfall. These findings are useful in determining appropriate breeding

strategies, especially for selection of test sites for the multi-environment trials in a target population of environments. The approach potentially could also be used for other crops and for other target environments.

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Manuscripts under preparation

C. Putto, A. Patanothai*, S. Jogloy, K. Pannangpetch, K.J. Boote and G. Hoogenboom. Determination of plant traits that affect G x E interaction in peanut using the CSM-CROPGRO-Peanut model.

C. Putto, A. Patanothai*, S. Jogloy, K. Pannangpetch, K.J. Boote and G. Hoogenboom. Determination of environmental factors causing G x E interaction in peanut using the CSM-CROPGRO-Peanut model.

4.5 Designing peanut ideotype for Thailand using the CROPGRO-Peanut model

B. Suriharn, A. Patanothai , K. J. Boote, and G. Hoogenboom

Breeding for higher yield through empirical selection for yield *per se* though has been successful in the past, but further progress is becoming increasingly difficult. Great effort has gone to the identification of traits which breeders might select for in order to increase yield indirectly. Although the concept of plant ideotype has long been proposed (Donald, 1968), it has never been used directly (Rasmusson, 1991; Sedgley, 1991). This was because the desired trait or combinations of traits have to be actually incorporated into a genotype to be able to evaluate their effects on yield performance (Mashall, 1991). Crop simulation models offer an opportunity to evaluate the effects on yield of a trait or a combination of traits by model simulation without actual incorporation of these traits into a genotype. With simulation models, effect of traits could be assessed in sensitivity analyses where the coefficients determining traits are varied, and the effects on simulated growth or yield observed (White, 1998).

In peanut, a process-oriented Cropping System Model (CSM) CROPGRO-Peanut has also been developed and used for various applications (Hoogenboom et al., 1994; Jones et al. 2003). Thus, it should be possible to use this model for evaluating the effects of various traits on peanut yield and for designing a peanut ideotype for a specific environment. Boote and Jone (1988) successfully used the PNUTGRO model, an earlier version of the CSM-CROPGRO-Peanut model, in comparing the effects of 16 parameters on peanut yield under rainfed conditions over 21 years in Florida. However, the use of this model in designing a peanut ideotype for a specific area has not yet been explored. The objective of this study was to design a large-seeded and a small-seeded peanut ideotypes with high yield potential for environmental conditions of Thailand using the CSM-CROPGRO-Peanut model.

Materials and Methods

The sensitivity analysis using the CSM-CROPGRO-Peanut model was employed in designing and evaluating the peanut ideotype. The CSM-CROPGRO-Peanut model uses 15 cultivar coefficients that define the growth and development characteristics or traits of a peanut cultivar (Hoogenboom et al., 1994). These include the life cycle parameters, the vegetative growth parameters and the reproductive growth parameters. The life cycle parameters consist of the critical daylength for photoperiod (CSDL), the sensitivity to photoperiod (PPSEN), and the number of photothermal days from emergence to flowering (EMFL), from first flowering to first pod (FLSH), from first flowering to first seed (FLSD), from first flowering to end of leaf expansion (FLLF) and from first seed to physiological maturity (SDPM) (life cycle phase). The vegetative growth parameters include the maximum leaf photosynthetic rate (LFMAX), specific leaf area (SLAVR), and the maximum size of a full leaf (SIZELF) (vegetative traits). The reproductive growth parameters include the maximum fraction of daily growth that is allocated to seed and shell (XFRT), individual seed size (WTPSD), seed filling duration for an individual pod cohort (SFDUR), average seed number per pod (SDPDV) and the photothermal time required for a cultivar to reach final pod load (PODUR) (reproductive traits) (Hoogenboom et al., 1994).

The sensitivity analysis was performed by first defining the target environment and management practices and selecting the initial values of the cultivar coefficients to

be used for model simulation, then changing the value of a particular genetic coefficient defining a particular trait within a certain limit for consecutive runs of model simulation over the target environment for three seasons and 30 years (1972-2001). The resulting simulated pod yield, seed yield and final biomass were compared to determine the yield responses to changing values of that particular trait parameter. The value that resulted in the highest average pod yield or seed yield or biomass over a specified number of seasons and years in the target area was selected to represent the best value for that particular trait parameter. Sensitivity analysis was conducted sequentially by first adjusting the duration of the developmental stages to obtain the maximum pod and seed yields, then adjusting the growth parameters to obtain the maximum biomass, and finally adjusting the partitioning parameters to obtain the maximum pod and seed yields. The final outcome was a set of values for the trait parameters that characterize the peanut ideotype for the target environment. The designed ideotype was then evaluated for yield improvement compared to the initial peanut cultivar.

Results

The first step in the sensitivity analyses for the ideotype design was adjusting the flowering date while keeping the crop duration as desired. The results indicated that shortening the days to flowering (EMFL) increased biomass as well as pod and seed yields for the large-seeded peanut line. For the small-seeded line, however, shortening the days to flowering from 16.5 to 15.0 days decreased biomass to some extent with little effect on pod and seed yields, but further shortening of EMFL resulted in a continuous increase in all the three parameters. The value for EMFL selected was 11.5 PDs with SDPM being 58.5 PDs for both the L-line and the S-line, as this value gave the maximum average pod and seed yields.

The next step in the sensitivity analysis was the adjustment of growth parameters to obtain the maximum biomass. The growth parameters adjusted were time between first flower and the end of leaf expansion (FLLF), size of full leaf (SIZLF), specific leaf area (SLAVR) and the maximum leaf photosynthesis rate (LFMAX). Results of the sensitivity analysis indicated that increasing FLLF gave an increase in biomass as well as pod and seed yields. The FLLF value of 90.0 PDs was chosen to be the value for the modified genotypes at this step. Increasing SIZLF increased biomass and pod and seed yields, and the SIZLF value of 40.0 cm^2 was chosen. Increasing SLAVR also resulted in an increase in biomass as well as pod and seed yields, and the value for SLAVR selected was 300 cm^2 g^{-1} for both peanut lines. Increasing maximum leaf photosynthesis rate (LFMAX) increased biomass, pod yield and seed yield, and the LFMAX value of 2.00 mg CO_2 m^{-2} s^{-1} was chosen to be the value for the modified genotypes at this step.

The parameters further adjusted were maximum fraction of daily growth that is partitioned to seed and shell (XFRT), time required to reach final pod load (PODUR) and seed filling duration for a pod cohort (SFDUR). From the sensitivity analysis, increasing XFRT gave an increase in pod and seed yields but a decrease in final biomass and the XFRT value of 1.0 was selected for both the L- and the S-modified genotypes. Increasing PODUR increased pod and seed yields only at low PODUR values but gave a yield decline at higher PODUR values. The PODUR of 19.0 and 18.0 PDs were selected for the L- and the S-modified genotypes, respectively. Increasing seed filling duration (SFDUR) gave an increase in pod and seed yield but a decline in

biomass. The selected SFDUR values were 32.0 and 34.0 PDs for the L- and the S-modified genotypes, respectively. The modified genotypes at the end of this step are the designed ideotypes.

Substantial yield improvements over the corresponding original lines were shown for both the L- and the S-ideotypes and in all seasons (Table 1). For the L-ideotype, improvements in the three seasons ranged from 13.3-18.8 % for pod yield, 11.0-18.0 % for seed yield and 7.1-11.1 % for biomass. Improvements obtained for the S-ideotype were considerably higher, being 25.7-29.0 % for pod yield, 28.4-32.0 % for seed yield and 6.1-11.1 % for biomass. Averaging over the three seasons, pod and seed yields of the L-ideotype were 4,019 kg ha^{-1} and 2,885 kg ha^{-1}, respectively, compared to 2,939 kg ha^{-1} and 2,180 kg ha^{-1} for the original L-line, representing an increase of 16.4 and 15.1%. The S-ideotype gave the average pod and seed yields of 3,558 kg ha^{-1} and 2,726 kg ha^{-1}, respectively, compared to 2,020 kg ha^{-1} and 1,456 kg ha^{-1}, respectively, for the original S-line, representing an increase of 27.0% and 29.7 %. Apparently, the designed peanut ideotypes were much more effective in reproductive growth than the original lines.

Table 1. Means over ten locations and 30 years for pod yield, seed yield and biomass of the original lines and the designed ideotypes in the early-rainy, the mid-rainy and the dry seasons, and percentages of yield improvement.

Category	Early-rainy season	Mid-rainy season	Dry season	Mean
Pod yield				
L † (kg ha^{-1})	2,049	2,016	4,753	2,939
L-ideotype (kg ha^{-1})	2,900	2,947	6,210	4,019
Improvement (%)	**17.2**	**18.8**	**13.3**	**16.4**
S ‡ (kg ha^{-1})	1,443	1,465	3,153	2,020
S-ideotype (kg ha^{-1})	2,470	2,478	5,726	3,558
Improvement (%)	**26.2**	**25.7**	**29.0**	**27.0**
Seed yield				
L † (kg ha^{-1})	1,504	1,495	3,542	2,180
L-ideotype (kg ha^{-1})	2,090	2,149	4,417	2,885
Improvement (%)	**16.3**	**18.0**	**11.0**	**15.1**
S ‡ (kg ha^{-1})	1,045	1,063	2,258	1,456
S-ideotype (kg ha^{-1})	1,878	1,907	4,392	2,726
Improvement (%)	**28.5**	**28.4**	**32.1**	**29.7**
Biomass				
L † (kg ha^{-1})	4,728	4,667	8,617	6,004
L-ideotype (kg ha^{-1})	5,818	5,829	9,940	7,196
Improvement (%)	**10.3**	**11.1**	**7.1**	**9.5**
S ‡ (kg ha^{-1})	4,466	4,356	8,463	5,762
S-ideotype (kg ha^{-1})	5,511	5,474	9,571	6,852
Improvement (%)	**10.5**	**11.4**	**6.1**	**9.3**

† L = ((Nc.Ac.17090 x B1)–25 x China 97-2) F6-2-2.

‡ S = KK 5

Conclusion

The results of this study demonstrated that the CSM-CROPGRO-Peanut model could be used for determining the effects of different traits on performance of the peanut genotype and in designing peanut ideotypes for a target environment. The characteristics of the designed peanut ideotypes are early flowering (11.5 PDs), long time between first flower and end of leaf expansion (90 PDs), large leaf size (40 cm^2) and high SLA (300 cm^2 g^{-1}), high leaf photosynthesis rate (2.0 mg CO_2 m^{-2} s^{-1}), determinate growth habit and high partitioning of assimilates to pods and seeds (high XFRT of 1.00), rapid pod adding period (PODUR of 19.0 PDs and 18.0 PDs for L-ideotype and S-ideotype, respectively), and moderately long seed filling durations (SFDUR; 32.0 and 34.0 PDs for L-ideotype and S-ideotype, respectively). Substantial improvements in yield performance over the original lines were shown for the designed ideotypes. Among all these traits, adjusting LFMAX, XFRT and SFDUR appeared to give the highest impact on yield improvement. While combining all these desirable traits into a single genotype will take a long time, greater attention should be given to selection for these three traits in breeding peanut for higher yield. The approach used in the present study can also be applied to other crops in other areas as well.

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Submitted manuscript

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Sub-Project 5

Utilization of Peanut Stover and Other Crop Residues to Improve Crop Productivity

Dr. Banyong Toomsan

Sub-project Leader

Legumes are widely known through their ability to fix atmospheric nitrogen when they are symbiotically living with bacteria in genera *Allorhizobium, Azorhizobium Rhizobium, Bradyrhizobium, Mesorhizobium and Sinorhizobium* (Giller, 2001). The fixed N can reduce the amount of N fertilizer requirement of the hosts and thus help conserve soil nitrogen (sparing effect). The sparing N and residue N (when ploughed under) can benefit the succeeding crops and lowering their N fertilizer requirements. Inclusion of legumes in cropping systems is, thus, desirable for long-term sustainability of land productivity.

The amount of N_2 fixed by legumes varied widely ranging from 34 to 897 kg N ha^{-1} $year^{-1}$ depending on types of legumes (FAO, 1984), cultivars of legumes (Giller *et al.*, 1987; McDonagh *et al.*, 1995; Kucey and Toomsan, 1988; Toomsan *et al.*, 1995), rhizobia strains (Kucey *et al.*, 1988) and environments (Giller, 2001; Sprent and Sprent, 1990). Not all legumes are soil fertility (nitrogen) builder. This depends on the amount of N removed in economic yield (% nitrogen harvest index, % NHI) and the amount of nitrogen fixed (% nitrogen derived from air, % Ndfa). A grain legume can be a soil fertility building crop only when its % NHI is lower than its % Ndfa, providing that its stover be returned to the soil. In this context, peanut is a soil fertility building crop because the amount of N removed in economic yield (%NHI) is less than the amount of fixed nitrogen (%Ndfa) (McDonagh *et al.*, 1993; McDonagh *et al.*, 1995; Toomsan *et al.*, 1995). Peanut stover yields of 3-6 tons ha^{-1} were reported to contain 60–160 kg N ha^{-1}, and when returned to the soil could increase growth and yield of succeeding crops such as maize (Suwanarit *et al.*, 1986; McDonagh *et al.*, 1993; Toomsan *et al.*, 1993; McDonagh *et al.*, 1995; Toomsan *et al.*, 1995) and cassava (Toomsan *et al.*, 2002).

Management of nutrient release from crop residues to meet crop demands is a major challenge for research. Most of the studies that showed benefit of peanut stover to succeeding crops were conducted either by growing succeeding crops immediately after peanut harvest or by storing peanut stover and applying them just before planting the succeeding crop. The practice of growing succeeding crops immediately after peanut harvest can be done only in areas where rainy season is long. However, in areas where the rainy season is short and only one crop can be grown, the peanut stover must be either left in the field or stored in the shade. Storing peanut stover requires extra labor and space due to its bulkiness. Management of stover during the period from peanut harvesting to planting the succeeding crop in the following year is, therefore, important. What happens to the nutrients in peanut stover when left in the field? Would it still be beneficial to succeeding crop when left in the field for 6-8 months? What is the role of weeds in nutrient conservation during the fallow period? What is the fate of N in peanut residue during the fallow period? How much is it absorbed by the succeeding crop? These are the questions that need to be answered in order to help us utilize peanut residue N effectively

and thus help conserve the environment. Research conducted under this sub-project has been aiming at finding answers to these questions. The cropping systems investigated include peanut in sugarcane system and peanut in paddy system.

Three studies were conducted under this sub-project:

5-1. Effect of peanut and other legumes for sugarcane production under dry season planting system.

5-2. Effect of sugarcane stover management on growth and yield of some grain legumes and their residual N effect on growth and yield of succeeding sugarcane.

5-3. Effect of peanut residues and rice straw management on growth and yield of succeeding rice.

5.1 Effects of peanut and other legumes for sugarcane production under dry period planting system

Srisuda Thippayarugs and Banyong Toomsan

Sugarcane is an important economic crop of Northeast Thailand. In this area, sugarcane is generally grown in October and harvested 14 months later for the cane-planted crop. Generally, one more ratoon crop can be harvested, and harvesting time is in December to April of the following year. After harvesting the ratoon crop, there is a lag period of 4–8 months in rainy season before planting the new sugarcane crop in the end of rainy season. During the lag period, the fields are generally left idle and are vulnerable to erosion. A logical approach is to grow legumes during this period to gain benefit not only from nitrogen fixed by the legumes but also the improvement of soil physical properties from legume residues.

Many studies have shown that the use of green manure legumes can increase the yield of a subsequent field crop and reduce the requirements for inorganic N fertilizer. There are many legumes that can be grown during fallow periods as green legumes. Peanut is one of the few grain legumes that grown successfully in this area because of sandy soil and long dry spell. After harvesting it leaves significant amount of green residues as well as economic yield. Other legumes that can be used for green manure are pigeonpea, sunnhemp, jackbean and leguminous weeds (hairy indigo and *Crotaralia striata*) that grow vigorously in the farmers' fields.

The goal of this study was to investigate the benefit of introducing grain or green manures during the fallow period to improve soil fertility. The objectives were:

1. To study nodulation, N_2 fixation and yield of peanut, pigeon pea, sun hemp, jackbean, indigo and *C. striata* grown after sugarcane.
2. To study the residual N benefits of different legumes on growth and yield of sugarcane under field and glasshouse conditions.
3. To study decomposition and nitrogen release patterns of different legume residues under both field and laboratory conditions.
4. To quantify the amount of N in legume residues taken up by succeeding sugarcane.

Materials and methods

This study consists of three experiments. The first two experiments (Experiment 1: Utilization of residues of peanut and other legumes to partially supply nitrogen to the succeeding sugarcane; Experiment 2: Interactions in decomposition and N mineralization between tropical legume residue components) had already been reported in Phase II. Only the third experiment is presented here.

Experiment 3. Effects of tropical legume residues on N dynamics, CO_2 evolution, sugarcane growth and soil nutrient contents

The residue of leguminous crops including grain legume, peanut (*Arachis hypogaea* L.), common green manure legumes, pigeonpea (*Cajanus cajan* (L.) Millsp.), jackbean (*Canavalia ensiformis* L.), sunnhemp (*Crotalaria juncea* L.) and two local leguminous weeds, *Crotalaria striata* and hairy indigo (*Indigofera hirsuta*) (Harvey) were investigated for their contribution to mineral N dynamic and C emission. Nutrient contents and growth of sugarcane were also investigated in pots using the green manure residues and mineral N application as $(NH_4)_2SO_4$ equivalent to 49 kg ha^{-1}.

The experiment was conducted in 28 L pots with two rates of legume residues. All of the legume residues were added at the rate equivalent to 6.25 Mg ha^{-1} and only peanut, pigeonpea and hairy indigo residues were additionally added at the rate equivalent to 12.50 Mg ha^{-1}. Altogether, there were nine treatments of incorporating legume residues, one treatment with chemical fertilizer N, and a non-treated control. All the treatments were replicated four times and received chemical P_2O_5 and K_2O fertilizers at the rate equivalent to 49 kg ha^{-1}.

Results

Peanut residues had good chemical quality for decomposition and N mineralization with high N concentration but low lignin and polyphenol concentration as well as C:N ratio. At 53 days after mixing the soil with legume residues and chemical fertilizers, mineral N was lower in the pot with peanut residues than those with sunnhemp and chemical N fertilizer. After this period, mineral N was the highest in the treatment with peanut residues equivalent to 12.50 Mg ha^{-1}.

Sugarcane biomass was highest in the pot receiving 12.50 Mg ha^{-1} of peanut residues, but the amount obtained was not significantly different from those pots that received peanut, sunnhemp, or *C. striata* residues at the rate of 6.25 Mg ha^{-1}. On the other hand, yield obtained from peanut residue application at the rate equivalent to 12.50 Mg ha^{-1} was significantly different from those of other treatments including the treatment with chemical N fertilizer (Table 1).

Application of peanut residues resulted in the highest levels of N, K and Mg in the sugarcane plant, while application of the hairy indigo gave the highest P content, and chemical N fertilizer application gave the highest in Ca content. CO_2 emission was significantly higher after adding legume residues than with mineral fertilizer N. The emissions increased with rate of residue incorporation. Pigeonpea residues at 12.50 Mg ha^{-1} showed the highest CO_2 emission.

Table 1. Effects of adding legume residues on biomass production and nutrient contents of sugarcane.

Legume residues	Added legume dry wt. (kg ha^{-1})	Sugarcane biomass DW (g pot^{-1})	N	P	K	Ca	Mg
			(mg pot^{-1})				
Peanut	6.25	296 abc	942 bc	334 abcd	2155 bc	334 bc	452 bc
Pigeonpea	6.25	259 bc	864bc	320 bcd	1787 cd	331 bc	413 bc
Hairy indigo	6.25	258 bc	848 bc	315 cd	1844 cd	310 bc	413 bc
sunnhemp	6.25	292 abc	955 bc	338 abc	1664 cde	362 abc	493 ab
jackbean	6.25	279 bc	899 bc	300 cd	1546 de	337 bc	441 bc
C. striata	6.25	280 abc	829 bc	313 cd	1923 cd	272 c	428 bc
Peanut	12.5	348 a	1340 a	389 ab	2784 a	401 ab	577 a
Pigeonpea	12.5	285 abc	977 bc	369 abc	2066 bcd	296 bc	449 bc
Hairy indigo	12.5	312 ab	1011 b	401 a	2561 ab	250 c	459 abc
Soil+N	0	252 bc	951 bc	275 d	963 f	475 a	432 bc
Soil	0	233 c	722 c	266 d	1172 ef	328 bc	367 c
F-test		**	**	**	**	*	**
CV%		12	15	11	15	23	14

$^{ns, *, **}$ Non-significant and significant at $P < 0.05$ and $P < 0.01$, respectively.

Conclusion

Application of peanut residue at the rate equivalent to 12.50 Mg ha^{-1} gave higher mineral N after 53 days from incorporation to the soil and higher final sugarcane biomass than those of other treatments including chemical N fertilizer. Application of peanut residues resulted in the highest levels of N, K and Mg in the sugarcane plant. However, CO_2 emission was significantly higher after adding legume residues than with mineral fertilizer N.

Published paper

Thippayarugs, S., B. Toomsan, P. Vitayakon, V. Limpinuntana, A. Patanothai and G. Cadisch. 2009. Effects of tropical legume residues on N dynamics, CO_2 evolution, sugarcane growth and soil nutrient contents. Thai J. Soils Fert. (in press). (No impact factor).

5.2 Effect of sugarcane stover management on growth and yield of some grain legumes and their residual N effect on growth and yield of succeeding sugarcane

Saowakon Hemwong and Banyong Toomsan

Burning of sugarcane residues either before or after sugarcane harvest is widely practiced in many tropical countries. However, residue burning may adversely affect soil fertility due to the fact that it causes losses of some nutrients and organic matter over time. Recently, sugarcane producers have been required to adopt alternative sugarcane stover management practices (e.g. stover retention).

Sugarcane is usually grown under rainfed conditions. In northeast of Thailand only one or two ratoon crops may be harvested due to poor fertility of the very sandy soils. Here, sugarcane is generally planted during October to December and harvested in December to April of the following year. Thus, there is a time gap of 6-8 months between the last ratoon crop harvest and the next sugarcane planting. Farmers are now being encouraged not to burn sugarcane residues and to grow green manure legumes instead during this fallow period. However, green manure legumes do not generate an economic return and are not generally accepted by small farmers who have limited resources. Grain legumes such as peanut and soybean are other alternatives because they can provide cash income as well as improve soil fertility.

This study hypothesizes that mulching and incorporation of high C:N ratio sugarcane residues instead of burning can play a significant role in improving soil fertility, growth and yield and N_2 fixation of peanut and soybean during the fallow phase between two sugarcane cycles. These, in turn could contribute to increases in yield and N use efficiency of a following sugarcane crop when peanut and soybean residues are subsequently recycled.

The objectives of this study were:

1. To assess the effects of burning, mulching and incorporation of sugarcane stover on soil N dynamics and then upon soil preparation (ploughing), its residual effect on N_2 fixation, growth and yield of peanut and soybean grown between two sugarcane cycles.
2. To assess the effects of mulching or incorporation of sugarcane stover as alternative to burning on soil N dynamics after sugarcane planting and sugarcane yield.
3. To assess residual nitrogen effect of previous fallow period treatments, i.e. grain legumes vs. natural weed fallow, on N dynamics, growth and yield of succeeding sugarcane crop.
4. To compare the effectiveness of legume residues to mineral N fertilizer on N use efficiency, N recovery (and losses) from the system (^{15}N balance), soil fertility and sugarcane yield.

Material and methods

The experiment consisted of three phases: the sugarcane stover management phase, the legume crop phase and the sugarcane crop phase.

The sugarcane stover management phase included burning, mulching and incorporating sugarcane stover. In this phase, the experiment was conducted using a randomized complete block design with 4 replicates. In the legume crop phase, the experiment was a split-plot in RCBD with 4 replications. The main-plot treatments were 3 sugarcane stover management and the sub-plot treatments included of 2 grain legumes, i.e., peanut and soybean, and a fallow (control) treatment (Table 1). The sugarcane crop phases consisted of two periods, i.e., from planting to 6 months and from 6 months to harvesting. For the first period, the experiment conducted was a split plot in RCBD, with the main-plot treatments being 3 sugarcane stover management methods and the sub-plot treatments being 2 grain legumes and a fallow plot. For the secondary period, the experiment was a split-split-split plot in RCBD. The main-plot treatments were 3 sugarcane stover management methods, the sub-plot treatments were 2 grain legumes and a fallow, and the sub-sub plot treatments were with and without N chemical fertilizer.

Table 1. Treatments in the experiment during the legume crop phase.

Treatment	Main-plot	Sub-plot
1	Burned	Soybean
2	Burned	Peanut
3	Burned	Fallow
4	Mulched	Soybean
5	Mulched	Peanut
6	Mulched	Fallow
7	Incorporated	Soybean
8	Incorporated	Peanut
9	Incorporated	Fallow

After sugarcane harvest, 9.38 t DW ha^{-1} of sugarcane stover was applied to the field during February 19-20, 2004. Weeds were allowed to grow during sugarcane stover management (dry season), according to the treatments. They were harvested, weighed and incorporated into the soil before maize, groundnut and soybean planting. Samples were taken to determine dry weight and nitrogen contents.

Following the sugarcane stover management phase, the field was ploughed and seedbed prepared for the subsequent legume phase. Peanut cultivar Khon Kaen 60-1 and soybean cultivar SJ. 5 were planted during July 12, 2004 to October 2004. Peanut and soybean were sampled to assess nodulation and growth at 45 and 60 DAP.

After legume harvest, the field was ploughed and seedbed prepared for the subsequent sugarcane phase. The sugarcane variety UT 1 was planted in 8 rows, 6 m long, 1.2 m apart each of 9.6 x 6 m^2. For the experiment during the period from sugarcane planting to 6 months (early November 2004 to May 2005), the main-plot treatments were the three previous sugarcane stover management treatments (burned, mulched and incorporated), and the sub-plot treatments were the previous soybean, peanut, and fallow plots. At the beginning of the sugarcane phase, the two fallow plots were divided into

fallow without mineral N and fallow with mineral N fertilizer (47 kg N ha^{-1} as $NH_4(SO_4)_2$). Legume treatments did not receive mineral N in order to test if they can substitute the commonly used basal mineral N fertilizer application at sugarcane planting. At the time of planting, phosphorus (P) at 21 kg P ha^{-1} and potassium (K) at 39 kg K ha^{-1} were applied to all plots as recommended for sugarcane.

At 6 months after sugarcane planting (May 2005), groundnut and soybean treatment plots were divided in half (4 rows, 6 m long) and half of plots received a topdressing of 47 kg N ha^{-1} as $NH_4(SO_4)_2$. Phosphorus (P) at 21 kg P ha^{-1} and potassium (K) at 39 kg K ha^{-1} were again applied to all plots as recommended for sugarcane. Total stalk numbers and height of sugarcane in each plot were recorded at 6, 8, 10, 12 and final harvesting (14 months after planting). Also, percentage of N and SPAD reading (Minolta SPAD-502 meter) of fully expanded functional leaves of sugarcane (4^{th} from the apex) were collected at 9 and 10 months after planting. Leaf, stem and tiller dry weights were determined at 6 months after planting and taken 4 hills $plot^{-1}$. The plants were cut at the ground level; tops were taken to determine dry weight and N, P, K and Ca contents. The final harvest was done on January 9-11, 2006. Sugarcane plant was separated into cane stem, dead leaves, top leaves and tiller to determined dry weight and N, P, K and Ca contents. Also, ten stalk samples were taken for determination of the commercial content of sugar (CCS) value. Soils were sampled during and after sugarcane stover application, legume crop and sugarcane crop phases to determine mineral N and microbial biomass N.

Results

Increased soil microbial biomass N was observed when sugarcane stover was incorporated compared to burning or surface application at 14 days after stover management initiation. Thereafter, high N mineralization associated with stover incorporation was accompanied by a reduction in microbial biomass N, indicating that mineralized N was derived from microbial N turnover. However, upon ploughing after 96 days the different previous sugarcane stover management strategies had no significant ($P>0.05$) effect on net mineral N content and microbial biomass N during the subsequent legume period (Figure 1).

Although ^{15}N enrichment in control reference plants and plant N uptake indicated significant N immobilization effects due to stover retention persisting into the legume crop phase, the proportion of N derived from N_2 fixation (% Ndfa) or amount of N_2 fixed were not significantly different between sugarcane stover management treatments (Table 2). Soybean fixed more N_2 (78 %Ndfa, 234 kg N fixed ha^{-1}) than peanut (67 % Ndfa, 170 kg N fixed ha^{-1}) probably due to its larger N demand and a poorer utilization of soil N (64 vs. 85 kg N ha^{-1}). Peanut led to a positive soil N balance while that of soybean was negative due to its high nitrogen harvest index. Legume residues returned 61 and 146 kg N ha^{-1} to the soil for soybean and peanut respectively, compared to only 34–39 kg N ha^{-1} by fallow weeds (Table 2). At the end of the legume phase, sugarcane stover retention improved soil organic matter and N content.

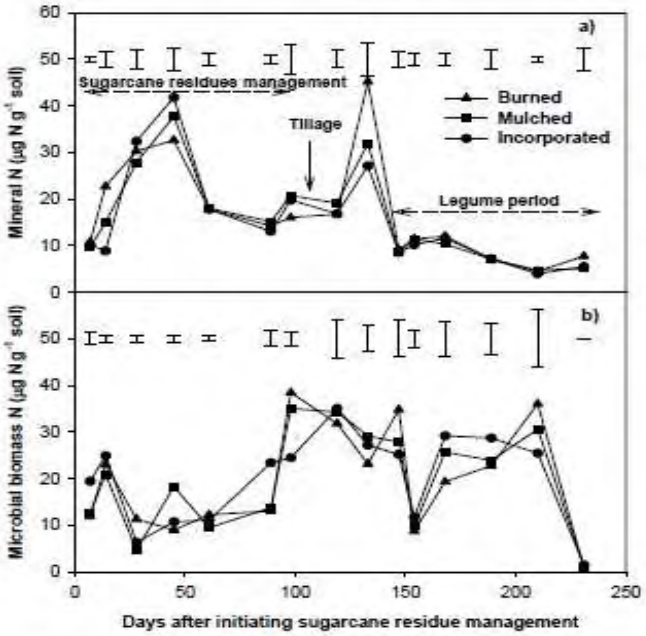

Figure 1. Pattern of mineral N (a) and microbial biomass N (b) dynamics under different sugarcane residue managements at 0-15 cm depth during sugarcane residue management period and legume period (average of legume/fallow treatments). Vertical bars represent LSD.

Table 2. Percentage atom^{15}N excess, N derived from air (%Ndfa),total soil N uptake, N balance and Net N benefit from N_2 fixation by soybean and groundnut as affected by sugarcane stover management at final harvest

Sugarcane residues management /Legume	%^{15}N excess	%Ndfa	Total N_2 fixed	Total soil N uptake[a]	N balance[b]	Net N benefit from N_2 fixation[c]
			(kg N ha^{-1})			
Burned	0.1128	69	178	67	81	13
Mulched	0.0902	78	223	55	83	28
Incorporated	0.1025	71	204	69	84	15
LSD_{05}	0.0138*	13^{ns}	50^{ns}	22^{ns}	10^{ns}	20^{ns}
C.V. (%)	13	14	20	34	11	104
Soybean	0.0432	78	234	64	61	-3
Groundnut	0.0659	67	170	85	146	60
Non-nod groundnut	0.1964	na	na	na	na	na
Fallow	na	na	na	41	41	0[d]
LSD_{05}	0.0304***	4***	29***	15***	15***	16***
C.V. (%)	35	6	15	27	20	97

na = not applicable

[a] = Total N – Total N_2 fixed, [b] = Total N – N removed, [c] = Total N_2 fixed – Seed N, [d] = assuming no major inputs from legume weeds.

LSD = Least Significant Difference. *** = significantly different at P<0.001, * = significantly different at P< 0.05 and ns = not significantly different (P>0.05)

The results suggested that although a change from burning to sugarcane stover retention led to alterations in N cycling and improved soil organic matter, it did not significantly affect N_2 fixation due to the uniform action of ploughing and the extended time gap between stover incorporation and legume planting.

Higher dry matter yield and N uptake were observed under stover burning than other mulching and incorporation treatments at 6 months after planting. However, the millable cane and sugar yield were positively affected by sugarcane residue mulching and incorporation (Table 2) suggesting remobilization of previously immobilized N during the later stages of plant development. Legumes had a positive residual effect on sugarcane yield compared to the fallow-N treatment. However, the two legumes induced a different response patterns due to their inherent different quality characteristics and the different amounts of residue N recycled. Both legume residues treatments resulted in higher tiller number and dry matter yield than the fallow–N and even the fallow+N treatments. However, the soybean treatment also improved subsequently sugarcane performance due to the extended later decomposition. This delayed decomposition led to a better conservation of soybean N in the system as less N losses, presumably due to leaching, and this was confirmed by the larger proportional residue ^{15}N recovery in the system.

Table 2. Effect of sugarcane stover management, plant residue and N fertilizer during the previous fallow phase on yield of sugarcane at final harvest (14 months after sugarcane planting).

Treatment	Stalk number ha^{-1}	Tiller number ha^{-1}	Millable cane (t ha^{-1})	Stalk DW (t ha^{-1})	Total DW (t ha^{-1})	Sugar yield (t ha^{-1})
Sugarcane stover management method						
Burned	7726	967	112	34	42	16
Mulched	8888	1127	125	39	48	18
	8666	1463	118	35	44	18
Incorporated						
SED	355*	41**	3*	1**	1*	0.6*
C.V. (%)	15	12	10	10	13	12
Plant residue						
Soybean	8111	1326	116	36	44	17
Peanut	8914	1379	127	37	47	19
Fallow	8255	852	112	35	44	17
SED	235*	58**	3**	0.8^{ns}	1^{ns}	0.5**
C.V. (%)	10	17	9	9	11	10
N fertilizer						
+ N	8553	1259	123	37	46	18
- N	8300	1112	114	35	44	17
SED	244^{ns}	42**	2*	0.8*	1^{ns}	0.5**
C.V. (%)	12	15	10	10	15	12

SED = standard error of the differences between means.
$^{ns, *, **}$ non-significant and significant at $P<0.05$ and $P< 0.01$, respectively.

The lower response of soybean compared to peanut can be explained by the lower amount of N recycled by soybean. Sugarcane dry matter yields under legume residues treatments were not different from that in the N fertilizer application treatment during the 6 months after planting. However, sugarcane yields under N fertilizer application treatment were greater than the non-N fertilizer treatment at final harvest. This suggests that legume residue N was available and enough to get good sugarcane growth until 6 months after planting, but thereafter it was not enough for sugarcane need until final harvest. ^{15}N recovery data indicated that sugarcane could absorbed more N from the N fertilizer application treatment than the residue retention treatments at both 6 months after planting and final harvest (Table 3). This might be due to N in the fertilizer treatment was readily available to the plant after application while those in plant residues need to be decomposed before becoming available.

Table 3. ^{15}N recoveries of labeled soybean and groundnut residues or mineral N fertilizer in sugarcane and associated weed plants as well as ^{15}N recoveries in soil (0-100 cm) at 6 and 14 months after sugarcane planting in relation to sugarcane stover management (average crop systems) and plant residue/fertilizer type (average sugarcane stover management) .

Treatment	6 months				14 months			
	Total recovery		Losses		Total recovery		Losses	
	%	kg N ha^{-1}	%	kg N ha^{-1}	%	kg N ha^{-1}	%	kg N ha^{-1}
Burned	58	46	42	38	56	44	44	56
Mulched	65	51	35	34	61	49	39	51
Incorporated	60	50	40	35	49	39	51	61
SED	6^{ns}	5^{ns}	6^{ns}	5^{ns}	6^{ns}	4^{ns}	6^{ns}	4^{ns}
C.V. (%)	25	26	39	36	25	24	31	19
Soybean	83	50	17	11	85	52	15	9
Groundnut	50	73	50	73	43	63	57	83
Fallow +N	50	22	50	23	37	17	63	75
SED	6^{***}	5^{***}	6^{***}	5^{***}	4^{***}	3^{***}	4^{***}	3^{***}
C.V. (%)	22	24	35	33	17	14	21	11

SED = standard error of the differences between means. *** = significantly different at $P<0.001$, **= significantly different at $P<0.01$, *= significantly different at $P< 0.05$ and ns=not significantly different ($P>0.05$). (Losses = N applied minus total recovery).

Conclusion

Soil microbial biomass N increased when sugarcane residues were incorporated instead of burned or surface applied at 14 days after initiation of cane residue management. Thereafter, high net N mineralization was accompanied by a reduction in microbial biomass N, indicating that mineralized N was derived from microbial N turnover. However, upon ploughing after 96 days the different previous sugarcane residue management strategies had no significant effect on net mineral N and microbial biomass N during the subsequent legume period. Sugarcane residue retention improved soil organic carbon and N content. The results suggested that although a change from burning to sugarcane residues retention led to alterations in N cycling and improved soil organic matter it did not significantly affect N_2 fixation due to the uniform action of ploughing and the extended time gap between sugarcane residue incorporation and legume planting.

The millable cane and sugar yield were positively affected by sugarcane residue mulching and incorporation compared to burning suggesting microbial remobilization of previously immobilized N. Residual effects of legumes increased sugarcane tillering and yield compared to the fallow treatment without N fertilizer. Soybean residues of higher C:N ratio and lignin content compared to groundnut residues decomposed slower and improved N synchrony with cane N demand. This led to a better conservation of residue N in the system with proportionally less ^{15}N losses compared to the large losses from groundnut residues or from mineral N fertilizer. ^{15}N recoveries in soil were larger from residues than from fertilizer at final harvest. Recycled legume residues were able to substitute basal fertilizer N application but not topdressing after 6 months.

Published papers

Hemwong, S., G. Cadisch, B. Toomsan, V. Limpinuntana, P. Vityakon and A. Patanothai. 2008. Dynamics of residue decomposition and N_2 fixation of grain legumes upon sugarcane stover retention instead of burning. Soil and Tillage Res. 99: 84-97.

Hemwong, S., B. Toomsan, G Cadisch, V. Limpinuntana, P. Vityakon and A. Patanothai. 2009. Sugarcane residue management and grain legume crop effects on N dynamics, N losses and growth of sugarcane. Nutrient Cycling in Agroecosyst. 83: 135-151.

5.3 Effects of peanut residues and rice straw management on growth and yield of succeeding rice

Wanwipa Kaewpradit and Banyong Toomsan

Groundnut as a pre-rice crop is usually harvested 1-2 months before rice transplanting during which much of legume residue N released could be lost and might not be beneficial to succeeding rice crop. Studies were, therefore, conducted to search the potential groundnut management that might be suitable to actual farmer practice during the time gap. Base on high- and low-quality organic matter mixing concept, rice straw was mixed with groundnut residues in different proportions and applied to the field immediately after harvest with the aims to investigate

(i) the effect of mixing groundnut residues and rice straw on N release and greenhouse gas emission under field conditions

(ii) its residual effect on growth, nutrient uptake dynamics, yield of succeeding rice and N lost (^{15}N balance) from the plant-soil system as well as fate of residue N in soil fractions.

(iii) the impact of addition of groundnut residues-rice straw mixtures and weed management on changes in soil N dynamics, methane emission and growth and yield of succeeding rice.

(iv) the effects of mixing groundnut residue with rice straw on N dynamics (gross N mineralization, N immobilization and net mineralization), microbial activities (carbon dioxide, nitrous oxide emissions, invertase and xylanase) and ergosterol content.

Material and methods

This research consisted of three experiments i.e. (1) a field experiment during June to December 2004, (2) a pot experiment during July to December 2005 and (3) an incubation experiment during December 2006 to April 2007.

The first experiment, a field experiment, was conducted in a farmer's field at Kalasin province, Thailand. There were 6 treatments: (i) control (no residues), (ii) NPK (at recommended rate, 38 kg N ha^{-1}), (iii) groundnut residues 5 Mg ha^{-1} (120 kg N ha^{-1}), (iv) rice straw 5 Mg ha^{-1} (25 kg N ha^{-1}) (v) 1:0.5 mixed (groundnut residues 5 Mg : rice straw 2.5 Mg ha^{-1}), and (vi) 1:1 mixed (groundnut residues 5 Mg : rice straw 5 Mg ha^{-1}). The objectives were (1) to investigate the effect of mixing groundnut residues (GN, 5 Mg ha^{-1}) with rice straw (RS) in different proportions (GN:RS, i.e. 1:0, 1:0.5, 1:1, 0:1) on a) regulating N dynamics, b) potential microbial interactions during decomposition, and c) associated nitrous oxide (N_2O) and methane (CH_4) emissions at weekly intervals during the lag phase until rice transplanting (a, b) or harvest (c). (2) Its residual effect on succeeding KDML 105 rice was investigated and compared with chemical fertilizer (at recommended rate, 38 kg N ha^{-1}) on a) growth and yield of succeeding rice, b) groundnut residue N use efficiency and c) N unaccounted for (^{15}N balance) from the plant-soil system and fate of residue N in soil fractions.

The second experiment was a pot trial conducted at Khon Kaen University. There were 7 treatments in the study i.e. i) no residue application plus weed control (-R-W); ii) no residue application plus no weed control (-R+W); iii) groundnut residues 5 Mg ha^{-1} plus weed control (GN-W); iv) groundnut residues 5 Mg ha^{-1} plus no weed control (GN+W); v) groundnut residues 5 Mg ha^{-1} and rice straw 5 Mg ha^{-1} plus weed control (Mixed-W); vi) groundnut residues 5 Mg ha^{-1} and rice straw 5 Mg ha^{-1} plus no weed control (Mixed+W) and vii) no residue application plus no weed control and NPK application at recommended rate to rice crop after planting (-R+W+NPK). The objectives of this study were to assess the impact of addition of groundnut residues-rice straw mixtures and weed management on: a) change in soil N dynamics, b) methane emission and c) growth and yield of succeeding rice

The third experiment was an incubation experiment conducted under laboratory condition at Institute of Soil Science, Department of Soil Biology and Institute of Plant Production and Agroecology in the Tropics and Subtropics (380a), University of Hohenheim Stuttgart, Germany. It consisted of 2 sub-experiments run in parallel i.e. microbiota activity and ^{15}N gross mineralization. There were five treatments in the microbiota activity experiment i.e. (i) control, (ii) GN (groundnut stover 5g kg^{-1} soil), (iii) RS (rice straw 5g kg^{-1} soil), (iv) 1:1 (GN 5g kg^{-1} soil : RS 5g kg^{-1} soil) and (v) 1:4 (GN 5g kg^{-1} soil: RS 20g kg^{-1} soil) while there were three treatments in ^{15}N gross mineralization i.e. (i) GN (groundnut stover 5g kg^{-1} soil), (ii) RS (rice straw 5g kg^{-1} soil) (iii) 1:1 (GN 5g kg^{-1} soil : RS 5g kg^{-1} soil). The objectives of this experiment were (i) to study carbon dioxide and nitrous oxide emissions from groundnut stover alone and mixing groundnut stover with rice straw after incubation, (ii) to study decomposition for understanding the community structure of the microbiota responsible for interaction of groundnut stover alone and mixing groundnut stover with rice straw, (iii) to study the effects of mixing groundnut residue with rice straw on gross N mineralization.

Results

The first experiment

Decomposition (dry weight and N loss) was fastest in groundnut residues (i.e. 64% N lost) with a negative interaction for N loss when mixed 1:1 with rice straw. Adding groundnut residues increased mineral N initially, while added rice straw led to initial microbial N immobilization. Mineral N of mixed residue treatments remained significantly ($P < 0.01$) higher than that of the control and single residue treatments at the beginning of rice transplanting. Soil microbial biomass N and apparent microbial efficiency were higher, while absolute and relative microbial C were lowest, in groundnut and mixing treatments at most sampling dates. Microbial C:N ratio increased with increasing proportion of added rice straw suggesting a change in microbial community or microbial efficiency. N_2O losses were largest ($P < 0.05$) in the groundnut treatment (12.2 mg N_2O-N m^{-2} day^{-1}) in the first week after residue incorporation and reduced by adding rice straw (9.0 mg N_2O-N m^{-2} day^{-1}) (Fig 1). N_2O-N emissions till rice harvest amounted to 0.73 g N_2O-N m^{-2}. CH_4 emissions were largest ($P < 0.01$) in mixed residue treatments (155.9 g CH_4 m^{-2} in the 1:1 (GN:RS) treatment till rice harvest) (Table 1).

Mixing residues resulted in a significant interaction in that observed gaseous losses were greater than predicted from a purely additive effect. The results suggest that it is possible to regulate N dynamics by mixing rice straw with groundnut residues leading to a delayed mineral N release and potentially decreased leaching and N_2O losses during the pre-rice phase, however at a trade-off of increased CH_4 emissions.

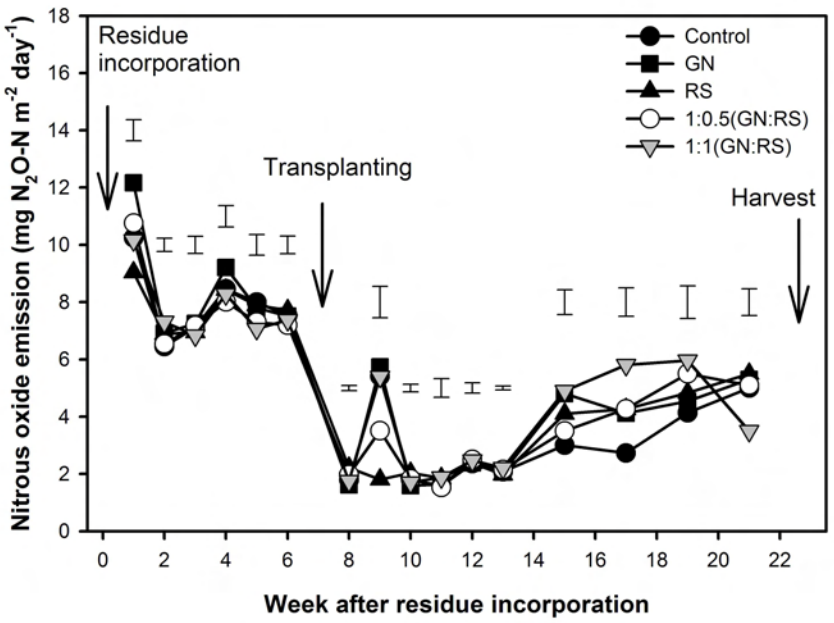

Fig. 1. Nitrous oxide emission rate (mg N_2O-N m^{-2} day^{-1}) patterns as affected by different residue treatments (groundnut (GN), rice straw (RS)) or unamended control from residue incorporation to rice harvest. Vertical bars represent SED.

Table 1. Cumulative (21 weeks) nitrous oxide (N_2O) and methane (CH_4) emissions and their respective global warming potential (N_2O=310, CH_4=21 CO_2 equivalents, IPPC 2001) after residue incorporation until rice harvesting. Values in brackets are for the period 1-6 weeks.

Residue management	Cumulative emissions (21 weeks) N_2O-N /g N_2O-N m^{-2}	Cumulative emissions (21 weeks) CH_4 /g CH_4 m^{-2}	Global warming potential N_2O /g CO_2 equiv. m^{-2}	Global warming potential CH_4 /g CO_2 equiv. m^{-2}
Control	0.66 (0.33)	50.3 (5.9)	322	1056
Groundnut (GN)	0.73 (0.36)	72.6 (27.4)	357	1525
Rice straw (RS)	0.71 (0.33)	97.4 (50.4)	346	2045
1:0.5 (GN:RS)	0.69 (0.33)	131.0 (87.5)	338	2751
1:1 (GN:RS)	0.73 (0.33)	155.9 (108.6)	356	3274
SED	0.023 (0.014)	7.0 (7.1)	11	154
Significance	$P < 0.10$ ($P>0.1$)	$P < 0.01$	$P < 0.10$	$P < 0.01$

After rice transplanting, samples of the lowland rice cultivar KDML 105 were periodically collected to determine growth and nutrient uptake. At final harvest, dry weight, nutrient contents and ^{15}N recovery of labeled groundnut residues were evaluated. Significant effects of residue management treatments on rice growth and nutrient uptake dynamics were observed from 30 days after transplanting onwards. At final harvest, the highest grain yield and N content of rice were obtained in the 1:1 (3.7 Mg ha^{-1}) and 1:0.5 mixed treatments followed by sole groundnut residues > rice straw > NPK > control (2.8 Mg ha^{-1}) treatments. There was a significant relationship between mineral N content in soil before transplanting and rice yield at harvest (Table 2).

Table 2. Yield, N uptake and nutrient efficiency.

Residue treatment	Yield (t ha^{-1})	N uptake (kg N ha^{-1})	IE_N (kg kg^{-1})	RE_N (%)	AE_N (kg kg^{-1})	PEP_N (kg kg^{-1})
Control	2.8 d	39 c	71.0 a	na	na	na
NPK	2.9 cd	45 c	65.1 ab	19 ab	5.1 b	90 b
GN	3.1 b	51 bc	61.2 bc	10 b	2.9 b	26 c
RS	3.0 bc	46 c	65.4 ab	28 a	10.0 a	120 a
1:0.5 (GN:RS)	3.5 a	65 a	55.8 c	20 ab	5.8 b	27 c
1:1 (GN:RS)	3.7 a	60 ab	61.7 bc	15 b	6.5 ab	26 c
SED	0.1**	4**	3.5*	5**	1.7**	2**

SED = Standard error of the differences between means.
*,** Significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively.
Numbers in the same column followed by the same letter are not significantly different at P = 0.05.

Mixed residue treatments had significantly ($P < 0.1$) higher groundnut-derived ^{15}N recoveries (13%) in rice than in the sole groundnut treatment (10%) but they were lower than the recovery of labeled mineral N fertilizer in the NPK treatment (29%) (Table 3). The highest ^{15}N recoveries in all treatments were observed in the topsoil (0-15

cm) where over 80% of ^{15}N was located. Systems N unaccounted for from groundnut residues (32%) were similar to fertilizer N losses (30%), but they were the lowest in the 1:0.5 mixed treatment (15%). The highest $\delta^{13}C$ value occurred in residue treatments and ^{13}C was positively related to N uptake while negatively related to internal N use efficiency. The result from our study demonstrated that mixing groundnut residues and rice straw could delay N release during the pre-rice lag phase leading to an improved synchrony in N demand/supply and increased growth and yield of the succeeding rice and reduced N losses from the soil-plant system.

Table 3. ^{15}N recovery of rice, weed, remaining residue, soil, total and loss

Residue treatment	Rice (%)	Weed (%)	Residues (%)	Total (%)		Loss (%)
				soil	recovery	
NPK	29.1 a	4.3	4.8 c	32.2 a	70.3 b	29.7 a
GN	10.0 b	4.0	22.4 b	31.2 a	67.7 b	32.3 a
1:0.5 (GN:RS)	12.3 b	5.6	27.3 ab	39.5 a	84.7 a	15.3 b
1:1 (GN:RS)	12.7 b	6.7	31.9 a	20.9 b	72.7 b	27.3 a
SED	2.5**	1.3^{ns}	2.2**	5.6*	5.8*	5.8*

SED = Standard error of the differences between means.
^{ns,*,**} Non-significant and significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively.
Numbers in the same column followed by the same letter are not significantly different at P = 0.05.

The second experiment

Mixed-W treatment had the highest mineral N ($P < 0.01$) and followed by GN+W, Mixed+W, GN-W and control treatments at the end of the pre-rice phase (Fig. 2). Peak methane emission rates in all treatments were observed at 2 week after residues incorporation. However, cumulative methane emissions were not significantly different between treatments. Weed management did not have significant effects on rice dry weight, grain yield and nutrient contents at final harvest. The result indicated that single groundnut residues and mixed residue incorporation treatments required different weed management during pre-rice phase. When groundnut residues were solely incorporated to the field, weed should be encouraged to grow in order to conserve more available N at upper soil depth before rice transplanting. However, the effect of weed on available N conservation was not observed in the mixed residue treatments.

The third experiment

The 1:4 mixed residue treatment gave the highest CO_2 emission rate, qCO_2, microbial biomass C, microbial biomass N, enzyme activities (invertase and xylanase) and ergosterol content and then followed by the 1:1 mixed residue treatment. However, the ^{15}N isotope technique indicated that mixing groundnut residue and rice straw at 1:1 proportion could delay N release as shown by lower net mineralization rate than the sole groundnut residue treatment at 14 DAI and had a tendency to do so at 56 DAI (Table 4). Thus, mixing groundnut residues and rice straw management not only could increase microbial activities but also mineralization-immobilization turnover.

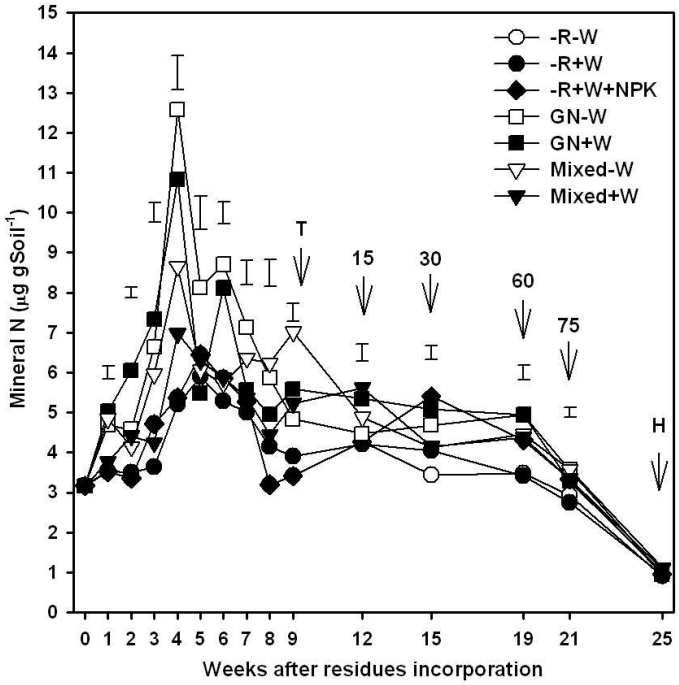

Fig. 2. Mineral N dynamics as affected by different residue treatments (groundnut (GN), rice straw (RS)) or unamended control in 0–15 cm soil depth.

T = rice transplanting. 15, 30, 60, 75 = sampling date at 15, 30, 60, 75 DAT respectively and H = harvest. Vertical bars represent SED.

Table 4. Gross N mineralization, immobilization and net mineralization rates of different treatments at different sampling dates.

Incubation time (days)	Treatments	Gross mineralization rate		Gross immobilization rate (µg gsoil^{-1} day^{-1})	Net mineralization rate	
3	RS	6.83	ab	10.75	-3.93	
	GN	4.35	b	3.90	0.45	
	1:1(RS: GN)	10.45	a	7.37	3.08	
	SED	2.0*		3.2^{ns}	2.9^{ns}	
14	RS	1.39	b	2.06	-0.67	b
	GN	8.51	a	1.12	7.40	a
	1:1(RS: GN)	4.40	ab	1.41	2.99	ab
	SED	1.9**		0.5^{ns}	2.0**	
56	RS	0.36		0.98	-0.62	
	GN	2.22		0.90	1.32	
	1:1(RS: GN)	2.79		1.52	1.27	
	SED	1.4^{ns}		0.3^{ns}	1.2^{ns}	

Treatment results in a column followed by a common letter are not significantly different according to Duncan Multiple Range Test with $\alpha = 0.01$.
** = significantly different at $P \leq 0.05$, * = significantly different at $P \leq 0.1$ and
^{ns} = not significantly different ($P > 0.1$).

Conclusion

Adding groundnut residues increased mineral N initially, while added rice straw led to initial microbial N immobilization. Soil microbial N and apparent efficiency were higher, while absolute and relative microbial C were often lowest in groundnut and mixed treatments. Microbial C:N ratio increased with increasing proportion of added rice straw. N_2O losses were largest in the groundnut treatment in the first week after residue incorporation and reduced by adding rice straw. N_2O-N emissions till rice harvest amounted to 0.73 g N_2O-N m^{-2} in the groundnut treatment. CH_4 emissions were largest in mixed treatments. Mixing residues resulted in a significant interaction in that observed gaseous losses were greater than predicted from a purely additive effect. It appears possible to regulate N dynamics by mixing rice straw with groundnut residues; however, at a trade-off of increased CH_4 emissions.

Significant effects of residue management treatments on rice growth and nutrient uptake dynamics were observed from 30 days after transplanting onwards. At final harvest, the highest grain yield and N content of rice were obtained in the 1:1 and 1:0.5 mixed treatments. There was a significant relationship between mineral N content in soil before transplanting and rice yield at harvest. Mixed residue treatments had significantly higher groundnut-derived ^{15}N recoveries in rice than in the sole groundnut treatment but they were lower than the recovery of labeled mineral N fertilizer in the NPK treatment. The highest ^{15}N recoveries in all treatments were observed in the topsoil (0–15 cm). The highest $\delta^{13}C$ value occurred in residue treatments and ^{13}C was positively related to N uptake while negatively related to internal N use efficiency. The result from our study demonstrated that mixing groundnut residues and rice straw could delay N release during the pre-rice lag phase leading to an improved synchrony in N demand/supply and increased growth and yield of the succeeding rice and reduced N losses from the soil–plant system.

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Sub-Project 6:
Varietal Improvement of Kaentawan (Jerusalem Artichoke) for High Inulin

Associate Professor Dr. Sanun Jogloy
Sub-Project Leader

Kaentawan or jerusalem artichoke (*Helianthus tuberosus* L.) originates in the North America continent (Cosgrove et al., 1991). It has been currently grown in many countries including the United States, Canada, France, Italy, Germany, Russia, China, India, Australia and Thailand. Inulin in tuber of kaentawan is the most important harvestable yield accounting for 75-80% of tuber dry matter. Unlike other tuber crops that normally have starch-containing tubers, kaentawan stores carbohydrate in tubers in the form of inulin and its derivatives - fructo-oligosacharide (Suzuki, 1993). Inulin is used in various ways and is high in economic value. It is a fructan that contains a long chain of fructose molecules flanked at each end of the chain by a molecule of glucose. Inulin is a dietary fiber (Orafti, 2005) that is not soluble in digestive system, but it is a carbon source for useful bacteria such as lactobacillus and bifidobacteria in colon. The bacteria can synthesize vitamin B and compete well with harmful bacteria such as Coliform, Salmonella, *E. coli* and Clostridium. Inulin is well recognized as a prebiotic food, and, as it has low caloric value, can be consumed safely without increasing blood sugar and obesity. Inulin is also suitable for patients with diabetes mellitus, high blood pressure and coronary artery disorders as it can reduce serum triglycerides, total cholesterol, LDL and VLDL. Moreover, inulin can increase immunity and reduce the risk for colorectal cancer (Farnworth, 1993). As it has various useful properties, inulin is good for health and can reduce several health problems.

Inulin and its chemical derivatives are also useful for animal health and help reduce bad smell and ammonia in facets and droppings of swine, cattle and poultry. The use of inulin to replace antibiotics in animal feed can reduce the import of antibiotic and prebiotic and is more environmental friendly for animal industry.

As a carbohydrate-producing crop, kaentawan is also used as a raw material for renewable energy. Fernandez (2006) found that ethanol yield of 80-100 l could be obtained from 1,000 kg fresh weight of kaentawan tubers. In yield trials conducted in Thailand, tuber yield of 19-25 tons ha^{-1} could be obtained from the crop grown for less than 120 days. The short duration makes kaentawan suitable for many cropping systems in Thailand. The crop can be grown in three growing seasons, i.e., early-rainy season from May to August, late-rainy season from August to November with partial irrigation, and dry season with irrigation from January to May.

Kaentawan flowers in the fields are more attractive than stand alone flowers. It looks like mini-sunflowers or Bua Tong flowers in the North. Kaentawan fields can be important tourist attractions and also important for bee keeping industry.

Special properties and versatility of inulin and its derivatives from kaentawan arouse the interest of private entrepreneurs to enter into kaentawan business by producing and marketing value-added products from kaentawan for human consumption and livestock industry. Most of the products are currently imported from abroad. As kaentawan can serve a wide range of functions and uses from human food to animal feed

and from the kitchen to gas station, the production systems and downstream industry should be established in Thailand to produce raw materials and value-added products from kaentawan to reduce imports and increase exports.

To establish kaentawan as a multi-purpose crop in Thailand, various research and promotion activities have been carried out at Khon Kaen University. The ongoing research comprises of varietal improvement, production, animal feed and ethanol production. Future plans have been set up for molecular characterization of germplasm, product development for kaentawan powder and pure inulin and dietary fiber. Promotion activities that had been done included tourism promotion, demonstration of preparation food from kaentawan by food expert (Muek Daeng), production training and publicity. Kaentawan field festival has been held at Khon Kaen university in May 2007, and the provincial governor presided over the festival. Photo contest was the highlight of the festival, enjoying about 5,000 participants. These activities are also planned to be held in other tourist attraction provinces in the country. Food introduction was another activity that was highly welcome by consumers. Currently, kaentawan is well known in Khon Kaen and nearby provinces as a vegetable for good health. Kaentawan Biotech Company Limited has been established to do business on kaentawan in Thailand. Kaentawan fresh tubers are now available in leading supermarkets in Bangkok. Just search for “kaentawan” (Thai font) in Google, the information on kaentawan will appear. It is now available in more than 1,000 websites in the internet.

Suitability varieties are an important component for the success of kaentawan production in Thailand. Several varieties have been introduced and tested for productivity. Although yield potential is favorable, the varieties used still have drawbacks such as susceptible to stem rot disease, long crop duration, slow growth rate at early growth stages, and more importantly low inulin content. Therefore, varietal improvement for high inulin content in kaentawan is of high priority.

The development of high inulin varieties requires broad genetic base of germplasm through the introduction of genetic materials and testing them for the traits under improvement to identify the best parents for developing segregating populations for further selection. Molecular markers associated with high inulin content might facilitate the effective selection for high inulin and speed up the progress in breeding. These were investigated under this sub-project.

This sub-project consists of four studies:

Study 6-1. Genotype by environment (G x E) interactions for yield components of Jerusalem artichoke

Study 6-2. Diversity and characterization of Jerusalem artichoke using agronomic and morphological characters

Study 6-3 Genetic analysis of kaentawan germplasm using standardized molecular marker arrays

Study 6-4. Development of an appropriate extraction and a simple analytical method for determination of inulin from kaentawan

The details of each study are presented below.

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6.1 Genotype by environment (G x E) interactions for yield components of Jerusalem artichoke

W. Pimsaen, S. Jogloy, B. Suriharn, V. Pensuk and A. Patanothai

Jerusalem artichoke (*Helianthus tuberosus* L.) is native to North America and its tubers are rich in inulin (Cosgrove et al., 2000), a natural soluble fiber that is useful for health (Monti *et al*.,2003). Although the crop is well adapted to temperate regions, it can be grown successfully for commercial production under growing conditions of Thailand. As a newly-introduced crop in Thailand, information on the responses of different varieties to varying environments is not available. It is still not known which varieties have wide adaptation, which varieties have specific adaptation to niche environments and what are niche environments for specific genotypes. Such information is important for breeding and varietal recommendations.

Objective

The objective of this study was to evaluate the effects of genotype, environment and genotype x environment interaction on tuber fresh weight, tuber number plant^{-1} and weight of individual tubers of Jerusalem artichoke grown under rainfed conditions with supplemental irrigation in Northeast Thailand.

Materials and Methods

Fifteen Jerusalem artichoke genotypes (JA 37, JA 38, JA 67, JA 89, HEL 53, JA 102, HEL335, HEL 231, HEL 69, HEL 61, HEL 65, HEL 68, HEL 66, CN 52867 and KKU Ac 001) were evaluated in 9 environments in Northeast Thailand that included seven environments in Khon Kaen, one environment in Udon Thani and one environment in Chaiyaphum. The environments covered all possible growing seasons, i.e., early-rainy season, late-rainy season and dry season. The experiment was laid out in a randomized complete block design with four replications. Plot size was 2.5 x 5 m with spacing of 50 cm between rows and 50 cm between plants within row.

Data were recorded on tuber fresh weight, tuber number plant^{-1} and weight of individual tubers (tuber size). Analysis of variance was first performed for each

environment, followed by a combined analysis of variance to determine genotype and environment main effects and genotype x environment interactions (Gomez and Gomez, 1984). Differences among the tested factors were determined by LSDs. Where genotype x environment effects were significant, stability parameters were calculated, using the method described by Eberhart and Russell (1966). Standard errors associated with stability parameters were also calculated.

Results

The environmental differences contributed to the large portion of variations in tuber fresh weight, tuber number and tuber size, whereas genotypic differences accounted for a smaller portion to variations in tuber fresh weight, tuber number and tuber size (Table 1). The contributions of genotype x environment interactions to the differences in tuber fresh weight, tuber number and tuber size were significant for all characters but were much smaller than those caused by genotypic differences. The significant G x E interactions indicated that multi-environment trials are necessary to determine superior genotypes.

Table 1. Mean squares for tuber fresh weight, tuber number plant^{-1} and weight of individual tubers of 15 Jerusalem artichoke clones evaluated at 9 environments during 2005-2008.

Source	DF	Tuber fresh weight	Tuber number plant^{-1}	Weight of individual tubers
Environment (E)	8	2686.5**	2537.0**	152868**
Rep/E	27	36.2	100.3	1621
Genotypes (G)	14	165.9**	1642.0**	15529**
G x E	112	55.9**	151.2**	3930**
Pooled error	378	13.7	58.4	1052
CV (%)		23.7	31.4	33.2

** significant at 0.01 probability level

The three top yielding varieties across environments were JA 89, CN 52867 and JA 37 with yields of 18.9, 18.8 and 18.0 t ha^{-1}, respectively (Table 2). The best genotypes for tuber number plant^{-1} were JA 37, CN 52867 and JA 89 with tuber numbers plant^{-1} of 36, 35 and 34, respectively whereas HEL 65, JA 102 and HEL 53 were the best performers for tuber size with tuber weight of 124, 119 and 114 g, respectively.

Based on tuber yield and stability parameter for tuber yield, JA 37 and JA 89 are more promising for possible release for growing in a wide range of environments because of their high and stable yield (Table 3). There were 10 cultivars showing stability for tuber number. These included JA 38, JA 67, HEL 53, JA 102, HEL 335, HEL 231, HEL 61, HEL 65, HEL 66 and KKU Ac 001. However, tuber numbers of JA 38, JA 67 and KKU Ac 001 were more fluctuated. For tuber size, there were seven cultivars having stability coefficients that were not different from 1 (JA 37, JA 67, JA 89, HEL 231, HEL 65, HEL 66 and CN 52867) but HEL 65 was more fluctuated because the standard deviation associated with the stability coefficient was higher than 0.

Table 2. Tuber fresh weight (t ha^{-1}), tuber number plant^{-1} and weight of individual tubers (g) of 15 Jerusalem artichoke clones grown in 9 environments during 2005-2008.

Genotype	Tuber fresh weight (t ha $^{-1}$)	Tuber number plant^{-1}	Weight of individual tubers (g)
JA 37	18.0	36	69
JA 38	15.3	33	62
JA 67	10.5	22	62
JA 89	18.9	34	84
HEL 53	17.0	21	114
JA 102	15.4	19	119
HEL 335	14.2	20	96
HEL 231	17.4	20	112
HLE 69	15.8	25	95
HEL 61	15.7	21	107
HEL 65	16.7	19	124
HEL 68	16.1	24	96
HEL 66	15.6	20	105
CN 52867	18.8	35	79
KKU Ac 001	15.6	18	117
Mean	16.1	24	96
LSD	2.57	5.0	21.0

Table 3. Estimates of stability parameters for tuber fresh weight, tuber number plant^{-1} and Weight of individual tubers of 15 Jerusalem artichoke clones evaluated at 9 environments during 2005-2008.

Genotype	Tuber fresh weight (b_i)		Tuber number plant^{-1} (b_i)		Weight of individual tubers (b_i)	
JA 37	0.83	(0.05)	1.63**	(0.29)**	0.54	(0.03)
JA 38	0.52*	(0.07)	0.98	(0.12)**	0.38**	(0.04)
JA 67	0.47*	(0.03)	0.61	(0.14)*	0.61	(0.04)
JA 89	0.78	(0.06)	1.18**	(0.42)**	0.98	(0.05)
HEL 53	0.66*	(0.08)**	0.80	(0.06)	1.36**	(0.10)**
JA 102	0.96*	(0.05)	0.85	(0.06)	1.47**	(0.06)
HEL 335	0.99	(0.04)	1.07	(0.07)	1.21**	(0.03)
HEL 231	1.11**	(0.05)	0.73	(0.04)	0.71	(0.05)
HLE 69	0.92	(0.05)	1.23**	(0.08)	1.49**	(0.05)
HEL 61	0.90	(0.04)	0.86	(0.05)	1.23**	(0.05)
HEL 65	0.97	(0.08)**	0.88	(0.07)	1.19	(0.10)**
HEL 68	0.86**	(0.05)	1.18**	(0.06)	1.21**	(0.06)
HEL 66	1.24*	(0.04)	0.71	(0.08)	0.92	(0.07)*
CN 52867	0.65*	(0.09)**	1.72**	(0.20)**	0.49	(0.05)
KKU Ac 001	0.55**	(0.05)	0.56	(0.09)*	1.21**	(0.07)*

b_i = regression coefficient, *,** Significantly different from 1.0 at 0.05 and 0.01 level of probability, respectively. Number in parenthesis indicates standard deviation and *, ** indicates significant difference from 0 at 0.05 and 0.01probability levels, respectively

Conclusion

Environment effects contributed to a larger portion of variations in fresh tuber yield, tuber number and tuber size, and genotype x environment interactions were also significant but were much smaller than genotype main effects in their contribution to the

variations of all characters. JA 89 was the most promising cultivar for wide adaptation and high tuber yield, whereas HEL 65 was the most promising cultivar for its bigger tubers and acceptable yield.

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6.2 Diversity and characterization of Jerusalem artichoke using agronomic and morphological characters

R. Puttha, S. Jogloy, P.P. Wangsomnuk, T. Kesmala and A. Patanothai

Jerusalem artichoke is a promising crop for inulin production in the tropical climates, and genetic improvement should increase crop productivity and product quality. Jerusalem artichoke, however, is an under-utilized crop that, so far, has received little interest of researchers for crop improvement. Consequently, information on economically important traits and genetic diversity of this species is lacking. A better understanding on genetic diversity and characterization of germplasm are priority in any breeding program. Morphological characterization of the germplasm can reveal their genetic relationships which will help breeders in select suitable parents for their breeding programs. The objectives of this study were to investigate genetic diversity in 87 accessions of Jerusalem artichoke germplasm from different sources in Europe and America and to characterize these accessions using agro-morphological characters.

Materials and Methods

Eighty seven Jerusalem artichoke accessions were evaluated for yield and morphological traits. Most of them were received from three institutions i.e., the North Central Regional Plant Introduction Station (NCRPIS) of the US, the Leibniz Institute of

Plant Genetics and Crop Plant Research (IPK) of Germany and the Plant Gene Resource of Canada (PGRC). One accession was generated in our breeding program and one accession of unknown origin has long been cultivated in Thailand.

These germplasm accessions were grown in a randomized complete block design with two replications in the late rainy season 2008 and early rainy season 2009 at Khon Kaen University. Each plot consisted of four rows with a spacing of 50 cm between rows and 50 cm between plants. The crop was well managed for optimum growth and yield, including weed and disease control and supplementary irrigation. At harvest, days to maturity, tuber yield, inulin content and others morphological characters were recorded.

The agronomic and morphological data were analyzed statistically for their variations by using computer software MSTAT-C (Bricker, 1989). The relationships among traits were investigated using simple correlation by using STATISTIX8.

Results

Significant differences among accessions were observed for days to harvest, fresh tuber yield, biomass and inulin content (Table 1). Days to harvest ranged from 92 to 104 days; fresh tuber yields varied from 2.3 to 25.1 t ha^{-1}; biomass ranged from 1.4 to 11.9 t ha^{-1}; and inulin contents varied from 53.9 to 75.4 % of dry weight (Table 2).

Table 1. Mean squares for days to harvest, fresh tuber yield, biomass and inulin content of 87 Jerusalem artichoke accessions in late rainy season 2008 and early rainy season 2009.

Source	D.F.	Days to harvest	Fresh tuber yield	Biomass	Inulin content
Environments (E)	1	24150.0 ^{ns}	264.5 ^{ns}	1167.1*	16.6**
Rep/Envi	2	129.9	7.5	1.5	121.0
Accessions (A)	86	55.0**	116.6**	28.1**	96.3**
E x A	86	41.0**	77.7**	13.0**	32.3**
Error	172	1.0	16.7	3.1	10.9

ns = non significant and ** = significant at 1%.

Days to harvest was not associated with fresh tuber yield (r = 0.19^{ns}, Fig. 1a) but significantly associated with biomass (r = 0.49**, Fig. 1b). The relationship between days to harvest and inulin content was negative (r = -0.36**, Fig. 1c). Biomass and fresh tuber yield were well correlated (r = 0.78**, Fig. 1e), but they were not associated with inulin content (Fig. 1d and 1f).

Table 2. Values for the 10 selected accessions, minimum and maximum values and mean ± standard deviation for days to maturity, fresh tuber yield, biomass and inulin content of 87 Jerusalem artichoke accessions in late rainy season 2008 and early rainy season 2009.

Accessions	Characters			
	Days to harvest (days)	Fresh tuber yield (t ha^{-1})	Biomass (t ha^{-1})	Inulin content (%wt)
HEL69	97	15.5	9.3	70.6
CN52867	92	25.1	11.9	68.0
JA37	92	23.1	10.8	70.5
JA92	99	22.5	8.3	57.2
HEL65	99	14.5	8.2	62.0
JA89	99	13.6	9.0	58.5
HEL308	102	13.7	8.7	56.5
JA6	92	10.0	6.0	75.4
JA30	92	4.8	3.2	72.0
HEL62	101	9.6	6.5	55.5
Minimum	92	2.3	1.4	53.9
Maximum	104	25.1	11.9	75.4
Mean	96±3.7	12.0±5.4	6.9±2.6	63.4±4.9

Means were calculate from 87 accessions.

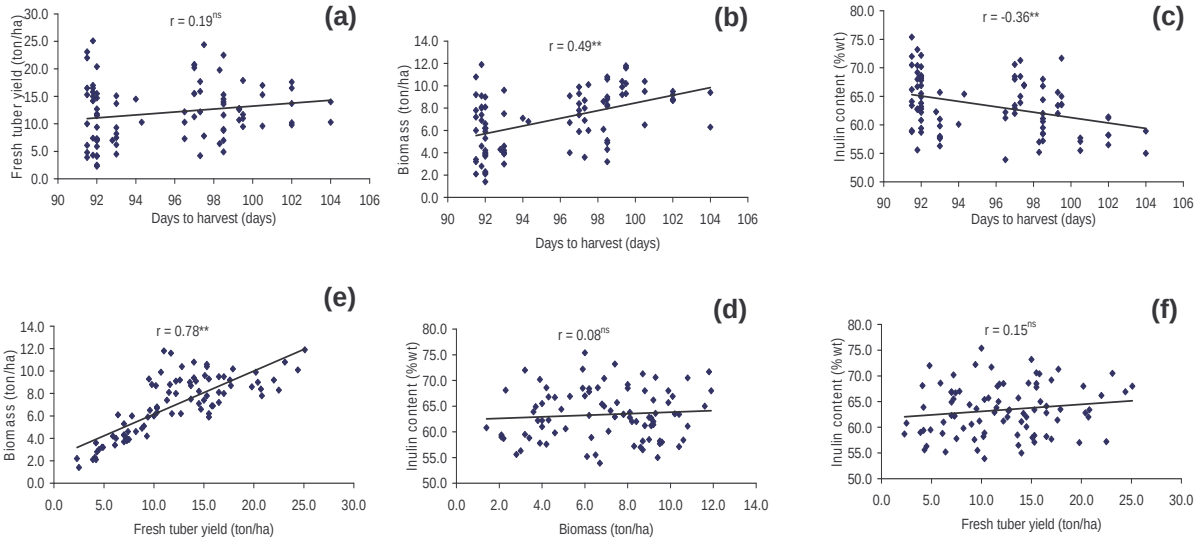

Fig. 1 Correlation between agronomic traits, days to harvest, fresh tuber yield, biomass and inulin content of 87 Jerusalem artichoke accessions.
ns = non significant and *,** = significant at 5 and 1%, respectively.

Conclusion

Jerusalem artichoke germplasm accessions had high variation in maturity, fresh tuber yield, biomass and inulin content, indicating that selection of superior genotypes should be possible among these accessions. Selection for early maturity and high yield should also be possible because of their weak correlations. There was no relationship between fresh tuber yield and inulin content, suggesting that these two traits are segregated independently making possible to select for both high fresh tuber yield and high or low inulin content. CN52867 and JA37 were identified as high yield and high inulin content. Genetic dissimilarity was high and they might be useful for generating breeding populations for further selection. These data would enable breeders to make decisions about suitable parents for their breeding programs.

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6.3 Genetic analysis of kaentawan germplasm using standardized molecular marker arrays

Preeya Puangsomlee Wangsomnuk, Sudarat Khampa and Sanun Jogloy

Phenotypic variation is positively associated with genetic diversity, but is also dependent on environmental factors as well as on the interaction between genotypes and environment. Thus, determining genetic diversity through variation between genotypes, genotype groups, or populations is essential to plant breeding programs. These measures are all important for the identification of genetically distant parental combinations, aiming to use distinct gene sets in crossings for superior hybrids. Determining genetic diversity can be based on agronomic, morphological, biochemical, and molecular types of information, among others. However, molecular markers have advantages over other kinds, where they show genetic differences on a more detailed level and without interferences from environmental factors.

This research was aimed to gain a better understanding of the diversity among Jerusalem artichoke germplasm accessions from three sources using ISSR and RAPD, since the combined results from different markers can provide more reliable information about genetic diversity than those obtained from one marker.

Materials and methods

One hundred and forty seven accessions of *H. tuberosus* obtained from the North Central Regional Plant Introduction Station, Iowa State University, U.S.A, the Gatersleben Genebank Department at the Foundation Leibniz, Institute of Plant Genetics and Crop Plant Research (IPK), Germany, and the Plant Gene Resources of Canada (PGRC), and *Helianthus annus and Tithonia diversifolia* were used in this study.

Total cellular DNA was extracted from young leaves using the methodology described by Wangsomnuk et al. (2006). After DNA extraction, DNA quantity and quality were determined. An initial selection of primers was performed to obtain ISSR and RAPD fragments. Six out of 25 ISSR primers and 13 out of 30 RAPD primers were selected to quantify genetic diversity among *H. tuberosus* accessions. The DNA

quantities to be used in the ISSR and RAPD amplification reactions were standardized, as was the concentration of $MgCl_2$ for the amplifications.

Amplification reactions were carried out in a thermal cycler. DNA denaturation was performed at 95°C for 2 min. This step was followed by 45 amplification cycles. After that, a final extension of 7 min at 72°C was performed. Amplification products were separated on a 1.2% agarose gel. The molecular weight marker used in the gels was 100bp DNA ladder Plus (Vivantis). Gels were stained with 0.5 µg/mL ethidium bromide, and the image was visualized with a High-Performance Ultraviolet Transilluminator.

ISSR and RAPD markers across the 147 accessions were scored for their presence '1' or absence '0' of bands for each primer. By comparing the banding patterns of genotypes for a specific primer, genotype-specific bands were identified and faint or unclear bands were not considered. Pairwise similarity matrices were generated by Jaccard's coefficient of similarity (Jaccard 1908). A dendrogram was constructed by using the unweighted pair group method with arithmetic average (UPGMA) with the SAHN module of PC-ORD 5.1 to show a phonetic representation of genetic relationships.

Results

A total of 141 markers were produced out of which 135 were polymorphic. The polymorphism percentage was 95.74. The number of markers produced by different primers ranged from 7 to 32. The maximum number of amplified product (32) was observed in the ISSR profiles of the primer P818. The Jaccard's similarity coefficient for the ISSR data set varied from 0.617 to 0.957. The level of polymorphism produced by 6 ISSR primers was high. This was proven by the present ISSR study, in which the accessions of *H. tuberosus* were groups into four clusters (Fig. 1). Clusters I and III were highly heterogeneous. Cluster I consisted of *H. tuberosus* accessions from the North Central Regional Plant Introduction Station, Iowa State University U.S.A and the Gatersleben Genebank Department at the Foundation Leibniz, Institute of Plant Genetics and Crop Plant Research (IPK), Germany. Clusters II and VI consisted of most cultivars from the Plant Gene Resources of Canada (PGRC).

A total of 381 RAPD markers were produced out of which 374 were polymorphic. The polymorphism percentage was 98.16. The number of markers from 147 *H. tuberosus* accessions produced by different primers ranged from 20 to 33. The maximum number of amplified product (33) was observed in the RAPD profiles of the primer PPS2. The Jaccard's similarity coefficient for the RAPD data set varied from 0.533 to 0.929. The level of polymorphism produced by 13 RAPD primers was high (Fig. 2). Cluster I was the most heterogeneous, consisting of cultivars from all three sources. Clusters II and VI consisted of only accessions from the Plant Gene Resources of Canada (PGRC), Canada, while Hel 324 and Hel 327 were the only two accessions which were not from this source. *Helianthus annus and Tithonia diversifolia* formed one cluster separated from 147 *H. tuberosus* cultivars using either ISSR or RAPD markers.

Conclusion

ISSR and RAPD analysis indicated the genetic diversity among the 147 accessions of *H. tuberosus* obtained from the three sources. These accessions were groups into four clusters by each marker. It was also clear that some of the genotypes, which were from the same germplasm, were closely related or were duplicates. These need to be eliminated from the core collection.

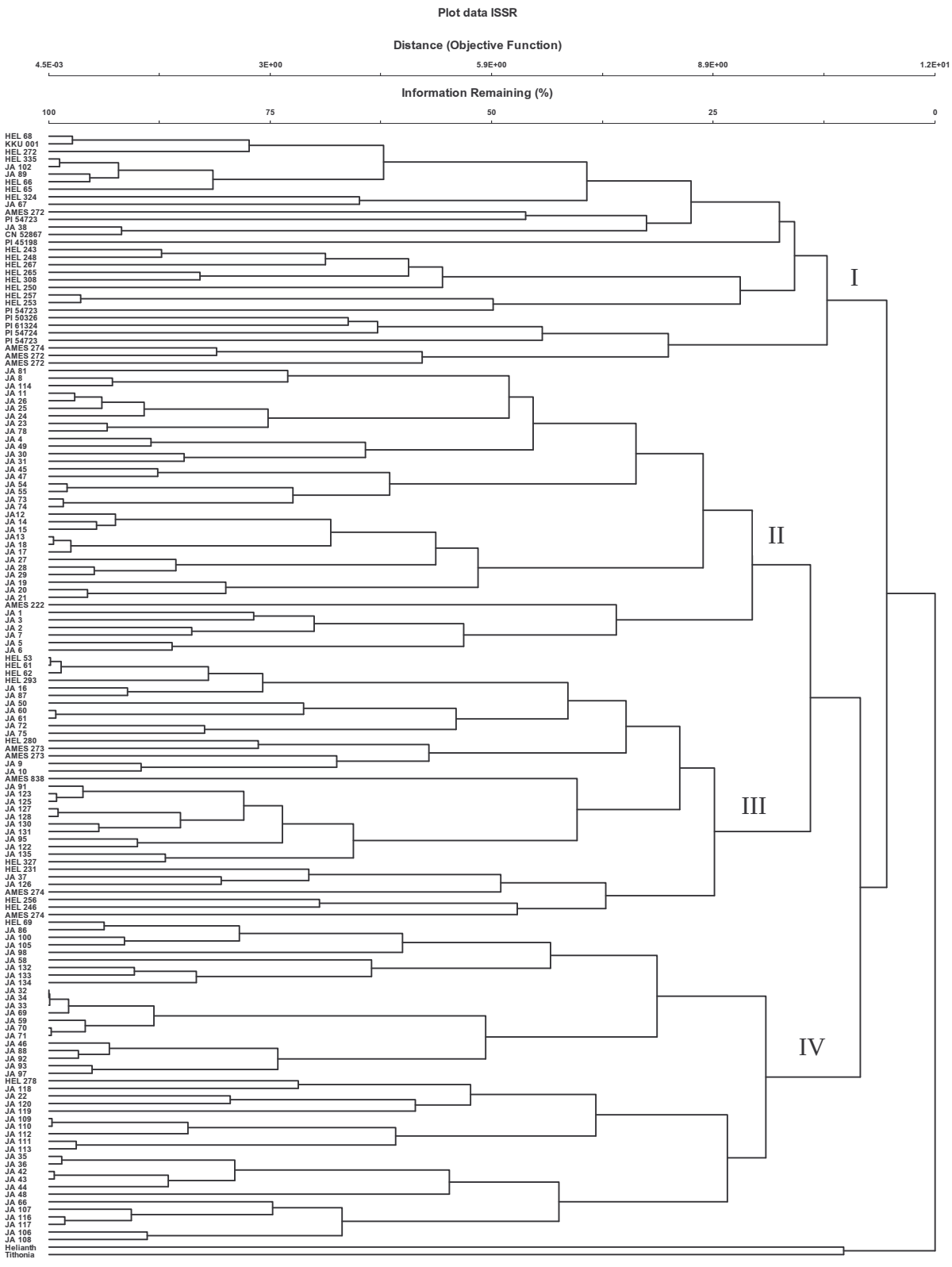

Fig. 1. Dendrogram of 147 *H. tuberosus* accessions based on Jaccard's similarity coefficient for ISSR data.

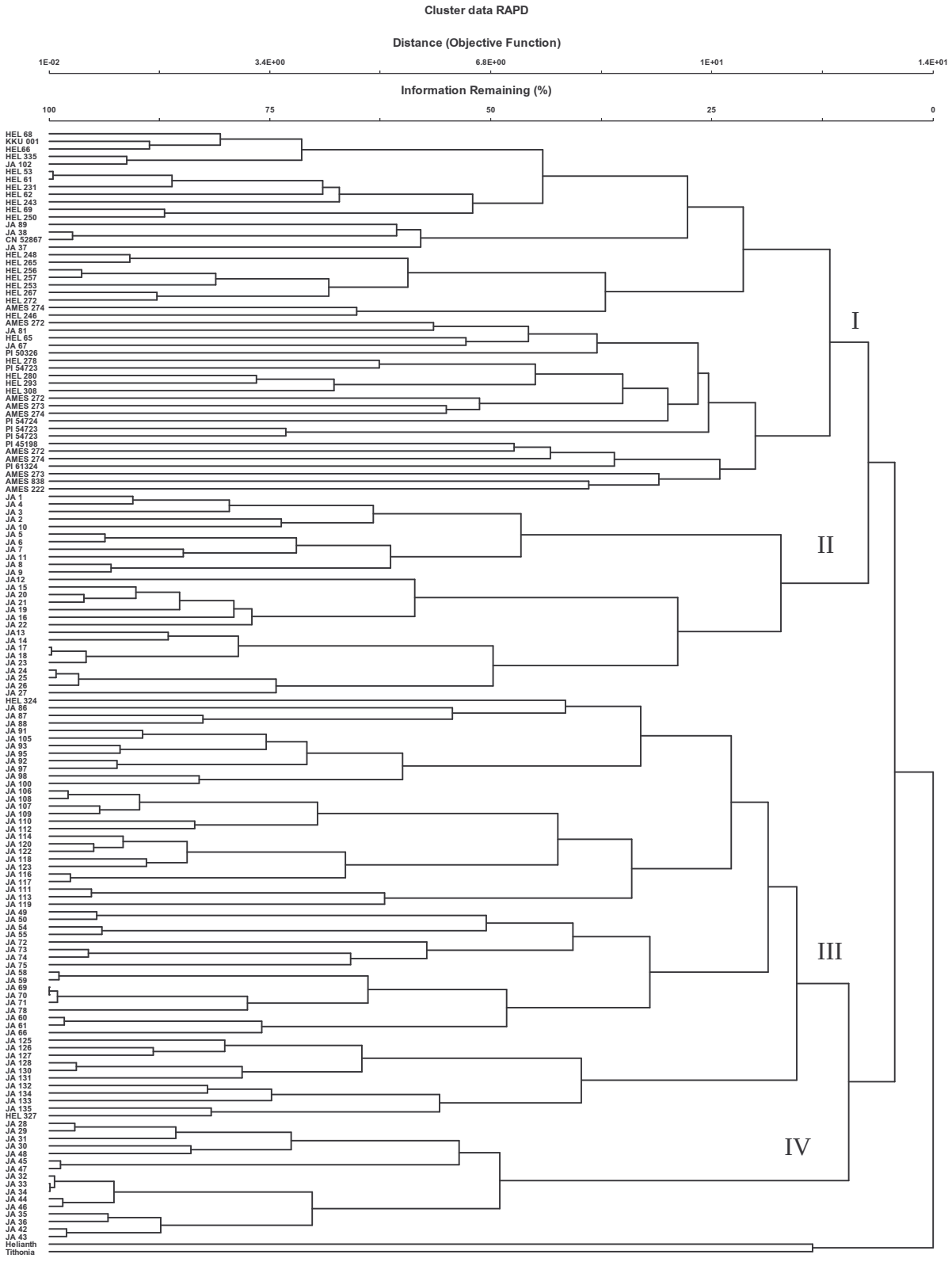

Fig. 2. Dendrogram of 147 *H. tuberosus* accessions based on Jaccard's similarity coefficient for RAPD data**.**

References

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6.4 Development of an appropriate extraction and a simple analytical method for determination of inulin from kaentawan

S. Srijaranai, S. Nuchadomrong, A. Saengkanuk and S. Jogloy

Inulin is the most studied and well-established prebiotic which has beneficial effects on health. It is present in many regularly consumed vegetables, fruits and cereals. A variety of products containing inulin and/or oligofructose formulations are becoming prevalent in the market (Kolida et al., 2002). Jerusalem artichoke (Kaentawan) is considered a new economical vegetable. It has been used in various forms, e.g., as fresh or cooked vegetable for human consumption, as an ingredient to substitute antibiotic in animal feed, as a raw material for sugar and ethanol production, and as a primary source of inulin. Each form of utilization requires different quality of inulin which is determined by its chain length profiles. Time of harvest and length of storage time were reported to affect inulin profile of Jerusalem artichoke tubers (Saengthongpinit and Sajjaanantakul, 2005). Planting season is likely to influence inulin content in the tubers because inulin is characterized as a plant secondary metabolite.

In a Jerusalem artichoke breeding program information on both quality and quantity of inulin in different genotypes are required. Although several techniques are available for the analysis of inulin, most of them are involved with equipments that are complicated and require expert chemists to operate. These techniques are not suitable for screening a large number of genotypes. Thus, the aim of the present work is to develop a simple spectrometric method for the determination of inulin. The proposed method is based on the extraction of inulin from Jerusalem artichoke tubers and the indirect determination of inulin using spectrophotometry. The objectives were to determine the optimum conditions for extraction and spectrophotometry.

Materials and Methods

Spectrophotometric determination of fructose was done by introducing a standard solution of fructose (5 mg/L) into a test tube containing 5.00 mL of citrate buffer (pH 6.0) and 4.65 mL of water. Thereafter 0.10 mL of periodate reagent was added, shaken and left for 5 min. Afterwards, potassium iodide (0.15 mL) was added, shaken and left for 5 min. The absorbance was measured at wavelength 350 nm.

To obtain the optimum condition for the spectrophotometric determination of fructose, the parameters influencing the reaction of fructose and periodate were studied as follows: pH (2-11), reaction time (1-15 min), concentration of sodium periodate (0.050-0.250 mM) and concentration of potassium iodide (1.00-2.75 mM).

Analysis of inulin from Jerusalem artichoke tubers was performed by extracting an accurate weight (2.0 g) of Jerusalem artichoke tuber powder with water at 80 ^{o}C under the pressure of 1500 psi using a pressurized liquid extractor for 20 min. The extract (1.00 mL) was hydrolyzed using 3% HCl at 100^{o}C for 45 min. The hydrolysate (1.00 mL) was placed into a test tube containing 5.00 mL of citrate buffer (pH 6.0) and 4.65 mL of water. Sodium periodate (0.1 mM) 0.10 mL was added, shaken and left for 5 min, potassium iodide (0.15 mL) was then added, shaken and left for 5 min. The absorbance was measured at wavelength 350 nm. The concentration of fructose in sample was evaluated using calibration plot of standard fructose. The content of inulin from Jerusalem artichoke tuber was then deduced from fructose content.

Results

The optimum condition for spectrophotometric detection of fructose is 0.1 mM sodium periodate and 1.5 mM potassium iodide at pH 6.0. The extraction of inulin from Jerusalem artichoke tuber is obtained quantitatively with water at 80^{o}C for 20 min under pressure of 1500 psi. The content of inulin from Jerusalem artichoke tuber was calculated indirectly by the content of fructose. Seven varieties of Jerusalem artichoke were studied. The results are summarized in Table 1. In addition, a conventional anion exchange chromatography was used for comparison. It can be seen from Table 1 that the results obtained from both methods are not significantly different.

Table 1. Inulin content of from Jerusalem artichoke lines determined by spectrophotometric and chromatographic methods.

Lines	Inulin content (% of dry wt.)	
	Spectrophotometric method	Chromatographic method
HEL 61	65.05	64.98
HEL 65	74.51	74.50
HEL 66	65.89	65.51
HEL 69	73.19	75.19
HEL 231	75.43	75.19
HEL 335	64.14	62.77
KKU AC 001	63.34	62.92

Conclusion

A simple spectrometric method for determining inulin content in Jerusalem artichoke tuber was developed. The process involved extracting the tubers using pressurized liquid extraction. The obtained inulin extracts were hydrolyzed with acid. The amount of inulin-derived fructose in the hydrolysates were then spectrophotometric detected using a periodate reaction. The content of inulin was deduced on the basis of the fructose content. The proposed method was found to give more or less the same results as the chromatographic method for the determination of plant inulin after its hydrolysis.

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Bruggink, C., R. Maurer, H. Herrmann, S. Cavalli and F. Hoefler F. 2005. Analysis of carbohydrates by anion exchange chromatography and mass spectrometry. J. Chromatography 1085:104-109.

Carneiro, J.M.T., A.C.B. Dias, E.A.G. Zagatto, J.L.M. Santos and J.L.F.C. Lima. 2005. An improved sampling approach in multi-pumping flow systems applied to the spectrophotometric determination of glucose and fructose in syrups. Anal. Chimica Acta 531:279-284.

Kolida, S., K. Tuohy and G.R. Gibson. 2002. Prebiotic effects of inulin and oligofructose. British J. Nutrit. 87: S193-S197.

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Project outputs

1) Capacity building of researchers

Research team:

Number of researchers: 14

Number of Ph.D. students: 13

Number of M.S. students: 8

Number of research assistants: 3

Improved research capacity of researchers

Researchers in the team have gained their experiences in conducting quality research and in supervising thesis research of graduate students both at the M.S. and Ph.D. levels.

All of the researchers in the team have the capability of seeking their own research funds, and have their own projects in which they are the project leaders. All of them have research projects that received supports from the government budget through their research systems of their organizations, e.g., the university or the Department of Agriculture of the Ministry of Agriculture and Cooperatives. In additions, Dr. Sanun Jogloy also have projects supported by the Thailand Research Fund, the National Research Council and some private enterprises; Dr. Chutipong Akkasaeng and Dr. Napaporn Tantisuwichwong each has a project supported by the National Center for Genetic Engineering and Biotechnology; and Dr. Sanun Jogloy, Dr. Banyong Toomsan and Dr. Chutipong Akkasaeng also are grantees of the RGJ-PhD program of the TRF.

Training of graduate students

Thirteen Ph.D. and 8 M.S. students are trained under the project. Ten of the Ph.D. students have finished their program, and 3 are still ongoing. Seven of the M.S. students have graduated and one is still on going. Names of students and their current status are listed below:

No.	Degree	Name of student	Major advisor	Expected date of completion
1	Ph.D.	Mr. Samran Pimratch	Dr. Aran Patanothai	Graduated
2	Ph.D.	Mr. Jakarat Anothai	Dr. Aran Patanothai	Graduated
3	Ph.D.	Miss Chanakarn Putto	Dr. Aran Patanothai	Graduated
4	Ph.D.	Mr. Patcharin Songsri	Dr. Sanun Jogloy	Graduated
5	Ph.D.	Miss Darunee Puangbut	Dr. Sanun Jogloy	Graduated
6	Ph.D.	Mr. Anuruck Arunyanark	Dr. Sanun Jogloy	Graduated
7	Ph.D.	Mr. Teerayoot Girdthai	Dr. Sanun Jogloy	Graduated
8	Ph.D.	Ms. Srisuda Thippayarags	Dr. Banyong Toomsan	Graduated
9	Ph.D.	Miss Saowakon Hemwong	Dr. Banyong Toomsan	Graduated
10	Ph.D.	Miss Wanwipa Kaewpradit	Dr. Banyong Toomsan	Graduated
11	Ph.D.	Mr. Nuttawut Singkham	Dr. Sanun Jogloy	December 2010
12	Ph.D.	Miss Ratchanee Puttha	Dr. Sanun Jogloy	April 2012
13	Ph.D.	Mr. Nuntawoot Jongrungklang	Dr. Banyong Toomsan	April 2011

No.	Degree	Name of student	Major advisor	Expected date of completion
1	M.S.	Miss Santira Chaiyadee	Dr. Nimitr Voorasut	Graduated
2	M.S.	Mr. Wunna Htoon	Dr. Sanun Jogloy	Graduated
3	M.S.	Mr. Montree Painawadee	Dr. Sanun Jogloy	Graduated
4	M.S.	Miss Wirongrat Pimsaen	Dr. Sanun Jogloy	Graduated
5	M.S.	Mr.Suwutchai Misuna	Dr. Prasan Swatsitang	Graduated
6	M.S.	Miss Sudarat Khampa	Dr. Preeya Wangsomnuk	Graduated
7	M.S.	Miss Araya Saengkanuk	Dr. Supalax Srijaranai	Graduated
8	M.S.	Miss Dolrat Sudlah	Dr. Preeya Wangsomnuk	December 2010

2) Published papers in accredited journals (as of 2 September 2010):

2.1 Number of published or accepted papers: 27.

(1) Arunyanark, A., S. Jogloy, C. Akkasaeng, N. Vorasoot, T. Kesmala, R.C. Nageswara Rao, G.C. Wright and **A. Patanothai**. 2008. Chlorophyll stability is an indicator of drought tolerance in peanut. J. Agron. Crop Sci. 194:113-125. (Impact factor 2009 = 2.283).

(2) Songsri, P., S. Jogloy, N. Vorasoot, C. Akkasaeng, **A. Patanothai** and C.C. Holbrook. 2008. Root distribution of drought resistant peanut genotype in response to drought stress. J. of Agron. Crop Sci. 194:92-103. (Impact factor 2009 = 2.283).

(3) Phakamas, N., **A. Patanothai**, K. Pannangpetch, S. Jogloy and G. Hoogenboom. 2008. Seasonal responses and genotype-by-season interactions for the dynamic growth and development traits of peanut. J. Agric. Sci. 146:1-13. (Impact factor 2009 = 1.658).

(4) Suriharn, B., **A. Patanothai,** K. Pannangpetch, S. Jogloy and G. Hoogenboom. 2008. Yield performance and stability evaluation of peanut breeding lines using the CSM-CROPGRO-Peanut model. Crop Sci. 48:1365-1372. (Impact factor 2009 = 1.735).

(5) Putto, W., **A. Patanothai**, S. Jogloy and G. Hoogenboom. 2008. Determination of mega-environment for peanut breeding using the CSM-CROPGRO-Peanut model. Crop Sci. 48:973-982. (Impact factor 2009 = 1.735).

(6) Hemwong, S., G Cadisch, B. Toomsan, V. Limpinuntana, P. Vityakon and **A. Patanothai**. 2008. Dynamics of residue decomposition and N_2 fixation of grain legumes upon sugarcane stover retention instead of burning. Soil and Tillage Res. 99:84-97. (Impact factor 2009 = 2.883).

(7) Kaewpradit, W., B. Toomsan, G. Cadisch, P. Vitayakon, V. Limpinuntana, P. Sanjun, S. Jogloy and **A. Patanothai**. 2008. Regulating mineral N release by mixing groundnut residues and rice straw under field conditions. Eur. J. Soil Sci. 59:640-652 (Impact factor 2009 = 2.131).

(8) Anothai, J., **A. Patanothai**, S. Jogloy, K. Pannangpetch, K.J. Boote and G. Hoogenboom. 2008. A sequential approach for determining the cultivar coefficients of peanut lines using end-of-season data of crop performance trials. Field Crops Res. 108(2):169-178. (Impact factor 2009 = 2.336).

(9) Pimratch. S., S. Jogloy, N. Vorasoot, B. Toomsan, T. Kesmala, **A. Patanothai** and C.C. Holbrook. 2008. Effect of drought stress on traits related to N2 fixation in eleven peanut (Arachis hypogaea L.) genotypes differing in degrees of resistance to drought. Asian J. Plant Sci. 7(4): 334-342. (ISI, No impact factor).

(10) Songsri P., S. Jogloy, N. Vorasoot, C. Akkasaeng, **A. Patanothai**, and C.C. Holbrook. 2008. Responses to drought on reproductive characters of drought resistant peanut genotypes. Asian J. Plant Sci. 7(5): 427-439. (ISI, no impact factor).

(11) Anothai, J., **A. Patanothai**, S. Jogloy, K. Pannangpetch, K.J. Boote and G. Hoogenboom. 2008. Multi-environment evaluation of peanut lines by model simulation with the cultivar coefficients derived from a reduced set of observed field data. Field Crops Res. 110:111-122. (Impact factor 2009 = 2.336).

(12) Kaewpradit, W., B. Toomsan, G. Cadisch, P. Vitayakon, V. Limpinuntana, P. Sanjun, S. Jogloy and **A. Patanothai**. 2009. Mixing groundnut residues and rice straw to improve rice yield and N use efficiency. Field Crops Res. 110:130-138 (Impact factor 2009 = 2.336).

(13) Phakamas, N., **A. Patanothai**, K. Pannangpetch, S. Jogloy and G. Hoogenboom. 2008. Physiological determinants for pod yield of peanut lines. Crop Sci. 48: 2351-2360. (Impact factor 2009 = 1.735).

(14) Hemwong, S., B. Toomsan, G. Cadisch, V. Limpinuntana, P. Vityakon and **A. Patanothai.** 2009. Sugarcane residue management and grain legume crop effects on N dynamics, N losses and growth of sugarcane. Nutr. Cycl. Agroecosyst. 83:135-151. (Impact factor 2009 = 1.35).

(15) Songsri, P., S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, **A. Patanothai**, and C. C. Holbrook. 2008. Heritability of drought resistance traits and correlation of drought resistance and agronomic traits in peanut. Crop Sci. 48:2245-2253. (Impact factor 2009 = 1.735).

(16) Songsri P., S. Jogloy, N. Vorasoot, C. Akkasaeng, **A. Patanothai**, and C.C. Holbrook. 2009. Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. Agr. Water Manage. 96: 790-798. (Impact factor 2009 = 2.016).

(17) Aranyanark, A., S. Jogloy, N. Vorasoot, C. Akkasaeng,T.Kesmala and **A. Patanothai**. 2009. Stability of relationship between chlorophyll density and SPAD chlorophyll meter reading across different drought stress conditions in peanut. Asian J. Plant Sci. 8:102-110. (ISI, no impact factor).

(18) Gerdthai, T, S.Jogloy, T.Kesmala,N. Vorasoot, C. Akkasaeng, S. WongKaew, C.C. Holbrook, and **A. Patanothai**. 2010. Relationship between root characteristics of peanut grown in hydroponics and pot conditions. Crop Sci. 50:159-167. (Impact factor 2009 = 1.735).

(19) Putto, C., **A. Patanothai**, S. Jogloy, K. Pannangpetch , K.J. Boote and G. Hoogenboom. 2009. Determination of efficient test sites for evaluation of peanut breeding lines using the CSM-CROPGRO-Peanut model. Field Crops Res. 110:272-281. (Impact factor 2009 = 2.336).

(20) Puangbut, D., S. Jogloy, C. Akkasaeng, T. Kesmala, N. Vorasoot and **A. Patanothai.** 2009. Variability in yield responses of peanut (*Arachis hypogaea* L.) Genotypes under Early Senson Drought. Asian J. Plant Sci. 8: 254-264. (ISI, no impact factor).

(21) Puangbut, D., S. Jogloy, N. Vorasoot, C. Akkasaeng, T. Kesmala, Rao C.N. Rachaputi, G.C. Wright and **A. Patanothai**. 2009. Association of root dry weight and transpiration efficiency of peanut genotypes under early season drought. Agr. Water Manage. 96:1460-1466. (Impact factor 2009 = 2.016)

(22) Aranyanark, A., S. Jogloy, S. Wangkaew, C. Akkasaeng, N. Vorasoot, G.C. Wright, Rao.C.N. Rachaputi and **A. Patanothai**. 2009. Association between aflatoxin contamination and drought tolerance traits in peanut. Field Crops Res. 114:14-22 (Impact factor 2009 = 2.336)

(23) Gerdthai, T, S. Jogloy, T. Kesmala,N. Vorasoot, C. Akkasaeng, S. Wongkaew, C.C. Holbrook, and **A. Patanothai**. 2010. Association between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought. Plant Breeding. (In press) (Impact factor 2009 = 1.026)

(24) Pimsaen, W., S. Jogloy, B. Suriharn, T. Kesmala, V. Pensuk and **A. Patanothai.** 2010. Genotype by environment interactions of yield components of Jerusalaem Artichock. Asian J. Plant Sci. 9(1): 11-19. (ISI, no impact factor).

(25) Phakamas, N., **A. Patanothai**, K. Pannangpetch, S. Jogloy and G. Hoogenboom. Determination of adaptive responses of peanut genotypes and patterns of genotype x location interaction using the CSM-CROPGRO-Peanut model. Inter. J. Plant Prod. (Impact factor 2009 = 0.635).

(26) Puangbut, D., S. Jogloy, B. Toomsan, N. Vorasoot, C. Akkasaeng, T. Kesmala R.C. Nageswara Rao, G.C. Wright and **A. Patanothai**. Physiological basis for genotypic variation in tolerance to and recovery from pre-flowering drought of peanut. J. Agron. Crop Sci.. (In press) (Impact factor 2009 = 2.325).

(27) Singkham, N., S. Jogloy, T. Kesmala, P. Switsitang, P. Jaisil, N. Puppala and A. **Patanothai**. Genotype by environment interaction for oil and fatty acids in high, medium and low oleic fatty acids peanut genotypes. J. Agric. Food Chem. 58: 6257-6263. (Impact factor 2009 = 2.469)

2.2 Number of first-submitted papers: 1.

(1) Suriharn, B., **A**. **Patanothai,** K. Pannangpetch, S. Jogloy, K.J. Boote, and G. Hoogenboom. 2009. Designing a peanut ideotype for a target environment using the CSM-CROPGRO Peanut model. Submitted to Crop Sci. (Impact factor 2009= 1.735)

3) New crop cultivars

3.1 New peanut cultivar

A new large seeded early-maturing peanut line (Luhua 11 x Chaina 97-2) F6-11-4 with outstanding performance will be recommended to farmers. This cultivar has yield and seed size comparable to the standard check, KK 6, but is 11 days earlier in maturity duration.

3.2 New kaentawan cultivars

Three outstanding cultivars (JA 89, HEL 65 and CN 52867) were recommended for commercial production.

JA 89 has high and stable yield, and large tubers. However, the tubers are rather branched and are more suitable for industrial use.

HEL 65 has high and stable yield, and large tubers. The tubers are less branched and more palatable than JA 89. Therefore, it is recommended for fresh vegetable.

CN 52867 has high tuber yield and high inulin yield. Its tubers are less branched and slightly smaller than those of JA 89 and HEL 65. Its maturity is much earlier than JA 89 and HEL 65. Therefore, it is suitable for cropping systems that require short growing duration and it can also be grown two consecutive crops in the rainy season.

4) Annual technical seminars

Three annual seminars had been held during the period of the project. All of these had been organized together with the Senior Research Scholar projects of Prof. Dr. Peerasak Srinives of Kasetsart University and Prof. Dr. Benjawan Rerkasem of Chiang Mai University. Three of such a joint seminar had been held previously.

The forth was held at the Ubolrat Dam, Khon Kaen, during 14-15 November 2007.

The fifth was held at the Golden Beach Hotel, Phetchaburi, during 21-22 November 2008.

The sixth was held at the Bhumibol Dam, Tak, during 18-19 November 2009.

5) Linkages with foreign institutes

Linkages have been established and collaborative research on peanut has been undertaken with the following foreign researchers at different institutes:

4.1 Dr. C.C. Holbrook, Crop Genetics and Breeding
Institute: USDA-ARS
Address: USDA-ARS, Coastal Plain Experiment Station
P.O. Box 748, Tifton, Georgia 31793 USA
E-mail : Holbrook@tifton.usda.gov

4.2 Dr. G.C. Wright, Principle Scientist (Agronomy)
Dr. N.C. Rachaputi, Senior Research Agronomist
Institute: Queensland Department of Primary Industries
Address: Queensland Department of Primary Industries
P.O. Box 23 Kingaroy Q4610, Australia
E-mail : Graeme.wright@dpi.gld.gov.au
E-mail : Rao.Rachaputi@dpi.gld.gov.au

4.3 Dr. G. Hoogenboom, Professor
Institute: University of Georgia
Address: Department of Biological and Agricultural Engineering
University of Georgia 165 Gordon Fotral Court Griffin,
Georgia 30223-1797, USA.
E-mail : gerrit@griffin.uga.edu

4.4 Dr. K.J. Boote, Professor
Institute: University of Florida
Address: Agronomy Department, University of Florida, Gainesville, FL
32611-0500, USA.
E-mail : kjb@mail.ifas.ufl.edu

4.5 Dr. R.K. Varshney, Senior Researcher
Institute: International Crop Research Institute for the Semi-arid Tropic
(ICRISAT)
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6) Linkages with private enterprises

Linkages have been established with the following enterprises:

1. **Tipco Asphalt Public Company Limited** located in Pran buri, Prachuap Khiri Khan. The co-operation includes the test of Jerusalem artichoke varieties, multiplication of seed tubers, and research in inulin extraction for commercial production of inulin. Industrial production is expected to be commenced soon.

2. **Chokechai-Farm** located in Nong Nam Daeng, Pak Chong, Nakhon Ratchasima. The farm uses Jerusalem artichoke for improving landscape for tourist attractions and sells seed tubers and fresh vegetable tubers.

3. **Phumarn Mek Resort** located in Pak Chong, Nakhon Ratchasima. The farm also uses Jerusalem artichoke for improving landscape for tourist attractions and sells tubers.

4. **Nopphawan Khanom Thai Company Limited** located in Ladyuo, Jatujack, Bangkok. The company uses kaentawan as an ingredient of bakery products.

5. **Jim Thompson Farm** located in Pak Thong Chai, Nakhon Ratchasima. The farm produces and sells fresh vegetable tubers of kaentawan.