Final Report

CHE-TRF-KKU Distinguished Research Professor Project of Professor Dr. Aran Patanothai

Basic Research for Supporting Varietal Improvement of Functional Food Crops Project

งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์พืชอาหารเพื่อสุขภาพ

By

Professor Dr. Aran Patanothai

Supported by

The Thailand Research Fund,
Office of the Higher Education Commission
and Khon Kaen University

December 2014

สัญญาเลขที่ DPG5480001

Final Report

CHE-TRF-KKU Distinguished Research Professor Project of Professor Dr. Aran Patanothai

Basic Research for Supporting Varietal Improvement of Functional Food Crops Project

งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์พืชอาหารเพื่อสุขภาพ

By

Professor Dr. Aran Patanothai

Supported by

The Thailand Research Fund,
Office of the Higher Education Commission
and Khon Kaen University

December 2014

Acknowledgment

This project received financial supports from the Thailand Research Fund (TRF), the Office of Higher Education (CHE) and Khon Kaen University. 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, the Department of Chemistry, the Department of Biochemistry and the Department of Biology of the Faculty of Science, Khon Kaen University, and the Faculty of Technology, Udon Thani Rajabhat 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.

Contents

Project Code: DPG5480001

Basic Research for Supporting Varietal Improvement of Functional Food Crops

Project leader: Prof. Dr. Aran Patanothai

Department of Plant Science and Agricultural Resources,
Faculty of Agriculture, Khon Kaen University

Abstract

Peanut can be improved for its oleic and phenolic contents. Adding these functional food qualities to peanut improve its market value and health benefit. Jerusalem artichoke is mainly grown for its edible tubers which has high inulin. The chemical compound is beneficial to health. Sweet gourd is a local crop which is a premier source of carotenoids and lycopene. Purple corn is a rich and economic source of anthocyanin colorants. This project aimed to provide basic knowledge to support the development of these new functional foods for Thailand.

This Basic Research for Supporting Varietal Improvement of Functional Food Crops Project is essentially a continuation of the Thailand Research Fund (TRF) Senior Research Scholar Project of the project leader which had been carried on for three phases. The project covered the period from 1 June 2011 – November 2014. The research team included 13 researchers, 4 post-doctoral researchers, 13 Ph.D. students, 7 M.S. students and 2 research assistants.

Research was organized into four sub-projects: (1) Basic research for supporting varietal improvement of peanut for drought tolerance, low aflatoxin contamination and high phenolic compounds, (2) Basic research for supporting varietal improvement of Jerusalem artichoke for drought tolerance, stem rot resistance and high inulin yield, (3) Basic research for supporting varietal improvement of sweet gourd for high fruit yield and high lycopene and β-carotene content and (4) Basic research for supporting varietal improvement of purple waxy corn for high anthocyanin content. The numbers of studies conducted were 15, 10, 2 and 2 for Sub-projects 1, 2, 3 and 4, respectively.

The project has strengthened the research capacity of the 13 researchers and 4 post-doctoral researchers in team; all have gained their experience in conducting quality research and in supervising M.S. and Ph.D. students and can seek their own research funds. For the 13 Ph.D. students, seven have finished their program. Four of the seven M.S. students have graduated. As of October 2014, 28 papers had been published or accepted for publication in accredited international journals, and three had been submitted. Three annual seminars had been held during the period of the project. Linkages have also been established with 14 foreign institutes and 9 private enterprises.

Research findings of the project have been utilized in the Kaentawan (Jerusalem artichoke) breeding program of Khon Kaen University. As a result, one new high tuber yield and high inulin content cultivar, Kaentawan50-4, has been released to farmers.

สัญญาเลขที่ DPG5480001

งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์พืชอาหารเพื่อสุขภาพ

หัวหน้าโครงการ: ศ.ดร. อารันต์ พัฒโนทัย
ภาควิชาพืชศาสตร์และทรัพยากรการเกษตร คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น

บทคัดย่อ

ถั่วลิสงเป็นแหล่งโปรตีนที่สำคัญของคนไทย การปรับปรุงคุณค่าเชิงอาหารสุขภาพจะเป็นการเพิ่มมูลค่าทางการตลาดและเป็นประโยชน์ต่อสุขภาพ แก่นตะวันเป็นพืชหัวที่มีสารสำคัญ คือ อินนูลิน ซึ่งเป็นสารที่มีประโยชน์ต่อสุขภาพ ฟักข้าวเป็นพืชท้องถิ่นที่เป็นแหล่งของสารคาโรทีนอยด์และไลโคปีน ซึ่งเป็นประโยชน์ต่อสุขภาพ ข้าวโพดข้าวเหนียวสีม่วงเป็นแหล่งของสารที่สำคัญคือแอนโธไซยานิน โครงการนี้มุ่งที่จะสร้างองค์ความรู้พื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์พืชอาหารเพื่อสุขภาพเหล่านี้สำหรับประเทศไทย

โครงการวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์พืชอาหารเพื่อสุขภาพนี้ เป็นโครงการต่อเนื่องจากโครงการเมธีวิจัยอาวุโส สกว. ที่มีการดำเนินเสร็จสิ้นแล้วทั้ง 3 ระยะ โครงการฯมีระยะเวลาในการดำเนินงาน 3 ปี เริ่มตั้งแต่ 1 มิถุนายน 2554 ถึง 30 พฤศจิกายน2557 ทีมวิจัยประกอบด้วยนักวิจัย 13 คน นักวิจัยหลังปริญญาเอก 4 คน นักศึกษาปริญญาเอก 13 คน นักศึกษาปริญญาโท 7 คน และผู้ช่วยวิจัย 2 คน

งานวิจัยแบ่งออกเป็นโครงการย่อย 4 โครงการ คือ (1) งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์ถั่วลิสงเพื่อให้ทนแล้ง มีการปนเปื้อนสารอะฟลาทอกซินต่ำ และมีสารฟีนอลิคสูง (2) งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์แก่นตะวันให้ทนแล้ง ต้านทานต่อโรคโคนเน่า และมีสารอินนูลินสูง (3) งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์ฟักข้าวให้มีผลผลิตสูง และมีสารไลโคปีนและสารเบต้าแคโรทีนสูง และ (4) งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์ข้าวโพดข้าวเหนียวสีม่วงให้มีสารแอนโธไซยานินสูง จำนวนหัวข้อที่ศึกษาในแต่ละหัวข้อย่อย คือ 15, 10, 2 และ 2 สำหรับโครงการย่อยที่ 1, 2, 3 และ 4 ตามลำดับ

ในการสร้างนักวิจัย โครงการฯ ได้เพิ่มขีดความสามารถของนักวิจัยในทีมทั้ง 13 คน และนักวิจัยหลังปริญญาเอก 4 คน กล่าวคือ นักวิจัยมีประสบการณ์ในการทำงานวิจัยที่มีคุณภาพ และสามารถให้คำปรึกษาและควบคุมดูแลการทำวิจัยที่เป็นวิทยานิพนธ์ของนักศึกษาปริญญาโทและเอก และทุกคนมีความสามารถในการหาทุนวิจัยได้เอง ในบรรดานักศึกษาปริญยาเอก 13 คน 7 คนสำเร็จการศึกษาแล้ว ส่วนนักศึกษาปริญญาโท 4 ใน 7 คนสำเร็จการศึกษาแล้ว จนถึงเดือนตุลาคม 2557 โครงการฯ มีงานวิจัยที่ได้รับการตีพิมพ์หรือได้รับการตอบรับให้ตีพิมพ์ในวารสารวิชาการระดับนานาชาติที่เชื่อถือได้ 28 เรื่อง

และมีงานวิจัยที่ส่งไปตีพิมพ์อีก 3 เรื่อง ได้มีการจัดสัมมนาวิชาการประจำปีแล้ว 3 ครั้ง โดยได้เชื่อมโยงกับสถาบันในต่างประเทศ 14 สถาบัน และบริษัทเอกชน 9 บริษัท

ผลงานของโครงการฯ ได้นำไปใช้ในโครงการปรับปรุงพันธุ์แก่นตะวันของมหาวิทยาลัยขอนแก่น เป็นผลให้ได้พันธุ์แก่นตะวันที่ให้ผลผลิตและมีสารอินนูลินสูง 1 พันธุ์ ได้แก่พันธุ์แก่นตะวัน 50-4 ที่ได้เผยแพร่ออกสู่เกษตรกรแล้ว

Project Code: DPG5480001

Project Title: Basic research for supporting Varietal Improvement of Functional Food Crops

ชื่อโครงการ: งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์พืชอาหารเพื่อสุขภาพ

Executive Summary

Project leader:

Prof. Dr. Aran Patanothai
Department of Plant Science and Agricultural Resources,
Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002
Tel: (043) 364 637
Fax: (043) 364 636
E-mail: aran@kku.ac.th

Type of funding support: Distinguished Research Professor Grant

Field of study: Agronomy, Biology and Chemistry

Project duration: 3 years (June 2011 – November 2014)

Research team:

Researcher 13
Postdoctoral 4
Ph.D. Student 13
M.S. Student 7
Research assistant 2
B.S. Student 5

Objectives:

1. To generate basic knowledge for supporting varietal improvement of functional food crops.
2. To obtain new breeding lines of functional food crops.
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 varietal improvement of functional food crops between Khon Kaen University and other institutes both within the country and overseas.

Expected outputs:

The project aims to produce the following outputs:

1. New breeding lines of food crops with high quality as functional food: 3
2. Number of published papers in accredited journals: 22
3. Number of researchers with improved capability: 11
4. Number of post doc: 1
5. Number of M.S. and Ph.D. graduates produced: 16
6. Number of research assistants: 2
7. Annual meetings held: 3
8. Number of Thai and foreign institutes that linkage established: 10
9. Number of private companies that linkage established: 6

Background and scope:

Despite a substantial progress has been made in agricultural development, Thailand is still faced with the problem of natural resource degradation and high risks of agricultural production from price fluctuation and crop losses from pests diseases, drought and floods. These have resulted in low income of the farmers who are the majority of the population. Thailand is also going into the era of the emerging health problems relating to modern lifestyle and aging society in which chronic diseases (e.g., cardiovascular disease, dementia and Alzheimer's disease, cancer, arthritis, and diabetes) will be a major threat to life. Functional foods have the physiologically-active components that are beneficial to health, thus, are high in market demand and command high prices as the view of consumers on diet have changed towards health and well-being rather than just a basic nutrition need. Functional foods can, therefore, play a vital role in alleviating these problems and provide more income to farmers at the same time, as the products will also be beneficial to the health of consumers. This project, through basic research, intends to provide support for the development of new functional foods in Thailand.

Under this project, we have chosen four crops to work on because they have great potential to be functional food crops for Thailand. They are peanut (*Arachis hypogaea*), Jerusalem artichoke (*Helianthus tuberosus* L.) or "kaentawan", sweet gourd (*Momordica cochinchinensis* Spreng) and purple corn (*Zea mays* L.). Peanut is a well established crop in the country; kaentawan is a new crop which its production is beginning to be established, sweet gourd is a local crop widely grown but underutilized, and purple corn is a new crop for future production.

Peanut can be improved for its oleic and phenolic contents. Adding these functional food qualities to peanut will improve its market value and health benefit, making the crop more attractive to growers, product manufacturers and consumers. Jerusalem artichoke is mainly grown for its edible tubers. The crop produces tubers which have a high amount of insulin. This substance is recognized as a prebiotic food, and 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. Sweet

gourd is a premier source of carotenoids, especially β-carotene and lycopene. Its lycopene content is 70 times higher than that of tomato, and its β-carotene also has good bioavailability. Purple corn is a rich and economical source of anthocyanin colorants and functional ingredients that have antioxidant and anticarcinogenic properties. Performances of purple corn on health benefits, product differentiation and value added to agricultural products make the crop attractive for the nutraceutical and functional food market. These crops, thus, have a great potential for functional food industry in Thailand. In addition, both sweet gourd and purple corn can also add functional food quality to rice, making the main crop of the country more health benefit to consumers and command higher prices, benefitting the farmers.

This project is essentially a continuation of the Thailand Research Fund (TRF) Senior Research Scholar Project of the project leader which had been carried on for three phases. Under the TRF Senior Research Scholar Project, we had undertaken basic research to support the improvement of production efficiency and quality of peanut and peanut products. Research had been conducted on resistance to peanut bud necrosis disease, utilization of peanut residues for soil improvement, the use of a crop simulation model in assisting peanut breeding, and on drought resistance in a holistic approach. The latter is not only to support breeding for drought resistance/tolerance, but also to provide a means for reducing the problem of aflatoxin contamination in which direct breeding for its resistance has not yet been successful. Research to support breeding for high oleic to linoleic ratio (O/L ratio) had also been initiated, setting a new direction of peanut breeding to improving functional food quality of the crop.

To develop a new crop for Thailand in order to provide a new alternative to farmers and diversify the products, we have looked for the crop which is high value and possesses quality as functional food to be in line with the trend in market demand. We have chosen Jerusalem artichoke, which we named “kaentawan”, to work on as it meets the above criteria. To establish kaentawan as a multi-purpose crop in Thailand, various promotion activities have been carried out. Currently, the crop has become widely known and various products are available on the market. Several varieties have been introduced and tested for their productivity under Thailand conditions. From our initial breeding work, we have released three cultivars for commercial production. However, further improvements are still needed, particularly in increasing inulin content, stem rot resistance and drought tolerance, and these are the current priority areas of our kaentawan breeding program. Research under this proposed project is aimed to support these breeding objectives.

Currently, several products from sweet gourd have been commercially produced, but the crop is still grown naturally. Commercial production of the crop is required to provide a regular supply of uniform and high quality raw materials to the industry. However, to make sweet gourd a commercial crop, varietal improvement is strongly needed. We have started collecting sweet gourd germplasm from various sources, and the introduced lines are being initially evaluated for their performance and basic agronomic characters. More basic information is needed to determine appropriate breeding strategy.

Purple corn is a type of waxy corn found in the germplasm collection of the waxy or glutinous corn varietal improvement program under the Plant Breeding Research Center for Sustainable Agriculture of the Faculty of Agriculture, Khon Kaen University. In Thailand, a waxy corn cultivar with high anthocyanin has not been released to corn growers. However, there are pre-commercial varieties of waxy corn with high anthocyanin of some seed companies that are being tested. Khon Kaen University has just

started a breeding program on purple corn for functional food quality. Research under this proposed project is aimed to support such a breeding work.

All research under this project aimed to support the breeding work of the four crops which is done concurrently under the Plant Breeding Research Center for Sustainable Agriculture of Khon Kaen University. The project also links directly to the Food and Functional Food Cluster, a research cluster of Khon Kaen University under the National Research University Program, in which research on product development and utilization and linkages with private enterprises are being undertaken.

The project also aimed to strengthen the capability of researchers, particularly young researchers, in doing quality research, and generate new researchers through graduate study. This should contribute to the improvement of the research capacity of the country and eventually raise the country's competitiveness in the global market.

Research in the project was organized into four sub-projects:

Sub-project 1. Basic research for supporting varietal improvement of peanut for drought tolerance, low aflatoxin contamination, and high phenolic compounds

Sub-project 2. Basic research for supporting varietal improvement of Jerusalem artichoke for drought tolerance, stem rot resistance and high inulin yield

Sub-project 3. Basic research for supporting varietal improvement of sweet gourd for high fruit yield and high lycopene and β-carotene contents

Sub-project 4. Basic research for supporting varietal improvement of purple waxy corn for high anthocyanin content

Highlights of research results:

Highlights of results for the individual sub-projects are as follows:

Sub-project 1. Basic research for supporting varietal improvement of peanut for drought tolerance, low aflatoxin contamination, and high phenolic compounds

1.1 Drought mechanism is explained by high water uptake of the root systems that provide sufficient water for normal transpiration. This could conserve more water by reducing transpiration to maintain high relative water content. The increasing peanut productivity to pre-flowering drought was contributed by the improvement of the assimilate proportion to economic part in the reproductive phase. This knowledge will be useful for breeding of peanut for pre-flowering drought environment.

1.2 The genotypes with greater production of reproductive parts, number of mature pods, also had enhanced pod yield under pre-flowering drought conditions. The number of mature pods was the major factor contributing to pod yield. Therefore, selecting for enhanced number of mature pods would be expected to increase peanut yield.

1.3 Forty peanut genotypes were categorized as either high or low percent root length density (%RLD) based on the mean of %RLD in each of the three soil layers. The relationship between %RLD in the lower soil layer and yield was significant and positive, indicating that %RLD in the lower layer is an important trait that affects pod yield under mid-season drought conditions. Harvest index was found to be an important trait associated with maintaining pod yield under mid-season drought.

1.4 Mid-season drought significantly reduced the uptake of all nutrient elements. Peanut genotypes with higher levels of drought tolerance took up more nutrients than those with lower levels. The uptake of all nutrient elements contributed to biomass production, pod yield, and number of pods per plant. ICGV 98305 was the best genotype with the highest uptakes of all observed nutrient elements.

1.5 Peanut genotypes having high percent root length density (%RLD) at deeper layers, high stomatal conductance, high water use efficiency and high harvest index could maintain pod yield under terminal drought. Percent RLD at deeper layer could be a useful selection criterion for yield under terminal drought conditions.

1.6 Terminal drought significantly reduced nutrient uptake, and peanut genotypes differed considerably with respect to nutrient uptakes under well-watered and terminal drought conditions. ICGV 98324 and ICGV 98348 were the best genotypes for nutrient uptake under terminal drought. Tainan 9 and Tifton 8 had low nutrient uptake under both conditions. Significant correlations between nutrient uptakes and biomass and pod yield were mostly observed under well-watered and drought conditions. Based on nutrient uptake, ICGV 98324 and ICGV 98348 were identified as drought-tolerant lines.

1.7 Terminal drought greatly reduced nitrogen fixation and nodule dry weight. Peanut genotypes differ considerably for these two traits. ICGV 98324 was the best genotype as it could maintain high nitrogen fixation and had a lower reduction in nitrogen fixation under terminal drought. Nitrogen fixation highly contributed to biomass production and pod yield under well-watered conditions, and consistently contributed to biological yields, but gave no contribution to economic yields under terminal drought condition.

1.8 Enhanced root surface area, root volume and percent root length density could maintain nodule growth and nitrogen fixation under terminal drought conditions. Peanut genotypes with deep root systems would improve nutrient uptake and nitrogen fixation under terminal drought conditions.

1.9 The relationships between physiological traits and yield components under terminal drought conditions were observed in ICGV 98324, ICGV 98348 and Tifton-8. The ability to maintain physiological traits and yield components under stress condition could aid peanut genotypes in maintaining high pod yield under terminal drought conditions.

1.10 The heritability estimates for physiological traits were higher than for agronomic traits, and varied among the crosses. Significant and positive correlations between harvest index (HI) and SPAD chlorophyll meter reading (SCMR) with most of agronomic traits were found. Specific leaf area (SLA) was also negatively

correlated with agronomic traits. These results indicated that HI, SLA, and SCMR are potentially useful as indirect selection traits for terminal drought resistance because of high heritability and good correlation with pod yield. Using these traits in selection might be effective and valuable for improving terminal drought tolerance in peanut.

1.11 Drought reduced the uptakes of N, P, K and Ca in peanut. Peanut genotype with high uptake of one nutrient seemed to be high in the uptake of other nutrients. Peanut genotypes performed consistently across water regimes for these traits and peanut lines with high nutrient uptake would be useful parents for further crossing program.

1.12 Drought stress reduced total nitrogen content and N_2 fixation, but increased kernel infection of *Aspergillua flavus* and aflatoxin contamination. Total nitrogen content, N_2 fixation and its related traits had significant negative effects on kernel infection and aflatoxin contamination especially under drought conditions. The ability to maintain high N_2 fixation under drought conditions of peanut genotypes can result in better resistance to aflatoxin contamination.

1.13 The general combining ability (GCA) effects were significant for oleic acid and oleic to linoleic ratio in both F_2 and F_3 generations. The specific combining ability (SCA) and reciprocal effects were also significant, but their relative contributions to variation among crosses were much smaller than those of the GCA effects. Genotypes with high oleic concentration can be selected in crosses with Sun Oleic 97R or Georgia-02C as parents because they carry the two recessive mutant genes (*ol1* and *ol2*) for high oleic acid.

1.14 The virulence of *A. flavus* in pod invasion and gene expression involving aflatoxin synthesis were studied in correlation with peanut genotypes, water statuses, and growth characteristics. All drought tolerant genotypes in this trial, especially ICGV 98303 exhibited the good vegetative trait, but the relative resistance to *A. flavus* seed invasion was found in ICGV 98300 and 98305. The tolerant genotypes expressed nor-1 gene at a comparable low level under irrigated condition, indicating a low potential for aflatoxin synthesis.

1.15 The growth inhibitory effect on five human cancer cell lines (HeLa, HT29, HCT116, Jurkat and MCF-7 cells) of both ICG15042 and KK4 testa extracts is in accordance with their capability to induce cancerous cell apoptosis. ICG15042 and KK4 testa extracts contained similar type and amount of phenolic acids and their antiproliferative activity seemed to correspond with the phenolic acid profiles.

Sub-project 2. Basic research for supporting varietal improvement of Jerusalem artichoke for drought tolerance, stem rot resistance and high inulin yield

2.1 Variations of seventy-nine accession of Jerusalem artichoke were high for number of tubers per plant, tuber width and tuber size. The accessions were clearly grouped into 4 clusters by using 9 qualitative traits and 20 quantitative traits. These results will enable breeders to make decisions about possible heterotic groups for their breeding programs and germplasm conservation.

2.2 Variations in water use efficiency for biomass and tubers were observed among 40 Jerusalem artichoke genotypes, and the use of water use efficiency as a surrogate trait for drought tolerance is promising. Genotypes with high water use efficiency in all water levels were HEL 231, HEL 65 and [JA 102×JA89] -8.

2.3 Drought reduced tuber dry weight and biomass of Jerusalem artichoke, and the reductions in both traits were greater under severe drought than under moderate drought conditions. CN 52867, HEL 53, HEL 231, HEL 335, JA 76, HEL 65 and [JA 102 × JA 89]-8 had high tuber dry weight, and these genotypes are promising parents in breeding for drought resistance.

2.4 Jerusalem artichoke genotypes responded differently to varying growing degree days (GDD) and photoperiod for harvest index (HI), shoot dry weight, leaf area, number of tubers and tuber size due largely to high variation in planting dates within years and between years. High GDD was positively associated with high shoot dry weight, high leaf area and the high number of tubers, but had negative correlations with HI and tuber size.

2.5 Spectrophotometric method can be used as an alternative for chromatographic analysis for the determination of inulin in plant samples. The proposed method is rapid, simple, and reliable for the determination of inulin in Jerusalem artichoke samples. The method is suitable for routine analysis of Jerusalem artichoke, especially for plant breeding purposes.

2.6 The efficient DNA and RNA extraction protocols for Jerusalem artichoke tissue were developed and proved to be useful for other plant species. Genetic relatedness among 147 Jerusalem artichoke accessions from nine countries of origin was elucidated.

2.7 Inoculation with three sorghum seeds gave the highest variation in Jerusalem artichoke genotypes and provided replicable results for most genotypes. This method will be further used to evaluate Jerusalem artichoke genotypes for resistance to stem rot disease caused by *Sclerotium rolfsii*.

2.8 Mature plant resistance is common in many hosts and pathogen systems. *Sclerotium rolfsii* inoculum derived from serial *in vitro* subculture caused more severe stem rot symptoms than inoculum derived by re-isolated from symptomatic host plants. The inoculum culture on the sorghum-based medium resulted in a higher incidence of stem rot than PDA. *Sclerotium rolfsii* from re-isolation had higher potential to infect plant part (sorghum seed) than *Sclerotium rolfsii* from serial subculture.

2.9 The correlations of stem rot resistance between seedling stage and adult stage were low for all traits. The results, thus, pointed out that the mechanisms controlling resistance to *Sclerotium rolfsii* at seedling and at adult growth stages might be different. Therefore, selection only at seedling stage will not be effective.

2.10 The combination of cv. HEL 246 with the addition of both *Glomus clarum* and *Trichoderma harzianum* had the lowest stem rot disease incidence and required the longest time to permanent wilt. Inoculation of cv. JA 37 and HEL 246 with *G. clarum* alone gave better control of the disease than did inoculation with *T. harzianum* alone.

Sub-project 3. Basic research for supporting varietal improvement of sweet gourd for high fruit yield and high lycopene and *β*-carotene contents

3.1 RAPD markers could clearly separate source and sex of 25 spiny bitter gourd genotypes. RAPD markers, therefore, could be used as an effective tool for germplasm management, such as fingerprinting, diversity study, and selection of parents and progenies with desired characters in breeding programs. The markers may also be useful in marker-assisted selection programs for economically important traits.

3.2 Genetic variation was found for agronomic traits and phytochemical contents in spiny bitter and these traits could be used a parameter for evaluation of genetic diversity. This result will enable breeders to make decisions about possible heterotic groups for their breeding programs and germplasm conservation.

Sub-project 4. Basic research for supporting varietal improvement of purple waxy corn for high anthocyanin content

4.1 Purple waxy corn had the highest total anthocyanin content (TAC) at immaturity and physiological maturity stages, while field corn had the highest total carotenoid content (TCC) followed by super sweet corn. For fresh kernel stage, super sweet corn had the highest TPC. Purple waxy corn also had the highest antioxidant activity, cyanidin-3-glucoside and pelargonidin-3-glucoside at both maturity stages. This information could be used for consumers' selection of a specialty corn, for production planning, for development of health food products and for pharmaceutical industries.

4.2 Location (L), genotype (G) and G × L interaction were highly significant for all characters of purple corn. Nakhon Ratchasima was the location that gave the highest values for total anthocyanin content (TAC) and cyanidin-3-glucoside (C3G). The KNDM4 genotype performed well under unfavorable environments for all traits. This information is useful for breeding programs and production of anthocyanins from purple waxy corn.

Project outputs

1) Capacity building of researchers

Research team:

Number of researchers: 13
Number of post-doctoral: 4
Number of Ph.D. Students: 13
Number of M.S. Students: 7
Number of research assistants: 2

Improved research capacity of researchers

2) Published papers in accredited journals

Number of published or accepted papers: 28
Number of first-submitted papers: 3

3) Annual technical seminars

Three annual seminars had been held during the period of the project.

4) New crop cultivar

One new cultivar of Jerusalem artichoke, KT 50-4, was recommended for commercial production.

5) Linkages with private sector and with Thai and foreign institutes

Linkages have been established and collaborative research on peanut, Jerusalem artichoke, sweet gourd and purple waxy corn have been undertaken with the following foreign institutes.

5.1 Linkage with private industry

1) The Lily Industry Co., TTD: Cooperated with the project in testing the quality of promising peanut lines for making various industrial products.

2) Tipco Asphalt Public Company Limited located in Pran buri, Prachuap Khiri Khan has a strong co-operation with the project. The co-operation includes the test of Jerusalem artichoke varieties, multiplication of seed tubers, and research in inulin extraction for commercial production of inulin

3) Chokechai-Farm located in Nong Nam Daeng, Pak Chong, Nakhon Ratchasima has a strong co-operation with project. The farm uses Jerusalem artichoke for improving landscape for tourist attractions and sells seed tubers and fresh vegetable tubers.

4) Phumarn-mek Resort located in Pak Chong, Nakhon Ratchasima also uses Jerusalem artichoke for improving landscape for tourist attractions and sells tubers.

5) Rai Piriya located in Pak Chong, Nakhon Ratchasima, uses Jerusalem artichoke for improving landscape for tourist attractions and sells seed tubers and fresh vegetable tubers.

6) RaiKaentawan@Wangnumkeaw located in Wangnamkeaw, Nakhon Ratchasima, uses Jerusalem artichoke for improving landscape for tourist attractions and sells seed tubers and fresh vegetable tubers.

7) Nopphawan Khanom Thai Company Limited located in Ladyuo, Jatujack, Bangkok, uses Jerusalem artichoke as an ingredient of bakery products.

8) Jim Thompson Farm located in Pak Thong Chai, Nakhon Ratchasima, produces fresh vegetable tubers and markets the tubers.

9) Chia Tai Co., Ltd. (Choncharoen Farm) located in Kanchanaburi-Saiyok Rd, Wangdong, Muang, Kanchanaburi, produces the highest of quality seed tubers and fresh vegetable tubers.

5.2 Linkages with Thai and foreign institutes

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

5.2.1 Thai institutes:

1) Dr. Bung-orn Sripanidkulchai, Professor (Plant medicinal chemistry)
Institute: Khon Kaen University
Address: Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, Khon Kaen Universit, Khon Kaen, Thailand
E-mail: bungorn@kku ac.th

2) Dr. Kasem Nuntachai, Assistant Professor (Food product development)
Institute: Khon Kaen University
Address: Department of Food Technology, Faculty of Technology, Khon Kaen Universit, Khon Kaen, Thailand
E-mail: kasem@kku ac.th

3) Dr. Juntanee Uriyapongson, Assistant Professor (Food product development)
Institute: Khon Kaen University
Address: Department of Food Technology, Faculty of Technology, Khon Kaen Universit, Khon Kaen, Thailand
E-mail: juntanee@kku.ac.th

4) Chanchana Siripanwattana, (Food technology and product development)
Institute: Suan Dusit Rajabhat University
Address: Faculty of Science and Technology Suan Dusit Rajabhat University, Bangkok, Thailand
E-mail: chtnuch@hotmail.com

5) Dr. Jintanaporn Wattanathorn, Associate Professor (Neuroscience, Physiology)
Institute: Khon Kaen University
Address: Department ofPhysiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
E-mail: jintan_w@kku.ac.th

6) Sukrichaya Hemathulin, Lectuere (Food product development)
Institute: Rajamungala University of Technology ISAN, Sakon Nakhon Campus
Address:Faculty of Natural Resources, Rajamungala University of Technology ISAN, Sakon Nakhon Campus, Sakon Nakhon, Thailand
E-mail: sukrichaya@hotmail.com

5.2.2 Foreign institutes:

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, USA.
E-mail: Holbrook@tifton.usda.gov

2) Dr. G. Hoogenboom (Professor)
Institute: Washington State University
Address: Weather Net, Washington State University, Prosser, WA, USA.
E-mail: gerrit.hoogenboom@wsu.edu

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

4) Dr. R.K. Varshney: Senior Researcher
Institute: International Crop Research Insitute for the Semi-arid Tropic (ICRISAT)
Address: Applied genomic lab, International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru, Andar Pradesh, India.
E-mail: r.k.varshney@cgiar.org, varshney.raj@gmail.com

5) Dr. Ramesh S. Kanwar (Environment and Water Resource Engineering)
Institute: Iowa State University
Address: Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, Iowa, USA.
E-mail: rskanwar@iastate.edu

6) Dr. Bill Davies (Environmental Physiology)
Institute: Lancaster University
Address: The Lancaster Environment Centre, Department of Biological Sciences, Lancaster University, Lancaster, UK.
E-mail: w.davies@lancater.ac.uk

7) Dr. Ian Dodd (Root Systems)
Institute: Lancaster University
Address: The Lancaster Environment Centre, Department of Biological Sciences, Lancaster University, Lancaster, UK.
E-mail: i.dodd@lancater.ac.uk

8) Dr. Naveen Puppala (Plant breeding)
Institute: New Mexico State University
Address: Agricultural Experiment Station College of Agriculture and Home Economics, New Mexico State University, New Mexico, USA
E-mail: npuppala@nmsu.edu

9) Dr. Mark Gleason (Plant pathologist)
Institute: Iowa State University
Address: Department of Pathology, Iowa State University, Ames, Iowa, USA.
E-mail: mgleason@iastate.edu

10) Dr. Susana Goggi (Seed Science)
Institute: Iowa State University
Address: Department of Agronomy, Seed Science Center, Iowa State University, Ames, Iowa, USA.
E-mail: Susana@iastate.edu

11) Dr. Thomas Sinclair (Plant Physiologist)
Institute: North Carolina State University
Address: Department of Crop Science, North Carolina State University, North Carolina State, USA.
E-mail: trsincla@ncsu.edu

12) Dr. Ratchaputi Nageswara Rao (Plant Physiologist)
Institute: The University of Queensland
Address: Centre for Plant Science, Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Queensland, Australia
E-mail: rao.rachaputi@uq.edu.au

13) Dr. Marvin Scott (Research Geneticist (Plants))
Institute: Iowa State University
Address: Corn Insects and Crop Genetics Research, Iowa State University, Ames, Iowa, USA
E-mail: paul.scott@ars.usda.gov

14) Dr. Jay-lin Jane (Food Science)
Institute: Iowa State University
Address: Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa, USA
E-mail: jjane@iastate.edu

Project Code: DPG5480001

Project Title: Basic Research for Supporting Varietal Improvement of Functional Food Crops

ชื่อโครงการ : งานวิจัยพื้นฐานเพื่อสนับสนุนการปรับปรุงพันธุ์พืชอาหารเพื่อสุขภาพ

Project leader:

Prof. Dr. Aran Patanothai
Department of Plant Science and Agricultural Resources,
Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002
Tel: (043) 342 949
Fax: (043) 364 636
E-mail: aran@kku.ac.th

Type of funding support: Distinguished Research Professor Grant

Field of study: Agronomy, Biology and Chemistry

Project duration: 3 years (June 2011 –November 2014)

Research team:

1. Associate Prof. Dr. Kamol Lertrat
   Department of Plant Science and Agricultural Resources,
   Faculty of Agriculture, Khon Kaen University

2. Associate Prof. Dr. Sanun Jogloy
   Department of Plant Science and Agricultural Resources,
   Faculty of Agriculture, Khon Kaen University

3. Associate Prof. Dr. Nimitr Vorasoot
   Department of Plant Science and Agricultural Resources,
   Faculty of Agriculture, Khon Kaen University

4. Assist. Prof. Dr. Bhalang Surihan
   Department of Plant Science and Agricultural Resources,
   Faculty of Agriculture, Khon Kaen University

5. Assist. Prof. Dr. Patcharin Songsri
   Department of Plant Science and Agricultural Resources,
   Faculty of Agriculture, Khon Kaen University

6. Dr. Wanwipa Kaewpradit
   Department of Plant Science and Agricultural Resources,
   Faculty of Agriculture, Khon Kaen University

7. Assoc. Prof. Dr. Supalax Srijaranai
   Department of Chemistry, Faculty of Science, Khon Kaen University

8. Assist. Prof. Dr. Suporn Nuchadomrong
   Department of Biochemistry, Faculty of Science, Khon Kaen University

9. Assist. Prof. Dr. Preeya Wangsomnuk
   Department of Biology, Faculty of Science, Khon Kaen University

10. Assist. Prof. Dr. Thanaset Senawong
    Department of Biochemistry, Faculty of Science, Khon Kaen University

11. Assist. Prof. Dr. Khomsorn Lomthaisong
    Department of Biochemistry, Faculty of Science, Khon Kaen University

12. Dr. Jirawat Sanitchon
    Department of Plant Science and Agricultural Resources,
    Faculty of Agriculture, Khon Kaen University

13. Dr. Viboon Pensuk
    Faculty of Technology, Udonthani Rajabhat University

Research Assistant:

1. Mr. Thawan Kesmala
2. Miss Chutima Sanunphaibool

Post doc:

1. Dr. Darunee Puangbut
2. Dr. Junya Junjittakarn
3. Dr. Nattawut Singkham
4. Dr. Nuntawoot Jonglangklang

Ph.D.Student:

1. Mr. Surasak Boontang
2. Mr. Wunna Htoon
3. Miss Rattikarn Sannoi
4. Miss Rattanajira Ruttanaprasert
5. Miss Rutchanee Puttha
6. Miss Rattanaporn Koolachart
7. Miss Chokaew Aninbon
8. Miss Waraluck Senakoon
9. Miss Chatsuda Junsopa
10. Miss Nuengsap Thangthong
11. Miss Wanalai Viriyasuthee
12. Miss Wanwipa Pinta
13. Mr. Somprasong Khaopha

M.S. Student:

1. Mr. Anon Janket
2. Mr. Dinh Thai Hoang
3. Miss Supattra Mahakoosee
4. Miss Cholada Aduldecha
5. Miss Sujitra Khampas
6. Miss Natthayaporn Nanta
7. Mr. Tanupat Mornkham

B.S. Student:

1. Mr. Nuttawoot Wannakool
2. Mr. Teerayoot Sarathee
3. Miss Ratchadaporn Kulduang
4. Mr. Waranyoo Photpairao
5. Mr. Montree Khruathong

Overall objectives:

1. To generate basic knowledge for supporting varietal improvement of functional food crops.
2. To obtain new breeding lines of functional food crops.
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 varietal improvement of functional food crops between Khon Kaen University and other institutes both within the country and overseas.

Expected outputs:

The project aimed to produce the following outputs:

1. New breeding lines of food crops with high quality as functional food: 3
2. Number of published papers in accredited journal: 22
3. Number of researchers with improved capacity: 11
4. Number of post-doctoral: 1
5. Number of M.S. and Ph.D. graduates produced: 16
6. Number of research assistants: 2
7. Annual meetings held: 3
8. Number of Thai and foreign institutes that linkage established: 10
9. Number of private companies that linkage established: 6

Background

Agriculture has long been the fundamental economic sector of Thailand, and even currently the majority of the Thai people are still engaged in agriculture. Achievements in agricultural development in the past have made Thailand one of the world export leaders of a number of agricultural commodities. However, agricultural development in the past has also resulted in a serious degradation of natural resources. In reality, farmers always face with the risks of price fluctuation and crop failure from pests, diseases, drought and flood, but their benefit shares are rather small. As a consequence, most farmers are still poor, and income disparity is even wider. These problems still remain and will even be

more serious as market competition for agricultural products will be stronger from trans-boundary trade agreements and new forms of non-tariff trade barriers imposed by buyers.

With the advancement in national economic development, Thailand has also followed the track of the developed countries in terms of lifestyle, eating habits and increased life expectancy of the people. Like what has happened in those countries, Thailand is going into the era of the emerging health problems relating to modern lifestyle and aging society. A study conducted by the World Health Organization and the World Bank predicted a very large increase in disability caused by increases in age-related chronic diseases in all regions of the world. In a few decades, the loss of health and life worldwide will be greater from chronic diseases (e.g., cardiovascular disease, dementia and alzheimer's disease, cancer, arthritis, and diabetes) than from infectious diseases, childhood diseases and accidents.

Thailand is, thus, facing with both the old un-resolved agricultural problems and the emerging health problems relating to modern lifestyle and aging society. Functional foods can play a vital role in alleviating these problems at the same time.

Functional foods have been defined as foods that, by virtue of the presence of physiologically-active components, provide a health benefit beyond basic nutrition. In the last decade of the 20th century, consumers began to radically change their view on diets. Food is no longer viewed merely as a mean to satisfy hunger, prevent diet-deficiency diseases or to provide the essential nutrition for maintenance and/or repair of body tissue; it has become the primary vehicle toward optimum health and wellness. In this regard, food is viewed as a first line of defense in the prevention of various chronic diseases of aging, including cancer, heart disease, osteoporosis, arthritis and age-relating macular degeneration. Under this "positive" or "pro-active" eating paradigm, the kitchen cabinet is now being viewed as the medicine cabinet. This "changing face" of food has provided a greater market opportunity for the functional food industry (Hasler, 2000). In addition, increased consumer understanding of the relationship between diet and health is driving demand for functional foods, making them command a premium price. Within the UK, the functional food market has experienced substantial development activity and is set to become one of the most successful in northern Europe (Gray *et al.*, 2003). As a major food producing country, Thailand is in a great position to take advantage of this rapidly increasing market demand for functional foods.

The development of functional foods requires raw materials that have high physiologically-active components beneficial to health and could be produced commercially within the country. Although there are a number of crops that have the required chemical components, most of them have not been improved in functional food quality or are under-utilized crops. Under this project, we have chosen four crops to work on because they have great potential to be functional food crops for Thailand. They are peanut, Jerusalem artichoke or "kaentawan", sweet gourd and purple corn. Peanut is a well-established crop in the country; kaentawan is a new crop which its production is beginning to be established, sweet gourd is a local crop widely grown but underutilized, and purple corn is a new crop for future production.

Peanut is grown as a secondary crop by small farmers in all parts of the country. The crop provides a significant source of supplementary income to a large number of rural people, being a safeguard for the risk of failure or low price of the main crop. It is consumed in various forms – as boiled and roasted peanut, various types of confectionery, and food ingredients. Peanut is also an important source of protein for Thai people and a major source of edible oil and protein for animal feed. The peanut crop also plays an important role in soil improvement through its nitrogen fixing ability. Under the current situation in which agricultural land has greatly degraded, incorporation of peanut in cropping systems is strongly needed for sustainable production.

Our research team at Khon Kaen University has long been engaged in peanut research, particularly in varietal improvement. In fact, this proposed project is a continuation of the Thailand Research Fund (TRF) Senior Research Scholar Project of the project leader which had been carried on for three phases. Under the TRF Senior Research Scholar Project, we had undertaken basic research to support the improvement of production efficiency and quality of peanut and peanut products, based on the expertise of researchers in the team. The main research topics in Phase I 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 in assisting the peanut variety evaluation. In Phase II, work was continued on peanut bud necrosis; the utilization of crop residues was expanded to cover the entire cropping systems, and 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 had also embarked upon a new area of research on drought resistance in a holistic approach. The aim was not only to support breeding for drought resistance/tolerance, but also to provide a means for reducing the problem of aflatoxin contamination in which direct breeding for its resistance has not yet been successful. These lines of research had been continued into Phase III. The findings from the above basic research had been utilized in the peanut breeding work that had also been carrying on in parallel under a separate project. New peanut cultivars had been released, particularly the large-seeded peanut cultivar with erect plant type and early maturity. New large-seeded peanut lines that are early in maturity, resistant to peanut bud necrosis disease, and drought resistant were in the final stage of yield evaluation.

A new direction of peanut breeding to improve the functional food quality of peanut had also been initiated. During Phase III of the TRF Senior Research Scholar Project, research to support peanut breeding for high oleic to linoleic ratio (O/L ratio) had been conducted. In this project, research to support breeding for high phenolic compounds was done to add more functional food quality to peanut. Work on drought tolerance and low aflatoxin contamination was also continued as these are essential for stable production and market acceptability of the crop.

To develop a new crop for Thailand in order to provide a new alternative to farmers and diversify the products, we have looked for the crop which is high value and possesses quality as functional food to be in line with the trend in market demand. During

Phase II of the TRF Senior Research Project, we had selected Jerusalem artichoke (*Helianthus tuberosus* L.) to work on as it fits the above criteria, and we had named the crop in Thai as "kaentawan". The crop produces tubers which contain a high amount of inulin (Suzuki, 1993). This compound is used in various ways and is high in economic value. It is a dietary fiber (Orafti, 2005) that is not soluble in the digestive system, but it is a carbon source for useful bacteria such as lactobacillus and bifidobacteria in the colon that synthesize vitamin B. Inulin is well recognized as a prebiotic food, and 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).

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 is 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.

The crop is not new in the world. It 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 and has never been grown commercially in the country. We have tested a number of introduced lines and found that they grew quite well and tuber yield of 19-25 tons ha^{-1} could be obtained. The duration of this crop is less than 120 days, thus, 2-3 crops could be grown in a year. Such a short duration makes kaentawan suitable for many cropping systems in Thailand.

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 these 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 the gas station, the production systems and downstream industry should be established in Thailand to produce value-added products from kaentawan for reducing the imports and increasing the exports.

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, and most importantly low inulin content. From our initial breeding work, we had released three cultivars for commercial production. However, further improvements are still needed, particularly in high inulin content and drought tolerance, and these are the current priority areas of our kaentawan breeding program. Research under this project was aimed to support these two breeding objectives.

The other two crops that we worked on under this project are sweet gourd (*Momordica cochinchinensis* Spreng) and purple corn. Sweet gourd is an indigenous crop of Southeast Asia and is normally consumed for dietary as well as medicinal purposes.

The crop is becoming to be known as a premier source of carotenoids, especially β-carotene and lycopene (Vong, *et al.*, 2006; Voung and King., 2003; Vuong, *et al.*, 2002; Aoki, et al., 2002; Ishida *et al.*, 2004; Ishida *et al.*, 2009). Sweet gourd aril has over 70 times of lycopene/gram higher than that of tomato and this has important implications for prostate health. Its β-carotene is also more bioavailable than that of the synthetic form (Vuong *et al.*, 2002). Thus, sweet gourd provides an acceptable source of high levels of valuable antioxidants that have good bioavailability (Burke, *et al.*, 2005).

Currently, several products from sweet gourd have been produced commercially. To further expand the market and diversify the products to meet the growing demand of consumer for functional foods, the crop needs to be grown commercially to regularly supply uniform and high quality raw materials to the industry. However, to make sweet gourd a commercial crop, varietal improvement is strongly needed as this has not been done before. The improvement of sweet gourd for high fruit yield and high lycopene and *β*-carotene genotypes requires a broad genetic base of germplasm. We have started collecting sweet gourd germplasm from various sources, and the introduced lines are being initially evaluated for their performance and basic agronomic characters. To determine appropriate breeding strategy, basic information is needed, particularly on the genetic differences and the effects of environments and genotype x environment interaction on fruit yield and lycopene and *β*-carotene contents of sweet gourd. Such research were carried out in this project.

Purple corn is a type of waxy corn found in our germplasm collection. Waxy or glutinous corn (*Zea mays* L. *var. ceratina*) is a type of corn wildly grown as a cash crop in several countries in Eastern Asia, including China, Myanmar, Laos, Vietnam, Cambodia, Taiwan , Korea and Thailand (Thongnarin *et al.*, 2008; Kesornkeaw *et al.*, 2009). Currently, Thailand is the exporter of waxy corn hybrid seeds and frozen waxy corn. Khon Kaen University has long been working on varietal improvement of waxy or glutinous corn. The program is operating under the Plant Breeding Research Center for Sustainable Agriculture, Faculty of Agriculture, Khon Kaen University. Some open-pollinated varieties and hybrids have been released by the program.

For breeding purposes, the program has collected germplasm of waxy corn from various sources. These germplasm lines have different kernel colors ranging from white, yellow, black and purple. The purple corn is of special interest because it is a rich and economical source of anthocyanin colorants and functional ingredients (Jing and Giusti, 2007). Li *et al.* (2008) found that purple corn produced anthocyanin pigment throughout the plant, particularly high in the husk and cob parts. The reports on positive effects of anthocyanin in normal purple corn on antioxidant and anticarcinogenic properties make the crop attractive for the nutraceutical and functional food market (Cevallos-Casals *et al.*, 2003; 2004). Yang and Zhai (2010) suggested that the seed and cob of purple corn possessed excellent antioxidant activity, which could lead to increased application of these natural food colorants by the food industry.These researchers showed performance of purple corn in term of health benefits, product differentiation and value added for

agricultural products. This type of corn, thus, has a great potential for functional food industry in Thailand.

In Thailand, waxy corn cultivar with high anthocyanin has not been released to corn growers. However, there are pre-commercial varieties of waxy corn with high anthocyanin of some seed companies that are being tested in Thailand. Khon Kaen University has started a breeding program on purple corn for functional food quality. Research under this proposed project was aimed to support such a breeding work.

All research under this project provides supports to the breeding works of the four crops which were done concurrently under the Plant Breeding Research Center for Sustainable Agriculture. The project also links directly to the Food and Functional Food Cluster, a research cluster of Khon Kaen University under the National Research University Program, in which research on product development and utilization and linkages with private enterprises were being undertaken.

References

Aoki, H., N.T. Kieu, N. Kuze, K. Tomisaka, and N. Van Chuyen. 2002. Carotenoid pigments in gac fruit (*Momordica cochinchinensis* Spreng). Biosci. Biotechnol. Biochem. 66(11): 2479-2482.

Burke, D.S., C.R. Smidt, and L.T. Vuong. 2005. *Momordica cochinchinensis, Rosa Roxburghii*, wolfberry, and sea buckthorn-Highly nutritional fruit supported by tradition and science. Curr. Top. Nutraceut. R. 3(4): 259-266.

Cevallos-Casals, B.A., and L. Cisneros-Zevallos. 2004. Stability of anthocyanin-based aqueous extracts of andean purple corn and redfleshed sweet potato compared to synthetic and natural colorants. Food Chem. 86: 69-77.

Farnworth, E.R. 1993. Fructans in human and animal diets. *In:* M. Suzuki and N.J. Chatterton, Eds. Science and Technology of Fructans. CRC Press, London.

Gray, J., G. Armstrong and H. Farley. 2003. Opportunities and constraints in the functional food market. Nutrition and Food Sci. 33: 213-218.

Hasler, C.M. 2000. The changing face of functional foods. J. American College of Nutrition 19: 499S-506S.

Ishida, B.K., C. Turner, M.H. Chapman, and T. McKeon. 2004. Fatty acid and carotenoid composition of gac (*Momordica cochinchinensis* Spreng) fruit. J. Agric. Food Chem. 52: 274-279.

Ishida, B.K. and M.H. Chapman. 2009. Carotenoid extraction from plants using a novel, environmentally friendly solvent. J. Agric. Food Chem. 57: 1051-1059.

Jing, P., and M.M. Giusti. 2007. Effects of extraction conditions on improving the yield and quality of an anthocyanin-rich purple corn (*Zea mays* L.) color extract. J. Food Sci. 72: 363-368.

Kesornkeaw, P., K. Lertrat, and B. Suriharn. 2009. Response to four cycles of mass selection for prolificacy at low and high population densities in small ear waxy corn. J. Plant Sci. 8(6): 425-432.

Li, C.Y., H.W. Kim, S.R., Won, H.K. Min, K.J. Park, J.Y. Park, M.S. Ahn, and Rhee, H.I. 2008. Corn husk as a potential source of anthocyanins. J. Agric. Food Chem. 56: 11413-11416.

Orafti. 2005. Active food scientific monitor. An orafti Newsletter, 12.

Suzuki, M. 1993. Fructans in crop production and preservation. *In:* M Suxuki and N.J. Chatterton, Eds. Science and Technology of Fructans. CRC Press. London.

Thongnarin, N., K. Lerteat, and S. Techawongstien. 2008. Combining ability study in waxy corn (*Zea mays* var. *ceratina*) inbred lines. Acta Hortic 769: 151-156.

Vuong, L.T., S.R. Dueker, and S.P. Murphy. 2002. Plasma *β*-carotene and retinol concentrations of children increase after a 30-d supplementation with the fruit *Momordica cochinchinensis* (gac). Am. J. Clin. Nutr. 75: 872-879.

Vuong, T.L., and J.C. King. 2003. A method of preserving and testing the acceptability of gac fruit oil, a good source of *β*-carotene and essential fatty acids. Food Nutr. Bull. 24 (2): 224-230.

Vuong, L.T., A.A. Franke, L.J. Custer, and S.P. Murphy. 2006. *Momordica cochinchinensis* Spreng. (gac) fruit carotenoids reevaluated. J. Food Compos. Anal. 19: 664–668.

Yang Z., and Zhai, W. 2010. Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (*Zea mays* L.). Innovative Food Sci. Emerging Technol. 11: 169-176.

Scope of research:

The project consisted of 4 sub-projects:

Sub-project 1. Basic research for supporting varietal improvement of peanut for drought tolerance, low aflatoxin contamination, and high phenolic compounds

Sub-project 2. Basic research for supporting varietal improvement of Jerusalem artichoke for drought tolerance, stem rot resistance and high inulin yield

Sub-project 3. Basic research for supporting varietal improvement of sweet gourd (*Momordica cochinchinensis* Spreng) for high fruit yield and high lycopene and *β*-carotene content

Sub-project 4. Basic research for supporting varietal improvement of purple waxy corn for high anthocyanin content

Results

Sub-project 1

Basic research for supporting varietal improvement of peanut for drought tolerance, low aflatoxin contamination, and high phenolic compounds

Associated Prof. Dr. Sanun Jogloy

Sub-project leader

Peanut is grown as a secondary crop by small farmers in all parts of the country. The crop provides a significant source of supplementary income to a large number of rural people, being a safeguard for the risk of failure or low price of the main crop. It is consumed in various forms – as boiled and roasted peanut, various types of confectionery, and food ingredients. Peanut is also an important source of protein for Thai people and a major source of edible oil and protein for animal feed. The peanut crop also plays an important role in soil improvement through its nitrogen fixing ability (Toomsan *et al.*, 1995). Under the current situation in which agricultural land has greatly degraded, incorporation of peanut in cropping systems is strongly needed for sustainable production.

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. Various types of confectionery products have been developed with a wide variety of packaging. The demand for these products is high in both internal and overseas markets. There is also a great export potential of peanut products if their quality meet the international standard, especially on the level aflatoxin contamination. 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. Peanut can be improved for its oleic and phenolic contents (Singkham *et al.*, 2010; Kornsteiner *et al.*, 2006). Adding these functional food qualities to peanut will improve its market value and health benefit, making the crop more attractive to growers, product manufacturers and consumers.

A new direction of peanut breeding to improve the functional food quality of peanut has also been initiated. During Phase III of the TRF Senior Research Scholar Project, research to support peanut breeding for high oleic to linoleic ratio (O/L ratio) had been conducted. In this project, research to support breeding for high phenolic compounds were done to add more functional food quality to peanut. Work on drought tolerance and low aflatoxin contamination was continued as these are essential for stable production and market acceptability of the crop.

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

1.1 Drought tolerance mechanisms for yield responses to pre-flowering drought stress of peanut genotypes with different drought tolerant levels.

1.2 Response of reproductive parts of peanut genotypic variation and their contributions to yield after pre-flowering drought.

1.3 Classification of root distribution patterns and their contributions to yield in peanut genotypes under mid-season drought stress.

1.4 Nutrient uptake of peanut genotypes with different levels of drought tolerance under mid-season drought.

1.5 Rooting traits of peanut genotypes with different yield responses to terminal drought.

1.6 Nutrient uptakes and their contributions to yield in peanut genotypes with different levels of terminal drought resistance.

1.7 Responses of peanut (*Arachis hypogaea* L.) genotypes to N_2-fixation under terminal drought and their contributions to peanut yield.

1.8 Relationship between root traits and nutrient uptake and nitrogen fixation in peanut under terminal drought.

1.9 Relationships between physiological traits and yield components of peanut genotypes with different levels of terminal drought resistance.

1.10 Inheritance of the physiological traits for drought resistance under terminal drought conditions and genotypic correlations with agronomic traits in peanut.

1.11 Nutrient uptake of peanut genotypes under different water regimes.

1.12 Association between aflatoxin contamination and N_2 fixation in peanut under drought conditions.

1.13 Combining ability for oleic acid in peanut (*Arachis hypogaea* L.).

1.14 *Aspergillus flavus* invasion and relevant activity in aflatoxin synthesis in seeds of different drought tolerant peanut genotypes.

1.15 HDAC inhibitory activity of peanut testa extracts against human cancer cell lines.

These studies are reported in this section.

References

Kornsteiner, M., K-H.Wagner and I. Elmadfa. 2006. Tocopherols and total phenolics in 10 different nut types.Food Chem. 98: 381-387

Singkham, N., S. Jogloy, T. Kesmala, P. Switsitang, P. Jaisil and N. Puppala. 2010.Genotypic variability and genotype by environment interactions in oil and fatty acids in high, intermediate and low oleic fatty acid peanut genotypes. J.Agric. Food Chem. 58: 6257-6263.

Toomsan, B., J.F. McDonagh, V. Limpinuntana and K.E. Giller. 1995. Nitrogen fixation by groundnut and soyabean and residual nitrogen benefits to rice in farmers field in Northeast Thailand. Plant Soil 17: 45-56.

1.1 Drought tolerance mechanisms for yield responses to pre-flowering drought stress of peanut genotypes with different drought tolerant levels

N. Jongrungklang, B. Toomsan, N. Vorasoot, S. Jogloy, K.J. Boote,
G. Hoogenboom and A. Patanothai

The improvement of peanut to extract water from the whole soil profile might increase drought tolerance (Songsri *et al.*, 2008). Peanut genotypes that have a high root length density in deeper soil layers have enhanced drought tolerance, which can result in a higher pod yield and harvest index under pre-flowering drought (PFD) conditions (Jongrungklang *et al.*, 2011). Even though the responses of root traits were reported that connect to yield under PFD, the mechanism by which peanut subjected to early drought stress can increase yield is not clearly understood. As yield is a complex trait, several mechanisms may be involved. The responses of rooting traits alone without considering the association with physiological response may not distinctly explain the responses of yield to PFD.

Several studies have reported on the response of physio-morphological characters of above ground plant components concerning with increased yield after PFD stress. These include chlorophyll concentration, stomatal conductance, photosynthesis, transpiration efficiency, relative growth rate, crop growth rate and pod growth rate (Nageswara Rao *et al.*, 1985; Nautiyal *et al.*, 1999; Puangbut *et al.*, 2009). However, these reports did not reveal the relationship between the physio-morphological and rooting character for contributing to pod yield under PFD.

The ability of plants to tolerate periods without significant rainfall while maintaining a high water status is dehydration postponement, which includes reducing water loss or maintaining water uptake (Turner 1986). Plants may use one or more mechanisms at the same time for resisting drought. However, peanut responses to drought are complex, and the mechanisms of drought tolerance are under different genetic controls. A better understanding of the mechanisms of peanut adaptation to drought would be very useful for increasing pod yield productivity under PFD.

Objective

The objective of this study was to investigate the mechanism for drought tolerance of diverse peanut genotypes with different pod yield responses under PFD conditions.

Materials and Methods

Field experiments were conducted during February to July, 2007 and during February to July, 2009. A split-plot experiment in a randomized complete block design with four replications was used. Two water management treatments were assigned in main plots, consisting of field capacity (FC) and PFD. The FC treatment was maintained at FC from planting to harvest. For PFD treatment, irrigation was withheld from 1 day after emergence (DAE) to 25 DAE. After a stress period, PFD treatment was irrigated to FC, and the soil water was maintained at FC until harvest. Six peanut genotypes, i.e. KK 60-3, Tainan 9, Tifton-8, ICGV 98305, ICGV 98324 and ICGV 98330 were assigned to sub plots. ICGV 98305, ICGV 98324 and ICGV 98330 have been reported to be drought resistant and were provided by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). Tifton-8 is a drought resistant Virginia-type peanut from the United States Department of Agriculture (USDA; Coffelt *et al.*, 1985). KK 60-3 and

Tainan 9 are high yielding cultivars released in Thailand that perform well under sufficient water conditions.

Relative water content (RWC) and stomatal conductance were recorded at 5, 10, 15, 20, 25, 30, 35 and 40 DAE. Leaf area index was measured at 25 DAE, R5 and R7. Total dry matter samples, including shoots, roots and pods, were obtained at 25 DAE, R5, R7 and harvest. Shoot growth rate, root growth rate and pod growth rate were then calculated.

Results

The test peanut genotypes were classified into three groups, i.e., genotypes with pod yield increase (ICGV 98305), genotypes with pod yield decrease (ICGV 98330) and genotypes showing no response (ICGV 98324, Tainan 9, KK 60-3 and Tifton-8). These three groups showed different responses to the change in the assimilate proportion at various growth stages (Fig. 1).

Under the PFD treatment, the increasing pod yield genotypes, represented by ICGV 98305, gave a higher assimilate portion to promote more root growth to acquire more soil moisture during the drought period compared to that grown under sufficient water conditions. As a consequence, this group of genotype had significantly higher root growth rate during the stress period than during the FC period. On the contrary, the decreased pod yield genotypes, represented by ICGV 98330, had no change in the root growth rate (Fig. 1).

After re-watering, the fraction of assimilates supplied to root decreased in ICGV 98305, as shown by the reduced root growth rate during the re-watering to R5 period. At the seed filling stage, the peanut genotypes with a yield increase in response to PFD had lower shoot growth rate than when they had adequate moisture. Under the PFD treatment, ICGV 98305 had higher pod growth rates at R5-R7 and R7-harvest periods than under the FC treatment. On the opposite, at these stages, ICGV 98330 had lower pod growth rate under the PFD treatment than under the FC treatment (Fig. 1).

Moreover, at the seed filling period, ICGV 98305 under PFD had considerably lower shoot growth rate than under FC, indicating that under PDF assimilate might be partitioned more to the economic part. At this stage, the shoot growth rate of ICGV 98330 under PFD was not different than that under FC. For the non-responsive group, represented by KK60-3, shoot and pod growth rates under different soil moisture treatments were not different at all growth stages. Although, at the stress period, the root growth rate of KK60-3 under the two treatments differed significantly in the 1st season, such a difference was not observed in the 2nd season, and also was not shown in other growth stages (Fig. 1).

Leaf RWC of irrigated and pre-flowering stress treatments at 5 and 10 DAE were not different among peanut genotypes. However, at 20 and 25 DAE, RWC of non water-stress and pre-flowering drought stress treatments differed for all genotypes (Fig. 2 and 3). After re-watering (30, 35 and 40 DAE), the differences in leaf RWC between the two water management treatments of all peanut genotypes disappeared. Reductions in RWC were small for ICGV 98305, ICGV 98324 and ICGV 98330, whereas large reductions in RWC were found in both seasons for Tainan 9, KK 60-3, and in one season for Tifton-8.

ICGV 98305 and ICGV 98324 showed only small reduction in stomatal conductance, indicating their sustained high transpirational water use. ICGV 98330,

Tainan 9, KK 60-3 and Tifton-8, however, showed larger reductions, indicating their reduced transpirational water use (Fig. 2 and 3).

These results suggested that, during the stress period, peanut genotypes might use different mechanisms in response to pre-flowering drought. After the relief of water stress, the response of physiological traits might importantly determine the reaction of pod yield to these conditions. Two drought resistant mechanisms might exist in peanut genotypes evaluated in this and previous studies. The first mechanism is explained by high water uptake of the root systems, providing sufficient water for normal transpiration, as shown by the response of ICGV 98305 to PFD. This would induce higher pod growth rate at the pod filling stage due to the change in the assimilate proportion that went to seed, resulting in the increase in pod yield under PDF compared to that under adequate water. The second mechanism is the ability of the genotype to save more water during the stress period by reducing transpiration without changing root traits, thus, maintaining high RWC, as shown by the response of ICGV 98330. However, this mechanism could not explain the yield increase under the PFD condition.

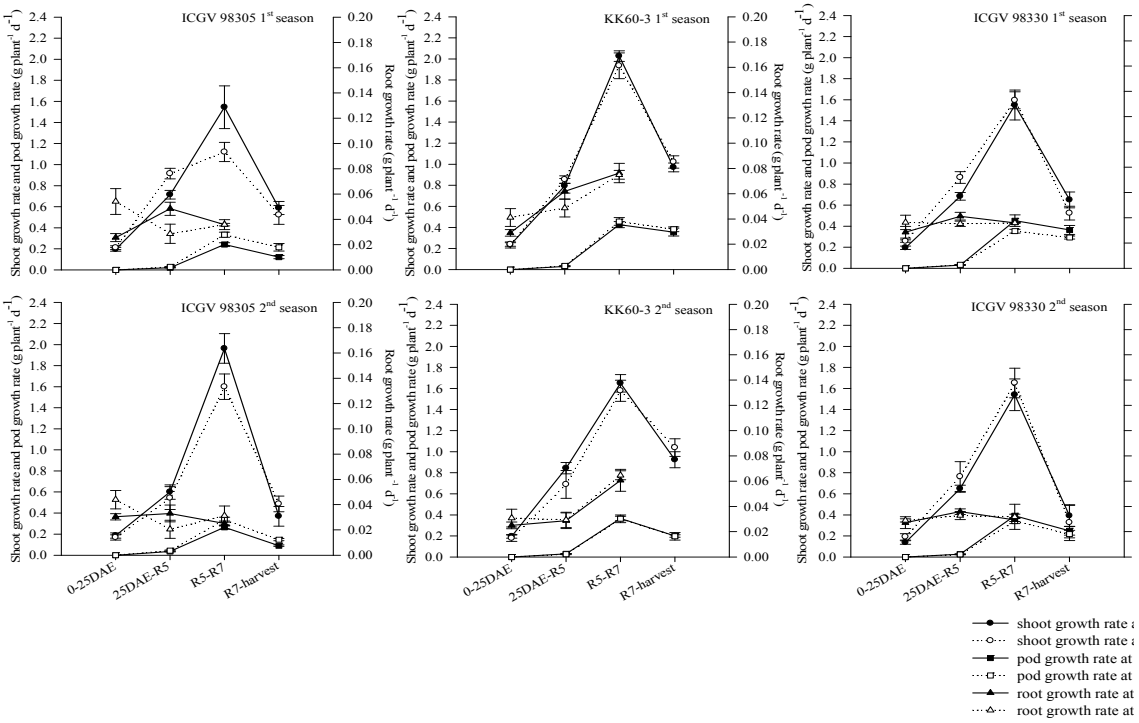


Fig. 1. Shoot growth rate, pod growth rate and root growth rate of three peanut genotypes with different yield responses to pre-flowering drought at 0-25 (days after emergence) DAE, 25 DAE-R5, R5-R7and R7-harvest in 2007 (1st season) and 2009 (2nd season).

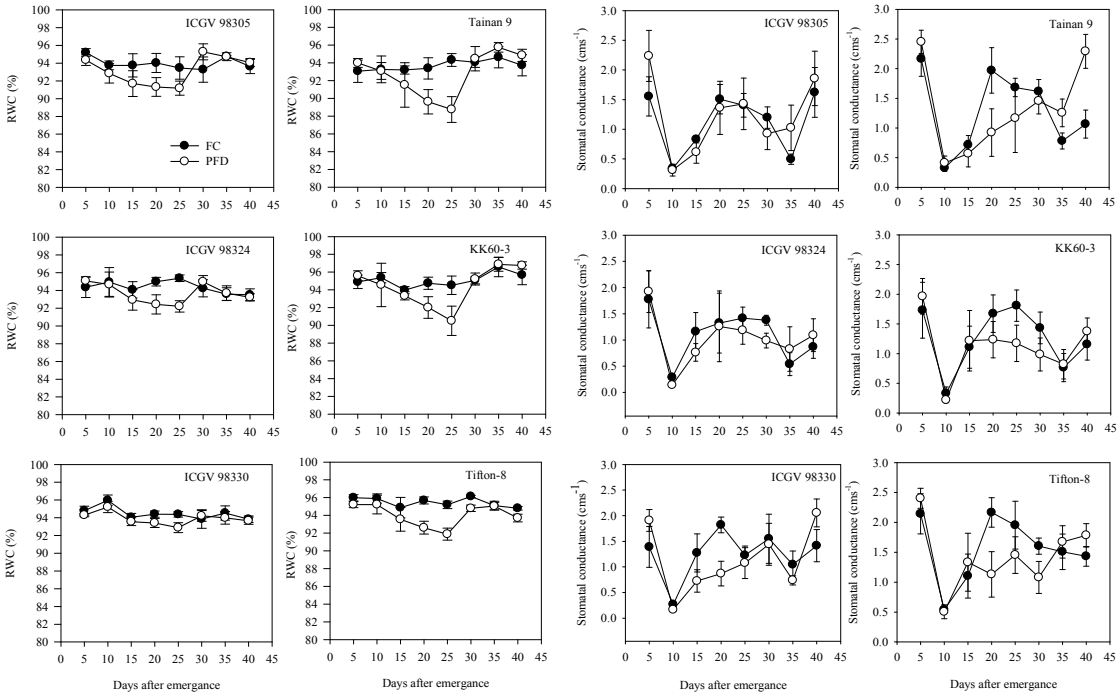


Fig. 2. Relative leaf water content (%) and stomatal conductance (cm/s) of six peanut genotypes at 5, 10, 15, 20, 25, 30, 35 and 40 days after

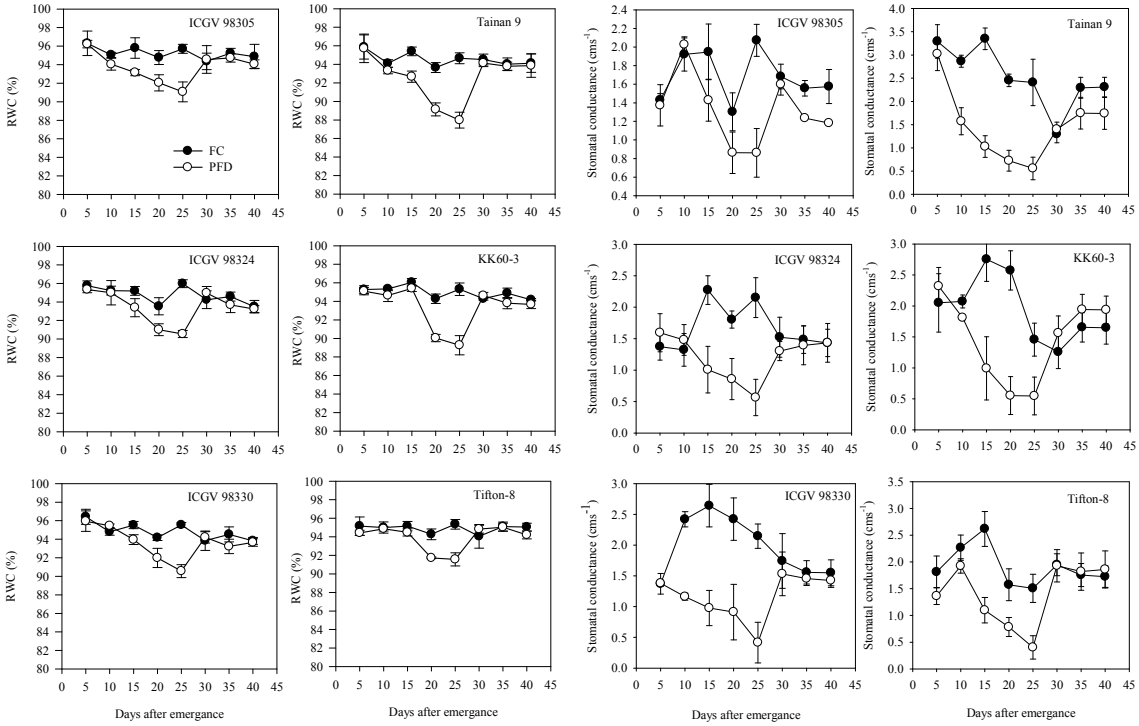


Fig. 3. Relative leaf water content (%) and stomatal conductance (cm/s) of six peanut genotypes at 5, 10, 15, 20, 25, 30, 35 and 40 days after emergence (DAE).

Conclusion

The mechanism for the increased in pod yield under PFD of certain peanut genotypes is likely be the high proportions of roots in lower soil layers that still have high soil moisture during the drought period, and the transpiration capability of the plants is unlimited. After re-watering, the plants under PFD gave higher pod growth rate than those with adequate water, resulting in higher pod yield. An understanding of this adaptive mechanism is important for peanut breeding to exploit positive interaction for pod yield under pre-flowering drought.

References

Coffelt, T.A., R.O.Hammons, W.D. Branch, R.W. Mozingo, P.M. Phipps, J.C. Smith, R.E. Lynch, C.S. Kvien, D.L. Ketring, D.M. Porter and A.C. Mixon.1985. Registration of Tifton-8 peanut germplasm. Crop Sci. 25:203.

Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, K.T. Boote, G. Hoogenboom and A. Patanothai. 2011. Rooting traits of peanut genotypes with different yield responses to pre-flowering drought stress. Field Crops Res. 120: 262-270.

Nageswara Rao, R.C., S. Singh, M.V.K. Sivakumar, K.L. Srivastava and J.H. Williams. 1985. Effect of water deficit at different growth phase of peanut. I. Yield response. Agron. J. 77: 782-786.

Nautiyal, P.C., V. Ravindra, P.V. Zala and Y.C. Joshi. 1999. Enhancement of yield in groundnut following the imposition of transient soil-moisture stress during the vegetative phase. Exp. Agric. 35: 371-385.

Puangbut, D., S. Jogloy, N. Vorasoot, C. Akkasaeng, T. Kesmala and A. Patanothai. 2009. Variability in yield responses of peanut (*Arachis hypogaea* L.) genotypes under early season drought. Asian J. Plant Sci. 8: 254-264.

Songsri, P., S. Jogloy, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook. 2008. Root distribution of drought-resistant peanut genotypes in response to drought. J. Agron. Crop Sci. 19: 92-103.

Turner, N. C. 1986. Adaptation to water deficits: A changing perspective. Aust. J. Plant Physiol. 13: 175-190.

Publication

Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, K.J. Boote, G. Hoogenboom and A. Patanothai. 2013. Drought tolerance mechanisms for yield responses to pre-flowering drought stress of peanut genotypes with different drought tolerant levels. Field Crops Res. 144:34-42. (Impact factor = 2.474).

1.2 Response of reproductive parts of peanut genotypic variation and their contributions to yield after pre-flowering drought

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

Previous studies indicated that drought during flowering or pod formation and prolonged drought can substantially reduce yield of peanut (Songsri *et al.*, 2008; Awal and Ikeda 2002). On the contrary, drought during the vegetative phase or pre-flowering has only small effects on yield (Puangbut *et al.*, 2010). In a greenhouse study, plants recovered from drought by initiating a flush of flowering after re-watering (Awal and Ikeda 2002). This study suggested that the initiation of a strong flowering flush after recovery may compensate for physiological drought damage. However, the contributions of conversions of flowers to pegs, and pods to mature pods is not well understood; similarly, there is limited information on genotypic variation for reproductive development after pre-flowering drought.

Recent reports have demonstrated that physiological traits (such as leaf area, transpiration efficiency, N_2 fixed, root dry weight) were important traits contributing to yield under pre-flowering drought (Puangbut *et al.*, 2011; Songsri *et al.*, 2009). However, very limited information has been available for the contribution of reproductive development to yield following pre-flowering drought conditions. Selection of superior peanut genotypes with the ability to maintain these physiological traits coupled with increased production of reproductive parts might help to improve yield under pre-flowering drought. A better understanding of their contribution to yield is important for peanut breeding. Therefore, the objective of this study was to investigate the variability in reproductive response of peanut genotypes subjected to pre-flowering drought and their contributions to yield.

Objective

The objective of this study was to investigate the variability in reproductive response of peanut genotypes subjected to pre-flowering drought and their contributions to yield.

Materials and Methods

The experiment was conducted in the rainy season from June to October 2005 and in the dry season from December 2005 to April 2006 at the Field Crop Research Station of Khon Kaen University located in Khon Kaen province.

In the rainy season, rainout shelters were available if necessary, but in the dry season the experiment was carried out under field conditions without rainout shelters. A split-plot in a randomized complete block design with four replications was used in both seasons. Main-plots were two water treatments [field capacity (FC) and1/3 available water from emergence to 40 days after emergence] and sub-plot treatments were 11 peanut genotypes. Plot sizes were 2.5 x 2.1 m in the rainy season and 3 x 3 m in the dry season, with a spacing of 30 cm between rows and 10 cm between plants.

The reproductive parts were recorded at harvest, including number of flowers, number of pegs (hanging pegs + pods), number of total pods (immature + mature pods) and number of mature pods per plant. Conversion of flowers to pegs and pegs to pods and pods to mature pods were calculated based on the formula suggested by Nautiyal (1999).

Multiple-linear regression was used to determine the relative contribution of number of flowers, number of pegs, number of pods, and mature pods to pod yield under irrigated and pre-flowering drought treatments. The analysis was based on the following statistical model (Hoshmand, 2006):

$$Y_i = \alpha + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \delta_i$$

where Y_i is pod yield of genotype i, α is the Y intercept, X_{1i}, X_{2i}, X_{3i} and X_{4i} are number of flowers, number of pegs, number of pods and number of mature pod of genotype i, respectively, $\beta_1, \beta_2 \beta_3$ and β_4 are regression coefficients for the independent variables X_1, X_2, X_3 and X_4 and δ_i is the associated deviation from regression.

Results

PFD significantly increased number of mature pods in 7 of 11 genotypes in both seasons, and ICGV 98303 and Tainan 9 had the highest number of mature pods under PFD in both seasons (Table 1). The number of mature pods of these genotypes also increased significantly under PFD compared to irrigated conditions. Genotypes differed significantly in seed size under PFD, with KK 60-3 and Tifton-8 having the highest seed size in both seasons.

In the rainy season, ICGV 98303, ICGV 98330 and Tainan 9 had the highest number of flowers, number of pegs and number of pods under PFD conditions, while ICGV 98308 and ICGV 98353 had the lowest (Table 2). In the dry season, the greatest numbers of flowers, pegs, and pods were found in ICGV 98300, ICGV 98303 and Tainan 9, while ICGV 98305 and ICGV 98353 had the lowest under PDF conditions.

In the rainy season, PFD increased conversion of flowers to pegs by 11%, of pegs to pods by 7% and of pods to mature pods by 6%, compared with irrigated conditions (Table 3). The greatest conversion of flowers to pegs under PFD conditions was observed in ICGV 98348 followed by ICGV 98303 and ICGV 98330, while ICGV 98305 followed by ICGV 98353 had the lowest. The greatest conversion of pegs to pods under PFD conditions was found in IGCV 98303, while ICGV 98353 had the lowest. Under PFD conditions, Tainan 9 exhibited the greatest conversion of pods to mature pods, and ICGV 98353 showed the lowest. In the dry season, PFD increased conversion of flowers to pegs by 34%, of pegs to pods by 10% and of pods to mature pods by 10%, compared to the irrigated conditions (Table 3). The greatest conversion of flowers to pegs was observed in KK 60-3 and Tifton-8, while ICGV 98353 had the lowest conversion under PFD conditions. The greatest conversion of pegs to pods was found in IGCV 98303, while ICGV 98353 had the lowest under PFD conditions. Under PFD conditions, ICGV 98303 and Tainan 9 exhibited the greatest conversion of pods to mature pods and ICGV 98305 showed the lowest.

In the rainy season, the number of mature pods was the major contributing factor to yield under irrigated (63.55%) and PFD conditions (74.92%), while the number of pegs and the number of pods showed the least contribution to yield under irrigated and PFD conditions, respectively (Table 4). In the dry season, the number of mature pods was still the major contributing factor to yield under both PFD (80.45%) and irrigated conditions (78.55%).

Table 1. Number of mature pods per plant, number of seed per pod and 100-seed weight by 11 peanut genotypes under irrigated and pre-flowering drought (PFD) in the rainy (2005) and dry season (2005/06).

	Rainy season			**Dry season**		
Genotypes	Irrigated	PFD	LSD	Irrigated	PFD	LSD
Number of mature pods plant^{-1}						
ICGV 98300	12.7 cd [1/]	17.0 bc	*	14.0 ab	22.0 ab	**
ICGV 98303	10.9 d	21.1 a	**	15.0 ab	30.0 a	**
ICGV 98305	14.2 b	10.2 de	*	14.2 ab	10.2 d	*
ICGV 98308	13.4 bc	12.6 d	ns	13.4 b	15.0 c	*
ICGV 98324	15.3 a	14.0 cd	ns	11.3 b	16.0 bc	**
ICGV 98330	13.0 c	18.5 b	*	13.0 b	18.5 b	*
ICGV 98348	7.9 e	15.3 c	**	9.2 c	15.3 c	**
ICGV 98353	10.2 d	7.5 e	*	10.2 c	8.2 d	*
Tainan 9	11.2 d	20.5 a	**	18.0 a	26.5 a	**
KK 60-3	8.6 e	14.0 cd	**	8.6 c	14.0 c	**
Tifton – 8	9.5 de	13.5 d	**	9.5 c	14.5 c	**
Mean	11.5	14.9		12.4	17.3	
Number of seed pod^{-1}						
ICGV 98300	1.4	1.8	ns	1.9	2.1	ns
ICGV 98303	1.6	1.8	ns	1.8	2.0	ns
ICGV 98305	1.7	1.8	ns	1.8	1.9	ns
ICGV 98308	1.7	2.0	ns	1.9	1.9	ns
ICGV 98324	1.4	2.1	ns	2.1	1.9	ns
ICGV 98330	1.6	1.7	ns	1.9	1.8	ns
ICGV 98348	1.5	1.7	ns	1.9	1.8	ns
ICGV 98353	1.6	1.8	ns	2.1	1.8	ns
Tainan 9	1.6	1.8	ns	1.7	1.8	ns
KK 60-3	1.6	1.9	ns	1.7	1.8	ns
Tifton – 8	1.2	1.8	ns	1.7	2.0	ns
Mean	1.5	1.8		1.9	1.9	
100 seed weight (g)						
ICGV 98300	38.4 b	45.2 b	**	45.7 d	47.5 d	ns
ICGV 98303	39.3 b	47.0 b	**	54.8 c	56.9 b	ns
ICGV 98305	38.6 b	36.5 c	ns	54.2 c	50.6 c	*
ICGV 98308	36.2 b	35.6 c	ns	55.2 b	57.0 b	*
ICGV 98324	38.7 b	37.2 c	ns	55.6 b	58.6 b	*
ICGV 98330	34.4 c	44.1 b	**	56.4 b	57.4 b	ns
ICGV 98348	32.3 c	42.3 b	**	50.2 c	43.4 d	**
ICGV 98353	36.8 b	36.3 c	ns	53.5 c	50.6 c	*
Tainan 9	35.4 c	47.4 b	**	55.9 b	57.1 b	ns
KK 60-3	46.1 a	50.0 b	*	71.1 a	74.4 a	*
Tifton – 8	39.6 b	58.6 a	**	68.9 a	70.8 a	ns
Mean	37.8	43.7		56.5	56.8	

[1/] Means in the same column with the same letters are not significantly different (at $p< 0.05$) by DMRT.
ns , * and ** = non significant, and significant at p <0.05 and p <0.01, respectivelyby LSD.

Table 2. Numbers of flowers, pegs and pods per plant produced by 11 peanut genotypes under irrigated and pre-flowering drought (PFD) conditions in the rainy (2005) and dry seasons (2005/06)**.**

	Rainy season			Dry season		
Genotypes	Irrigated	PFD	LSD	Irrigated	PFD	LSD
Number of flowers plant^{-1}						
ICGV 98300	92 a [1/]	104 b	**	93 a	124 b	**
ICGV 98303	87 b	140 a	**	80 b	136 a	**
ICGV 98305	94 a	85 c	*	68 cd	59 e	*
ICGV 98308	90 a	72 d	**	63 d	76 d	*
ICGV 98324	90 a	87 d	ns	62 d	80 d	**
ICGV 98330	88 b	120 ab	**	62 d	79 d	*
ICGV 98348	80 bc	110 b	**	64 d	74 d	*
ICGV 98353	86 b	69 d	**	66 d	52 e	*
Tainan 9	76 c	136 a	**	75 bc	128 b	**
KK 60-3	62 d	102 b	**	68 cd	87 c	*
Tifton – 8	63 d	83 c	**	64 d	85 c	*
Mean	83	101		70	89	
Number of pegs plant^{-1}						
ICGV 98300	39 ab	51 c	*	45 a	76 b	**
ICGV 98303	44 a	78 a	**	47 a	86 a	**
ICGV 98305	40 b	36 d	ns	41 a	38 e	ns
ICGV 98308	38 ab	38 d	ns	28 c	48 d	**
ICGV 98324	42 a	48 c	*	25 c	54 d	**
ICGV 98330	44 a	72 a	**	32 bc	56 d	**
ICGV 98348	37 ab	68 b	**	34 bc	44 d	*
ICGV 98353	37 ab	34 e	ns	32 bc	29 f	ns
Tainan 9	36 ab	80 a	**	38 b	78 b	**
KK 60-3	26 c	50 c	**	28 c	68 c	**
Tifton – 8	28 c	41 c	**	25 c	62 c	**
Mean	37	54		34	58	
Number of pods plant^{-1}						
ICGV 98300	19 a	24 b	*	25 a	33 b	*
ICGV 98303	20 a	30 a	**	26 a	38 a	**
ICGV 98305	19 a	16 c	ns	25 a	22 c	ns
ICGV 98308	19 a	14 c	ns	22 b	25 c	ns
ICGV 98324	21 a	19 d	ns	16 c	28 b	*
ICGV 98330	18 b	26 b	**	20 b	30 b	**
ICGV 98348	16 c	22 bc	*	23 b	25 c	ns
ICGV 98353	16 c	14 c	ns	21 b	16 d	*
Tainan 9	17 b	25 b	*	28 a	34 b	*
KK 60-3	16 c	21 bc	*	20 b	26 c	*
Tifton – 8	15 c	21 bc	*	20 b	24 c	*
Mean	18	21		22	27	

[1/] Means in the same column with the same letters are not significantly different (at $p< 0.05$) by DMRT. ns , * and ** = non significant, and significant at $p <0.05$ and $p <0.01$, respectively, by LSD.

Table 3. Conversion of flowers to pegs and pods to mature pods of 11 peanut genotypes under irrigated and pre-flowering drought (PFD) in the rainy (2005) and dry seasons (2005/06).

	Rainy season			Dry season		
Genotypes	Irrigated	PFD	LSD	Irrigated	PFD	LSD
Flowers to pegs (%)						
ICGV 98300	43.5 c [1/]	49.9 c	*	48.4 bc	61.3 c	**
ICGV 98303	50.6 b	60.0 b	**	58.8 a	63.2 b	*
ICGV 98305	42.6 c	42.4 c	ns	60.3 a	64.4 b	*
ICGV 98308	51.1 b	52.8 c	ns	44.4 c	63.2 b	**
ICGV 98324	55.6 a	55.2 c	ns	40.3 c	67.5 bc	**
ICGV 98330	50.0 b	60.0 b	**	51.6 ab	70.9 b	**
ICGV 98348	50.0 b	70.9 a	**	53.1 ab	59.5 c	*
ICGV 98353	46.5 c	49.3 c	*	48.5 b	55.8 d	**
Tainan 9	47.4 c	58.8 bc	**	50.7 ab	60.9 c	*
KK 60-3	54.8 b	49.0 c	**	41.2 c	78.2 a	**
Tifton – 8	44.4 c	49.4 c	*	39.1 d	72.9 a	**
Mean	48.8	54.3		48.8	65.2	
Pegs to pods (%)						
ICGV 98300	31.8 ab	33.3 ab	*	42.7 b	52.0 ab	*
ICGV 98303	31.6 ab	39.6 a	**	46.1 a	56.9 a	*
ICGV 98305	32.5 ab	28.3 bc	**	44.6 ab	40.2 c	*
ICGV 98308	29.1 b	29.0 b	ns	39.0 c	42.8 c	*
ICGV 98324	30.6 b	29.2 b	ns	38.0 c	44.0 c	**
ICGV 98330	29.5 b	35.7 ab	**	40.2 c	46.1 bc	**
ICGV 98348	27.8 c	29.5 b	*	46.6 a	50.9 b	*
ICGV 98353	25.0 c	24.0 c	*	38.3 c	37.6 d	ns
Tainan 9	31.1 ab	35.6 ab	*	46.4 a	50.5 b	*
KK 60-3	25.3 c	31.8 b	**	44.8 ab	46.3 bc	*
Tifton – 8	33.9 a	34.4 ab	*	40.2 c	44.5 c	*
Mean	29.1	31.2		42.4	46.5	
Pods to mature pods (%)						
ICGV 98300	66.8 b	70.8 b	*	56.0 c	66.7 b	**
ICGV 98303	54.5 c	70.3 b	**	57.7 c	78.9 a	**
ICGV 98305	74.7 a	63.8 c	**	56.8 c	46.4 e	**
ICGV 98308	70.5 a	60.0 c	**	60.9 bc	60.0 bc	ns
ICGV 98324	73.9 a	72.7 b	ns	70.6 a	57.1 c	**
ICGV 98330	62.2 b	74.2 b	**	65.0 b	61.7 bc	*
ICGV 98348	49.4 d	69.5 b	**	40.0 e	61.0 bc	**
ICGV 98353	63.8 b	53.6 d	**	48.8 d	51.3 d	*
Tainan 9	65.9 b	82.0 a	**	64.3 b	77.9 a	**
KK 60-3	53.8 c	66.7 bc	**	43.0 e	53.8 d	**
Tifton – 8	63.3 b	64.3 c	ns	47.5 d	60.4 bc	**
Mean	64.3	68.0		55.5	61.4	

[1/] Means in the same column with the same letters are not significantly different (at $p < 0.05$) by DMRT. ns , * and ** = non significant, and significant at $p < 0.05$ and $p < 0.01$, respectively, by LSD.

Table 4. Contributions of reproductive parts to pod yield under irrigated and pre-flowering drought (PFD) conditions in the rainy season (2005) and dry season (2005/06).

Traits	Explained by regression (%)			
	Rainy season		Dry season	
	Irrigated	PFD	Irrigated	PFD
Regression	70.22**	81.35**	81.60 **	83.78**
Flower No. plant^{-1}	1.46*	5.04**	0.95*	0.02**
Peg No. plant^{-1}	0.02	0.97**	2.61**	0.15**
Pod No. plant^{-1}	5.19**	0.41**	2.10**	3.16**
Mature pod No. plant^{-1}	63.55**	74.92**	78.55**	80.45**

* and ** = significant at $p<0.05$ and $p<0.01$, respectively; PFD- Pre-flowering drought.

Conclusion

The results demonstrated that PFD increased the number of reproductive parts produced following drought in both seasons. The variation among peanut genotypes in production of reproductive parts after PFD was consistent across seasons. The genotypes with greater production of reproductive parts, i.e., the number of mature pods, also had enhanced pod yield under PFD conditions. The result revealed that number of mature pods was the major factor contributing to yield. Therefore, selecting for enhanced number of mature pods would be expected to increase peanut yield.

References

Awal, M.A. and T. Ikeda. 2002. Recovery strategy following the imposition of episodic soil moisture deficit in stands of peanut (*Arachis hypogaea* L.). J. Agron. Crop Sci. 188: 185-192.

Hoshmand, A.R. 2006. Design of Experiments for Agriculture and the Natural Sciences. Chapman & Hall, Florida.

Nautiyal, P.C., V. Ravindra, P.V. Zala and Y.C. Joshi. 1999. Enhancement of yield in groundnut following the imposition of transient soil-moisture stress during the vegetative phase. Exp Agric. 35: 371-385.

Puangbut, D., S. Jogloy, B. Toomsan, N. Vorasoot, C. Akkasaeng, T. Kesmala, R.C. Rachaputi Rao, G.C.Wright and A. Patanothai. 2010. Physiological basis for genotypic variation in tolerance to and recovery from pre-flowering drought in peanut. J. Agron. Crop Sci. 196: 358-367.

Puangbut, D., S. Jogloy, N. Vorasoot, C. Akkasaeng and A. Patanothai. 2011. Association of transpiration and N_2 fixation of peanut under early season drought. Int. J. Plant Prod. 4: 381-394.

Songsri, P., S. Jogloy, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook. 2008. Response of reproductive characters of drought resistant peanut genotypes to drought. Asian J. Plant Sci. 7: 427-539.

Songsri, P., S. Jogloy, C.C. Holbrook, T. Kesmala, N. Vorasoot, C. Akkasaeng and A. Patanothai. 2009. Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. Agric. Water Manage. 96: 790-798.

Publication

Puangbut, D., S. Jogloy, N. Vorasoot, T. Kesmala, C.C. Holbrook and A. Patanothai. 2013. Response of reproductive parts and their contributions to yield of peanut under pre-flowering drought. Aust. J. Crop Sci. 7: 1627-1633 (Impact factor = 1.632).

1.3 Classification of root distribution patterns and their contributions to yield in peanut genotypes under mid-season drought stress

N. Jongrungklang, B. Toomsan, N. Vorasoot, S. Jogloy, K.J. Boote, G. Hoogenboom and A. Patanothai

The mechanisms of drought resistance in relation to above ground part have been demonstrated in the literature. However, there is limited information on the root responses of peanut under water deficit environment. Drought resistance may be enhanced by improving the ability of the crop to extract water from the soil (Wright and Nageswara Rao 1994). Deep rooting, root length density (RLD) and root distribution have been identified as drought adaptive traits (Turner 1986; Matsui and Singh 2003; Taiz and Zeiger 2006).

Peanut genotypes that have a high RLD at deeper soil depths may have enhanced drought tolerance which could help maintain a high pod yield and harvest index. These genotypes are classified as drought responsive as their RLD increases in deeper soil layers in response to drought (Songsri *et al.*, 2008). Pandey *et al*. (1984) reported that drought increased RLD in the lower soil profile of the peanut genotype "Kidang". However, Robertson et al (1980) found no effect of water management strategies on RLD of Florunner peanut cultivar. There are different magnitudes of RLD with different peanut genotypes (Songsri *et al.*, 2008). Although this trait is used to express the capability for water uptake of peanut genotypes (Taiz and Zeiger 2006), it has not been used in the classification of root distribution patterns of different peanut genotypes.

Peanut root distribution patterns are not well understood and have not been studied extensively. The results reported so far have been limited to experiments under chamber conditions and with few peanut genotypes. Even though the responses of RLD were investigated for six peanut genotypes under pre-flowering drought by Jongrungklang et al (2011), the root distribution pattern of diverse peanut genotypes under severe mid-season drought stress have not been reported. Classification of root distribution patterns for peanut genotypes under mid-season drought would be useful for breeding peanut to enhance drought tolerance.

Objective

The aim of this study was to classify the root distribution patterns of peanut genotypes under mid-season drought, and to determine the relationships between RLD in different soil depths and yield under these conditions.

Materials and Methods

The experiment was conducted under field conditions at the Field Crop Research station of Khon Kaen University from December 2007 to May 2008, and was repeated from November 2008 to April 2009. A randomized complete block design with four replications was used in both years. The plot size was 3 x 5 m, with a spacing of 50 cm between rows and 20 cm between plants.

The peanut genotypes compared in this study included 40 peanut genotypes that differed in the level of drought tolerance and the source of origin. Nine genotypes with different levels of drought tolerance were obtained from the United State Department of Agriculture (USDA) (Jongrungklang *et al*., 2008). Eleven are commercially released cultivars in Thailand (KKU 40, KKU 60, KKU 1, KKU 72-1, KK 6, KK 4, KK5, KS 2, KK 60-2, KK 60-3 and Tainan 9) (Table 1). KK 60-3 is a Virginia-type peanut cultivar sensitive to drought for pod yield, while Tainan 9 is a Spanish-type peanut cultivar having low dry matter production under drought conditions (Vorasoot *et al.*, 2003). Eight elite drought resistant lines (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348 and ICGV 98353) were provided by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) in India. These lines had been selected based on high total dry matter and pod yield under drought stress conditions (Nigam *et al.*, 2003; Nigam *et al.*, 2005). One (Tifton-8) was a Virginia-type drought-resistant line (Coffelt *et al.*, 1985) introduced from USDA. Eleven genotypes were selected based on their differences in dry matter production, harvest index and specific leaf area under well-watered conditions (data from our previous study), and three genotypes were received from China.

A sprinkler irrigation system was installed prior to planting to supply water during the growing season. The water regime in this experiment was an imitated mid-season drought stress that would normally occur in a farmer's field. All plots were supplied with water to obtain field capacity moisture level to the depth of 60 cm from planting to 50 DAP. After 50 DAP, water was withheld until 83 DAP in the first season to mimic mid-season drought conditions. Thermal degree day accumulation was calculated in both seasons for predicting crop growth stage, and irrigation was withheld from 50 to 87 DAP for the second season. After the drought period, all plots were re-watered and maintained at FC level until harvest. Top dry weight was observed at the most water-stressed date and at harvest, while root data were measured at the most water-stressed date using the auger method. The soil was sampled to a depth of 90 cm and was separated into the upper (0 to 30 cm), middle (30 to 60 cm) and deeper (60 to 90 cm) soil layers. For each peanut genotype, the relative contribution to each layer was calculated and defined as root length density percentage (%RLD). Pod yield was observed at final harvest date and pod harvest index (PHI) wascalculated.

Results

Forty peanut genotypes were categorized into six combinative groups, based on the high (H) and low (L) %RLD in the upper, middle and lower soil layers. Five peanut genotypes were classified as HHL, four were HLL, five were LHL, 10 were LHH, seven were LLH, and two were HLH (Fig. 1). The drought tolerance levels of these genotypes agree well with the previous report. Most drought tolerant genotypes were in the LHH, LLH and HLH, susceptible genotypes were HHL, HLL and LHL, and moderately tolerant genotypes were found in all groups. Evidently, the mean %RLD in lower layers could be used as an index to indicate the level of drought tolerance of a peanut genotype.

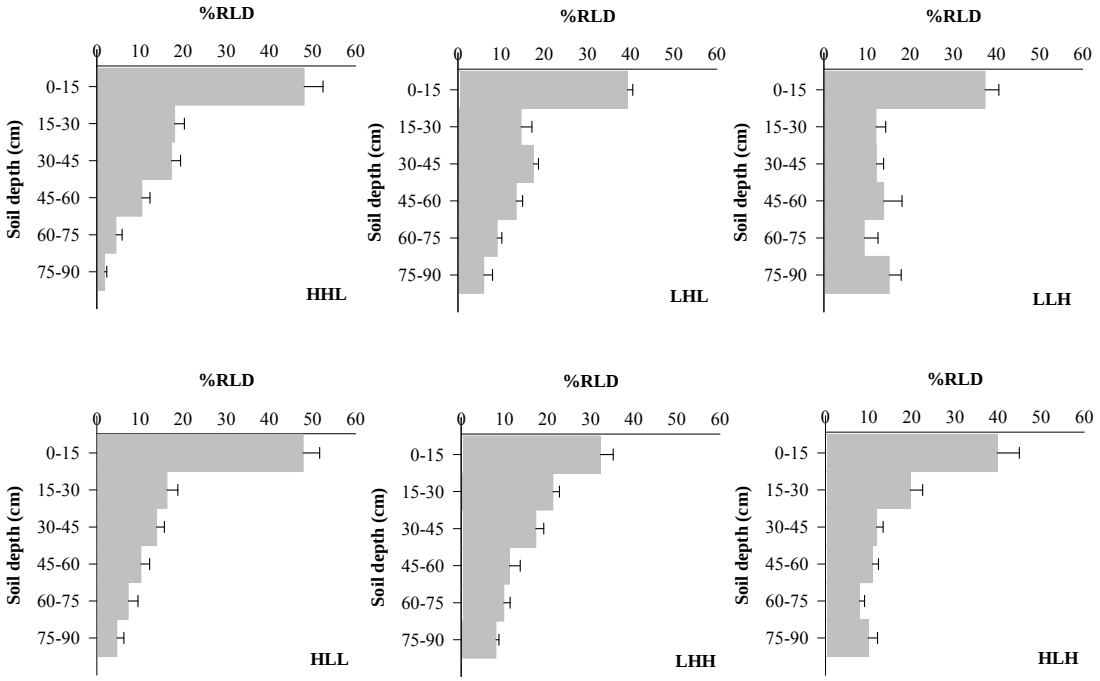


Fig. 1. Six root distribution patterns of 40 peanut genotypes from the experiments conducting during December 2007- May 2008 and during November 2008- April 2009 at Khon Kaen University, Khon Kaen, Thailand.

HHL= high RLD in upper and middle layers but low RLD in lower layer (a; representation by Tainan 9), HLL= high RLD at upper but low RLD at middle and lower layers (b; representation by 306 PI 430237), LHL= low RLD in upper and lower layers but high RLD in middle layer (c; representation by 187 PI 433352), LHH= low RLD in upper layer but high RLD in middle and lower layers (d; representation by Luhua 11), LLH= low RLD in upper and middle layers but high RLD in lower layer (e; representation by 106 PI 268949), HLH= high RLD in upper and lower layers but low RLD in middle layer (f; representation by 204 PI 442572

Under mid-season stress, the test peanut genotypes differed significantly in top dry weight at the most drought-stressed date, top dry weight at harvest, pod yield and pod harvest index (PHI). Simple correlation coefficients between %RLD and pod yield, top dry weight and PHI calculated for three soil depths showed that, in the upper layer (0-30 cm), %RLD and pod yield was negative correlated (Fig. 2 a, d). No relationship was found between pod yield and %RLD in the middle layer (30-60 cm) (Fig. 2 b, e), indicating that %RLD in middle soil depth layer did not affect pod dry weight. For the lower layer (60-90 cm), %RLD was positively correlated with pod yield ($r = 0.42$ and 0.58 for the first and the second seasons, respectively) (Fig. 2 c, f), indicating that the %RLD in the lower layer is an important trait for pod yield under mid-season drought conditions. The relationships between %RLD and top weight at the most drought stressed date, top dry weight at harvest, and PHI were negative in the upper layer, but no relationship was found among these traits for the middle layer. For both seasons, %RLD in the lower layer was positively correlated with top weight at the most drought-stressed date, top dry weight at harvest, and PHI.

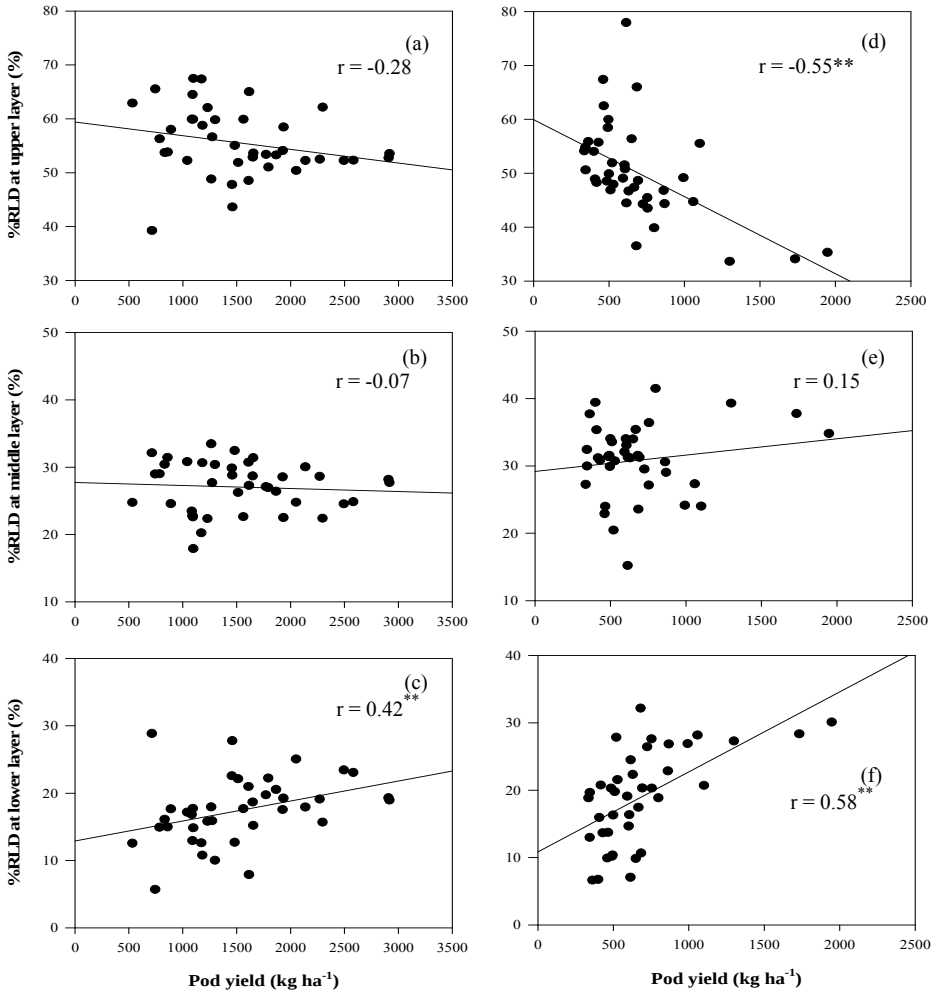


Fig. 2. Relationship between pod yield and % root length density (%RLD) for three layers, i.e., upper (a), middle (b) and lower (c) in the first season (December 2007-May 2008) and upper (d), middle (e) and lower (f) in the second season (November 2008- April 2009) at Khon Kaen University, Khon Kaen, Thailand. ** = significant at 1 % probability level.

Conclusion

Peanut genotypes were categorized into six combinative groups, based on %RLD in each of the three soil layers (upper, middle and lower layers). Percent RLD in the lower soil layer was positively correlated with yield traits, indicating its importance in determining yield under mid-season drought conditions, probably because of more sustained water extraction during drought. However, the %RLD in the middle soil layer did not affect yield under mid-season drought.

References

Coffelt, T.A., R.O. Hammons, W.D. Branch, R.W. Mozingo, P.M. Phipps, J.C. Smith, R.E. Lynch, C.S. Kvien, D.L. Ketring, D.M. Porter and A.C. Mixon.1985. Registration of Tifton-8 peanut germplasm. Crop Sci. 25: 203.

Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, T. Kesmala and A. Patanothai. 2008. Identification of peanut genotypes with high water use efficiency under drought stress conditions from peanut germplasm of diverse origins. Asian J. Plant Sci. 7: 628-638.

Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, K.J. Boote, G. Hoogenboom and A. Patanothai. 2011. Rooting traits of peanut genotypes with different yield responses to pre-flowering drought stress. Field Crops Res. 120: 262-270.

Matsui, T. and B.B. Singh.2003. Root characteristics in cowpea related to drought tolerance at the seedling stage. Exp. Agric. 39: 29-38.

Nigam, S.N., M.S. Basu and A.W. Cruickshank. 2003. Hybridization and description of the trait-based and empirical selection programs. *In*: Breeding for Drought-resistant Peanuts, Report of a workshop held at ICRISAT Centre, Andhra Pradesh, India, 25–27 February 2002. pp. 15–17. ACIAR, Canberra, Australia.

Nigam, S.N., S. Chandra, K. Rupa Sridevi, A. Manoha Bhukta, G.S. Reddy, R.C. NageswaraRao, G.C. Wright, P.V. Reddy, M.P. Deshmukh, R.K. Mathur, M.S. Basu, S. Vasundhara, P. Vindhiya Varman and A.K. Nagda. 2005. Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut. Ann. Appl. Biol. 146:433-439.

Pandey, P.K., W.A.T. Herrera, A.N. Villegas and J.W. Pendleton.1984. Drought response of grain legumes under irrigation gradient: III. Plant growth. Agron. J. 76: 557-560.

Robertson, W.K., L.C. Hammond, J.T. Johnson and K.J. Boote. 1980. Effects of plant water stress on root distribution of corn, soybeans, and peanuts in sandy soil. Agron. J. 72: 548-550.

Songsri, P., S. Jogloy, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook. 2008. Root distribution of drought resistant peanut genotypes in response to drought. J. Agron. Crop Sci. 19: 92-103.

Taiz, L. and E. Zeiger. 2006. Stress physiology. *In*: Taiz, L., Zeiger, E. (Eds.), Plant Physiology, 4th ed. Sinauer Associates, Inc., Sunderland, MA, pp. 671-681.

Turner, N.C. 1986. Adaptation to water deficits: a changing perspective. Aust. J. Plant Physiol. 13, 175–190.

Vorasoot, N., P. Songsri, C. Akkasaeng, S. Jogloy and A. Patanothai. 2003. Effect of water stress on yield and agronomic characters of peanut (*Arachis hypogaea* L.). Songklanakarin J. Sci. Technol. 25: 283-288.

Wright, G.C. and R.C. Nageswara Rao. 1994. Peanut water relations. *In*: Smartt, J. (Ed.), The Peanut Crop. Chapman & Hall, London, pp. 281–325.

Publication

Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, K.J. Boote, G. Hoogenboom and A. Patanothai. 2012.Classification of root distribution patterns and their contributions to yield in peanut genotypes under mid-season drought stress. Field Crops Res. 127: 181-190. (Impact factor = 2.608).

1.4 Nutrient uptake of peanut genotypes with different levels of drought tolerance under mid-season drought

D.T. Hoang, W. Kaewpradit, S. Jogloy, N. Vorasoot, and A. Patanothai

Peanut (*Arachis hypogaea* L.), like other agricultural crops, takes up essential nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), etc. to construct organic compounds of proteins, lipids, enzymes and other important chemicals. Most nutrients are taken up into the plant in forms of soluble inorganic fertilizers by the root system, and therefore, water stress reduces nutrient absorbability and nutrient uptake of plant (Fageria *et al.*, 2002). The reductions in nutrient uptake by drought during flowering, pegging, pod formation and pod-filling stage (Kulkarni *et al.*, 1988; Kolay 2008) were also reported in peanut. However, the studies conducted so far have been limited to one or two peanut genotypes.

Reduction in nutrient uptake as affected by drought can severely reduce plant growth and yield. Improvement of nutrient uptake, therefore, is necessary to maintain acceptable growth and yield under drought. Enrichment of tissue with Ca in groundnut and cowpea (Chari *et al.*, 1986), and with P in white clover (Singh and Sale 2000) by favorable additions improved tolerant ability to drought. Similarly, K supplement proved helpful in mitigating the adverse effects of water stress in peanut and sorghum (Umar 2006). Accumulation of mineral under drought condition might be an important trait of drought tolerance in tall fescue (Huang 2001), soybean (Samarah *et al.*, 2004) and chickpea (Gunes *et al.*, 2006). However, differential responses among species and genotypes for nutrient uptake under drought stress were observed (Garg 2003). It is still in doubt whether peanut genotypes with higher nutrient uptake under mid-season drought are more tolerant in term of productivity. A better understanding on peanut response to drought would be useful for determining appropriate breeding strategies for drought resistance in peanut.

Objective

The objective of this study was to investigate nutrients uptakes of peanut genotypes and the relationships between nutrients uptakes with biomass production, yield components and pod yield of peanut genotypes under mid-season drought.

Materials and Methods

The experiment was conducted during the dry seasons of 2011/12 and 2012/13. A split-plot in randomized complete block design with four replications was used. Main plots consisted of two water regimes W1 (well-watered at field capacity) and W2 (mid-season drought by withholding water from 30 to 60 days after planting (DAP)). Sub-plots comprised five peanut genotypes with different levels of drought tolerance. Tainan 9, KS 2 and KKU 60 are released cultivars in Thailand. Tainan 9 and KS 2 are susceptible to drought, whereas KKU 60 is rather tolerance to drought. ICGV 98305 is a drought tolerant line from the International Crops Research Institute for the Semi-Arid Tropics. Tifton 8 is a drought tolerant germplasm provided by the United States Department of

Agriculture. The data were recorded on contents of N, P, K, Ca and Mg in plant tissues, biomass production, yield components and pod yield at harvest

Results

Effect of mid-season drought on nutrient uptakes of peanut genotypes

Differences between years, water regimes and among peanut genotypes were significant for nutrient uptakes (Table 1). The interactions were significant between year and genotype, but not significant between water regime with year and genotype for all traits. The results indicated that various genotype was the main source of variations in uptake of nutrient elements. Genotypes with high potential for nutrient uptake under well-watered condition performed well under mid-season drought condition.

Table 1. Combined analysis of variance for nutrient uptake of observed elements of five peanut genotypes under well-watered and mid-season drought conditions during growing seasons of 2011/12 and 2012/13.

Source of variation	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
Year (Y)	18.69**	0.0270**	7.27**	11.30**	0.1125**
Reps. within year	0.02	0.0009	0.06	0.02	0.0005
Water regimes (W)	1.40**	0.0143**	1.08**	0.12*	0.0267*
Y*W	0.04	0.0002	0.01	0.06	0.0001
Error Y*R*W	0.07	0.0010	0.06	0.01	0.0020
Genotypes (G)	1.86**	0.0154**	0.97**	0.20**	0.0295**
Y*G	0.49**	0.0046**	0.52**	0.17**	0.0089*
W*G	0.02	0.0011	0.11	0.02	0.0032
Y*W*G	0.01	0.0001	0.05	0.02	0.0013
Error Y*R*W*G	0.10	0.0008	0.05	0.02	0.0015
CV (Y*R*W) %	12.6	16.1	18.5	18.7	15.6
CV (Y*R*W*G) %	15.8	14.4	17.0	23.2	13.3

*, **= significant at the 5% and 1% level, respectively.

Mid-season drought significantly reduced the uptakes of N, P and K ($P < 0.01$), Ca and Mg ($P < 0.05$) (Table 2). The differences in nutrient uptake among peanut genotypes were also considerable for all observed elements. In general, drought tolerant genotypes (DTG) (ICGV 98305, Tifton 8 and KKU 60) took up higher nutrient than did drought sensitive genotypes (DSG) (KS2 and Tainan 9), and the differences in N uptakes were much clearer in both years. ICGV 98305 had the highest uptakes, whilst Tainan 9 was the lowest.

In particularly, ICGV 98305, Tifton 8 and KKU 60 took up higher N than did KS 2 and Tainan 9 in both years. Similarly, DTG took up P higher than DSG in 2011/12. In 2012/13, ICGV 98305 and KKU 60 had higher P uptake than did both DSG, whereas Tifton 8 did higher than Tainan 9 only. All DTG took higher K than did Tainan 9 in both years, but only ICGV 98305 took up higher K than KS2 in 2011/12. ICGV 98305 was the highest genotype for Ca uptake, whilst other genotypes were rather similar. DTG had higher Mg uptake than DSG in 2011/12. However, in 2012/13 the Mg uptake of KKU 60 compared with those of KS 2 and Tainan 9 were not significant different.

Table 2. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) uptake of peanut genotypes across water regimes during growing seasons 2011/12 and 2012/13 (g plant^{-1}).

Source	N	P	K	Ca	Mg
Water regimes					
Well-watered	2.18 a	0.21 a	1.41 a	0.81 a	0.31 a
Mid-season drought	1.91 b	0.18 b	1.18 b	0.66 b	0.27 b
Genotypes			*Year 2011/12*		
ICGV 98305	3.04 a	0.27 a	2.30 a	1.27 a	0.43 a
Tifton 8	2.96 a	0.24 a	1.52 b	0.99 b	0.34 b
KKU 60	2.62 a	0.24 a	1.57 b	0.77 b	0.33 b
KS 2	2.08 b	0.17 b	1.39 bc	0.77 b	0.25 c
Tainan 9	1.92 b	0.16b	1.20c	0.85 b	0.28 c
Genotypes			*Year 2012/13*		
ICGV 98305	1.63 a	0.19 a	1.07 a	0.58 a	0.28 a
Tifton 8	1.71 a	0.18 ab	1.05 a	0.59 a	0.27 a
KKU 60	1.74 a	0.19 a	1.04 a	0.48 b	0.26 ab
KS 2	1.29 b	0.17 bc	0.97 ab	0.50 ab	0.23 b
Tainan 9	1.29 b	0.16 c	0.84 b	0.55 ab	0.23 b

Means followed by a lower case letter in a column are not significant different at the 5% level by LSD.

Relationships of nutrient uptakes with biomass production, yield components and pod yield

There were positive and significant correlations of all nutrient uptakes with biomass production and the number of pods per plant in both years. In fact, these correlations in 2011/12 were stronger than those in 2012/13. Moreover, the correlation coefficients among nutrient uptakes with biomass production were higher than with the number of pods per plant (Table 3).

Pod yield had significant correlations with uptakes of most nutrient elements except for Ca in 2011/12 ($r = 0.25$). The correlations between nutrient uptakes with pod yield were weaker in the first year, but somewhat stronger in the later year compared to correlations between nutrient uptakes with biomass production.

In general, 100-seed weight had positive correlations with uptakes of nutrient elements, whilst the correlations of the number of seeds per pod with nutrient uptakes were negative. However, the correlation coefficients were significant only between the number of seeds with N, P, Mg and Ca uptake in 2011/12 and N uptake in 2012/13; and between 100-seed weight with P uptake in 2011/12, and N, K and Ca uptake in 2012/13.

Table 3. Correlation between nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) uptake with biomass production, pod yield, number of seeds per pod, number of pods per plant and 100-seed weight, across water regimes during growing seasons 2011/12 and 2012/13 (n = 40).

Source	Dry season 2011/12					Dry season 2012/13				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
Biomass production	0.82**	0.88**	0.86**	0.78**	0.83**	0.49**	0.38*	0.50**	0.43**	0.32*
Pod yield	0.69**	0.73**	0.38*	0.25	0.48**	0.53**	0.45**	0.50**	0.43**	0.37**
No. of pod/plant	0.69**	0.77**	0.73**	0.63**	0.77**	0.38*	0.37*	0.37*	0.50**	0.36*
No. of seed/pod	-0.44**	-0.48**	-0.26	-0.35*	-0.55**	-0.41**	-0.31	-0.10	-0.21	-0.28
100-seed weight	0.20	0.33*	-0.04	0.13	0.16	0.42**	0.28	0.44**	0.33*	0.19

* and ** = significant at the 5% and 1% level, respectively.

Conclusions

Mid-season drought reduced nutrient uptakes in all peanut genotypes. Peanut genotypes with high potential for nutrient uptake under normal conditions performed well under mid-season drought conditions. Nutrient uptakes by peanut genotypes with higher levels of drought tolerance were higher than those with lower levels. Uptake of all nutrient elements contributed to biomass production, pod yield and the number of pods per plant. ICGV 98305 was the best genotype with the highest uptake of all observed nutrient elements, whereas Tifton 8 and KKU 60 were the good genotypes with high nutrient uptake across water regimes.

References

Chari, K.M., K. Gupta, T.G. Prasad, K.S.S. Sastry and M.U. Kumar. 1986. Enhancement of water stress by calcium treatment in groundnut and cowpea plants subjected to moisture stress. Plant Soil 91: 109-114.

Fageria, N.K., V.C. Baligar and R.B. Clark. 2002. Micronutrients in crop production. Adv. Agron. 77: 185-268.

Garg, B.K. 2003. Nutrient uptake and management under drought: nutrient-moisture interaction. Curr. Agr. 27: 1-8.

Gunes, A., N. Cicek, A. Inal, M. Alpaslan, F. Eraslan, E. Guneri and T. Guzelordu. 2006. Genotypic response of chickpea (*Cicer artietinum* L.) cultivars to drought stress implemented at pre- and post-anthesis stages and its relations with nutrient uptake and efficiency. Plant Soil Environ. 52: 368-376.

Huang, B. 2001. Nutrient accumulation and associated root characteristics in response to drought stress in Tall Fescue cultivars. Hort. Sci. 36: 148-152.

Kolay, A.K. 2008. Soil moisture stress and plant growth. In: Water and Crop Growth. pp. 43-48, Atlantic, New Delhi.

Kulkarni, J.H., V. Ravindra, V.K. Sojitra and D.M. Bhatt. 1988. Growth, nodulation and N uptake of groundnut (*Arachis hypogaea* L.) as influenced by water deficits stress at different phenophase. Oleagineus 43: 415-419.

Samarah, N., R. Mullen and S. Cianzio 2004. Size distribution and mineral nutrients of soybean seeds in response to drought stress. J Plant Nutr 27: 815-835.

Singh, D.K. and P.W.G. Sale. 2000. Growth and potential conductivity of white clover roots in dry soil with increasing phosphorus supply and defoliation frequency. Agron. J. 92: 868-874.

Umar, S. 2006. Alleviating adverse effects of water stress on yield of sorghum, mustard and groundnut by potassium application. Pak. J. Bot. 38: 1373-1380.

Publication

Dinh, H.T., W. Kaewpradit, S. Jogloy, N. Vorasoot and A. Patanothai. 2014. Nutrient uptake of peanut genotypes with different levels of drought tolerance under mid-season drought. Turk. J. Agric. For. 38: 495-505. (Impact factor = 0.914).

1.5 Rooting traits of peanut genotypes with different yield responses to terminal drought

R. Koolachart, S. Jogloy, N. Vorasoot, S. Wongkaew, C.C. Holbrook, N. Jongrungklang, T. Kesmala and A. Patanothai

Peanut (*Arachis hypogaea* L.) is a major legume in many developing countries and is widely cultivated in the semi-arid tropics (Latha *et al*., 2007). In areas with very low rainfall and uneven rain distribution, peanut is likely to suffer from drought that affects growth, development, pod yield and product quality. Drought during late growth stages not only affects peanut productivity, but also increases *A. flavus* infection and aflatoxin contamination (Diener *et al*., 1987; Girdthai *et al*., 2010). Yield reductions of 56-85% were recorded when peanut was exposed to drought at seed-filling stage (Del Rosario and Fajardo, 1988; Nageswara Rao *et al*., 1985), and yield reduction of 24 % was reported when the crop was subjected to drought at the end of the growing season (Boontang *et al*., 2010).

Access to sufficient irrigation can alleviate problems from drought. However, high investment is required and water is often insufficient because of competition for water with the industrial sector and urban consumption. When drought resistant varieties are available, the use of these varieties is a good and sustainable choice (Girdthai *et al*., 2010; Songsri *et al*., 2008). Breeding of peanut for resistance to late season drought requires information on morphological and physiological responses of peanut to drought and the mechanisms underlying the adaptability of the crop to minimize yield loss. Most studies have reported on the response of physio-morphological characters of above ground plant components (Girdthai *et al*., 2010; Puangbut *et al*., 2010), but there is limited information for root responses under drought conditions (Songsri *et al*., 2008).

Root traits associated with drought tolerance are important for identifying drought resistant mechanisms of plants. Root characteristics such as deep rooting, root length density (RLD) and root distribution have been identified as drought adaptive traits that can be used as selection criteria for drought resistance (Matsui and Singh, 2003; Taiz and Zeiger 2006; Turner 1986). Peanut genotypes with higher root length density in the deeper soil layers potentially have an enhanced drought tolerance and this could aid peanut genotypes to obtain higher pod yield and harvest index under long-term drought conditions (Songsri *et al*., 2008). There was a tendency of greater average RLD at the 30-90 cm soil profile in the group of genotypes that were subjected to pre-flowering drought, and RLD remained high even after re-watering, resulting in increased pod yield

(Jongrungklang *et al*., 2011). Large root system can maintain high plant water status and yield during drought stress (Rucker *et a*l., 1995), and the ability of a plant to change its root distribution in the deeper soil water was an important mechanism for drought avoidance (Benjamin and Nielsen 2006).

Root response to drought is another mechanism enhancing drought resistance as roots penetrate deeper into the drying soil to mine more water (Ludlow and Muchow 1990; Taiz and Zeiger 2006). Girdthai *et al*. (2010) observed that peanut genotypes were different in tolerance to late season drought possibly due to the differences in root responses of these peanut genotypes. If this is the case, root response to late season drought might be a factor contributing to higher yield under drought. However, this hypothesis has not been tested elsewhere.

Root responses at pre-flowering (Jongrungklang *et al*., 2011) at mid-season drought (Jongrungklang et al., 2012) and long-term drought (Songsri et al., 2008) were reported previously by our research project. Root response at the late period of growth stages can be an important mechanism to maintain high yield, and the character may be used as a selection tool for drought tolerance in peanut. The information on this trait is very scant in the literature and further investigations are required. As the continuation of previous studies, this study focuses on root response to terminal drought and will complete the responses of root for all drought conditions.

Objective

The objective of this study was to investigate the responses of root dry weight and %RLD of peanut genotypes having different yield responses to terminal drought stress and their relationships with biological and economic yield.

Materials and Methods

A field experiment was conducted at Khon Kaen University's Agronomy Farm in 2010/2011 and 2011/2012. The treatments were arranged in a split-plot design with four replications during the dry season. Two soil moisture levels [field capacity (FC) and 1/3 available water (1/3AW) at R7 growth stage through harvest] were assigned to main plots, and five peanut genotypes (ICGV 98308, ICGV 98324, ICGV 98348, Tainan 9 and Tifton 8) were assigned to sub-plots. Plot size was 5 x 5 m with spacing of 50 cm between rows and 20 cm between plants within a row. Rainout shelters were available if necessary.

Data for root dry weight, % root length density (% RLD) in deeper soil layer, stomatal conductance, water use efficiency (WUE), pod yield, biomass and harvest index (HI) were recorded at harvest.

Results

Drought significantly reduced biomass by 21 %, pod yield by 38 % and HI by 21 % for season 1 (Table 1). Significant differences among peanut genotypes were found for biomass, pod yield and HI under non-stress and stress conditions, therefore, the superior peanut genotypes for these traits under stress and non-stress conditions should be readily identified. ICGV98324 had the highest pod yield and HI under drought, whereas Tifton 8 was the best genotype for biomass. ICGV98348 had the highest drought tolerance index (DTI) for biomass, pod yield and HI.

Drought significantly reduced biomass by 29 %, pod yield by 42 % and HI by 18 % for season 2 (Table 2). Significant differences among peanut genotypes were found for biomass, pod yield and HI under non-stress and stress conditions. ICGV98324 was one of the highest genotypes for pod yield and HI under drought, whereas Tifton 8 was the best genotype for biomass. Although there were no statistically significant differences, ICGV 98348 gave the highest DTI for biomass and pod yield, whereas Tainan 9 and ICGV 98348 gave the highest DTI for HI.

ICGV 98324 and ICGV 98348 had the highest pod yield and HI, whereas Tifton 8 had the highest biomass under both stressed and non-stressed conditions and its pod yield under stressed conditions was also high in the trial in season 2 (Table 2).

Table 1. Biomass, pod yield, harvest index (HI) and drought tolerance index (DTI) at harvest stage of five peanut genotypes grown under field capacity (FC) and 1/3 available water (1/3 AW) in the dry seasons 2010/2011(season 1).

Genotypes	Biomass (kg ha^{-1})[1/]			Pod yield (kg ha^{-1})[1/]			HI[1/]		
	FC	1/3 AW	DTI[1/]	FC	1/3 AW	DTI	FC	1/3 AW	DTI
ICGV 98308	7387c	5587c	0.76	2354d	1238c	0.53	0.32bc	0.22b	0.69
ICGV 98324	10199b	7329b	0.72	4052a	2332a	0.57	0.40a	0.32a	0.81
ICGV 98348	7922c	7013b	0.89	2644c	2272a	0.87	0.34b	0.32a	0.96
Tainan 9	7421c	5638c	0.76	2024e	1006d	0.50	0.27d	0.18c	0.66
Tifton 8	11477a	9322a	0.81	3341b	2094b	0.63	0.29cd	0.22b	0.76
Mean	8881	978	0.79	2883	1788	0.62	0.32	0.25	0.79

Means within a column followed by the same letter are not significantly different (at $p < 0.05$) by DMRT

[1/] DTI = Drought tolerance index (stress (1/3 AW)/ non-stress (FC); more than 1 = increased, less than 1 = decreased)

Table 2. Biomass, pod yield,harvest index (HI) and drought tolerance index (DTI) at harvest stage of five peanut genotypes grown under field capacity (FC) and 1/3 available water (1/3 AW) in the dry seasons 2011/2012(season 2).

Genotypes	Biomass (kg ha^{-1})			Pod yield (kg ha^{-1})			HI		
	FC	1/3 AW	DTI[1/]	FC	1/3 AW	DTI	FC	1/3 AW	DTI
ICGV 98308	7142b	4508bc	0.63	1721c	973b	0.57	0.24c	0.22b	0.92
ICGV 98324	7924ab	5492b	0.69	3640a	1883a	0.52	0.47a	0.37a	0.79
ICGV 98348	6480b	5522b	0.85	2261b	1776a	0.79	0.35b	0.33ab	0.94
Tainan 9	6857b	4144c	0.60	1544c	933b	0.60	0.23c	0.22b	0.96
Tifton 8	9108a	6875a	0.75	3494a	1815a	0.52	0.39b	0.26ab	0.67
Mean	7502	5308	0.71	2532	1476	0.58	0.34	0.28	0.82

Means within a column followed by the same letter are not significantly different (at $p < 0.05$) by DMRT

[1/]DTI = Drought tolerance index (stress (1/3 AW)/ non-stress (FC); more than 1 = increased, less than 1 = decreased)

Significant differences among peanut genotypes in both seasons were found for root dry weight and % RLD under stressed and non-stressed conditions at harvest (Table 3). Most DTI values were higher than one. Most peanut genotypes were rather similar for root dry weight and % RLD under non-stressed conditions except for Tifton 8, which had the highest root dry weight under non-stressed conditions, but it had rather low % RLD under stressed conditions. In contrast to Tifton 8, other genotypes showed high % RLD under stressed conditions. In this study, drought increased %RLD at 30-90 cm soil layer for all peanut genotypes in season 2 when compared with non-stressed conditions and for most genotypes in season 1 except for Tifton 8 and ICGV 98308.

Table 3. Root dry weight and percent root length density (% RLD) in deeper soil layer (30–90 cm) at harvest stage of five peanut genotypes grown under field capacity (FC) and 1/3 available water (1/3 AW) in the dry seasons 2010/2011 (season 1)and 2011/2012(season 2).

Genotypes	Root dry weight (g plant^{-1})					
	season 1			season 2		
	FC	1/3 AW	DTI[1/]	FC	1/3 AW	DTI
ICGV 98308	5.84bc	6.26b	1.07	6.47	6.60b	1.02
ICGV 98324	5.16c	5.70b	1.10	3.76	5.29b	1.41
ICGV 98348	5.70c	5.09b	0.89	4.07	5.64b	1.39
Tainan 9	7.56b	5.56b	0.74	4.88	5.17b	1.06
Tifton 8	9.59a	9.35a	0.97	7.37	8.57a	1.16
Mean	6.70	6.39	0.96	5.31	6.26	1.18
Genotypes	%RLD					
	season 1			season 2		
	FC	1/3 AW	DTI	FC	1/3 AW	DTI
ICGV 98308	37.22ab	36.00a	0.98b	19.44b	36.09ab	1.86c
ICGV 98324	31.60b	36.60a	1.20b	29.39a	40.15a	1.37c
ICGV 98348	35.14b	39.10a	1.12b	28.73a	41.79a	1.45c
Tainan 9	20.92c	41.51a	2.01a	7.76c	32.44b	4.18a
Tifton 8	44.60a	18.45b	0.42c	9.89c	26.21c	2.65b
Mean	33.89	34.23	1.01	19.04	35.34	1.86

Means within a column followed by the same letter are not significantly different (at $p < 0.05$) by DMRT
% RLD = Percentage of root length density ((RLD 30to 90 cm/ RLD 30 to 90 cm +RLD 0 to 30 cm)x 100)
[1/] DTI = Drought tolerance index (stress (1/3 AW)/ non-stress (FC); more than 1 = increased, less than 1 = decreased)

Peanut genotypes were significantly different for stomatal conductance in season 2. Although the differences in stomatal conductance were not significant in season 1, the patterns were rather similar to those in season 2 (Table 4). Peanut genotypes were also significantly different for water use efficiency (WUE) under stressed conditions in season 1, and the ranking of peanut genotypes for this trait was in a similar pattern although the differences among peanut genotypes were not significant in season 2. Tifton 8, ICGV 98324 and ICGV 98348 had high stomatal conductance and WUE, whereas ICGV 98308 and Tainan 9 had low stomatal conductance and WUE in both seasons.

The relationships of root dry weight with biomass, pod yield, and HI under stress conditions for both seasons are presented in Figure 1. The relationship between root dry weight and biomass followed the same pattern in both seasons, showing that the relationships between the traits were dependent on genotypes, and Tifton 8 had the highest root dry weight and biomass (Figure 1a and d).

For the relationships between root dry weight and pod yield, Tifton 8 had high root dry weight and also had high pod yield under stress conditions in both seasons (Figure 1b and e). ICGV 98324 and ICGV 98348 did not have high root dry weight but had high pod yield, whereas Tainan 9 had low root dry weight and low pod yield. The patterns were similar in both seasons.

For the relationships between root dry weight and HI, Tifton 8 had high root dry weight and also had low HI in both seasons (Figure 1c and f). However, ICGV 98324 and ICGV 98348 did not have high root dry weight but also had high HI, whereas Tainan 9 had low root dry weight and low HI. The pattern was similar in both seasons.

For the relationships between % RLD and biomass, the genotypes with high % RLD did not necessarily have high biomass and *vice versa*, and Tifton 8 had the highest biomass and it also had the lowest % RLD (Figure 2a and d).

For the relationship between % RLD and pod yield, the patterns in both seasons were rather similar except for Tainan 9 (Figure 2b and e). Tifton 8 had a relatively high pod yield but had low % RLD, whereas ICGV 98324 and ICGV 98348 had high % RLD and high pod yield. ICGV 98308 and Tainan 9 had relatively high % RLD but had low pod yield in season 1, whereas Tainan 9 had relatively low % RLD and also had low pod yield in season 2.

For the relationships between % RLD and HI, the patterns in both seasons were similar except for Tainan 9 (Figure 2c and f). Tifton 8 had relatively low HI and had low % RLD, whereas ICGV 98324 and ICGV 98348 had high % RLD and high HI. ICGV 98308 and Tainan 9 had relatively high % RLD but had low HI in season 1, whereas Tainan 9 had relatively low % RLD and Tainan 9 had relatively low % RLD and also had low HI in season 2.

Table 4. Stomatal conductance and water use efficiency (WUE) at harvest stage of five peanut genotypes grown under 1/3 available water (1/3 AW) in the dry seasons 2010/2011 (season 1) and 2011/2012(season 2).

Genotypes	Stomatal conductance (cm s^{-1})[1/]		WUE (g l^{-1})[1/]	
	season 1	season 2	season 1	season 2
ICGV 98308	0.23	0.83 a	1.96 b	1.26
ICGV 98324	0.62	0.64 ab	2.62 a	1.62
ICGV 98348	0.54	1.19 a	2.51 a	1.59
Tainan 9	0.16	0.21 b	1.99 b	1.24
Tifton 8	0.47	0.98 a	2.71 a	1.71
Mean	0.40	0.77	2.36	1.48

[1/] = Means within a column followed by the same letter are not significantly different (at $p < 0.05$) by DMRT

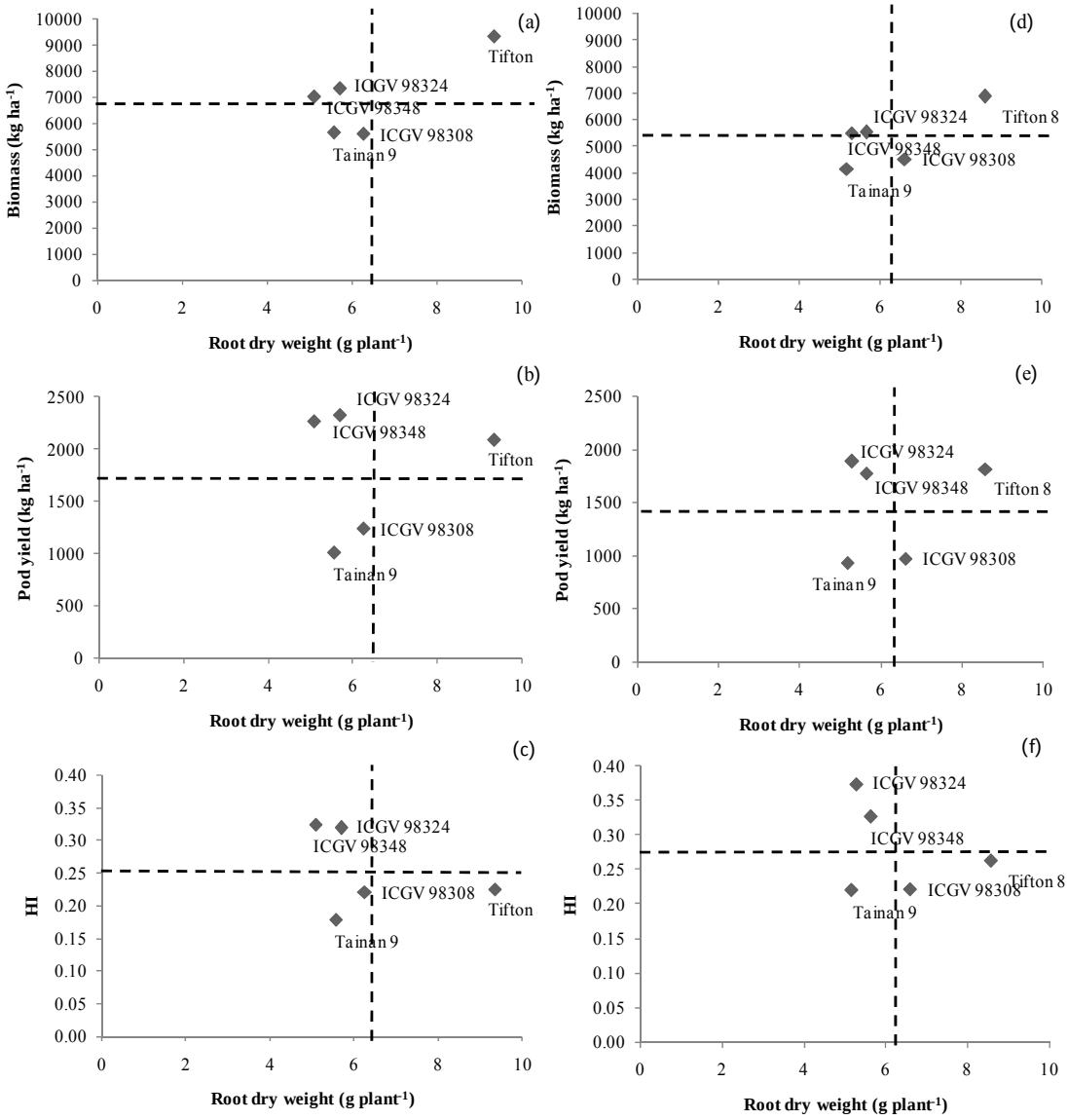


Fig. 1. Relationships between root dry weight and biomass (a), pod yield (b), HI (c) in 2010/2011 and relationships between root dry weight and biomass (d), pod yield (e) and HI (f) in 2011/2012 at harvest stage for five peanut genotypes grown under 1/3 available water (1/3 AW).

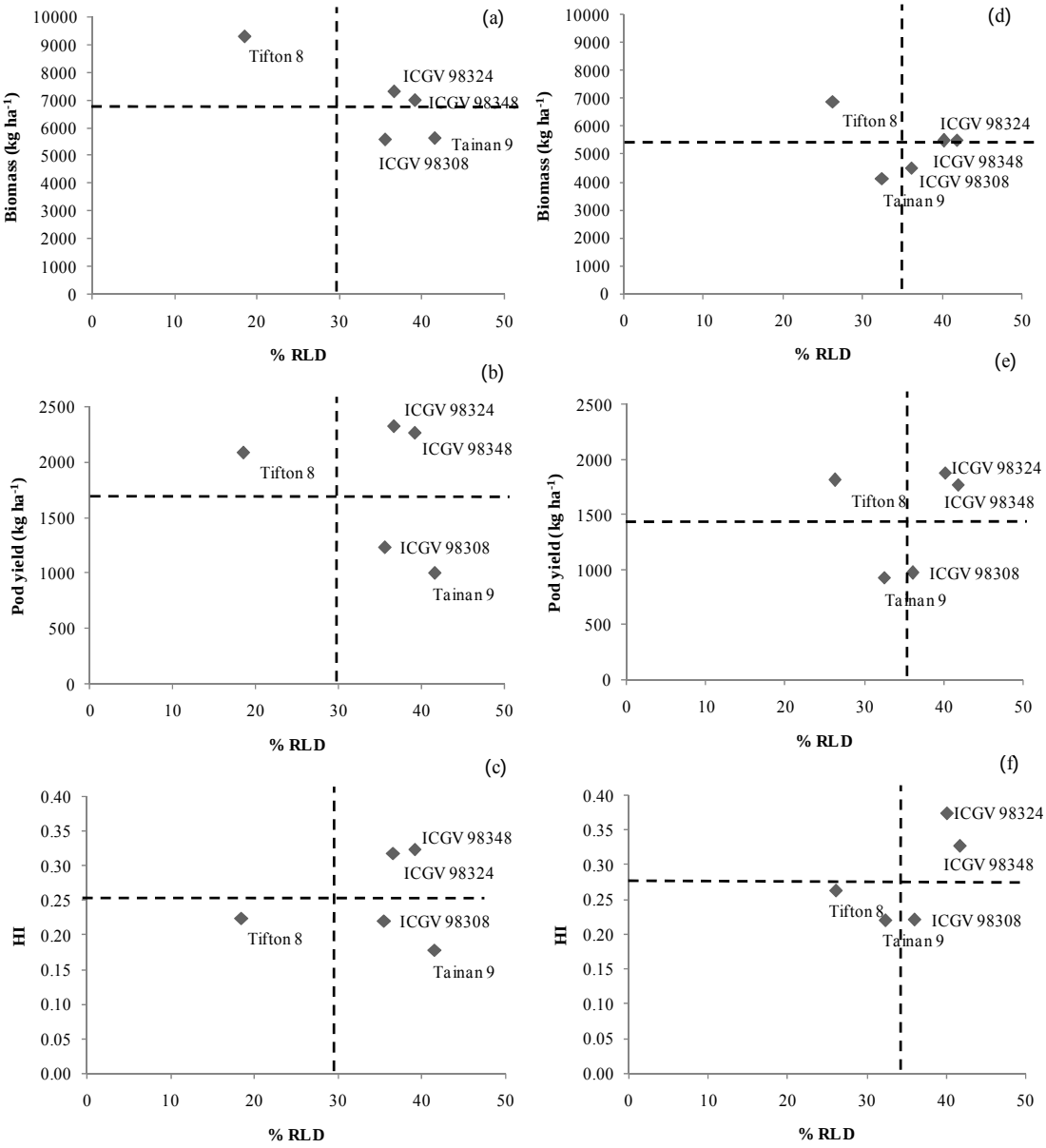


Fig. 2. Relationships between % RLD in deeper soil layer (30-90 cm) and biomass (a), pod yield (b), HI (c) in 2010/2011,and relationships between % RLD in deeper soil layer (30-90 cm) and biomass (d), pod yield (e) and HI (f)in 2011/2012 at harvest stagefor five peanut genotypes grown under 1/3 available water (1/3 AW).

Conclusion

Peanut genotypes may use different strategies to maintain pod yield under drought conditions. The genotypes having large root systems and high stomatal conductance, WUE and biomass could maintain pod yield under terminal drought. Peanut genotypes having high % RLD at deeper layers, stomatal conductance, WUE and HI could maintain pod yield under terminal drought. However, peanut genotypes with good deep root system were not always associated with high pod yield under terminal drought. This is because these genotypes had low stomatal conductance and high relative water content that supported survival under drought condition but did not contribute to pod yield. Some peanut genotypes did not respond to terminal drought for deep root system and pod yields of these peanut genotypes were also low. This study demonstrated that % RLD, stomatal conductance and WUE may be important traits related to pod yield. Breeding for maintaining yield under water limited conditions by deeper rooting may be successful for specific water limited conditions where water is available in deeper soil. The results indicated that % RLD at deeper layers may contribute to yield maintenance under terminal drought conditions, thus, % RLD could be useful as a selection criterion for yield under terminal drought conditions.

References

Benjamin, J.G. and D.C. Nielsen. 2006. Water deficit effects on root distribution of soybean field pea and chickpea. Field Crops Res. 97: 248–253.

Boontang, S., T. Girdthai, S. Jogloy, C. Akkasaeng, N. Vorasoot, A. Patanothai and N. Tantissuwichwong. 2010. Responses of released cultivars of peanut to terminal drought for traits related to drought tolerance. Asian J. Plant Sci. 9: 423-431.

Del Rosario, D.A. and F.F. Fajado. 1988. Morphophysiological responses of ten peanut (*Arachis hypogaea* L.) varieties to drought stress. Philipp. Agric. 71: 447–459.

Diener, U.L., R.J. Cole, T.H. Sanders, G.A. Payne, L.S. Lee and M.A. Klich. 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. Ann. Rev. Phytopathol. 25: 249-270.

Girdthai, T., S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew, C.C. Holbrook and A. Patanothai. 2010. Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought. Plant Breed. 129: 693-699.

Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, K.J. Boote, G. Hoogenboom and A. Patanothai. 2011. Rooting traits of peanut genotypes with different yield responses to pre-flowering drought stress. Field Crops Res.120: 262-270.

Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, K.J. Boote, G. Hoogenboomand A. Patanothai. 2012. Classification of root distribution patterns and their contributions to yield in peanut genotypes under mid-season drought stress. Field Crops Res. 127: 181-190.

Latha, P., P. Sudhakar, Y. Sreenivasulu, P.H. Naiduand P.V. Reddy. 2007. Relationship between total phenols and aflatoxin production of peanut genotypes under end-of-season drought conditions. Acta Physiol. Plant. 29: 563-566.

Ludlow, M.M. and R.C. Muchow. 1990. A critical evaluation of traits for improving crop yield in water limited environments. Adv. Agron. 43: 107-153.

Matsui, T. and B.B. Singh. 2003. Root characteristics in cowpea related to drought tolerance at the seedling stage. Expl. Agric. 39: 29-38.

Nageswara Rao, R.C., S. Singh, M.V.K. Sivakumar, K.L. Srivastava and J.H. Williams. 1985. Effect of water deficit at different growth phase of peanut. I. Yield response. Agron. J. 77: 782-786.

Puangbut, D., S. Jogloy, B. Toomsan, N. Vorasoot, C. Akkasaeng, T. Kesmala, C.N. Rachaputi, G.C. Wright and A. Patanothai. 2010. Physiological basis for genotypic variation in tolerance to and recovery from pre-flowering drought in peanut. J. Agron. Crop Sci. 196: 358-367.

Rucker, K.S., C.K. Kvien, C.C. Holbrook and J.E. Hook. 1995. Identification of peanut genotypes with improved drought avoidance traits. Peanut Sci. 22: 14-18.

Songsri, P., S. Jogloy, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook. 2008. Root distribution of drought-resistant peanut genotypes in response to drought. J.Agron.Crop Sci. 19: 92-103.

Taiz, L. and E. Zeiger. 2006. Stress physiology. In: Taiz, L. and E. Zeiger, editors, Plant physiology, Sinauer Associates, Inc, Sunderland, MA. pp. 671–678.

Turner, N.C. 1986. Adaptation to water deficits: A changing perspective. Aust. J. Plant Physiol. 13: 175-190.

Publication

Koolachart R., S. Jogloy, N. Vorasoot, S. Wongkaew, C.C. Holbrook, N. Jongrungklang, T. Kesmala and A.Patanothai. 2013. Rooting traits of peanut genotypes with different yield responses to terminal drought. Field Crops Res. 149: 366–378 (Impact factor 2013 = 2.608).

1.6 Nutrient uptakes and their contributions to yield in peanut genotypes with different levels of terminal drought resistance

W. Htoon, S. Jogloy, N. Vorasoot, B. Toomsan, W. Kaewpradit,
N. Puppala and A. Patanothai

Drought during the pod-filling phase of peanut is common and caused the greatest reduction in peanut pod yield (Ravindra *et al*., 1990). Girdthai *et al*. (2010) reported that terminal drought reduced pod yield up to 35% and reduced biomass by 21%.

More rapid progress in breeding foe drought resistance may be achieved by using physiological and morphological traits in selection (Nigam *et al*., 2005). Physiological traits such as SPAD chlorophyll meter reading (SCMR), specific leaf area (SLA) (Arunyanark *et al.*, 2009), biological N_2-fixation (Pimtrach *et al.*, 2008) and root length density (RLD) (Songsri *et al.*, 2008; Jongrungklang *et al.*, 2011) have been used as surrogate traits for selection. However, there is still a need to explore other mechanisms or traits conferring drought tolerance in peanut.

Drought is deleterious for plant growth, yield and mineral uptakes (Suther and Patel, 1992) and cultivars differ in their responses to environmental stress at different

growth stages (Garg 2003; Gunes *et al.*, 2006). Under water stressed conditions, there was a reduction in total nutrient uptake followed by reduction in concentrations of mineral nutrients in crop plants due to the decreased soil moisture (Gunes *et al.*, 2006). Fageria *et al*. (2002) indicated that drought stress may involve the uptakes of mineral elements in plant tissues by affecting root growth, nutrient mobility in soil and nutrient uptakes. Samarah *et al*. (2004) stated that nutrient uptakes in soybean under drought stress might play an important role in drought tolerance mechanisms. However, there is no information on the responses of peanut to terminal drought for nutrient uptakes. This information should provide a better understanding on how peanut genotypes could achieve high yield under terminal drought and could have important implications for breeding for drought resistance in peanut.

Objective

The objectives of this study were to characterize the effect of terminal drought on the nutrient uptakes and to investigate the genotypic variability and their interaction to terminal drought for nutrient uptakes.

Materials and Methods

Field experiments were conducted at the Field Crop Research Station of Khon Kaen University, Thailand, during the October 2010 to January 2011 and October 2011 to January 2012. A split-plot in a randomized complete block design with four replicates was used. Plot size was 5 × 5 m with 50 cm spacing of between rows and 20 cm between plants. Main-plot treatments were a well-watered condition and a terminal drought condition from R7 growth stage (Boote, 1982) to harvest. For the well-watered treatment, water was applied regularly since seed sowing to harvest. Similarly, terminal drought treatment sub-plots were regularly supplied with water before R7 growth stage, but afterwards, irrigation was withheld until reaching 1/3 available water (1/3 AW) of soil moisture content at R7 growth stage of each peanut genotype, and soil moisture was maintained at that level until harvest. The water stressed period in this experiment was defined as terminal drought (Girdthai *et al.*, 2010). Sub-plot treatments consisted of 6 peanut genotypes which are ICGV 98308, ICGV 98324, ICGV 98348, Tainan 9, Tifton-8 and a non-nodulating line which was used as a check variety for N_2-fixation. Data were recorded for uptakes of N, P, K, Ca, Mg, biomass (BM) and pod yields (PY) and the ratio of nutrient uptake under stress condition (RNS).

Results

In general, the results clearly showed that terminal drought stress reduced the uptake of N, P, K, Ca and Mg in both years. Peanut genotypes considerably differed with respect to uptakes of N, P, K, Ca and Mg at both well-watered and terminal drought conditions. ICGV 98308 had the highest uptake of N, K, Ca and Mg under the well-watered condition in both years. But under the terminal drought condition, nutrient uptakes of this genotype were reduced. ICGV 98308 was not the best genotype for uptake of P under both water regimes in both years (Table 1 and 2).

Tainan 9 had low uptakes of N, P, K, Ca and Mg under both well-watered and terminal drought conditions in both years. Although Tifton 8 had low uptake of N, P, K and Mg under both conditions, it showed the highest uptake of Ca under terminal drought in both years. (Tables 1 and 2).

The non-nodulating line was the best for the uptake of P, K, Ca and Mg under the well-watered condition, and also was the best for the uptake of P, Ca and Mg except K under terminal drought in 2010/2011 (Table 1 and 2). However, in 2011/2012, it had the highest uptake of only P and had high uptakes of K and Mg under the drought condition. This line performed poorly in the uptake of soil N not only under the well-watered condition but also under the terminal drought condition in both years.

The drought resistant genotype ICGV 98324 had the highest uptake of Ca and also had high K uptake under the well-watered condition in the first year, whereas the uptakes of other nutrients such as N, P and Mg were relatively low. But in 2011/2012, this line was high only in the uptake of N under the well-watered treatment. In this study, like other genotypes, nutrient uptakes of ICGV 98324 were decreased by terminal drought, but under this stress condition, it performed consistently well for all nutrient uptakes even though it was not the best genotype under well-watered condition. Similar performances were observed for IGCV 98348 which showed high uptakes of N, P, K, Ca and Mg under drought in both years compared to nutrient uptakes of other genotypes.

Effects of water stress on ratio of nutrient uptake under stress conditions (RNS)

In 2010/2011, ICGV 98348 had high RNS for P, K, Ca and Mg, being 0.82, 0.97, 0.99 and 0.90, respectively (Table 1 and 2). This genotype showed good performance for N uptake under terminal drought, with the N-RNS being 1.06. In second year, ICGV 98348 had the highest K-RNS (0.96) and also had good performances for uptakes of N, P, Ca and Mg under terminal drought, with the RNS for N, P, Ca and Mg being 1.28, 1.06, 1.33 and 1.08, respectively. The N-RNS of ICGV 98324 was comparatively high, i.e. 0.94 and 0.84 in 2010/2011 and 2011/2012, respectively. This line consistently had high rank for RNS of P, K, Ca and Mg in both years. In the first year, RNS for N, Ca and Mg of the non-nodulating line (non-nod) were relatively high, but they decreased in the second year. However, the non-nod genotype had medium RNS for P and K in both years. The RNS for K and Ca of Tifton 8 were high in 2010/2011, i.e. 0.85 and 0.78, respectively, but only Ca RNS was still high (0.85) in 2011-2012. This line had medium RNS for N, P and Mg in both years, as did ICGV 98308. Tainan 9 gave the lowest RNS for all nutrients in both years.

Correlations between uptakes of N, P, K, Ca, Mg and biomass (BM) and pod yield (PY)

Positive correlations between N, P, K, Ca, Mg uptakes and BM were observed both under well-watered and drought conditions across years ($P<0.01$) (Table 3). Similarly, there were significant correlations between uptakes of N, K, Ca, Mg and PY under both treatments. Uptake of P and PY was significantly correlated under well-watered condition ($r = 0.35$, $P<0.05$) but was not correlated under terminal drought. However, the correlation coefficients between N, K Ca and Mg uptakes and BM were higher in the well-watered treatment than in the terminal drought condition. Interestingly, under water stress, correlations between uptakes of N, K and PY were high, but correlations between uptakes of Ca, Mg and PY were lower.

Table 1. Effects of terminal drought stress on N, P and K uptakes of 6 peanut genotypes in 2010/2011and 2011/2012.

Genotype	N g plant-^{1}			P g plant^{-1}			K g plant^{-1}		
	Well-water	Drought	N- RNS	Well-water	Drought	P -RNS	Well-water	Drought	K-RNS
2010/2011									
98308	3.70 a	2.3 ab	0.63c	0.35b	0.21bc	0.61b	1.30a	0.83b	0.64b
98324	2.77b	2.61a	0.94a	0.27c	0.22b	0.83a	1.17ab	0.84ab	0.71b
98348	2.46b	2.62a	1.06a	0.27c	0.22b	0.82a	1.01c	0.98a	0.97a
Tainan 9	2.60b	1.53c	0.59c	0.27c	0.15d	0.58b	1.05bc	0.59c	0.56 b
Tifton 8	2.65b	2.01b	0.76b	0.27c	0.19cd	0.69a	0.86d	0.73bc	0.85ab
Non-nod	1.75c	1.41c	0.81b	0.44a	0.32a	0.73ab	1.19ab	0.82b	0.69b
Mean	2.66	2.09	0.80	0.31	0.22	0.71	1.10	0.80	0.74
2011/2012									
98308	2.36a	1.34b	0.57c	0.25b	0.14b	0.54b	1.94a	0.77bc	0.40c
98324	1.96ab	1.64a	0.84b	0.19c	0.14b	0.73b	1.42b	1.01a	0.71b
98348	1.38cd	1.77a	1.28a	0.13d	0.14b	1.06a	0.97c	0.93a	0.96a
Tainan 9	1.77bc	0.91c	0.52c	0.18cd	0.10c	0.53b	1.30b	0.62d	0.48c
Tifton 8	1.74bc	1.25b	0.72bc	0.17cd	0.09c	0.56b	1.31b	0.70cd	0.54bc
Non-nod	1.18d	0.87c	0.74bc	0.42a	0.21a	0.50b	1.46b	0.90ab	0.62b
Mean	1.73	1.30	0.78	0.22	0.14	0.65	1.40	0.82	0.62

Different letters adjacent to data in the same column show significant at $P < 0.05$ by Duncan's multiple range test.

Table 2. Effect ofs terminal drought stress on Ca and Mg uptakes of 6 peanut genotypes in 2010/2011and 2011/2012.

Genotype	Ca (g plant^{-1})						Mg (g plant^{-1})					
	Well-water		Drought		Ca-RNS		Well-water		Drought		Mg - RNS	
2010/2011												
98308	1.82	a	1.10	b	0.60	c	0.57	a	0.38	ab	0.66	b
98324	1.81	a	1.34	a	0.74	b	0.47	b	0.43	a	0.92	a
98348	1.38	c	1.37	a	0.99	a	0.37	c	0.33	bc	0.90	a
Tainan 9	1.53	bc	1.00	b	0.65	c	0.39	c	0.26	d	0.68	b
Tifton 8	1.80	a	1.40	a	0.78	ab	0.46	b	0.31	cd	0.66	b
Non-nod	1.76	ab	1.46	a	0.83	ab	0.51	ab	0.41	a	0.81	ab
Mean	1.68		1.28		0.77		0.46		0.35		0.77	
2011/2012												
98308	1.56	a	0.70	c	0.45	d	0.41	a	0.21	b	0.52	b
98324	1.27	b	0.89	a b	0.70	c	0.33	b	0.27	a	0.81	b
98348	0.76	c	1.01	a	1.33	a	0.21	c	0.23	ab	1.08	a
Tainan 9	1.12	b	0.70	c	0.62	cd	0.27	bc	0.17	c	0.62	b
Tifton 8	1.24	b	1.05	a	0.85	b	0.28	b	0.17	c	0.59	b
Non-nod	1.25	b	0.83	b c	0.66	cd	0.33	b	0.23	ab	0.71	b
Mean	1.20		0.86		0.77		0.31		0.21		0.72	

Different letters adjacent to data in the same column show significant at $P < 0.05$ by Duncan's multiple range test.

Table 3. Correlation coefficients between uptakes of N, P, K, Ca, Mg and biomass (BM) and pod yield (PY) at well-water and terminal drought.

Yield Yield	Nutrient uptake mg plant^{-1}				
	N	P	K	Ca	Mg
Well-water					
BM mg plant^{-1}	0.61**	0.52**	0.95**	0.94**	0.95**
PY mg plant^{-1}	0.67**	0.35*	0.75**	0.70**	0.68**
Terminal Drought					
BM mg plant^{-1}	0.50**	0.70**	0.90**	0.70**	0.92**
PY mg plant^{-1}	0.86**	0.26	0.84**	0.46*	0.59**

*, ** = significant at $P< 0.05$ and $P< 0.01$, respectively

Conclusions

Terminal drought reduced the uptakes of nutrients but the responses of peanut genotypes to nutrient uptakes under terminal drought varied based on their drought resistance levels. The drought resistant genotypes ICGV 98324 and ICGV 98348 were the best for the nutrients uptakes and RNS. Drought tolerant peanut genotypes could maintain high nutrient uptakes across water regimes, and their nutrient uptakes were well correlated with biomass and pod yield. Thus, nutrient uptakes and RNS could be new surrogate traits for selection for terminal drought tolerance in peanut.

References

Arunyanark, A., S. Jogloy, C. Akkasaeng, N. Vorasoot, T. Kesmala, R.C. NageswaraRao G.C. Wright and A. Patanothai. 2009. Association between aflatoxin contamination and drought tolerance traits in peanut. Field Crops Res. 114: 14-22

Boote, K.J. 1982. Growth stage of peanut (*Arachis hypogaea* L.). Peanut Sci. 9: 35-40.

Fageria, N.K., V.C. Baligar and R.B. Clark. 2002. Micronutrients in crop production. Adv. Agron. 77: 185-268.

Garg, B.K. 2003. Nutrient uptake and management under drought: nutrient–moisture interaction. Curr. Agric. 27: 1–8.

Girdthai, T., S. Jogloy, N.Vorasoot, C. Akkasaen, S. Wongkaew, C.C. Holbrook and A. Patanothai. 2010. Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought. Plant Breed. 129: 693-699.

Gunes, A., N. Cicek, A. Inal, M. Alpaslan, F. Eraslan, E. Guneri and T. Guzelordu. 2006. Genotypic response of chickpea (*Cicer arietinum* L.) cultivars to drought stress implemented at pre- and post- anthesis stages and its relations with nutrient uptake and efficiency. Plant Soil. Environ. 52: 368-376.

Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, K.T. Boote, G. Hoogenboom and A. Patanothai. 2011. Rooting traits of peanut genotypes with different yield responses to pre-flowering drought stress. Field Crops Res. 120: 262-270.

Nigam, S.N., S. Chandra, R.K.Sridevi, M.Bhukta, A.G.S. Reddy, R.C. Nageswara Rao, G.C. Wright, P.V. Reddy, M.P. Deshmukh, R.K. Mathur, M.S. Basu, S.Vasundhara, V.P. Varman and A.K. Nagda. 2005. Efficiency of physiological trait–based and empirical selection approaches for drought tolerance in groundnut. Ann. App. Biol. 146: 433-439.

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 N_2-fixation in eleven peanut (*Arachis hypogaea* L.) genotypes differing in degrees of resistance to drought. Asian J. Plant Sci. 7: 334-342.

Ravindra, V., P.C. Nautiyal and Y.C. Joshi. 1990. Physiological analysis of drought resistance and yield in groundnut (*Arachis hypogaea* L). Trop. Agric. Trinidad 67: 290-296.

Samarah, N., R. Mullen and S. Cianzio. 2004. Size distribution and mineral nutrients of soybean seeds in response to drought stress. J. Plant Nutr. 27: 815–835.

Songsri, P., S. Jogloy, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook. 2008. Root distribution of drought-resistant peanut genotypes in response to drought. J Agron. Crop Sci. 194: 92-103

Suther, D.M., M.S. Patel. 1992. Yield and nutrient absorption by groundnut and iron availability in soil as influenced by lime and soil water. J Indian Soc. Soil Sci. 40: 594-596

Publication

Htoon,W., S. Jogloy, N. Vorasoot, B. Toomsan, W. Kaewpradit, N. Puppala, and A. Patanothai. 2014. Nutrient uptakes and their contributions to yield in peanut genotypes with different levels of terminal drought resistance. Turk. J. Aric. For. 38: 781-791. (Impact factor 0.914)

1.7 Responses of peanut (*Arachis hypogaea* L.) genotypes to N_2-fixation under terminal drought and their contributions to peanut yield

W. Htoon, W. Kaewpradit, S. Jogloy, N. Vorasoot, B. Toomsan,
C. Akkasaeng, N. Puppala and A. Patanothai

Duration and intensity of drought and the growth stage at which the stress occurs have large effects on growth and productivity. Drought occurring during pre-flowering stage has a small effect on yield or, in some cases, was found to increase yield (Puangbut *et al.*, 2009) and drought at pod setting can reduce 15 to 88 % of yield (Vorasoot *et al.*, 2003). Drought resistance is a complex trait, the expression of which depends on action and interaction of different morphological and physiological traits. The direct use of pod yield as drought resistance trait had been limited by high resource investments and poor repeatability of the results due to large G x E (genotype x environment) interactions resulting in slow breeding progress (Araus *et al.*, 2002).

Improvement of nitrogen fixation (NF) under drought conditions is a promising strategy that would lead to yield improvement (Pimratch *et al.*, 2008). However, NF and its related traits such as biomass, nodule number, and nodule dry weight in peanut were

also affected by drought. The reduction of NF might vary according to the degree of stress, the period of stress and the stage of crop development (Giller 2001). Under drought stress conditions, NF is greatly reduced, leading to low N accumulation, dry matter production, and yield (Desilva *et al.*, 1996). Peanut genotypes were also a source of variation in NF traits. Unfortunately, information is still lacking on the responses of peanut genotypes to NF under terminal drought. Although NF attains a peak during the pod-filling stage and declines at maturity under normal condition (Nambiar and Dart, 1983), it is anticipated that the response and contribution of NF might play an important role in peanut productivity under a terminal drought condition.

Objectives

The objectives of this study were to characterize the effect of terminal drought on NF, to examine whether there were any major differences among peanut genotypes, and to investigate the contribution of NF to yield under terminal drought.

Materials and Methods

A field experiment was conducted during October 2010 to January 2011 and repeated during October 2011 to January 2012) using a split plot design with four replicates. Two water regimes (well-watered and terminal drought, i.e., 1/3 available water at R7 growth stage until final harvest) were assigned to main-plots, and five peanut genotypes were assigned to sub-plots. Three genotypes (i.e. ICGV 98308, ICGV 98324 and ICGV 98348) are drought resistant lines with different drought tolerance index (DTI). Tifton 8 is the highest biomass production line under terminal drought and low DTI. Tainan 9 performed poorly for total biomass and pod yield under terminal drought and had the highest reduction in total biomass.

Observation on relative water content (RWC) were recorded at the day irrigation was withdrawn, at R7 growth stage and at harvest. Nodule dry weight, biomass, pod yield, nitrogen fixation (NF) and percentage of reduction were measured at harvest.

Results

Significant differences were found between water regimes (W) for nitrogen fixation (NF) and nodule dry weight (NDW) ($P \leq 0.01$). Genotypic differences in NF responses to terminal drought were also observed ($P \leq 0.01$), indicating a genetic control for this trait (Table 1). The year effects (Y) were also significant for both NF and NDW ($P \leq 0.01$), but they were relatively low compared to W and G main effects. The W x G interaction effects were significant for both traits ($P \leq 0.01$).

The same RWC values were observed on the last day of irrigation in both years, but they were significantly lower in the stressed treatment than in the well-watered treatment at R7 and harvest in both years (Fig. 1). The highest RWC was observed at R7 under well-watered treatment in both years, followed by RWC of the well-watered treatment on the last day of irrigation and at harvest.

The results clearly showed that terminal drought reduced NF in every genotype (Table 2). ICGV 98324 maintained a high NF under terminal drought and had a relatively low reduction (13%). Tainan 9 had the lowest reduction in NF (9%) because it had lower NF (0.55 g N plant^{-1}). NF of Tifton 8 at the well-watered condition was 0.60 g N plant^{-1} and decreased to 0.45 g N plant^{-1} under terminal drought. ICGV 98308 had a 58%

reduction of the NF under terminal drought, although it was the highest (1.22 g N plant^{-1}) under the well-watered condition. ICGV 98348 also showed high NF reduction (74%).

Table 1. Mean square from the combined analyses of variance for fixed N_2 (NF) and nodule dry weight (NDW) of five peanut genotypes at final harvest grown under well-watered and terminal drought in the dry seasons of 2010-2011 and 2011-2012.

Source of variation	df	NF	NDW
Year (Y)	1	121680**	0.0180**
Reps within year (Y*R)	6	7046	0.0011
Water regimes (W)	1	2386353**	0.9176**
Y*W	1	6611	0.0018
Error Y*R*W	6	11351	0.0034
Genotypes (G)	4	371520**	0.2981**
Y*G	4	8121	0.0013
W*G	4	439822**	0.0146**
Y*W*G	4	4229	0.0022
Error Y*R*W*G	48	4209	0.0011

*, ** = significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. df = degrees of freedom. Well-watered = full irrigation since sowing until final harvest. Terminal drought = 1/3 AW at R7 until final harvest.

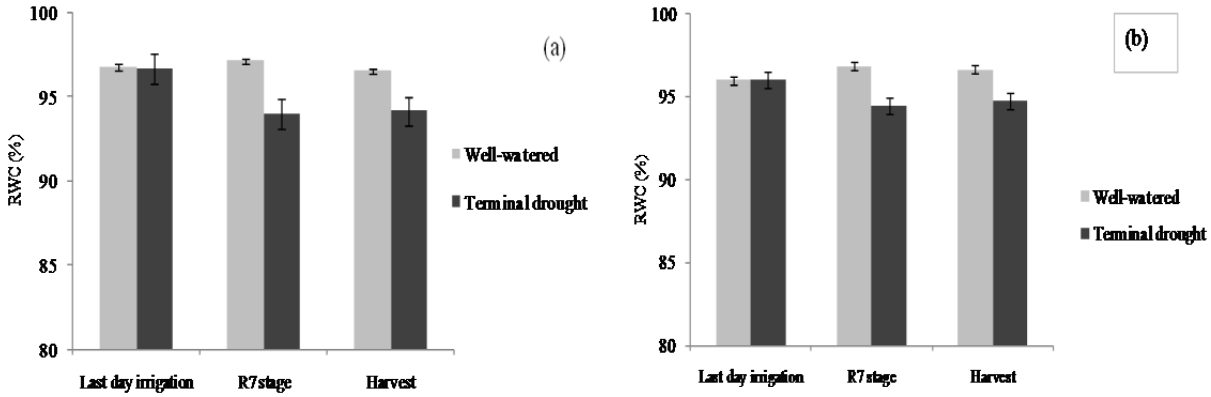


Fig. 1. Relative water content (RWC) on the last day of irrigation, at R7 stage and at harvest of five peanut genotypes grown under well-watered and terminal drought in (a) 2010/2011 and (b) 2011/2012.

*****Well-watered = full irrigation since sowing until final harvest. Terminal drought = 1/3 AW at R7 until final harvest.

NDW of all genotypes were reduced by terminal drought (Table 2). ICGV 98308 had the highest NDW under well-watered and terminal drought conditions. ICGV 98324 also had high NDW under both well-watered and terminal drought conditions. ICGV 98348 had medium NDW under both treatments. Tainan 9 and Tifton 8 showed the lowest NDW under both the well-watered and the terminal drought conditions.

NDW was significantly correlated to BM under both water regimes (Table 3). The correlations between NF and PY and between NDW and PY were significant only under the well-watered condition.

Table 2. Effects of terminal drought stress on fixed N_2 (NF) and nodule dry weight (NDW) and their reduction (%) of five peanut genotypes at final harvest grown under well-watered and terminal drought conditions, 2010-2011 and 2011-2012.

	NF (g N plant^{-1})			NDW (g plant^{-1})		
Genotypes	Well-watered	Drought	Reduction (%)	Well-watered	Drought	Reduction (%)
ICGV 98308	1.22 a	0.51 b	58 b	0.84 a	0.58 a	31 b
ICGV 98324	0.82 b	0.71 a	13 cd	0.66 b	0.48 b	27 b
ICGV 98348	0.94 b	0.24 c	74 a	0.59 c	0.36 c	39 ab
Tainan 9	0.55 c	0.50 b	9 d	0.55 c	0.38 c	31 b
Tifton 8	0.60 c	0.45 b	25 c	0.45 d	0.23 d	49 a
Mean	0.83	0.48	42	0.62	0.41	34

Different letters adjacent to data in the same column show significance at $P \leq 0.05$ by Duncan's multiple range test.
Well-watered = full irrigation since sowing until final harvest.
Terminal drought = 1/3 AW at R7 until final harvest.

Table 3. Correlation coefficients between fixed N_2 (NF), nodule dry weight (NDW), biomass (BM) and pod yield (PY) at well-watered and terminal drought treatments, 2010-2011 and 2011-2012.

Traits	BM	PY
Well-watered condition		
NF	0.80**	0.57*
NDW	0.79**	0.51*
Terminal drought		
NF	0.47*	0.10
NDW	0.61**	0.22

*, ** = significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. Well-watered = full irrigation since sowing until final harvest.
Terminal drought = 1/3 AW at R7 until final harvest.

Conclusion

Terminal drought during pod-filling stage until harvest greatly reduced the NF and NDW in peanut, but peanut genotypes did differ in NF and NDW under terminal drought condition. Drought tolerant genotypes ICGV 98308 and ICGV 98324 had high NF and NDW across water regimes. During terminal drought period, fixed N_2 contributed to biological yields only, but did not contribute to economic yields. Since the correlation coefficients of NF to economic yields were low under terminal drought, the ability to fix high N_2 under non-stress conditions could aid peanut genotypes in maintaining high yield under water-limited conditions.

References

Araus, J.L., G.A. Slafer, M.P. Reynolds and C. Royo. 2002. Plant breeding and drought in C3 cereals: what should we breed for? Ann. Bot.89: 925-940.

Desilva, M., L.C. Purcell and C.A. King. 1996. Soybean petiole ureide response to water deficits and decreased transpiration. Crop Sci.36: 611-616.

Giller, K.E. 2001. Nitrogen fixation in tropical cropping systems. 2nd edition. p. 423. CAB International, Wallingford.

Nambiar, P.T.C. and P.J. Dart. 1983. Factors influencing nitrogenase activity (acetylene reduction) by root nodules of groundnut, *Arachis hypogaea* L. Peanut Sci.10; 26-29.

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 N_2-fixation in eleven peanut (*Arachis hypogaea* L.) genotypes differing in degrees of resistance to drought. Asian J. Plant Sci.7: 334-342.

Puangbut, D., S. Jogloy, N. Vorasoot, C. Akkasaeng,T. Kesmala and A. Patanothai. 2009. Variability in yield response of peanut (*Arachis hypogaea* L.) genotypes under early season drought. Asian J. Plant Sci.8: 254-264.

Vorasoot, N., P. Songsri, C. Akkasaeng, S. Jogloy and A. Patanothai. 2003. Effect of water stress on yield and agronomic characters of peanut (*Arachis hypogaea* L.). Songklanakarin J. Sci. Technol.25: 283-288.

Publication

Htoon, W., W. Kaewpradit, S. Jogloy, N. Vorasoot, B. Toomsan, C. Akkasaeng, N. Puppala and A. Patanothai. 2013. Responses of peanut (*Arachis hypogaea* L.) genotypes to N_2fixation under terminal drought and their contributions to peanut yield. SABRAO J. Breed. Genet. 45: 296-310. (Impact factor 2010 = 0.227).

1.8 Relationship between root traits, nutrient uptake and nitrogen fixation in peanut under terminal drought

W. Htoon, W. Kaewpradit, S. Jogloy, N. Vorasoot, B. Toomsan,
C. Akkasaeng, N. Puppala and A. Patanothai

Breeding for drought resistance has been an important strategy for alleviating yield reduction under drought condition. While direct selection for pod yield under drought conditions could be effective, the limitations of this approach were the large genotype x environment (G x E) interaction and low heritability of such trait resulting in slow breeding progress.

Rooting depth, root distribution, root mass and root length density (RLD) have been identified as drought avoidance traits (Taiz and Zeiger, 2006). Root development is fundamentally involved in the response to many plant stresses, particularly drought and mineral deficiency (Maiti *et al.*, 2002), and drought resistance might be enhanced by improvements in soil water extraction capability (Wright and Nageswara Rao, 1994). Water stress affected root growth, nutrient mobility in the soil and nutrient uptake

(Fageria *et al.,* 2002). However, the mechanisms underlying the responses of root growth and nutrient uptake to drought stress are not well understood. Especially, information is lacking on the contribution of root traits to nutrient uptake during terminal drought conditions, which occurred at sensitive growth stages.

Moreover, drought affected nodulation, nodule growth and weight, as well as N_2-fixing activity in peanut (Pimratch *et al.*, 2008). The deep rooted legumes exploiting moisture in the lower soil layers could continue N_2 fixing when the soil is drying. Effective nodules are generally located with a higher density in tap roots than in lateral roots (Hungria and Bohrer 2000). Therefore, both lateral and vertical root growths play an important role in nodule growth and NF in peanut. Root traits and NF were found to have the potential to be drought tolerance indicators for peanut (Htoon *et al.*, 2009). Unfortunately, the relationships between root traits, nutrient uptake and NF under both well-watered and drought stress conditions are still in question.

Objective

The objective of this study was to investigate the relationships between root traits, nutrient uptake and NF under terminal drought.

Materials and Methods

A field experiment was conducted during October 2010 to January 2011 and repeated during October 2011 to January 2012, using a split-plot design with four replications. Five peanut genotypes (ICGV 98308, ICGV 98324, ICGV 98348, Tainan 9 and Tifton 8) were assigned to main-plots and two water regimes [well-watered condition and terminal drought (1/3 available water, 1/3 AW)] were assigned to sub-plots. For the well-watered treatment, water was applied daily from planting to harvest. For the terminal drought plots, irrigation was withheld and soil moisture was allowed to decrease gradually to meet the predetermined drought condition (1/3 AW) at the R7 growth stage of each genotype. From then on, the drought condition was maintained until harvest.

The relative water content (RWC) was measured on the last irrigation day, at the R7 stage and at harvest. Root samples were taken from a depth of 90 cm and analyzed with the Winrhizo program to determine the total root length per sample, root surface area (cm^2), root diameter (mm) and root volume (cm^3). Root dry weight (RDW) was also determined at R8 using the monolith method. Leaf, stem, pods and roots were harvested and analyzed for plant nutrient (N, P, K, Ca and Mg). Nodule dry weight (NDW) and nitrogen fixation (NF) were measured at harvest for each genotype.

Results

RWC decreased under terminal drought in both years (Figure 1). Values for RWC on the last day of irrigation were almost the same as those under well-watered and terminal drought conditions. RWC of the water-stressed treatment was significantly lower than that of the well-watered treatment in both years. The highest RWCs were observed at R7 under the well-watered treatment in both years, followed by RWC under the well-watered treatment on the last day of irrigation and at harvest.

The correlations between root surface area (cm^2) and nutrient uptake were not significant under the well-watered condition (Table 1). Root surface area significantly correlated with the uptakes of N, P, K and Mg, but there was no correlation with the uptakes of Ca under drought stress. The correlations between root diameter and nutrient

uptake were not significant under both the well-watered and the terminal drought conditions. Strong correlations between root volume and nutrient uptake were observed under terminal drought but not under well-watered condition. RLD significantly correlated with the uptakes of N, P, K and Mg under terminal drought but not under the well-watered condition. RDW had no relationship with nutrient uptake under well-watered conditions, but had significant correlation with the uptake of Ca under terminal drought.

Significant correlations were observed between NF and root surface area, root volume, RLD and %RLD under well-watered (Table 2). The correlations between NF and root surface area, root volume and %RLD were also found when peanut genotypes were subjected to terminal drought. There was no correlation between root dry weight and NF under well-watered condition. Root surface area significantly correlated with NDW under both well-watered and terminal drought conditions. The correlation coefficients between NDW with root volume, RLD, and % RLD were significant under terminal drought. RDW significantly correlated with NDW only under the well-watered condition.

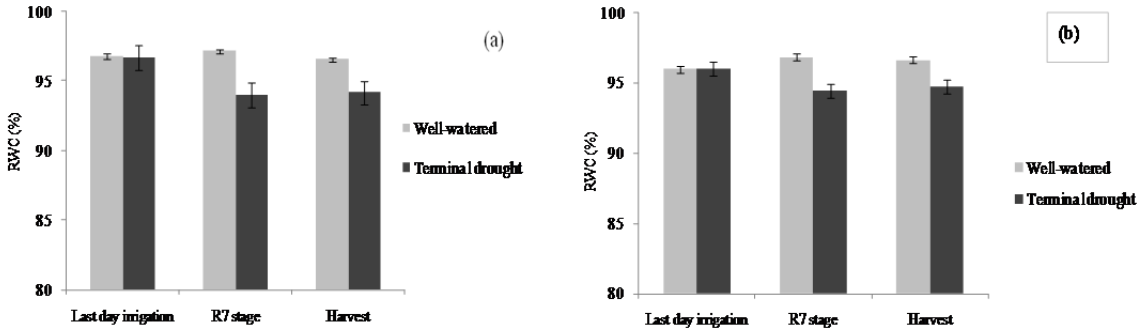


Fig. 1. Relative water content (RWC) on the last day of irrigation, at R7 stage and harvest stage of five peanut genotypes grown under well-watered and terminal drought during 2010/2011 (a) and 2011/2012 (b) Well-watered = Full irrigation since sowing until final harvest. Terminal drought = 1/3 available water (AW) at R7 until final harvest.

Table 1. Correlation coefficients between root surface area (cm^2), root diameter (mm), root volume (cm^3), root length density (RLD), percentage of root length density (% RLD) at 30-90 cm, root dry weight (RDW) (g $plant^{-1}$) at 0-50 cm, and uptake of N, P, K, Ca and Mg (mg $plant^{-1}$) across 2010/2011 and 2011/2012.

Nutrient uptake (mg $plant^{-1}$)	Root surface area (cm^2)	Root diameter (mm)	Root volume (cm^3)	RLD	%RLD	RDW (g $plant^{-1}$)
Well-watered						
N	0.28	-0.12	0.09	-0.03	0.05	0.08
P	0.17	-0.10	0.00	-0.06	0.06	0.14
K	0.12	-0.08	0.02	-0.08	0.05	0.16
Ca	0.12	-0.02	-0.01	-0.10	-0.09	0.13
Mg	0.3	0.06	0.25	0.14	0.20	0.20
Terminal drought						
N	0.51**	0.28	0.58**	0.81**	0.35*	-0.19
P	0.43**	0.06	0.51**	0.76**	0.40**	-0.20
K	0.33*	0.25	0.58**	-0.27	0.39*	-0.20
Ca	0.16	0.29	0.25	0.10	-0.27	0.36*
Mg	0.32*	0.16	0.46**	0.53**	0.33*	-0.26

*,** significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. Well-watered = Full irrigation since sowing until final harvest. Terminal drought = 1/3 available water (AW) at R7 until final harvest.

Table 2. Correlation coefficients between root surface area (cm^2), root diameter (mm), root volume (cm3), root length density (RLD), percentage of root length density (%RLD) at (30-90 cm), root dry weight (RDW) (g $plant^{-1}$) at (0-50 cm), and N2-fixation (NF) (mg $plant^{-1}$) and nodule dry weight (NDW) (mg $plant^{-1}$) across 2010/2011 and 2011/2012.

NF and NDW	Root surface area (cm^2)	Root diameter (mm)	Root volume (cm^3)	RLD	%RLD	RDW (g $plant^{-1}$)
Well-watered						
NF	0.63**	0.20	0.48**	0.46**	0.40**	0.3
NDW	0.47**	0.04	0.27	0.23	0.19	0.34*
Terminal drought						
NF	0.53**	-0.23	0.31*	0.65**	0.64**	-0.52**
NDW	0.32*	-0.50	0.31*	0.55**	0.35*	-0.24

*,** significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. Well-watered = Full irrigation since sowing until final harvest. Terminal drought = 1/3 available water (AW) at R7 until final harvest.

Conclusion

Deep root systems favored nutrient uptake under terminal drought conditions. A large root mass was important for Ca uptake only and it alone was not effective to take up other nutrients when large root systems in the upper soil were unable to mine soil water. Root diameter might not be an important trait for nutrient uptake and NF. Moreover, enhanced root surface area, root volume, RLD and % RLD were some mechanisms that helped peanut maintain nodule growth and NF under terminal drought conditions. These findings suggested that selection of peanut genotypes with deep root systems would improve nutrient uptake and NF under terminal drought conditions.

References

Fageria, N.K., V.C. Baligar and R.B. Clark. 2002. Micronutrient in crop production. Adv. Agron. 77: 185-268.

Htoon, W., S. Jogloy, B. Toomsan and J. Sanitchon. 2009. Response to early drought for traits related to nitrogen fixation and their correlation to yield and drought tolerance traits in peanut (*Arachishypogaea* L.). Asian J. Plant Sci. 8: 138-145.

Hungria, M. and T.R.J. Bohrer. 2000. Variability of nodulation and dinitrogen fixation capacity among soybean cultivars. Biol. Fert. Soils31: 45-52.

Maiti, R.K., P. Wesche-Ebeling, A. Nunez-Gonzalez and E. Sanchez-Arreola. 2002. Root system and mineral nutrition, In: R.K. Maiti, and P. Wesche-Ebeling, eds. The Peanut (*Arachishypogaea*) Crop. pp. 125–146. Science Publishers, Inc., New Hampshire, USA.

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: 334-342.

Wright, G.C. and R.C. Nageswara Rao. 1994. Groundnut water relations. In: J. Smartt, ed. The Groundnut Crop. A Scientific Basis for Improvement. pp. 281–325. Chapman and Hall, London, UK.

Taiz, L. and E. Zeiger. 2006. Stress physiology. In: L. Taiz and E. Zeiger, eds. Plant Physiology. pp. 671-681. 4th edition. Sinauer Associates, Inc., Massachusetts, USA.

Publication

Htoon, W., W. Kaewpradit, S. Jogloy, N. Vorasoot, B. Toomsan, C. Akkasaeng, N. Puppala and A. Patanothai. 2013. Relationship between root traits and nutrient uptake and nitrogen fixation in peanut under terminal drought. SABRAO J. Breed. Genet. 45: 311-323. (Impact factor 2010 = 0.227).

1.9 Relationships between physiological traits and yield components of peanut genotypes with different levels of terminal drought resistance

R. Koolachart, B. Suriharn, S. Jogloy, N. Vorasoot, S. Wongkaew, C.C. Holbrook, N. Jongrungklang, T. Kesmala and A. Patanothai

Peanut (*Arachis hypogaea* L.) is an important cash crop in the semi-arid tropics where drought is a major constraint (Drame *et al.*, 2007). Drought resistant varieties have been used to stabilize peanut productivity under drought conditions, and breeding for drought resistance has been an important strategy in alleviating the problem (Jongrungklang *et al.*, 2008; Songsri *et al.*, 2008). The response of plants to water stress depends on several factors such as developmental stage, severity and duration of stress and genotype (Beltrano and Ronco 2008). Information on physiological traits contributing to high yield under drought stress would reveal the underlying mechanism

from which improved strategies could be developed to enhance the effectiveness and progress in breeding for drought resistance in peanut (Pimratch *et al.*, 2008; Songsri *et al.*, 2009). Some physiological characters such as leaf area index (LAI), specific leaf area (SLA), relative water content (RWC), SPAD chlorophyll meter reading (SCMR), canopy temperature and stomatal conductance are related to drought tolerance in peanut. SLA was associated with variation in photosynthetic capacity and chlorophyll density expressed as high SCMR (Wright and Nageswara Rao 1994; Nageswara Rao *et al.*, 1995, 2001). SCMR was directly related to the amount of chlorophyll in the leaves of peanuts (Akkasaeng *et al.*, 2003). SCMR was also increased under severe drought (Jongrungklang *et al.*, 2008). Drought tolerant cultivars had lower water potential but higher RWC than drought susceptible cultivars (Joshi *et al.*, 1988). Canopy temperature was positively correlated with visual drought stress ratings (Rucker *et al.*, 1995). Stomatal conductance in peanut was closely related to water status (Bennet *et al.*, 1984). Moreover, yield components are important characters for sustaining pod yield under drought, and peanut genotypes with high pod yields under drought also had high numbers of mature pods under both non-stress and stress conditions (Songsri *et al.*, 2008). The reductions in seed size were significantly different among cultivars (Boontang *et al.*, 2010). SCMR under drought conditions was positively correlated with pod per plant (Puangbut *et al.*, 2011; Painawadee *et al.*, 2009). However, Boontang *et al*. (2010) found that SCMR was not related to number of pods plant^{-1} and number of seeds pod $^{-1}$.

Most of these investigations, however, were carried out under early season drought, long-term drought or intermittent drought, but not under late season drought. The contrasting results of different studies lead us to hypothesize that drought conditions may modify the relationships among these traits. The relationships between physiological traits and yield components for drought tolerance of peanut genotypes with different levels of terminal drought resistance are still lacking. Such information would be useful for the improvement of peanut for terminal drought resistance.

Objective

The objective of the current investigation was to determine the relationships between physiological traits and yield components for drought tolerance of peanut genotypes with different levels of terminal drought resistance.

Materials and Methods

The experiment was conducted under field conditions at the Field Crop Research Station of Khon Kaen University during the dry season for two years (2010/11 and 2011/12). The treatments were arranged in a split-plot design with four replications. Two soil moisture levels [field capacity (FC) and 1/3 available water (1/3AW) at R7 growth stage through harvest] were assigned to main-plots, and five peanut genotypes were assigned to sub-plots. Plot size was 5 x 5 m with a spacing of 50 cm between rows and 20 cm between plants within a row. Rainout shelters were available if necessary.

Data were recorded on physiological traits, including leaf area index (LAI), specific leaf area (SLA), relative water content (RWC), SPAD chlorophyll meter reading (SCMR), canopy temperature and stomatal conductance at the R7 growth stage and at harvest, and yield components consisting of number of pod plant^{-1}, number of seeds pod^{-1}and 100 seed weight were recorded at harvest.

Results

The relationships between physiological traits and yield components at the R7 growth stage and at harvest are presented in Figure 1-6. ICGV 98348 had high LAI and high DTI for number of pods plant^{-1} at the R7 stage, but no genotypes were high in both traits at harvest (Fig. 1a, d). Tifton 8 and ICGV 98348 had high LAI and high DTI for number of seeds pod^{-1} at the R7 stage, and Tifton 8, ICGV 98348 and ICGV 98324 had high LAI and high DTI for number of seeds pod^{-1} at harvest (Fig. 1b, e). Tifton 8, ICGV 98348 and ICGV 98308 had high LAI and high DTI for 100-seed weight at the R7 stage, and Tifton 8 and ICGV 98324 had high LAI and also high DTI for 100-seed weight at harvest (Fig. 1c, f).

ICGV 98348 had low SLA but high DTI for number of pods plant^{-1} at both stages (Figure 2a, d). ICGV 98324 and ICGV 98348 had low SLA but high DTI for number of seeds pod^{-1} at the R7 stage, and ICGV 98324 and Tifton 8 had low SLA but high DTI for number of seeds pod^{-1} at harvest (Fig. 2b, e). The same relationship was shown between SLA and DTI for 100-seed weight (Fig. 2c, f).

ICGV 98348 had high RWC and high DTI for number of pods plant^{-1} (Figure 3a, d). ICGV 98324 and ICGV 98348 had high RWC and also high DTI for number of seeds pod^{-1} (Figure 3b, e). All genotypes had high RWC and high DTI for 100-seed weight, except for Tainan 9 (Fig. 3c, f).

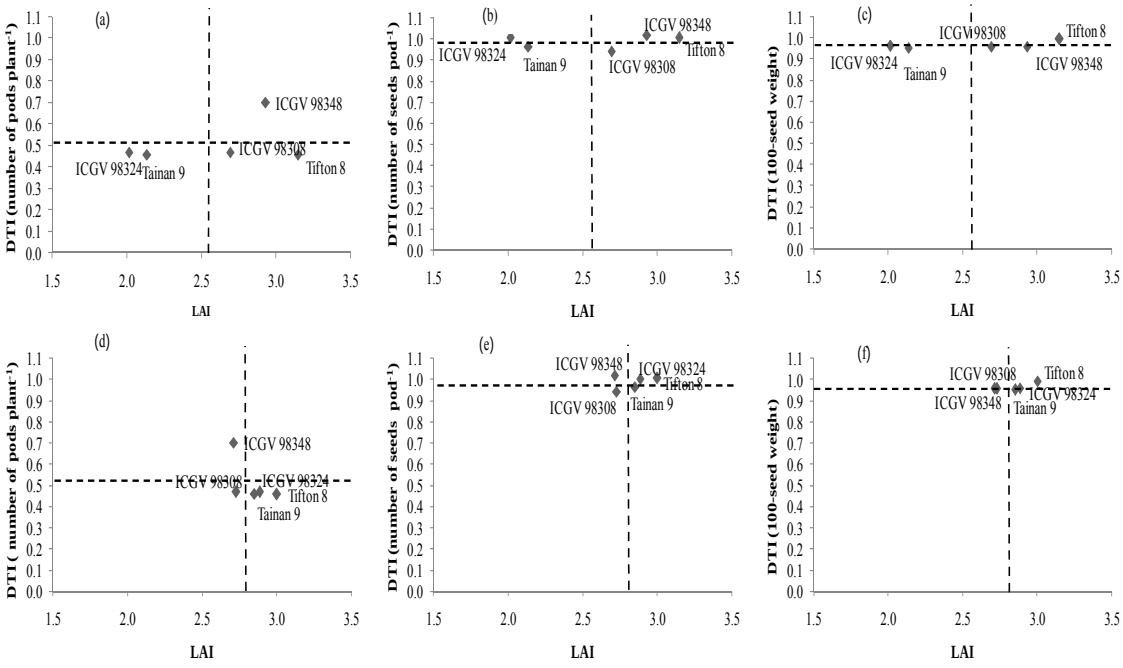


Fig. 1. Relationships between leaf area index (LAI) and DTI (number of podsplant^{-1}) (a), DTI number of seedspod^{-1}) (b) and DTI (100-seed weight)(c) at the R7 growth stage, and between leaf area index (LAI) and DTI (number of pods plant^{-1}) (d), DTI (number of seeds pod^{-1}) (e) and DTI (100-seed weight)(f) at harvest.

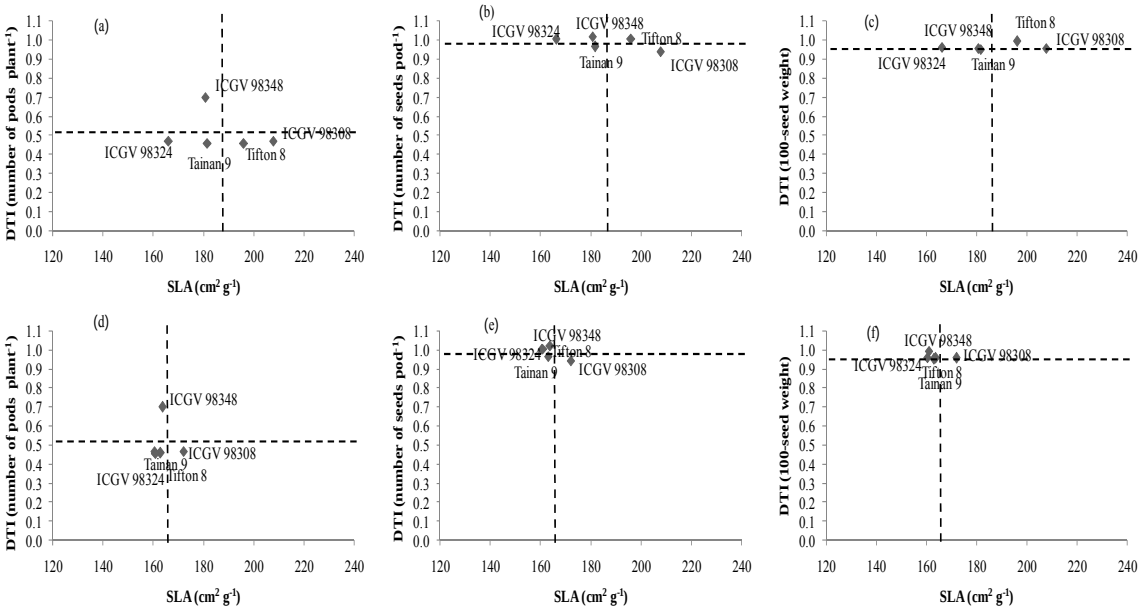


Fig. 2. Relationships between specific leaf area (SLA) and DTI (number of pods plant-1) (a), DTI (number of seeds pod-1) (b) and DTI (100-seed weight) (c) at the R7 growth stage, and between SLA and DTI (number of pods plant-1) (d), DTI (number of seeds pod-1) (e) and DTI (100-seed weight) (f) at harvest.

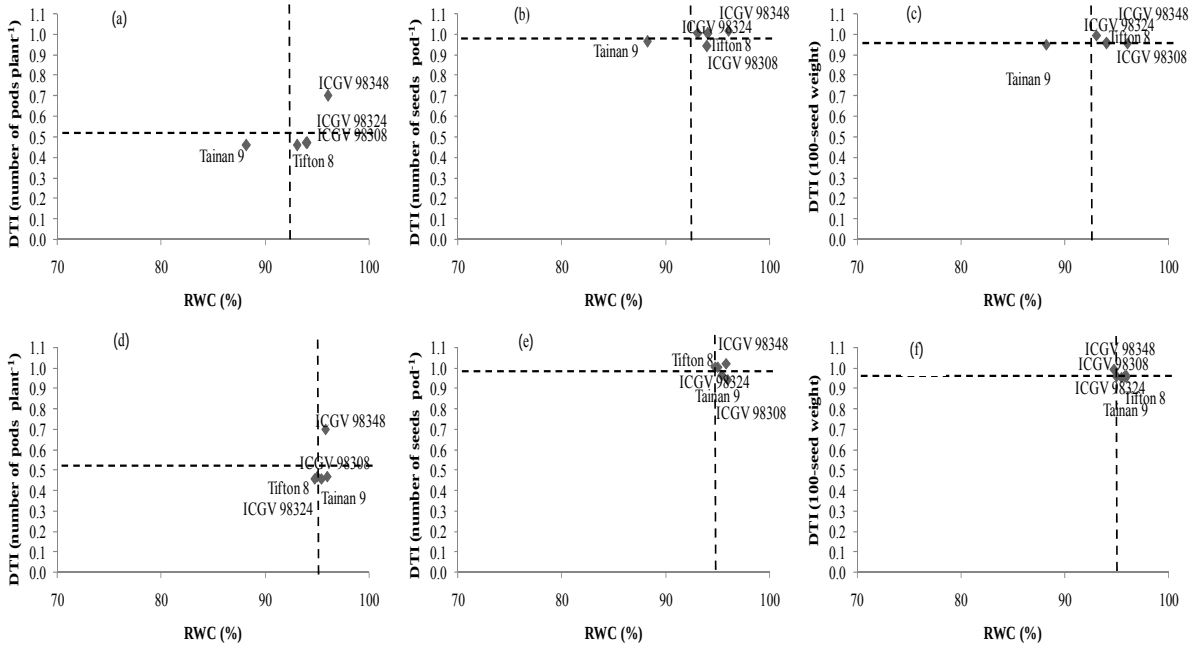


Fig. 3. Relationships between RWC and DTI (number of pods plant-1) (a), DTI (number of seeds pod-1) (b) and DTI (100-seed weight) (c) at the R7 growth stage, and between RWC and DTI (number of pods plant-1) (d), DTI (number of seeds pod-1) (e) and DTI (100-seed weight) (f) at harvest.

No genotype had high SCMR and high DTI for number of pods plant^{-1} at the R7 stage, whereas ICGV 98348 had high SCMR and also high DTI for number of pods plant^{-1} at harvest (Fig. 4a, d). ICGV 98324 and Tifton 8 had high SCMR and also high DTI for number of seeds pod^{-1} at the R7 stage, while ICGV 98324, ICGV 98348 and Tifton 8 had high SCMR and high DTI for number of seeds pod^{-1} at harvest, and the same result was found for the relationship between SCMR and DTI for 100-seed weight at the R7 growth stage and at harvest (Fig. 4b, e, c, f).

No relationship was found between canopy temperature and DTI for number of pods plant^{-1} at both stages (Fig. 5a, d). While, Tifton 8 and ICGV 98324 had low canopy temperature and high DTI for number of seeds pod^{-1} at R7, whereas only ICGV 98324 had low canopy temperature and high DTI for number of seeds pod^{-1} at harvest. The same was found for the relationship between canopy temperature and DTI for 100-seed weight at both stages (Fig. 5b, e, c, f).

The relationships between stomatal conductance and DTI for number of pods plant^{-1}, DTI for number of seeds pod^{-1} and DTI for100-seed weight followed the same pattern at the R7 stage (Fig. 6). At this stage, ICGV 98348 had high stomatal conductance and also high DTI for number of pods plant^{-1}, DTI for number of seeds pod^{-1} and DTI for 100-seed weight (Figure 6a, b, c, d). However, ICGV 98348, Tifton 8 and ICGV 98324 had high stomatal conductance and high DTI for number of seeds pod^{-1} and DTI for 100-seed weight at harvest (Fig. 6e, f).

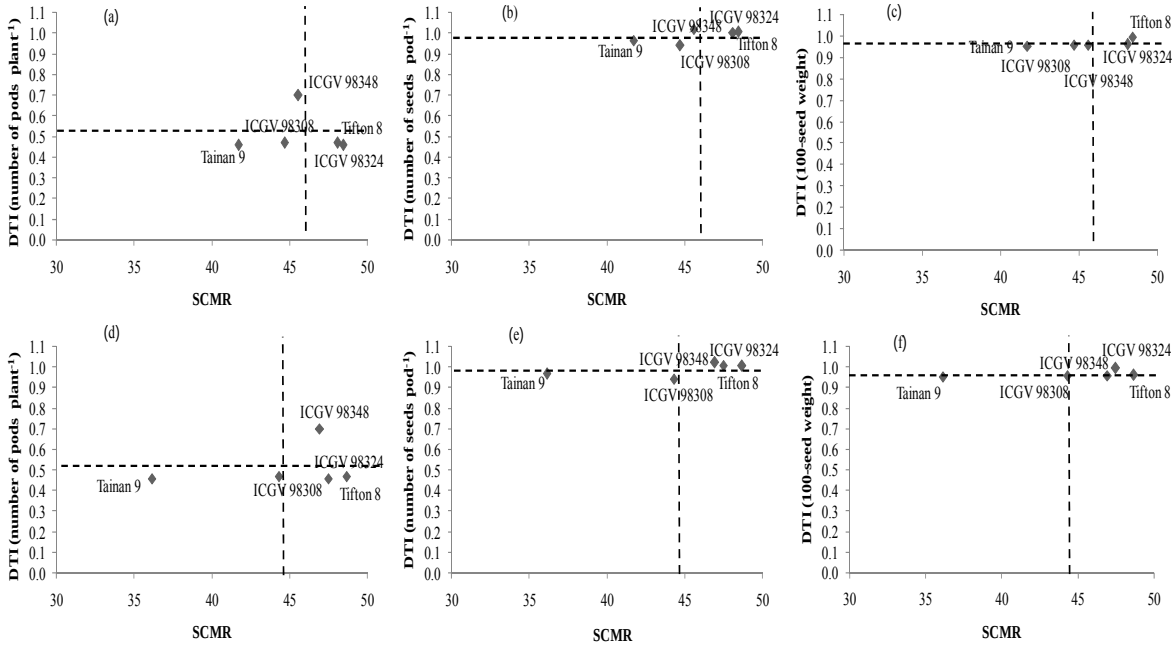


Fig. 4. Relationships between SPAD chlorophyll meter reading (SCMR) and DTI (number of pods plant-1) (a), DTI (number of seeds pod-1) (b) and DTI (100-seed weight) (c) at the R7 growth stage, and between SCMR and DTI (number of pods plant1) (d), DTI (number of seeds pod-1) (e) and DTI (100-seed weight) (f) at harvest.

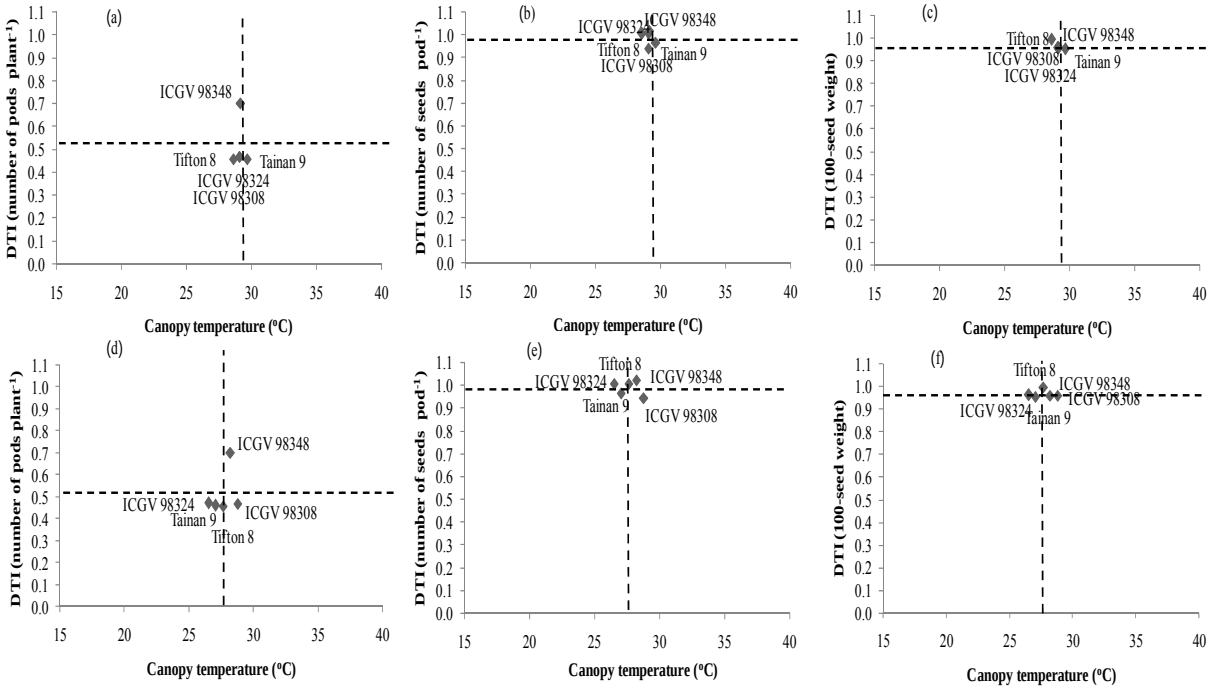


Fig. 5. Relationships between canopy temperature and DTI (number of pods plant^{-1}) (a), DTI (number of seeds pod^{-1}) (b) and DTI (100-seed weight) (c) at the R7 growth stage, and between canopy temperature and DTI (number of pods plant^{-1}) (d), DTI (number of seeds pod^{-1}) (e) and DTI (100-seed weight) (f) at harvest.

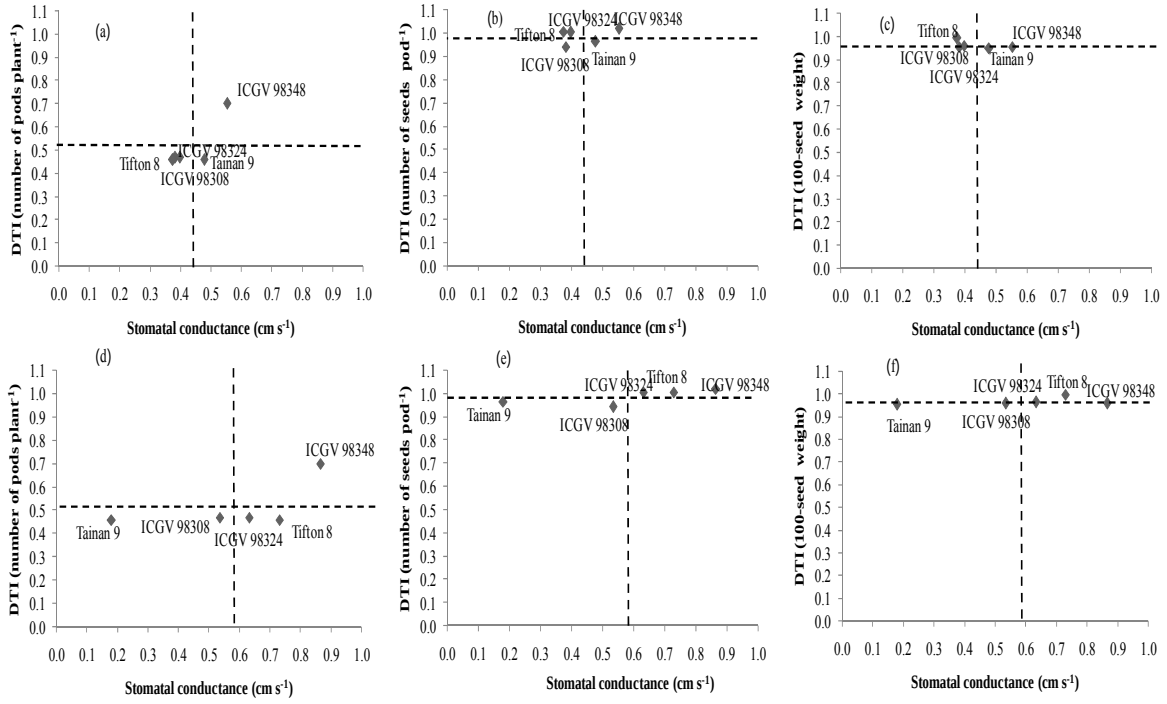


Fig. 6. Relationships between stomatal conductance and DTI (number of pods plant^{-1}) (a), DTI (number of seeds pod^{-1}) (b) and DTI (100-seed weight) (c) at the R7 growth stage, and between stomatal conductance and DTI (number of pods plant^{-1}) (d), DTI (number of seeds pod^{-1}) (e) and DTI (100-seed weight) (f) at harvest.

Conclusions

When all peanut genotypes were considered, the relationships between physiological traits and yield components were not clear. However, when the individual peanut genotypes were considered, clear relationships were observed. ICGV 98324, ICGV 98348 and Tifton 8, the drought tolerance genotypes, all gave high performances for both physiological traits and yield components. Thus, the ability to maintain physiological traits and yield components under stress condition could be the mechanism for peanut genotypes to maintain high pod yield under water limited conditions.

References

Akkasang, C., N. Vorasoot, S. Jogloy and A. Patanothai. 2003. Relationship between SPAD readings and chlorophyll contents in leaves of peanut (*Arachis hypogaea* L.). Thai J. Agri. Sci. 36: 279-284.

Beltrano, J.and M.G. Ronco. 2008. Improved tolerance of wheat plants (*Triticum aestivum* L.) to drought stress and rewatering by the arbuscular mycorrhizal fungus *Glomus claroideum*: effect on growth and cell membrane stability. Braz. J. Plant Physiol.20: 29-37.

Bennet, J.M., K.J. Boote and L.C. Hammon. 1984. Relationships among water potential components, relative water content and stomatal resistance of field–grown peanut. Peanut Sci. 11: 31-35.

Boontang, S., T. Girdthai, S. Jogloy, C. Akkasaeng, N. Vorasoot, A. Patanothai and N. Tantissuwichwong. 2010. Responses of released cultivars of peanut to terminal drought for traits related to drought tolerance. Asian J. Plant Sci. 9:423-431.

Drame, K.N., D. Clavel, A. Repellin, C. Passaquet and Y. Zuily–Fodil. 2007.Water deficit variation in expression of stress-responsive genes in two peanut *(Arachis hypogaea* L.*)* cultivars with different tolerance to drought. Plant Physiol. Biochem. 45: 236-243.

Jongrungkrang, N., B. Toomsan, N. Vorasoot, S. Jogloy, T. Kesmala and A. Patanothai. 2008. Identification of peanut genotypes with high water use efficiency under drought stress conditions from peanut germplasm of diverse origins. Asian J. Plant Sci. 7: 628-638.

Joshi, Y.C., P.C. Naultiyal, V. Ravindra and R.S. Dwivedi. 1988. Water relations in two cultivars ofgroundnut (*Arachis hypogaea* L.) under soilwater deficit. Tropic.Agric. (Trinid). 65: 182-184.

Nageswara Rao, R.C., H.S. Talwar and G.C. Wright. 2001. Rapid assessment of specific leaf area and leaf N in peanut (*Arachis hypogaea* L.) using chlorophyll meter. J. Agron. Crop Sci. 189: 175-182.

Nageswara Rao, R.C., M. Udaykumar, G.D. Farquhar, H.S. Talwar and T.G. Prasad. 1995. Variation in carbon isotope discrimination and its relationship to specific leaf area and ribulose-1,5-bisphosphate carboxylase content in groundnut genotypes. Aust. J. Plant Physiol. 22: 545-551.

Painawadee, M., S. Jogloy, T. Kesmala, C. Akkasaeng and A. Patanothai. 2009. Identification of traits related to drought resistance in peanut (*Arachis hypogaea* L.). Asian J. Plant Sci. 8: 325-334.

Pimratch, S., S. Jogloy, N. Vorasoot, B. Toomsan, A. Patanothai and C.C. Holbrook. 2008. Relationship between biomass production and nitrogen fixation under drought–stress condition in peanut genotypes with different levels of drought resistance. J. Agron. Crop Sci. 194: 15-25.

Puangbut, D., S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, A. Patanothai and N. Puppala. 2011. Heritability of early season drought resistance traits and genotypic correlation of early season drought resistance and agronomic traits in peanut. SABRAO J. Breed. Genet. 43: 165-187.

Rucker, K.S., C.K. Kvien, C.C. Holbrook and J.E. Hook. 1995. Identification of peanut genotypes with improved drought avoidance traits. Peanut Sci. 22: 14-18.

Songsri, P., S. Jogloy, C.C. Holbrook, T. Kesmala, N. Vorasoot, C. Akkasaeng and A. Patanothai. 2009. Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. Agric. Water Manage. 96: 790–798.

Songsri, P., S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook. 2008. Response of reproductive characters of drought resistant peanut genotypes to drought. Asian J. Plant Sci.7: 427–439.

Wright, G.C. and R.C. Negeswara Rao. 1994. Groundnut water relation. *In* Smartt, J. (ed), pp. 281–325. The Groundnut Crop. Chapman and Hall, London.

Publication

Koolachart R., B. Suriharn, S. Jogloy, N. Vorasoot, S. Wongkaew, C.C. Holbrook, N. Jongrungklang, T. Kesmala and A.Patanothai. 2013. Relationships between physiological traits and yield components of peanut genotypes with different levels of terminal drought resistance. SABRAO J. Breed. Genet. 45: 422-446. (Impact factor = 0.227).

1.10 Inheritance of the physiological traits for drought resistance under terminal drought conditions and genotypic correlations with agronomic traits in peanut

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

Breeding peanut varieties for drought resistance is an important approach to solve the problem of drought stress to the crop. In addition, preharvest aflatoxin contamination induced by terminal drought in peanut may be reduced with improved resistance to drought (Girdthai *et al.*, 2010; Holbrook *et al.*, 2009). However, progress in breeding for drought tolerance in peanut using yield as a selection criterion has been slow due to large and uncontrollable genotype x environment (G x E) interactions. Utilization of surrogate physiological traits in the selection would be more effective.

Efficient utilization of the physiological traits for improving drought resistance in a breeding program requires an understanding of the inheritance and genetic relationships of the trait that is available for selection. Hubick *et al*. (1988) reported that heritability estimates were high for transpiration efficiency (TE), especially the carbon isotope

discrimination which has no significant G x E interaction. Songsri *et al*. (2008) found that heritability estimates of physiological traits for drought resistance in peanut were high (h^2> 0.50) under drought and well-watered conditions, and physiological traits like specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR), harvest index (HI), and drought tolerance index for pod yield and biomass were associated well with agronomic traits under long periods of drought. Cruickshank *et al*. (2004) also found that broad-sense heritability estimates for HI were high under rainfed conditions. Since the mechanisms of drought resistance are diverse under different timing and periods of drought conditions (Clavel *et al.*, 2004), the inheritance of terminal-drought resistance traits might be different. So far, relatively few studies have investigated the heritability and genotypic correlations of physiological traits for drought resistance in peanut, and none have been done under terminal drought conditions.

Objectives

The objectives of this study were to estimate the heritability of terminal-drought resistance traits, to determine genotypic and phenotypic correlations between drought resistance traits and agronomic traits, and the correlations among physiological traits in peanut under terminal drought conditions.

Materials and methods

Four peanut F_1 hybrids (ICGV 98348 x Tainan 9, ICGV 98348 x KK60-3, ICGV 98353 x Tainan 9, and ICGV 98353 x KK60-3) were generated from the hybridization of two drought resistant lines (ICGV 98348 and ICGV 98353) with two high yielding drought-susceptible cultivars KK60-3 and Tainan 9 (Girdthai *et al.*, 2010). The parental lines and the 140 families from these four crosses were evaluated in the $F_{4:6}$ and $F_{4:7}$ generations (F_4 – derived lines in the F_6 and F_7 generations, respectively) under two soil moisture levels (field capacity (FC) and 1/3 available soil water (1/3 AW) at 80 days after planting (DAP) to final harvest) in dry seasons of 2006/2007 and 2007/2008. A split plot design with four replications was used for both years. The trials were conducted at the Field Crops Research Station of the Faculty of Agriculture of Khon Kaen University.

For the stress treatment, water was withheld at 60 DAP for 20 days to allow soil moisture to gradually decline until reaching the predetermined levels of 1/3 AW at 80 DAP, and then the soil moistures were held fairly constant until harvest. Irrigation was applied regularly to prevent soil moisture from decreasing by more than 1 % in each plot.

SPAD chlorophyll meter reading (SCMR) and specific leaf area (SLA) were recorded at 80, 90 and 100 days after planting (DAP). Pod yields were weighed after air drying to approximately 7-8 % moisture content. The number of mature pods per plant, number of seeds per pod, and 100 seed weight were also recorded at final harvest. HI was computed by pod weight/total biomass. .

Broad-sense heritability for the four 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 as follows (Holland *et al.*, 2003):

$$h^2_b = \delta^2_G / \delta^2_P$$

$$\delta^2_P = \delta^2_G + \delta^2_{GE}/e + \delta^2_E/re$$

where, h^2_b = broad-sense heritability, δ^2_G = genotypic variation, δ^2_P = phenotypic variation, r = number of replications, and e = number of environments. The standard error (SE) of heritability (Singh *et al.*, 1993) for each trait was also calculated.

Phenotypic and genotypic correlations between drought tolerance traits and agronomic traits, and correlations among physiological traits were calculated following the methods of Falconer and Mackay (1996).

Results

Wide ranges of yield, biomass, and physiological traits of the four peanut crosses under different water regimes were observed. Differences among genotypes for pod yield and total biomass were greater under terminal drought than under well-watered conditions (Table 1). Means and ranges for SCMR and SLA were also different between crosses.

In this study, heritability estimates for physiological traits were higher than those for agronomic traits, and varied among crosses under both well-watered and terminal drought conditions (Table 2). The heritability estimates for pod yield (ranged from 0.25 to 0.79) and biomass (ranged from 0.17 to 0.66) were moderate, but high for HI (ranged from 0.58 to 0.85), SCMR at 80, 90, and 100 DAP (ranged from 0.72 to 0.91), and SLA at 80, 90, and 100 DAP (ranged from 0.61 to 0.90). The heritability estimates for all three physiological traits ranged from 0.61 to 0.91, and those for pod yield and biomass ranged from 0.17 to 0.79. Standard errors for physiological traits were also lower than those for pod yield and biomass, especially under non-stressed conditions. Thus, the expected genetic gain per cycle of selection under terminal drought conditions would be less for pod yield and biomass compared to HI, SCMR, and SLA. The high heritability estimates for HI and for SCMR and SLA indicated that selection for these traits should be effective. Heritability estimates for these traits were similar under the two water regimes, and correlations between traits under the two water regimes were positive and significant (r = 0.22-0.67, $P \leq 0.01$) (Table 2), indicating that these traits could be selected under either well-watered or terminal drought conditions.

Significant correlations between drought resistance traits and agronomic traits were observed (Table 3). Genotypic (r_G) and phenotypic (r_P) correlations were similar, hence, only r_G is reported herein. Positive correlations were found between HI and pod yield, number of mature pods per plant, and seeds per pod under non-stressed and terminal drought conditions (r_G = 0.49 to 0.78, $P \leq 0.01$). Positive correlations between SCMR at 80, 90, and 100 DAP and pod yield, biomass, and seed size were also significant (r_G = 0.11 to 0.47, $P \leq 0.01$), and the correlations were higher under stressed conditions. These results indicated that selection for higher HI and SCMR would result in higher pod yield in peanut. SLA at 80, 90, and 100 DAP were negatively correlated with agronomic traits (r_G = -0.07 to -0.56, $P \leq 0.05$ and $P \leq 0.01$, respectively), especially under terminal drought conditions. Correlations between SLA at 80, 90, and 100 DAP and pod yield were positive and highly significant (r_G = -0.19 to -0.56, $P \leq 0.01$) under stressed conditions, but were not significant under well-watered conditions. Weak correlations between SLA and the yield components, number of mature pods per plant and seed size, were also found (r_G = -0.25 to 0.21, $P \leq 0.05$ to $P \leq 0.01$, respectively). Thus, genotypes with low SLA tended to have high pod yield, biomass, and large number of mature pods per plant and seed size. Associations between SLA and agronomic traits were stronger under terminal drought conditions.

Table 1. Range and mean of pod yield, total biomass, and physiological traits [specific leaf area (SLA) (cm^2 g^{-1}) and SPAD chlorophyll meter reading (SCMR) at 80, 90, and 100 days after planting (DAP)] of 4 peanut crosses under well-watered and terminal drought conditions in the dry season of 2006/2007 and 2007/2008.

	ICGV 98348 x Tainan 9			ICGV 98348 x KK60-3		
Traits	Range	Mean	S.E.	Range	Mean	S.E.
Well-watered conditions						
Pod yield (kg h^{-1})	1,219 - 4,750	2,688	801	1,218 - 5,396	3,211	922
Biomass (kg h^{-1})	4,193 - 15,320	8,289	2,027	5,088 - 17,093	10,367	2,441
SCMR 80 DAP	29 - 51	41	3.80	34 - 51	43	3.57
SCMR 90 DAP	33 - 52	42	3.39	36 - 51	43	2.96
SCMR 100 DAP	30 - 58	44	4.16	35 - 55	45	3.64
SLA 80 DAP	106 - 198	148	18.56	104 - 194	146	18.17
SLA 90 DAP	100 - 245	148	33.01	96 - 237	146	31.98
SLA 100 DAP	98 - 199	142	19.14	111 - 205	141	17.05
Drought conditions						
Pod yield (kg h^{-1})	252 - 3,469	1,853	681	766 - 4,696	2,649	881
Biomass (kg h^{-1})	2,951 - 11,350	6,498	1,678	3,429 - 15,426	8,549	2,077
SCMR 80 DAP	34 - 53	43	3.88	37 - 54	44	3.54
SCMR 90 DAP	34 - 55	45	3.46	36 - 55	47	3.34
SCMR 100 DAP	38 - 57	47	4.01	37 - 60	49	3.84
SLA 80 DAP	109 - 206	142	29.46	106 - 195	142	26.55
SLA 90 DAP	104 - 198	139	18.88	103 - 195	135	19.99
SLA 100 DAP	101 - 181	138	16.56	99 - 175	133	14.39
	ICGV 98353 x Tainan 9			ICGV 98353 x KK60-3		
Traits	Range	Mean	S.E.	Range	Mean	S.E.
Well-watered conditions						
Pod yield (kg h^{-1})	1,077 - 4,823	2,220	761	1,117 - 5,470	2,528	792
Biomass (kg h^{-1})	3,347 - 14,813	7,420	1,911	4,644 - 15,542	9,617	2,421
SCMR 80 DAP	32 - 51	41	3.91	37 - 51	42	3.03
SCMR 90 DAP	32 - 54	42	4.03	34 - 50	43	2.83
SCMR 100 DAP	31 - 57	45	4.39	34 - 58	46	3.30
SLA 80 DAP	112 - 202	146	18.35	106 - 194	146	17.82
SLA 90 DAP	97 - 236	144	29.46	94 - 223	147	32.15
SLA 100 DAP	99 - 196	136	17.27	99 - 210	139	17.09
Drought conditions						
Pod yield (kg h^{-1})	441 - 3,540	1,733	681	718 - 4,020	2,128	670
Biomass (kg h^{-1})	1,825 - 10,534	6,079	1,532	3,273 - 14,030	8,003	1,968
SCMR 80 DAP	35 - 55	44	4.30	37 - 53	44	3.37
SCMR 90 DAP	32 - 55	46	4.38	38 - 55	46	3.31
SCMR 100 DAP	35 - 60	47	4.45	40 - 57	48	3.59
SLA 80 DAP	108 - 201	146	28.88	105 - 216	147	30.69
SLA 90 DAP	103 - 197	137	18.81	101 - 194	139	21.28
SLA 100 DAP	101 - 181	132	17.52	100 - 175	135	15.07

S.E., Standard error for genotypes means.

Table 2. Broad-sense heritability estimates for pod yield (PY), biomass(BIO), harvest index (HI), and physiological traits [SPAD chlorophyll meter reading (SCMR), and specific leaf area (SLA) at 80, 90, and 100 days after planting (DAP)] under well-watered and terminal drought conditions of 4 peanut crosses.

	Broad-sense heritability					
Peanut crosses	PY	BIO	HI	SCMR		
				80DAP	90DAP	100DAP
Well-watered conditions						
ICGV 98348 x Tainan 9	0.43±0.32‡	0.65±0.24	0.67±0.23	0.87±0.13	0.79±0.18	0.88±0.12
ICGV 98348 x KK60-3	0.73±0.20	0.52±0.29	0.77±0.18	0.86±0.14	0.81±0.14	0.87±0.13
ICGV 98353 x Tainan 9	0.60±0.26	0.49±0.30	0.65±0.25	0.91±0.10	0.85±0.13	0.85±0.14
ICGV 98353 x KK60-3	0.25±0.37	0.17±0.37	0.74±0.20	0.77±0.19	0.79±0.20	0.76±0.19
Drought conditions						
ICGV 98348 x Tainan 9	0.57±0.27	0.53±0.29	0.58±0.27	0.77±0.18	0.79±0.18	0.75±0.19
ICGV 98348 x KK60-3	0.75±0.19	0.66±0.23	0.85±0.14	0.72±0.20	0.77±0.19	0.77±0.15
ICGV 98353 x Tainan 9	0.79±0.17	0.32±0.34	0.74±0.20	0.90±0.11	0.80±0.15	0.82±0.16
ICGV 98353 x KK60-3	0.45±0.31	0.36±0.34	0.55±0.29	0.75±0.20	0.73±0.21	0.76±0.19
Correlation (r)†	0.43**	0.40**	0.42**	0.32**	0.26**	0.22**
Peanut crosses	SLA					
	80DAP	90 DAP	100 DAP			
Well-watered conditions						
ICGV 98348 x Tainan 9	0.84±0.16	0.84±0.14	0.89±0.11			
ICGV 98348 x KK60-3	0.75±0.20	0.83±0.15	0.74±0.24			
ICGV 98353 x Tainan 9	0.73±0.21	0.75±0.18	0.82±0.15			
ICGV 98353 x KK60-3	0.79±0.18	0.79±0.16	0.72±0.21			
Drought conditions						
ICGV 98348 x Tainan 9	0.61 ¡ 0.25	0.83±0.25	0.76±0.19			
ICGV 98348 x KK60-3	0.90 ¡ 0.10	0.84±0.25	0.68±0.23			
ICGV 98353 x Tainan 9	0.74 ¡ 0.20	0.78±0.27	0.82±0.15			
ICGV 98353 x KK60-3	0.74 ¡ 0.20	0.74±0.24	0.86±0.14			
Correlation (r)†	0.30 **	0.67**	0.48**			

* and ** significant at $P \leq 0.05$ and significant at $P \leq 0.01$, respectively.
† Correlations between well-watered conditions and drought conditions.
‡ Standard error

Table 3. Genotypic (r_G) correlations between drought tolerance at 80, 90, and 100 days after planting (DAP) and agronomic traits for the 140 progenies of peanut under well-watered and terminal drought conditions (degree of freedom = 556).

	Agronomic traits									
Drought tolerance traits	Pod yield		Biomass		PPP		Seed/pod		Seed size	
Well-watered conditions										
HI	0.66	**	-0.34	**	0.69	**	0.52	**	-0.05	
SCMR 80DAP	0.34	**	0.23	**	0.00		-0.27	**	0.32	**
SCMR 90DAP	0.16	**	0.27	**	-0.22	**	0.02		0.33	**
SCMR 100DAP	0.13	**	0.11	**	-0.34	**	-0.05		0.47	**
SLA 80DAP	-0.05		0.01		0.08	*	-0.01		-0.18	**
SLA 90DAP	-0.09	*	0.06		0.10	**	-0.15	**	-0.03	
SLA 100DAP	0.10	**	0.20	**	0.21	**	-0.25	**	-0.24	**
Drought conditions										
HI	0.74	**	-0.03		0.78	**	0.49	**	0.06	
SCMR 80DAP	0.37	**	0.34	**	-0.05		-0.12	**	0.31	**
SCMR 90DAP	0.34	**	0.22	**	0.10	**	-0.13	**	0.19	**
SCMR 100DAP	0.42	**	0.37	**	0.12	**	-0.07	*	0.25	**
SLA 80DAP	-0.56	**	-0.31	**	-0.24	**	-0.02		-0.42	**
SLA 90DAP	-0.22	**	-0.07	*	-0.09	*	-0.05		-0.04	
SLA 100DAP	-0.19	**	-0.07	*	0.09	*	0.05		-0.13	**

*, ** Significant at $P \leq 0.05$ and $P \leq 0.01$ probability levels, respectively.

For the 140 progeny lines, genotypic correlations among drought resistance traits were found under both well-watered and terminal drought conditions (Table 4). A positive and significant correlation between HI and SCMR at 80 DAP was found under non-stressed conditions ($r_G = 0.16$, $P \leq 0.01$), while the correlations between HI and SCMR at 80, 90, and 100 DAP were found under the stressed conditions ($r_G = 0.22$ to 0.30, $P \leq 0.01$). SLA was found to be inversely associated with SCMR and HI. The correlations between SLA and HI were stronger under water stress conditions. Under terminal drought, SLA at 80, 90, and 100 DAP were negatively correlated with HI (r_G = -0.20 to –0.50, $P \leq 0.01$) and SCMR (r_G = -0.34 to -0.45, $P \leq 0.01$). Under non-stressed conditions, a negative correlation between SLA and SCMR was also observed (r_G = -0.39 to 0.44, $P \leq 0.01$).

Table 4. Genotypic (r_G) correlation among drought tolerance traits for the 140 progenies of peanut under field capacity (FC) and 1/3 available water (1/3 AW) (degree of freedom = 556).

Drought tolerance traits	Well-watered conditions					
	SCMR			SLA		
	80 DAP	90 DAP	100 DAP	80 DAP	90 DAP	100 DAP
HI	0.16 **	-0.06	0.01	-0.05	-0.16 **	-0.07 *
SCMR 80DAP				-0.43 **	-	-
SCMR 90DAP					-0.39 **	-
SCMR 100DAP						-0.44 **
Drought tolerance traits	Terminal drought conditions					
	SCMR			SLA		
	80 DAP	90 DAP	100 DAP	80 DAP	90 DAP	100 DAP
HI	0.22 **	0.29 **	0.30 **	-0.50 **	-0.25 **	-0.20 **
SCMR 80DAP				-0.45 **	-	-
SCMR 90DAP					-0.34 **	-
SCMR 100DAP						-0.45 **

*, ** Significant at $P \leq 0.05$ and $P \leq 0.01$ probability levels, respectively.

Conclusions

The results suggest that HI, SLA, and SCMR are potentially useful as indirect selection traits for terminal drought resistance because of their low G x E interactions, high heritability and significant correlations with pod yield and the other agronomic traits under terminal drought conditions. Breeding approaches using these traits should be effective for improving terminal drought tolerance in peanut.

References

Clavel, D., B. Sarr, E. Marone and R. Ortiz. 2004. Potential agronomic and physiological traits of Spanish groundnut varieties (*Arachis hypogaea* L.) as selection criteria under end-of cycle drought conditions. Agronomie 24: 101-111.

Cruickshank A., A. Dowkiw, G.C. Wright, C.R. Nageswara Rao and S.N. Nigam. 2004. Heritability of drought-resistance traits in peanut. Proc. of the 4th International Crop Science Congress. Sept 26 - Oct 1, 2004. Brisbane. Australia.

Falconer, D.S. and T.F.C. Mackay. 1996. Introduction to Quantitative Genetics, p. 464, Longman, London.

Girdthai, T., S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew, C. C. Holbrook and A. Patanothai. 2010. Heritability of, and genotypic correlations between, aflatoxin traits and physiological traits for drought tolerance under end of season drought in peanut (*Arachis hypogaea* L.) Field Crops Res. 118: 169-176.

Gorbet, D.W. and D.A. Knauft. 2000. Registration of 'SunOleic 97R' peanut. Crop Sci. 40: 1190-1191.

Holbrook, C.C., B.Z. Guo, D.M. Wilson and P. Timper. 2009. The U.S. breeding program to develop peanut with drought tolerance and reduced aflatoxin contamination. Peanut Sci. 36: 50-53.

Holland, J.B., W.E. Nyquist, and C.T. Cervartes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: an update. Plant Breed. Rev. 22: 2-112.

Hubick, K.T., R. Shorter and G.D. Farquhar. 1988. Heritability and genotypic x environment interactions of carbon isotope discrimination and transpiration efficiency in peanut (*Arachis hypogaea* L.). Aust. J. Plant Physiol. 15: 799-813.

Singh, M., S. Ceccarelli and J. Hamblin. 1993. Estimation of heritability from varietal trials data. Theor. Appl. Genet. 86: 437-441.

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.

Publication

Girdthai, T., S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew, A. Patanothai and C.C. Holbrook. 2012. Inheritance of physiological traits for drought resistance under terminal drought condition and genotypic correlations with agronomic traits in peanut. SABRAO J. Breed. Genet. 44: 240-262. (Impact factor 0.227).

1.11 Nutrient uptake of peanut genotypes under different water regimes

J. Junjittakarn, S. Pimratch, S. Jogloy, W. Htoon, N. Singkham,
N. Vorasoot, B. Toomsan, C.C. Holbrook and A. Patanothai

Prolonged drought has been found to severely reduced yield of peanut (Songsri *et al.*, 2008) and increased aflatoxin contamination (Girdthai *et al.*, 2010). The use of drought resistant peanut varieties and sufficient irrigation can overcome the drought problem (Sankar *et al.*, 2008).

Drought generally reduces nutrient uptake in crop plants and concentrations of mineral nutrients in plant tissues (Fageria *et al.*, 2002). The reduction in nutrient uptake by plant under drought stress is due to reduced transpiration and impaired active transport and membrane permeability resulting in reduced root absorbing power (Tanguilig *et al.*, 1987). Water stress, thus, affects nutrient transportation to the root and root growth.

In peanut, water stress at flowering, pegging, pod formation and pod development stages was found to reduce pod yield and the uptakes of N, P, K, Ca, magnesium (Mg) and sulfur (S) (Kolay 2008). Under drought stress conditions, the available soil N (NO_3^- and NH_4^+) and N_2 fixation is greatly reduced and such a reduction leads to the low N accumulation and consequent low dry matter production and low crop yield (Pimratch *et al.*, 2008). Crop species and genotypes within a species are known to differ in their ability to take up nutrients under drought stress conditions. However, the information on the genotypic variation among peanut genotypes for nutrient uptakes across different water regimes is still lacking.

Objectives

The aims of this study were to investigate the responses of peanut genotypes to drought for nutrient uptake and to estimate the relationships between nutrient uptake and peanut yield parameters.

Materials and Methods

Pot experiment was conducted under greenhouse conditions during December 2002 to May 2003 and repeated from June to November 2003. The treatments were 3×11 factorial combinations of three water levels [field capacity (FC), 2/3 available soil water (2/3 AW) and 1/3 available soil water (1/3 AW)] and 11 peanut genotypes. The eight genotypes (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348 and ICGV 98353) had high total biomass and pod yield in screening tests under drought stress conditions. Another three genotypes were Tifton 8 (a Virginia-type drought resistant line received from USDA), KK 60-3 (a Virginia-type peanut cultivar being grown in Thailand) and Tainan 9 (a Spanish-type peanut cultivar having low N_2 fixation and low dry matter production). A split-plot in a randomized completed block design with six replications was used. Water levels were assigned to main-plots and peanut genotypes were assigned to sub-plots.

Data were recorded on relative water content (RWC) and leaf water potential (LWP) at 30, 60 and 90 days after emergence (DAE), and on total dry matter (biomass, pod dry weight and harvest index), total nitrogen, phosphorus, potassium and calcium contents at harvest.

Results

Leaf water potential (LWP) for FC treatment evaluated at 30, 60 and 90 DAE in the dry season and the rainy season were higher than those for 2/3 AW and 1/3 AW treatments (Fig. 1). The 1/3 AW treatment had the lowest relative water content (RWC).

Season × water regime interactions and the differences between seasons, water regimes and genotypes were highly significant for nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) (Table 1). The interactions between season and genotype were highly significant only for N and K, whereas the interactions between water regime and genotype and between seasons, water regimes and genotypes were not significant for these traits.

Drought at 2/3 AW and 1/3 AW in the dry season 2002/03 and the rainy season 2003 significantly reduced plant nutrient uptake compared to field capacity (Table 2). Tifton 8 had the highest nutrient uptake for N, P, K and Ca at all water regimes and in both seasons. Tainan 9 had the lowest N (144 mg $plant^{-1}$) in the rainy season (2003) and ICGV 98353 had the lowest N (72.88 mg $plant^{-1}$) in the dry season (2002/03). However, the means of all nutrients in the rainy season 2003 were higher than in the dry season 2002/03.

Positive and significant correlations were found between biomass (BM) and total N, P, K, Ca across water regimes in the dry season and the rainy season (Table 3). The correlations between pod dry weight and total nutrient were also positive and significant in the dry season and the rainy season. However, the correlations between N and HI and between P and HI were not significant in the dry season 2002/03, but they were significant in the rainy season 2003 ($r=0.36$ and $r=0.52$, respectively).

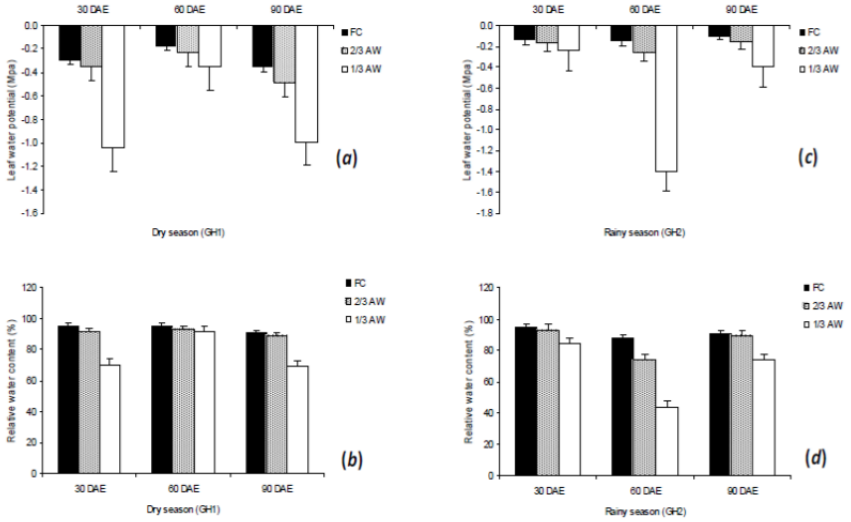


Fig. 1. Leaf water potential and relative water content at 30, 60 and 90 day after emergence (DAE) in dry season (*a* and *b* for greenhouse 1; GH1) and rainy season (*c* and *d* for greenhouse 2; GH2) in 2003.

Table 1. Mean squares for the combined analyses of variance for N, P, K and Ca under greenhouse conditions in the dry season (2002/03) and the rainy season (2003).

SOV	df	N	P	K	Ca
Season	1	483263**	5460.95**	555859**	490798**
Rep/season	10	4139	176.16	3830	9428
Water (W)	2	269262**	1569.55**	503994**	610331**
Genotype (G)	10	19390**	90.39**	8299**	15183**
S X W	2	28042**	401.41**	27699**	42315**
S X G	10	4762**	18.19	3515**	2148
W X G	20	2511	12.99	1832	1827
S X W X G	20	2307	10.82	1449	2065
Error	320	1632	10.79	1352	1391
C.V. (%)		29.03	30.85	22.19	24.09

** Significant at the 0.01

Table 2. N, P, K and Ca contents under three water regimes of 11 peanut genotypes in the dry season (2002/03) and the rainy season (2003).

	Dry season				Rainy season			
Factor	N	P	K	Ca	N	P	K	Ca
Water regime								
FC	139.59^{a}	8.93^{a}	182.62^{a}	176.96^{a}	242.34^{a}	20.33^{a}	290.18^{a}	288.20^{a}
2/3 AW	84.61^{b}	5.63^{b}	105.71^{b}	94.44^{b}	131.78^{c}	11.63^{b}	170.69^{b}	153.11^{b}
1/3 AW	88.41^{b}	6.24^{b}	96.20^{b}	84.41^{b}	148.10^{b}	11.12^{b}	148.45^{c}	128.73^{c}
Genotype								
ICGV 98300	101.45^{c}	6.36b^{cd}	116.24^{bc}	109.10^{cd}	166.00^{de}	14.52^{bcd}	194.08^{cdef}	179.00^{cd}
ICGV 98303	85.93^{cd}	5.73^{cd}	104.66^{c}	95.68^{d}	199.61^{b}	15.93^{ab}	220.43^{ab}	177.11^{cd}
ICGV 98305	83.23^{cd}	5.81^{cd}	120.55^{bc}	96.50^{d}	170.91^{cd}	13.81^{bcd}	205.93^{bcde}	184.77^{bc}
ICGV 98308	84.52^{cd}	6.18^{cd}	129.68^{b}	94.88^{d}	162.13^{de}	13.05^{d}	205.48^{bcde}	161.34^{d}
ICGV 98324	96.15^{cd}	6.17^{cd}	124.95^{bc}	119.71^{bcd}	155.85^{de}	12.49^{d}	192.77^{def}	186.65^{bc}
ICGV 98330	106.27^{bc}	7.00^{bcd}	139.92^{b}	131.51^{bc}	194.88^{bc}	15.50^{bc}	218.60^{abc}	207.97^{b}
ICGV 98348	101.63^{c}	6.34^{bcd}	129.91^{bc}	114.77^{cd}	166.02^{de}	13.30^{cd}	277.03^{f}	171.96^{cd}
ICGV 98353	72.88^{d}	5.20^{d}	117.04^{bc}	114.31^{cd}	156.13^{de}	13.93^{bcd}	215.58^{abcd}	207.53^{b}
Tainan 9	108.90b^{c}	7.16^{bc}	128.67^{b}	143.16^{ab}	144.06^{e}	13.83^{bcd}	179.37^{f}	205.72^{b}
KK 60-3	131.48^{b}	8.18^{b}	130.38^{b}	133.25^{bc}	173.52^{cd}	13.64^{bcd}	188.99^{ef}	170.42^{cd}
Tifton 8	173.79^{a}	12.12^{a}	173.94^{a}	162.76^{a}	225.66^{a}	17.94^{a}	235.93^{a}	237.66^{a}
Mean	104.20	6.93	128.18	119.60	174.07	14.36	203.11	190.01

Mean in the same column for each factor with the same letters are not significantly different by DMRT (at $P < 0.05$).

Table 3. Correlation coefficients among total N, P, K, Ca, biomass (BM), pod dry weight (PDW) and harvest index (HI) of 11 peanut genotypes across three water regimes grown in the dry season (2002/03) and rainy season (2003).

	Dry season			Rainy season		
Nutrient	BM	PDW	HI	BM	PDW	HI
N	0.74**	0.39*	0.00	0.82**	0.66**	0.36*
P	0.66**	0.38*	-0.11	0.91**	0.78**	0.52**
K	0.91**	0.70**	0.37*	0.93**	0.85**	0.64**
Ca	0.90**	0.71**	0.41*	0.92**	0.84**	0.64**

*,** Significant at $P < 0.05$ and $P < 0.01$, respectively.

Conclusion

Drought reduced the uptakes of N, P, K and Ca in peanut. High nutrient uptakes across water regimes were observed in Tifton 8 and KK 60-3. Performances for these traits of peanut genotypes were consistent across water regimes. Peanut genotype that showed the high uptake of one nutrient seemed to be high in the uptake of other nutrients. This information is useful for selecting parents for future crossing.

References

Fageria, N.K., V.C. Baligar and R.B. Clark. 2002. Micronutrient in crop production. Adv. Agron. 77: 185-268.

Girdthai, T., S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew, C.C. Holbrook and A. Patanothai. 2010. Heritability of and genotypic correlations between, aflatoxin traits and physiological traits for drought tolerance under end of season drought in peanut (*Arachishypogaea* L.). Field Crops Res. 118: 169-176.

Kolay, A.K., 2008. Soil moisture stress and plant growth. In: Water and Crop Growth. pp. 45-46, Atlantic, Delhi.

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 N_2 fixation in eleven peanut (*Arachis hypogaea*L.) genotypes differing in degrees of resistance to drought. Asian J. Plant Sci. 7: 334-342.

Sankar, B., C.A. Jaleel, P. Manivannan, A. Kishorekumar, R. Somasundaramand R. Panneerselvam. 2008. Relative efficacy of water use in five varieties of *Abelmoschus esculentus* (L.) Moench. under water-limited conditions. Colloids Surf. B: Biointerfaces. 62: 125-129.

Songsri, P., S. Jogloy, T. Kesmala, N. Vorasoot, C. Akkasaeng, A. Patanothai and C.C. Holbrook. 2008: Responses of reproductive characters of drought resistant peanut genotypes to drought. Asian J. Plant Sci. 7: 427-439.

Tanguilig, V.C., E.B. Yambao, J.C. O'Toole and S.K. De Datta. 1987. Water stress effect on leaf elongation, leaf water potential, transpiration and nutrient uptake of rice, maize and soybean. Plant Soil. 103: 155-168.

Publication

Junjittakarn, J., S. Pimratch, S. Jogloy, W. Htoon, N. Singkham,N. Vorasoot, B. Toomsan, C.C. Holbrook and A. Patanothai. 2013. Nutrient uptake of peanut genotypes under different water regimes. Int. J. Plant Prod.7: 691-706 (Impact factor = 1.028).

1.12 Association between aflatoxin contamination and N_2 fixation in peanut under drought conditions

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

Aflatoxin contamination is the most important quality problem in peanuts throughout the world as it is related to serious health problems in human as well as in livestock. Alleviation of aflatoxin contamination through genetic manipulation has been a long–term goal of peanut breeders. Research has suggested that drought tolerance in peanut may have the potential to be used as an indirect selection criterion for resistance to pre–harvest aflatoxin contamination as drought tolerant lines generally display lower levels of pre–harvest aflatoxin contamination (Arunyanark *et al.*, 2009; Arunyanark *et al.*, 2010; Girdthai *et al.*, 2010). Drought tolerance mechanisms can enhance the ability of genotypes to minimize aflatoxin production. Therefore, breeding of peanut for drought tolerance is seen as a promising strategy for reducing aflatoxin contamination.

The ability of peanut to maintain high N_2 fixation under water limited conditions could be a mechanism for tolerance to drought stress (Pimratch *et al.*, 2008b). Atmospheric nitrogen can be assimilated into useful forms through symbiotic nitrogen fixation by legumes including peanut, in association with specific *Rhizobium* spp. or *Bradyrhizobium* spp. (Giller 2001). As nitrogen is an essential nutrient for growth and yield of peanut, genotypes with high nitrogen fixation would be expected to give higher yield under drought.

Pimratch *et al.* (2008b) reported that the ability to maintain high nitrogen fixation under drought stress could aid peanut genotypes in maintaining high yield under water limited conditions. Moreover, it has been well demonstrated in peanut that nitrogen fixation is closely related to nodule traits and nitrogenase activity and they have been used as surrogate traits for nitrogen fixation (Pimratch *et al.*, 2008a). Nitrogen fixation and its related traits may be used as indirect selection tools for aflatoxin resistance.

Objective

The objective of this study was to investigate the relationship between N_2 fixation traits and aflatoxin contamination under different drought stress conditions.

Materials and Methods

A field experiment was conducted during November 2003 to March 2004, and October 2004 to February 2005, using a split–plot in a randomized complete block design with four replications. Three water regimes (field capacity (1 AW), 2/3 available water (AW) and 1/3 AW) were assigned to main plots and 11 peanut cultivars were arranged in sub–plots. Eleven peanut genotypes (ICGV 98300, ICGV 98303, ICGV 98305, ICGV 98308, ICGV 98324, ICGV 98330, ICGV 98348, ICGV 98353, Tifton–8, KK 60–3 and Tainan 9) were selected because of their superior drought resistance characteristics. A non–nodulating line (Non–nod) obtained from ICRISAT was also included as a reference plant in determining nitrogen fixation.

Data were collected on kernel infection by *Aspergillus flavus*, aflatoxin contamination, total nitrogen content, N_2 fixation and its related traits, i.e., nodule number, nodule dry weight and nitrogenese activity. Drought tolerance index (DTI) was

calculated for total nitrogen content, N_2 fixation and its related traits as the ratio of each parameter in the stress treatment (1/3 available water) to the well–watered treatment.

Results

Combined analysis of variance showed significant effects of seasons (S) on kernel infection by *A. Flavus* (P<0.01) and aflatoxin contamination (P<0.01) (Table 1). The effects of water regimes (W) and genotypes (G) were also significant (P<0.01) for kernel infection, aflatoxin contamination, total nitrogen content, N_2 fixation and percent of N derived from the atmosphere (%Ndfa). Interaction effects were significant for all parameters. Generally, G x E interactions such as S x W, S x G, W x G, and S x W x G were highest for aflatoxin contamination. Drought consistently increased kernel infection and aflatoxin contamination in both seasons (data not shown). However, drought stress consistently reduced total nitrogen content, N_2 fixation and % Ndfa (data not shown).

There were negative and significant correlations between total nitrogen content and N_2 fixation with kernel infection and aflatoxin contamination when data were pooled across irrigation treatments (Table 2) in each season (r = –0.37* to –0.55**) and pooled data (r = –0.28* to –0.45**). The reverse relationships were also found between nodule number, nodule dry weight and acetylene reduction assay (ARA) with kernel infection and aflatoxin contamination across water regimes in each season (r = –0.11 to –0.63**) and pooled data (r = –0.14 to –0.60**).

The correlations between kernel infection and aflatoxin contamination with nitrogen content, N_2 fixation and its related traits were evaluated across seasons under severe drought stress and non–stress conditions (Table 3). There were negative relationships between kernel infection and aflatoxin contamination with nitrogen content and N_2 fixation in the whole plant (shoot+shell+kernel) and shoot under drought conditions (r = –0.26 to –0.67**). However, such relationships with nitrogen content and N_2 fixation in kernels and nitrogen partitioning to kernels were positive (r = 0.22 to 0.54**). The negative correlations in shoots cancelled out the positive correlations in kernels and resulted in weak correlations in the whole plant. Moreover, correlations between kernel infection and aflatoxin contamination with nodule dry weight and ARA under drought conditions were negative (r = –0.43* to –0.86**). In addition, the negative correlations between kernel infection and aflatoxin contamination with drought tolerance index (DTI) of nitrogen content, N_2 fixation and its related traits were also found. In general, correlations between kernel infection and aflatoxin contamination with nitrogen traits were stronger under drought conditions than well–watered conditions.

Table 1. Pooled analysis of variance over two seasons for kernel infection by *A. flavus*, aflatoxin contamination, total nitrogen content, N_2 fixation and percent of N derived from the atmosphere (% Ndfa).

Source of variance	df	Mean squares									
		Kernel infection		Aflatoxin		Total nitrogen content		N_2 fixation		% N_2 fixation	
Season (S)	1	52920	**	41712	**	0.026		0.006		7	
Rep. within season	6	877		687		0.180		0.341		1973	
Water regimes (W)	2	4708	**	38578	**	2.857	**	3.786	**	11806	**
S x W	2	552		9024	*	0.423	**	0.228		348	
Error A	12	240		1833		0.028		0.118		1162	
Genotypes (G)	10	859	**	11142	**	0.097	**	0.096	**	178	**
S x G	10	173	*	4612	**	0.021		0.024	*	23	
W x G	20	115		3303	**	0.028	**	0.030	**	66	**
S x W x G	20	59		1663	**	0.024	*	0.023	*	27	
Error B	180	81		631		0.013		0.012		26	

Significant at * P < 0.05 and ** P < 0.01 levels.

Table 2. Correlation coefficients between kernel infection and aflatoxin contamination with total nitrogen content, N_2 fixation and related traits of N_2 fixation across three water regimes.

Traits	Kernel infection						Aflatoxin contamination					
	2003/04 (n = 33)		2004/05 (n = 33)		Pooled (n = 66)		2003/04 (n = 33)		2004/05 (n = 33)		Pooled (n = 66)	
Total nitrogen content	–0.42	*	–0.50	**	–0.32	**	–0.37	*	–0.48	**	–0.45	**
N_2 fixation	–0.50	**	–0.55	**	–0.28	*	–0.48	**	–0.49	**	–0.43	**
Nodule number	–0.32		–0.48	**	–0.14		–0.52	**	–0.50	**	–0.41	**
Nodule dry weight	–0.11		–0.51	**	–0.45	**	–0.35	*	–0.45	**	–0.47	**
ARA	–0.59	**	–0.51	**	–0.60	**	–0.63	**	–0.49	**	–0.50	**

Significant at * P < 0.05 and ** P < 0.01 levels. ARA, Acetylene reduction assay.

Table 3. Correlation coefficients between kernel infection and aflatoxin contamination with total nitrogen content, N_2 fixation and related traits of N_2 fixation of 11 peanut genotypes in the three water regimes.

Triats	Kernel infection			Aflatoxin contamination		
	2/3 AW	1/3 AW	DTI	2/3 AW	1/3 AW	DTI
Total nitrogen content						
Plant (shoot + shell+kernel)	0.37	–0.67**	-0.74**	–0.09	–0.33	–0.28
Shoot	–0.34	–0.62**	–0.60**	–0.41	–0.48*	–0.56**
Kernel	0.47*	–0.07	–0.55**	0.21	0.29	0.05
Partitioning to kernel	0.41	0.34	–0.09	0.31	0.50*	0.48*
N_2 fixation						
Plant (shoot + shell+kernel)	0.30	–0.49*	–0.61**	–0.15	–0.26	–0.22
Shoot	–0.43*	–0.49*	–0.34	–0.46*	–0.44*	–0.52*
Kernel	0.49*	0.22	–0.19	0.22	0.45*	0.33
Partitioning to kernel	0.44*	0.43*	0.20	0.35	0.54**	0.61**
Related traits of N_2 fixation						
Nodule number	0.73**	–0.33*	–0.58**	0.40	–0.35	–0.41
Nodule dry weight	0.43*	–0.65**	–0.81**	0.16	–0.43*	–0.50*
ARA	–0.70**	–0.86**	0.13	–0.58**	–0.60**	0.11

Significant at * $P < 0.05$ and ** $P < 0.01$ levels. ARA, Acetylene reduction assay.
Partitioning to kernel = N of kernel / N of plant;
DTI = drought tolerance index of nitrogen parameters was calculated by
the ratio of stressed (1/3 available water) / non–stressed (1 available water) conditions.

Conclusion

Drought reduced total nitrogen content and N_2 fixation, but increased kernel infection by *A. flavus* and aflatoxin contamination. This is the first report showing evidence that total nitrogen content, N_2 fixation and its related traits such as nodule number, nodule dry weight and ARA had negative and significant effects on kernel infection and aflatoxin contamination. Therefore, peanuts with the ability to maintain high nitrogen content or N_2 fixation and its related traits under drought conditions would also exhibit better aflatoxin resistance.

References

Arunyanark, A., S. Jogloy, C. Akkasaeng, N. Vorasoot, R.C. Nageswara Rao, G.C. Wright and A. Patanothai. 2009. Association between aflatoxin contamination and drought tolerance traits in peanut. Field Crops Res. 114: 14-22.

Arunyanark, A., S. Jogloy, C. Akkasaeng, N. Vorasoot, T. Kesmala, R.C. Nageswara Rao, G.C. Wright and A. Patanothai. 2010. Heritability of aflatoxin resistance traits and correlation with drought tolerance traits in peanut. Field Crops Res. 117: 258-264.

Giller, K.E. 2001. Nitrogen Fixation in Tropical Cropping Systems. 2nd edition. CAB International, Wallingford.

Girdthai, T., S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew, C.C. Holbrook and A. Patanothai. 2010. Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought. Plant Breed. 129: 693-699.

Pimratch, S., S. Jogloy, N. Vorasoot, B. Toomsan, T. Kesmala, A. Patanothai and C.C. Holbrook. 2008a. Effect of drought stress on traits releated to N_2 fixation in eleven peanut (*Arachis hypogaea* L.) genotypes differing in degrees of resistance to drought. Asian J. Plant Sci. 7: 334-342.

Pimratch, S., S. Jogloy, N. Vorasoot, B. Toomsan, A. Patanothai and C.C. Holbrook. 2008b. Relationship between biomass production and nitrogen fixation under drought–stress conditions in peanut genotypes with different levels of drought resistance. J. Agron. Crop Sci. 194: 15-25.

Publication

Arunyanark, A, S. Pimratch, S. Jogloy, S. Wongkaew, N. Vorasoot ,C. Akkasang, A. Patanothai and C.C. Holbrook. 2012. Association between aflatoxin contamination and N_2 fixation in peanut under drought conditions. Int. J. Plant Prod. 6:161-172. (Impact factor 1.028).

1.13 Combining ability for oleic acid in peanut (*Arachis hypogaea* L.)

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

Peanut (*Arachis hypogaea* L.) is an important oil-bearing crop and the kernels contain 50% oil. Oleic and linoleic acids are the primary fatty acids in peanut oil accounting for 80% of fatty acid composition (Andersen and Gorbet, 2002). Oleic acid was positively correlated with the ratio of oleic to linoleic acids (O/L ratio), but was negatively associated with linoleic acid (Dwivedi *et al.*, 1993). High oleic acid peanut has much longer shelf life and flavor stability than normal-oleic peanut (Mugendi *et al.*, 1998).

Most studies supported a model that oleic acid is controlled by two recessive genes (*ol1* and *ol2*) (Isleib *et al.*, 1996). However, they were also studies that support the quantitative inheritance of this trait. The results of previous studies appeared to be dependent on materials used (with or without high oleic acid *ol* genes) and methods of studies (qualitative or quantitative), and genetic control of oleic acid may be beyond the two major genes with large effect (Isleib *et al.*, 2006). The O/L ratio in a segregating population with high oleic acid showed continuous variation ranging from 2.2 to 25.4 (López *et al.*, 2001). High oleic acid can improve peanut oil quality similar to that of olive oil, and is an important objective of most current breeding programs.

Since the discovery of high oleic acid in peanut, Georgia-02C (Branch, 2003) and SunOleic 97R (Gorbet and Knauft, 2000) were developed and used as parents in many

peanut breeding programs to transfer the high oleic character. However, a breeding line ([(NC17090 × B1)-9-1 × KK 60-3] F6-8-3) developed at Khon Kaen University has intermediate oleic acid concentration, and it may carry either *ol1* or *ol2* or another oleic modifying gene.

Objectives

The objective of this study were to determine GCA and SCA effects for oleic acid concentration in peanut crosses, and to determine the effect of the KKU intermediate oleic genotype when crossed with high oleic and normal oleic genotypes.

Materials and Methods

Five peanut genotypes differing in oleic acid concentration were tested in a field experiment in the rainy season 2008 and the dry season 2008/09, using a randomized complete block design with four replications. They included SunOleic 97R, Georgia-02C, [(NC17090 × B1)-9-1 × KK 60-3] F6-8-3, KKU 1 and KK 5. These lines were used in crossing to produce F1 hybrids. Twelve seeds for each of the 20 F_1 hybrids and the five parental lines were planted in the rainy season (2008) for producing F_2 seeds. The first set of F_2 seeds was analyzed for fatty acids, and the other set was used for planting plots to produce the F_3 generation. The remnant F_2 seeds of the 20 crosses and seeds of the parental lines were planted in the dry season (2008/09). The seeds from each plot were bulked for fatty acid analysis.

Each sample in the F2 and F3 generations was extracted for oil. The extracted oil was analyzed for fatty acid concentration by gas liquid chromatography (GLC) and Shimadzu Gas Chromatograph (SGE).

Results

Parents were significantly different for most characters in both seasons (rainy 2008 and dry 2008/09) except for % oil in the rainy season (Table 1). Georgia-02C and SunOleic 97R had the highest oleic, eicosenoic, lignoceric acid, O/L ratio and U/S ratio in both seasons. Georgia-02C and SunOleic 97R also had low palmitic, stearic, linoleic, arachidic acids and low IV in both seasons. KK 5 and KKU 1 had relatively low oleic acid and O/L ratio, and they had higher linoleic acid in both seasons. [(NC17090 × B1)-9-1 × KK 60-3]F6-8-3 had intermediate-oleic acid in both the rainy and dry seasons (71.6 and 67.3%, respectively).

GCA was significant for all characters in both the F_2 and F_3 generations (Table 2). SCA was also significant for palmitic, stearic, oleic, and linoleic acids, as well as % oil, O/L ratio, IV and U/S ratio in both F_2 and F_3 generations. The reciprocal effects were significant for palmitic, oleic, linoleic acids, % oil, O/L ratio and IV in both the F_2 and F_3 generations. The ratios of GCA/SCA mean squares for oleic acid and linoleic acid were high in both the F_2 and F_3 generations, and, therefore, GCA contributed greater to the variation in these characters than did SCA.

Table 1. Means for fatty acid concentration (% of total fatty acid), % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV), unsaturated to saturated fatty acids ratio (U/S ratio) of parental lines in the rainy season 2008 and the dry season 2008/09 in Thailand.

Parental line	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoceric acid	% oil	O/L ratio	IV	U/S ratio
						Rainy season 2008						
SunOleic 97R	6.3 d	3.1 d	78.7 a	4.5 d	1.4 d	1.8 a	2.8 b	1.7 a	47.2	18.0 b	76.8 d	5.5 a
Georgia-02C	6.5 d	2.8 d	79.8 a	3.1 e	1.3 d	1.8 a	2.7 b	1.7 a	46.5	27.1 a	75.4 d	5.7 a
F6-8-3[a]	7.3 c	4.5 b	71.6 b	11.0 c	1.9 b	1.0 b	2.7 b	1.1 b	45.0	6.6 c	81.3 c	4.8 b
KK 5	11.5 a	4.0 c	46.9 c	31.0 a	1.7 c	0.7 c	3.0 ab	1.3 b	47.6	1.5 c	94.6 a	3.7 c
KKU 1	10.6 b	5.4 a	47.5 c	28.4 b	2.2 a	0.6 d	3.2 a	1.2 b	50.3	1.7 c	90.5 b	3.4 d
Mean	8.6	4.0	63.8	16.4	1.7	1.2	2.9	1.4	10.5	47.3	84.1	4.5
F-test	**	**	**	**	**	**	**	**		**	**	**
						Dry season 2008/09						
SunOleic 97R	6.1 e	2.3 c	80.5 a	3.8 d	1.3 c	1.8 a	2.5 b	1.7 a	47.8 ab	21.8 a	77.1 c	6.2 a
Georgia-02C	6.9 d	2.6 c	78.3 a	4.8 d	1.3 c	1.8 a	2.7 b	1.6 a	51.4 a	17.1 b	77.2 c	5.6 b
F6-8-3[a]	8.6 c	4.1 b	67.3 b	14.4 c	1.8 b	1.0 b	2.6 b	1.2 b	45.8 b	4.7 c	83.5 b	4.5 c
KK 5	11.9 a	4.1 b	45.3 c	30.2 a	1.7 b	0.8 bc	3.2 a	1.3 b	43.6 b	1.5 c	91.8 a	3.4 d
KKU 1	10.7 b	5.2 a	49.2 c	27.0 b	2.1 a	0.7 c	3.3 a	1.3 b	45.4 b	1.9 c	89.7 a	3.4 d
Mean	8.8	3.6	64.1	16.0	1.6	1.2	2.9	1.4	9.4	46.8	83.8	4.7
F-test	**	**	**	**	**	**	**	**	**	*	**	**

[a] The variety [(NC17090 × B1)-9-1 × KK 60-3]F6-8-3.
* and ** significant at the 5% and 1% probability levels, respectively.
Means in the same column followed by the same letter (s) are not significantly different (at $P < 0.05$) by DMRT.

Table 2. Mean squares for fatty acid concentration, % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV), unsaturated to saturated fatty acids ratio (U/S ratio) in the F_2 and F_3 generations.

Source	d.f.	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoceric acid	% oil	O/L ratio	IV	U/S ratio
							F_2 generation						
GCA[a]	4	13.60**	1.96**	609.50**	393.69**	0.21**	0.67**	0.16**	0.14**	1.15**	219.78**	158.31**	2.51**
SCA[b]	10	0.26**	0.14**	5.75**	4.23**	0.01	0.02	0.03	0.01	7.22**	37.86**	2.46**	0.10*
Reciprocals	10	0.09*	0.06	8.58**	5.60**	0.01	0.01	0.01	0.01	4.93**	0.62**	2.94**	0.02
GCA/SCA[c]		52.26	14.07	105.98	93.1	21.47	34.5	5.72	22.62	0.16	5.8	64.38	24.97
Error	48	0.04	0.02	0.89	0.58	0.003	0.005	0.01	0.004	1.17	1.05	0.61	0.01
							F_3 generation						
GCA[a]	4	15.91**	3.34**	733.20**	434.55**	0.31**	0.64**	0.28**	0.15*	11.48**	217.39**	155.70**	3.64**
SCA[b]	10	0.80**	0.10*	17.10**	19.44**	0.01	0.03	0.05	0.01	6.14**	41.63**	17.93**	0.16**
Reciprocals	10	0.50**	0.09	22.53**	17.71**	0.02	0.01	0.05	0.02	3.32**	7.33**	12.42**	0.05
GCA/SCA[c]		19.86	31.93	42.28	22.35	44.26	21.88	5.96	15.25	1.87	5.22	8.68	23.43
Error	48	0.08	0.04	6.19	3.59	0.01	0.004	0.01	0.01	1.54	1.54	4.11	0.02

* and ** significant at the 5% and 1% probability levels, respectively.
[a] General combining ability.
[b] Specific combining ability.
[c] The ratio of general combining ability mean squares and specific combining ability mean squares.

SunOleic 97R, Georgia-02C and [(NC17090 × B1)-9-1 × KK 60-3] F6-8-3 had high GCA effects for oleic acid both in the F_2 and F_3 generations (Table 3). In contrast to oleic acid, these genotypes had low GCA effect for linoleic acid (Table 3). For O/L ratio, GCA effects were high for SunOleic 97R and Georgia-02C in both the F_2 and F_3 generations. The correlation coefficients between fatty acid composition of the parents and their progenies were positive and significant except for % oil in both the F_2 and F_3 generations.

For SCA effects in the F_2 generation, SunOleic 97R × Georgia-02C had the highest SCA effects for oleic acid and O/L ratio, whereas KKU 1 × Georgia-02C had the lowest SCA effect for oleic acid but it had the highest SCA effects for linoleic acid and IV (Table 4). For SCA effects in the F_3 generation, SunOleic 97R × Georgia-02C had the highest for oleic acid and for O/L ratio, but it had the lowest SCA for linoleic acid and IV (Table 5). KK 5 × SunOleic 97R had the highest SCA effect for linoleic acid but it had the lowest SCA effect for oleic acid.

The GCA effect of the intermediate oleic genotype [(NC 17090 x B1)-9-1 x KK60-3)F6-8-3] was positive and significant for olelic acid in both F_2 and F_3 (Table 3). The SCA effects of this line with high oleic genotypes (SunOleic 97R and Georgia-02C) were both negative for oleic acid concentration, and the crosses with normal oleic genotypes (KK 5 and KKU 1) also exhibited negative for oleic acid concentration in both F2 and F3 generations (Table 4 and 5).

Table 3. General combining ability effects for fatty acid concentration, % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV), unsaturated to saturated fatty acids ratio (U/S ratio) and correlation between fatty acid compositions of parental lines and their progenies in the F_2 and F_3 generations.

Parental line	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoceric acid	% oil	O/L ratio	IV	U/S ratio
F2 generation												
SunOleic 97R	-0.98**	-0.41**	6.41**	-5.01**	-0.15**	0.24**	-0.09**	0.11**	0.14	3.85**	-2.97**	0.47**
Georgia-02C	-0.98**	-0.52**	7.20**	-5.97**	-0.15**	0.30**	0.01	0.14**	-0.28	5.94**	-3.91**	0.46**
F6-8-3[a]	-0.56**	0.22*	3.16**	-2.46**	0.06**	-0.07**	-0.13**	-0.15**	0.01	-1.31**	-1.60**	0.13**
KK 5	1.41**	0.22*	-8.57**	7.14**	0.06**	-0.24**	0.01	-0.06**	-0.36	-4.30**	4.82**	-0.49**
KKU 1	1.11**	0.50**	-8.21**	6.30**	0.18**	-0.24**	0.20**	-0.05**	0.48	-4.19**	3.66**	-0.57**
r[b]	0.59**	0.47**	0.59**	0.58**	0.50**	0.56**	0.39*	0.47**	-0.09	0.47**	0.57**	0.56**
F3 generation												
SunOleic 97R	-1.12**	-0.64**	8.02**	-5.75**	-0.21**	0.26**	-0.16**	0.11**	0.41	5.58**	-2.87**	0.69**
Georgia-02C	-1.11**	-0.54**	7.72**	-6.33**	-0.14**	0.28**	-0.02	0.14**	1.30**	4.41**	-4.10**	0.50**
F6-8-3[a]	-0.43**	0.32**	2.16**	-1.85**	0.10**	-0.10**	-0.15**	-0.11**	0.52	-1.93**	-1.41**	0.02
KK 5	1.59**	0.15**	-9.48**	7.28**	0.04	-0.19**	0.13**	-0.11**	-1.11**	-3.86**	4.31**	-0.59**
KKU 1	1.07**	0.71**	-8.42**	6.65**	0.21**	-0.25**	0.21**	-0.04**	-1.12**	-4.21**	4.07**	-0.62**
r[b]	0.53**	0.54**	0.56**	0.54**	0.52**	0.55**	0.36*	0.44**	0.16	0.45**	0.47**	0.56**

* and ** significant different from zero at the 5% and 1% probability levels, respectively.
[a] The variety [(NC17090 × B1)-9-1 × KK 60-3]F6-8-3.
[b] Correlation coefficient between fatty acid contents of parents and their progenies.

Table 4. Specific combining ability effects for fatty acid concentration, % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV), unsaturated to saturated fatty acids ratio (U/S ratio) in the F_2 generation.

Cross	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoceric acid	% oil	O/L ratio	IV	U/S ratio
SunOleic 97R x Georgia-02C	-0.27	-0.14	2.16**	-1.87**	-0.05	0.11*	-0.09	-0.03	-1.68	5.30**	-1.29*	-0.06
SunOleic 97R x F6-8-3[a]	0.05	0.01	-1.18*	0.96	0.01	-0.01	-0.05	-0.07	1.87*	-2.12**	0.64	-0.20**
SunOleic 97R x KK 5	0.47**	0.49**	-2.48**	1.98**	0.12**	-0.18**	0.07	-0.03	1.54	-3.43**	1.15	-0.13*
SunOleic 97R x KKU 1	0.33	-0.35**	-0.75	0.97	-0.07	-0.07	0.03	0.02	-0.72	-3.22**	0.99	-0.02
Georgia-02C x SunOleic 97R	0.07	0.10	-0.09	-0.21	0.05	-0.01	0.002	-0.05	1.26	1.09	-0.45	0.24**
Georgia-02C x F6-8-3[a]	0.18	-0.01	-1.55*	1.44*	0.03	-0.04	0.23**	-0.01	2.84**	-3.63**	1.11	-0.09
Georgia-02C x KK 5	0.11	0.04	-0.99	0.73	0.01	-0.08	0.002	-0.03	-2.05**	-5.06**	0.36	-0.15*
Georgia-02C x KKU 1	0.39*	0.17	-1.40	1.18	0.08*	-0.07	0.12	0.07	1.79*	-4.94**	0.78	-0.10
F6-8-3[a] x SunOleic 97R	0.08	0.26*	-0.79	0.34	0.10*	0.08	0.11	0.09	1.08	-0.30	-0.04	0.01
F6-8-3[a] x Georgia-02C	0.02	0.20	-0.16	0.19	0.08*	-0.03	-0.02	0.12*	-1.70	-0.38	0.15	-0.14*
F6-8-3[a] x KK 5	0.06	0.003	1.13	-1.62*	-0.02	0.06	-0.08	0.03	0.46	1.84*	-1.80**	0.05
F6-8-3[a] x KKU 1	0.17	-0.13	-0.08	-0.16	-0.08*	0.03	-0.05	0.04	-2.17**	1.57	-0.31	-0.05
KK 5 x SunOleic 97R	0.26	-0.08	-3.48**	2.70**	0.07	-0.01	0.17*	0.05	-0.88	-0.56	1.68**	-0.35**
KK 5 x Georgia-02C	0.39*	0.14	-2.92**	2.31**	0.04	-0.06	0.07	-0.002	0.40	-0.58	1.43*	-0.14*
KK 5 x F6-8-3[a]	-0.05	-0.05	-0.28	0.75	0.004	-0.003	-0.05	0.01	-0.35	-0.13	1.08	-0.01
KK 5 x KKU 1	-0.43*	-0.24*	1.91*	-1.28*	-0.02	0.11*	0.05	0.02	-0.27	3.38**	-0.49	-0.13*
KKU 1 x SunOleic 97R	-0.16	0.16	-0.44	0.41	0.04	-0.02	0.02	-0.01	1.58	-0.99	0.32	-0.10
KKU 1 x Georgia-02C	0.39*	0.06	-4.13**	3.75**	-0.02	-0.18**	0.03	-0.02	-2.25**	-0.09	2.80**	-0.29**
KKU 1 x F6-8-3[a]	0.12	-0.17	-1.70*	0.57	-0.02	0.02	0.04	0.04	2.48**	-0.18	-0.48	-0.01
KKU 1 x KK 5	0.16	0.34**	-1.21	0.31	0.12**	-0.07	0.02	-0.04	-2.06**	-0.06	-0.54	0.26**

* and ** significant different from zero at the 5% and 1% probability levels, respectively.
[a] The variety [(NC17090 × B1)-9-1 × KK 60-3]F6-8-3.

Table 5. Specific combining ability effects for fatty acid concentration, % oil, the ratio of oleic to linoleic acids (O/L ratio), iodine value (IV),unsaturated to saturated fatty acids ratio (U/S ratio) in the F3 generation.

Cross	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Eicosenoic acid	Behenic acid	Lignoceric acid	% oil	O/L ratio	IV	U/S ratio
SunOleic 97R x Georgia-02C	-0.53**	-0.14	3.47	-3.68*	-0.05	0.15**	-0.07	-0.01	-2.57*	8.40**	-3.29*	0.32**
SunOleic 97R x F6-8-3[a]	0.03	-0.08	-1.58	0.92	-0.02	-0.10*	0.09	-0.09	-0.12	-4.08**	0.18	-0.07
SunOleic 97R x KK 5	0.47**	0.08	-1.92	1.46	-0.03	-0.07	0.18*	0.03	2.94**	-3.16**	0.81	-0.33**
SunOleic 97R x KKU 1	1.16	0.23	-3.38	5.24**	0.05	-0.12*	-0.16	-0.04	-0.55	-5.41**	6.10**	-0.34**
Georgia-02C x SunOleic 97R	0.00	-0.10	-0.54	0.69	-0.06	0.04	0.01	-0.05	-0.41	-4.85**	0.76	0.07
Georgia-02C x F6-8-3[a]	-0.01	0.08	1.14	-0.38	0.04	-0.03	0.04	0.00	1.1	-1.94	0.3	-0.03
Georgia-02C x KK 5	0.47**	0.31**	-2.89	3.31*	0.11	-0.18**	0.08	-0.02	0.44	-4.50**	3.12	-0.27**
Georgia-02C x KKU 1	0.44**	-0.28	-3.50	2.46	-0.04	-0.12*	0.08	0.09	-1.11	-3.87**	1.13	-0.24*
F6-8-3[a] x SunOleic 97R	0.46**	0.02	-2.59	2.10	0.01	-0.04	0.06	-0.04	-2.13*	-1.20	1.38	-0.18
F6-8-3[a] x Georgia-02C	-0.11	0.21	-0.17	-1.13	0.08	-0.05	0.00	-0.01	-0.36	0.65	-2.14	-0.13
F6-8-3[a] x KK 5	0.28	-0.12	-0.88	0.82	-0.02	0.04	-0.32**	-0.01	-1.18	1.62	0.72	0.05
F6-8-3[a] x KKU 1	-0.23	0.31*	-0.57	-0.23	0.05	0.04	0.14	0.05	2.08*	2.18**	-0.89	-0.05
KK 5 x SunOleic 97R	1.14	-0.04	-9.38**	8.54**	-0.16*	-0.20**	0.01	-0.21**	-0.89	-3.33**	6.58**	-0.21*
KK 5 x Georgia-02C	0.26	-0.13	-2.24	1.16	-0.03	0.01	0.03	0.08	0.07	-0.21	0.05	-0.10
KK 5 x F6-8-3[a]	0.85**	-0.43**	-1.74	1.35	-0.09	0.10*	0.11	0.02	0.25	-0.29	0.95	-0.10
KK 5 x KKU 1	-0.47*	-0.34*	2.47	-1.98	-0.07	0.11*	-0.05	-0.11	-1.38	3.16**	-1.22	0.35**
KKU 1 x SunOleic 97R	0.23	-0.05	-0.83	1.84	0.06	-0.01	0.23*	0.06	1.24	-0.19	2.45*	-0.06
KKU 1 x Georgia-02C	-0.09	0.11	1.23	0.69	0.03	-0.10*	0.08	0.05	-0.91	0.06	2.15	0.05
KKU 1 x F6-8-3[a]	0.38	0.41	-2.73	1.50	0.19**	-0.01	0.34**	0.08	-2.93**	-0.25	0.20	-0.31*
KKU 1 x KK 5	-0.09	0.08	-0.32	0.48	-0.05	-0.01	-0.20*	-0.18*	0.04	-0.03	0.51	0.08

* and ** significant different from zero at the 5% and 1% probability levels, respectively.
[a] The variety [(NC17090 × B1)-9-1 × KK 60-3]F6-8-3.

Conclusion

Additive gene action was more important than non additive gene action in determining fatty acid concentration. Genotypes with high oleic concentration can be selected in early generations, especially in crosses withSunOleic 97R or Georgia-02C as parents because they carry the two recessive mutant genes (*ol1* and *ol2*) for high oleic acid. The crosses SunOleic 97R ×[(NC17090 × B1)-9-1 × KK 60-3]F6-8-3 and Georgia-02C × [(NC17090 × B1)-9-1 ×KK 60-3]F6-8-3 might be promising in identifying genetic control of oleic acid concentration beyond two major *ol* genes.

References

Andersen, P.C. and D.W. Gorbet. 2002. Influence of year and planting date on fatty acid chemistry of high oleic acid and normal peanut genotypes. J. Agric. Food Chem.50: 1298-1305.

Branch, W.D. 2003: Registration of 'Georgia-02C' peanut. Crop Sci.43: 1883–1884.

Dwivedi, S.L., S.N. Nigam, R. Jambunathan, K.L. Sahrawat, G.V.S. Nagabhushanam and K. Raghunath. 1993. Effect of genotypes and environments on oil content and oil quality parameters and their correlation in peanut (*Arachis hypogaea.* L.). Peanut Sci.20: 84-89.

Gorbet, D.W. and D.A. Knauft. 2000. Registration of 'SunOleic 97R' peanut. Crop Sci. 40: 1190-1191.

Isleib, T.G., C.T. Young and D.A. Knauft. 1996. Fatty acid genotypes of five Virginia-type peanut cultivars. Crop Sci.36: 556-558.

Isleib, T.G., R.F. Wilso and W.P. Novitzky. 2006. Partial dominant, pleiotropism, and epistasis in the inheritance of the high-oleate trait in peanut. Crop Sci. 46: 1331-1335.

López, Y., O.D. Smith, S.A. Senseman and W.L. Rooney 2001. Genetic factor influencing high oleic acid content in Spanish market-type peanut cultivars. Crop Sci. 41: 51-56.

Mugendi, J.B., C.A. Sims, D.W. Gorbet and S.F. O'Keefe. 1998. Flavor stability of high oleic peanut stored at low humidity. J. Am. Oil Chem. Soc.75: 21-25.

Publication

Singkham, N., S. Jogloy, T. Kesmala, P. Swatsitang, P. Jaisil, N. Puppala and A. Patanothai. 2011. Combining ability for oleic acid in peanut (*Arachis hypogaea* L.). SABRAO J. Breed. Genet. 43: 59-72. (Impact factor 2010 = 0.227).

1.14 *Aspergillus flavus* invasion and relevant activity in aflatoxin synthesis in seeds of different drought tolerant peanut genotypes

W. Senakoon, Suporn Nuchadomrong, P. Jeeranaipepame, G. Senawong, S. Jogloy, P. Songsri and A. Patanothai

Drought tolerance is closely related to aflatoxin contamination resistance (Holbrook *et al.*, 2000). There have been no works for comparative evidences of pod *A. flavus* infection or invasion in terms of genotype by water status interaction. The goal of the present work is to use the histological method to better understand the correlation of resistance to *A. flavus* invasion in young peanut pod with two phenotypic traits (specific leaf area; SLA and SPAD chlorophyll meter reading; SCMR) at the beginning pod stage (R3) and the beginning seed stage (R5) of the drought sensitive and tolerant peanut genotypes. The route of *A. flavus* invasion was investigated using lactophenol cotton blue, a potent dye more specific to stain fungal cell (Rebecca *et al.*, 2012). The fungal infection was confirmed by *A. flavus* specific PCR detection. The *A. flavus* virulence was also focused at the synthesis of aflatoxin at the level of *nor-1* gene expression by RT-PCR.

Materials and methods

The study was on four drought resistant peanut genotypes (ICGVs 98300, 98303, 98305, and 98308) and two sensitive genotypes (KK 60-3 and Tainan-9). The *A. flavus* spore suspension was spread to the soil geocarposphere at the date of 50% flowering of the plants (37 DAS) when the early forming pegs were about to penetrate the soil surface. In parallel with control watering, drought stress at the soil water of 1/3 AW was imposed continuously since the R3 stage (57 DAS). SCMR and SLA were evaluated at 11-12 a.m. on the second fully expanded leaf on the main stem apex at the time between R3 and R5 stages (76 DAS). Pod at R3 and R5 stages were fixed and further processed for thin film sections. The sections were double stained (Marques *et al.*, 2013), firstly by lactophenol cotton blue specifically for the chitin in fungal cell wall (Rebecca *et al.*, 2012), and secondly by safranin O for plant cell wall stain. The stained sections were observed under a light microscope.

DNA extraction (CTAB method) and RNA extraction (Qiagen RNeasy Plant Mini kit) were undertaken using pods at the R5 stage. The DNA of *A. flavus* was isolated and used for a positive PCR control. A primer pair for PCR detection of *A. flavus* genomic DNA within the ITS2 of rDNA genes, as described by Sardiñas *et al.* (2011), was used. The *nor-1* primer pair was adopted from Scherm *et al.* (2005) for detection of *nor-1* gene expression by RT-PCR (Transcriptor High Fidelity kit, Roche). DNA sequence analysis was conducted for verification of the PCR and RT-PCR products.

Results

The basal values of SLA and SCMR were demonstrated in the control experiments with slight differences in all six genotypes. Lower SLA indicated a good character. Under drought stress conditions, the SLA reduction was found in the two drought sensitive genotypes, Tainan-9 and KK 60-3, together with one resistant genotype ICGV 98303. The genotypes having unchanged SLA value were ICGV98305 and ICGV 98308. For SCMR value, ICGV 98303 appeared to be the superior drought resistant genotype, compared to ICGV 98300, 98305 and 98308. Therefore, ICGV98303 was well adapted for both SLA and SCMR.

Taken together with the fungal stain in the R3 and R5 pod sections, the fungal invasion was more dense and occupied in more cell layers in Tainan-9 and KK 60-3 (both drought susceptible genotypes) samples even of normal watering condition. Pod maturation from R3 to R5 did not cause the pericarp of any genotype to become more distinguishable vulnerable to fungal invasion. The R5 pods showed that the fungus was captured at the testa region, although fungal invasion into kernel was apparent in a different manner (Fig. 1). The kernels of Tainan-9 and KK 60-3 were contaminated with a large number of fungal colonization. The drought tolerant ICGV 98303 was strikingly not resistant to fungal invasion. ICGV 98300 and ICGV 98305 were more tolerant to kernel invasion when compared to ICGV 98308. PCR and the DNA sequence analysis confirmed the *A. flavus* infection, however, the analyses could not differentiate the level of the fungal contamination (Fig. 2). The RT-PCR result (Fig. 3) demonstrated the difference in the expression level of *nor-1* gene of the aflatoxin synthesis. Under the well watering condition, the resistant genotypes except ICGV98308 exhibited lower expression compared to the susceptible genotypes (Tainan-9 and KK 60-3). ICGV98305 showed remarkable superior for this point. The high expression of *nor-1* under drought was found in all genotypes. However, the detection of high *nor-1* gene expression should not be the ultimate challenging approach for the genotypes tolerant to drought and aflatoxin contamination, because the aflatoxin synthesis is also served by the seed metabolites including amino acids and sugars (Feng and Leonard, 1995).

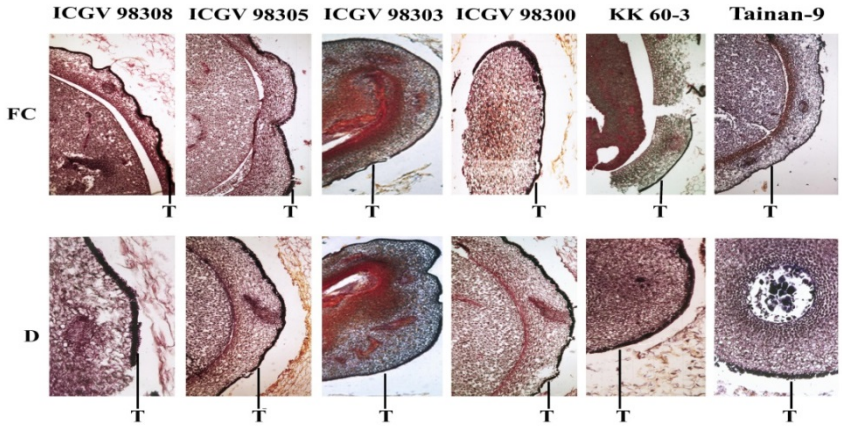


Fig. 1. Fungal stain in seeds of R5 pods from the control (FC) and drought-stressed (D) samples of peanut genotypes. The T letter indicates the testa layer. Observations were at magnification of 4×10.

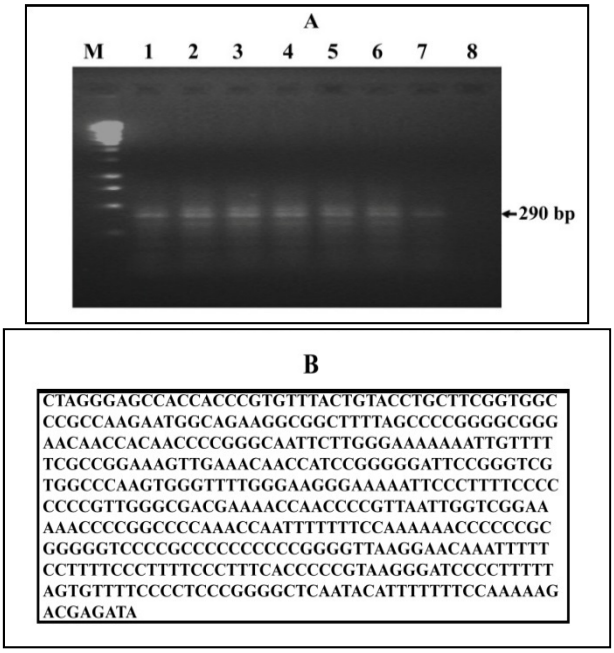


Fig. 2. PCR analysis for *Aspergillus flavus* infection in R5 seeds (A), and the sequence of the expected target 290-bp band (B). Lane 1-6 is represented for Tainan-9, ICGV98300, ICGV98303, ICGV98305 and ICGV98308. Lane 7 is the positive control by *A. flavus* DNA. Lane 8 is the negative control (no DNA templates).

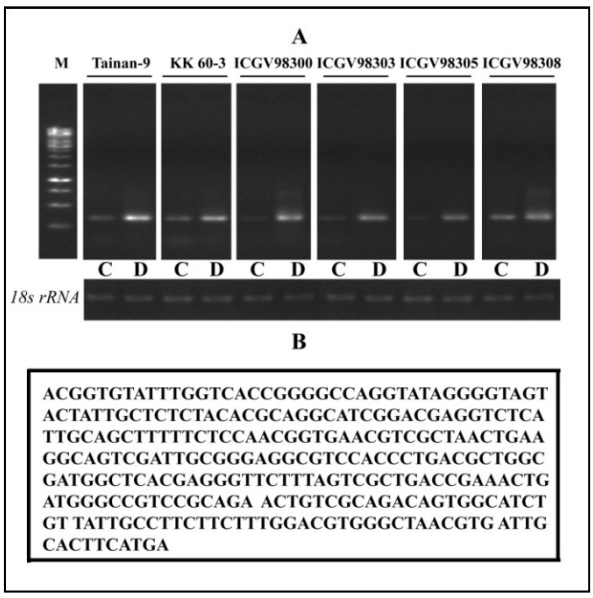


Fig. 3. RT-PCR in the analysis of *nor-1* expression in R5 seeds (A), and the sequence of the expected target 350-bp band (B). C is the control and D is the drought-stressed sample of all genotypes. RT-PCR of *18s rRNA* was normalized in all samples under both water conditions.

Conclusions

The virulence of *A. flavus* in pod invasion and gene expression involving aflatoxin synthesis was studied in correlation with peanut genotypes, water statuses, and growth characteristics. The simple and rapid SCMR measurement method was more beneficial to evaluate drought resistant peanut genotypes (ICGVs) and responses to drought stress than the SLA method. All drought tolerant genotypes tested, especially ICGV 98303, exhibited the good vegetative trait, but the relative resistance to *A. flavus* seed invasion was found in ICGV 98300 and 98305. The tolerant genotype, except ICGV 98308, expressed *nor-1* gene at comparable low level under irrigated condition, indicating its low potential for aflatoxin synthesis. Contrarily, all genotypes had high *nor-1* gene expression in the drought experiment.

References

Feng, G.H. and T.J. Leonard. 1995. Characterization of the polyketide synthase gene (pksL1)required for aflatoxin biosynthesis in *Aspergillus parasiticus*. J. Bacteriol. 177: 6246-6254.

Holbrook, C.C., C.K. Kvien, K.S. Rucker, D.W. Wilson and J.E. Hook. 2000. Preharvest aflatoxin contamination in drought tolerant and intolerant peanut genotypes. Peanut Sci. 27: 45–48.

Marques, J.P.R., M.K.M. Soares and B. Appezzato-da-Gloria. 2013. New stain technique for fungal-infected plant tissues. Turk. J. Bot. 37: 784-787.

Rebecca, L.J., V. Dhanalakshmi, S. Sharmila, G. Susithra, S. Kumar and S. Bala. 2012. Isolation, identification and characterization of fungi from rhizosphere soil of *Barleria cristata*. Inter. J. Hort. Crop. Sci. Res. 2: 1-6.

Sardiñas, N., C. Vazquez, J. Gil-Serna, M.T. Gonzalez-Jaen and B. Patino. 2011. Specific detection and quantification of *Aspergillus flavus* and *Aspergillus parasiticus* in wheat flour by SYBR® Green quantitative PCR. Inter. J. Food Microbiol. 145: 121-125.

Scherm, B., M. Palomba, D. Serra, A. Marcello and Q. Migheli. 2005. Detection of transcripts of the aflatoxin genes *aflD*, *aflO*, and *aflP* by reverse transcription polymerase chain reaction allows differentiation of aflatoxin-producing and nonproducing isolates of *Aspergillus flavus* and *Aspergillus parasiticus*. Inter. J. Food Microbiol. 98: 201-210.

Publication

Senakoon, W., S. Nuchadomrong, P. Jeeranaipepame, G. Senawong, S. Jogloy , P. Songsri and A. Patanothai. 2014. *Aspergillus flavus* invasion and relevant activity in aflatoxin synthesis in seeds of peanuts of different drought tolerant genotypes. Turk. J. Bot. (*Submitted*). (No impact factor)

1.15 HDAC inhibitory activity of peanut testa extracts against human cancer cell lines

S. Khaopha, S. Jogloy, A. Patanothai and T. Senawong

Condensation and decondensation of a chromatin regulate the access of the cellular machinery to specific DNA sequences to facilitate metabolic processes, including transcription, replication, and repair (Kouzarides, 2007). Acetylation is one of the most important post-translational modifications of the N-terminal tails of histones, which, in combination with phosphorylation, methylation and ubiquitination, contributes to a "histone code" determining the activity of target genes. Acetylation of core nucleosomal histones is regulated by a balance between the activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Based on the homology to their yeast analogues, HDACs are classified into four classes: (1) Class I (HDACs 1, 2, 3 and 8), located in the nucleus; (2) Class IIa (HDACs 4, 5, 7 and 9, located in the nucleus) and Class IIb (HDACs 6 and 10, located both in the cytoplasm and nucleus); (3) Class III (Sirtuins or SIRT 1-7, NAD^+-dependent homologues of the yeast Sir2 proteins); and Class IV (HDAC 11, exhibiting features of both Class I and II) (Mark and Xu, 2009). HDACs can be grouped into two distinct families as Zn^{2+}-dependent and NAD^+-dependent HDACs based on the mechanisms of removing the acetyl groups. The balance between HDACs and HATs is often impaired in cancer, which leads to aberrant expressions of tumor suppressor genes and/or proto-oncogenes (Kim and Bae, 2011).

During the last few decades, many types of HDAC inhibitors have been developed and classified as a new class of chemotherapeutic drug currently in several clinical trials with promising results as anticancer agents (Mark and Xu, 2009). The treatment with HDAC inhibitors usually resulted in growth arrest, apoptosis, and inhibition of angiogenesis in cancer cells (Mark and Xu, 2009). HDAC inhibitor treatments resulted in cancer cell apoptosis due to a shift in the balance of pro- and anti-apoptotic genes toward apoptosis (Carew *et al.*, 2008). However, some serious side effects, such as fatigue, nausea, anorexia, diarrhea, anemia, and myalgia, have been observed upon treatment with some HDAC inhibitors, especially pan-HDAC inhibitors (Rikiishi, 2011), indicating that discovering more safe and effective HDAC inhibitors is needed.

In recent years, the development and search for novel HDAC inhibitors have become a popular research focus on discovering safe and effective anticancer agents (Bora-Tatar et al, 2009). The phenolic compounds of some plants have been shown to possess HDAC inhibitory activity (Bora-Tatar *et al.*, 2009; Senawong *et al.*, 2013), indicating that plant phenolic compounds are promising new source of HDAC inhibitors. Interestingly, peanut (*Arachis hypogaea* L.), one of the most popular foods consumed worldwide, is a potential source of natural phenolic compounds, especially in peanut skin (seed coat or testa) (Khaopha *et al.*, 2012). The major phenolic acids found in peanut testa are *p*-coumaric acid and *p*-hydroxybenzoic acid (Khaopha *et al.*, 2012). Strikingly, the purified compound *p*-coumaric acid has been shown to exhibit anticancer activity (Jaganathan *et al.*, 2013; Janicke *et al.*, 2011), hence one can envision that a phenolic-rich testa extract may possess anticancer activity due to, at least in part, its active component *p*-coumaric acid. However, the possibility that other active components of the phenolic-rich testa extract may underpin its anticancer action cannot be excluded. To date, HDAC inhibitory and anticancer activities of peanut testa extracts have not yet been explored.

Objectives

The objective of this study were to (a) investigate HDAC inhibitory activity of 15 Valencia-type peanut testa extracts, (b) assess antiproliferative and apoptosis induction activities of two selected phenolic-rich testa extracts with the greatest HDAC inhibitory activity and greatest amount of phenolic content against 5 human cancer cell lines and one non cancer cell line (Vero cells), and (c) study HPLC profiles and phenolic acid compositions of the two selected extracts.

Materials and Methods

Vero and HCT116 cells were kindly provided by Dr. S. Barusrux (Khon Kaen University) and Dr. O. Tetsu (University of California, San Francisco), respectively. HT29 cells were kindly provided by Dr. P. Picha (National Cancer Institute, Thailand). Fifteen Valencia-type peanut kernels of *A. hypogaea* L. of the 2010 were obtained from the Field Crop Research Station of Khon Kaen University (KKU), deposited in plastic bags, and stored at 4°C until used. Testae were removed from kernels with a razor blade. All phenolic acid pure standards were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA), except for *m*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid purchased from Fluka (Buchs, Switzerland) and Acros Organics (Geel, Belgium), respectively. All cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) (Gibco-BRL). The cells were incubated in a humidified incubator with an atmosphere of 95% air and 5% CO_2 at 37°C.

Crude methanolic extraction using 100% methanol as a solvent was performed to obtain 15 "phenolic-rich" testa extracts. HeLa cells were used for the assessment of HDAC inhibition in mammalian cells due to their high level of HDAC expression. Hyperacetylation of histone H4 was analyzed using Acid-Urea-Triton (AUT) gel electrophoresis. The antiproliferative activity of two selected phenolic-rich extracts was determined by MTT assay.

Apoptosis was assayed according to the manufacturer's instructions using a Vybrant Apoptosis Assay Kit #2 (Molecular Probes, Invitrogen Corporation, Carlsbad, CA, U.S.A.). The Alexa Fluor 488 Annexin V and Propidium Iodide (PI) solutions were used to stain the apoptotic cells. The apoptotic cells were analyzed by flow cytometry using a BD FACSCantoII Flow Cytometer (Becton Dickinson, San Jose, CA).

Free form of phenolic acids was extracted as previously described (Khaopha *et al.*, 2012) and subjected to HPLC analysis for identification of individual phenolic acids. The amount of individual phenolic acids in samples was determined by using a standard curve between the concentration of phenolic acid standard (µg) (the axis of *X*) and the ratio between peak areas of the phenolic acid standard and the internal standard (*m*-hydroxybenzaldehyde; 1 µg) (the axis of *Y*).

Results

Inhibition of HDACs causes accumulation of acetylated forms of histone proteins. HDAC inhibition by methanolic crude (phenolic-rich) extracts of 15 Valencia-type peanut testae in HeLa cells was analyzed by AUT gel electrophoresis, whereby the cellular core histone H4 with different extent of acetylation can be separated. Herein, the profiles of histone H4 extracted from methanolic crude extract-, TSA- or DMSO-treated HeLa cells were demonstrated (Fig. 1). Interestingly, the histone H4 with all four acetylated lysine residues was markedly increased when treated the cells with phenolic-rich testa extracts

of ICG15042, KS2, and KK4 (Fig. 1B), indicating that the phenolic-rich testa extracts of these genotypes exhibited a greater HDAC inhibitory activity than those of the New Mexico varieties. The discrepancy on hyperacetylation pattern may be explained by a different sensitivity of specific HDAC enzymes to the inhibitor(s) (Khan *et al.*, 2008) and/or a different mechanism of HDAC inhibition (reversible or irreversible) by the inhibitor(s) (Mark & Xu, 2009). Among the greatest HDAC inhibitory genotypes (ICG15042, KS2, and KK4), ICG15042 and KK4 were also found to have the greatest amount of total phenolic content among all 15 genotypes tested (Khaopha *et al.*, 2012). Therefore, ICG15042 and KK4 were chosen for further study on their anticancer activity. According to our results, most of the phenolic-rich extracts of Valencia-type peanut testae appear to be the promising natural sources of HDAC inhibitors, which are undoubtedly edible. Nonetheless, identification of individual phenolic compounds acting as HDAC inhibitors in peanut skin is further needed.

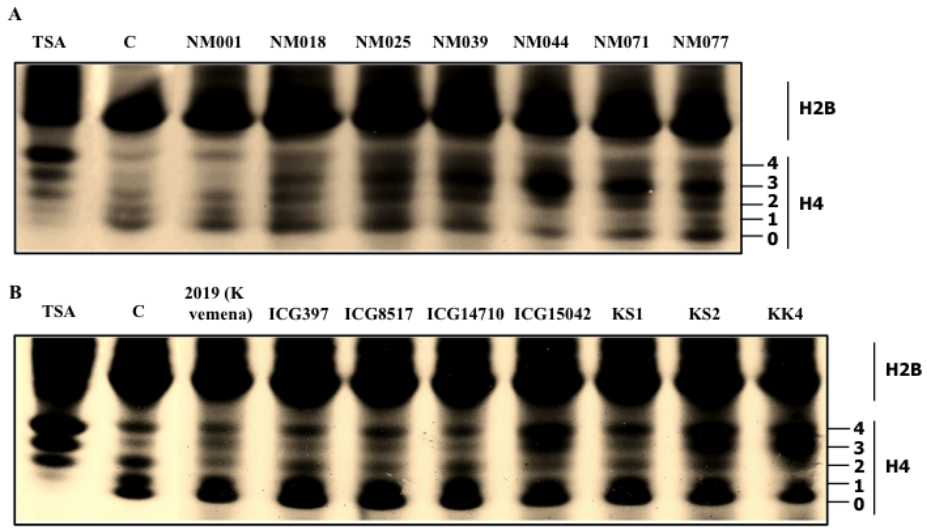


Fig. 1. HDAC inhibitory activity of 15 Valencia-type peanut testa extracts in mammalian cells (HeLa cells); Effect of (A) NM001, NM018, NM025, NM039, NM044, NM071, and NM077 testa extracts and (B) 2019 (K vemena), ICG397, ICG8517, ICG14710, ICG15042, KS1, KS2, and KK4 testa extracts on histone acetylation in HeLa cells. Control (C) represents the level of histone acetylation in vehicle control treatment. The Trichostatin A (TSA)-treatment was used as a positive control. The degree of histone acetylation of histone H4 is indicated as follows: 0, nonacetylated; 1, monoacetylated; 2, diacetylated; 3, triacetylated; and 4, tetraacetylated. The data shown are representative of two independent experiments performed in duplicate.

Two phenolic-rich extracts of peanut testae (KK4 and ICG15042) with the greatest HDAC inhibitory activity and phenolic content were investigated for their antiproliferative activity by MTT method. In this study, five human cancer cell lines (HeLa, HCT116, HT29, Jurkat, and MCF-7 cells) and a non-cancer cell line (Vero cells) were used. Both ICG15042 and KK4 testa extracts inhibited the proliferation of HeLa, HT29, HCT116, and Jurkat cells in a concentration- and time-dependent manner (Table 1). In contrast, a breast cancer cell line (MCF-7 cells) and a non cancer cell line (Vero cells) were less sensitive to both extracts (Table 1). The testa extracts of both ICG15042 and KK4 at concentrations less than 90 µg/ml were not cytotoxic to a non-tumorous cell line (Table 1). Based on an IC_{50} values at 24-, 48- and 72-h exposures, the human T-cell leukemia cell line (Jurkat cells) was considered the most sensitive cancer cell line to testa extracts of both ICG15042 and KK4 (Table 1).

Table 1. Half maximal inhibitory concentrations (IC_{50}) of ICG15042 and KK4 testa extracts after 24, 48 and 72 h exposures to cancer cell lines**.**

Peanut Testa Extracts	IC_{50} values (mean ± SD; *n* = 3; µg/ml)																	
	Vero cells[a]			HeLa cells			HT29 cells			HCT116 cells			MCF-7 cells			Jurkat cells		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
ICG15042	>90	>90	>90	82.5 ± 1.3	57.2 ± 0.2	55.6 ± 1.2	69.1 ± 3.8	55.6 ± 1.2	44.2 ± 1.1	>90	62.4 ± 5.1	56.8 ± 0.7	>90	>90	>90	57.4 ± 2.9	44.6 ± 5.4	30.0 ± 2.1
KK4	>90	>90	>90	>90	69.5 ± 0.3	51.4 ± 1.5	60.0 ± 0.0	46.2 ± 0.5	44.5 ± 2.0	>90	69.6 ± 0.3	50.7 ± 0.3	>90	>90	>90	54.5 ± 4.3	42.4 ± 3.1	28.8 ± 1.9

[a]Non cancer cells

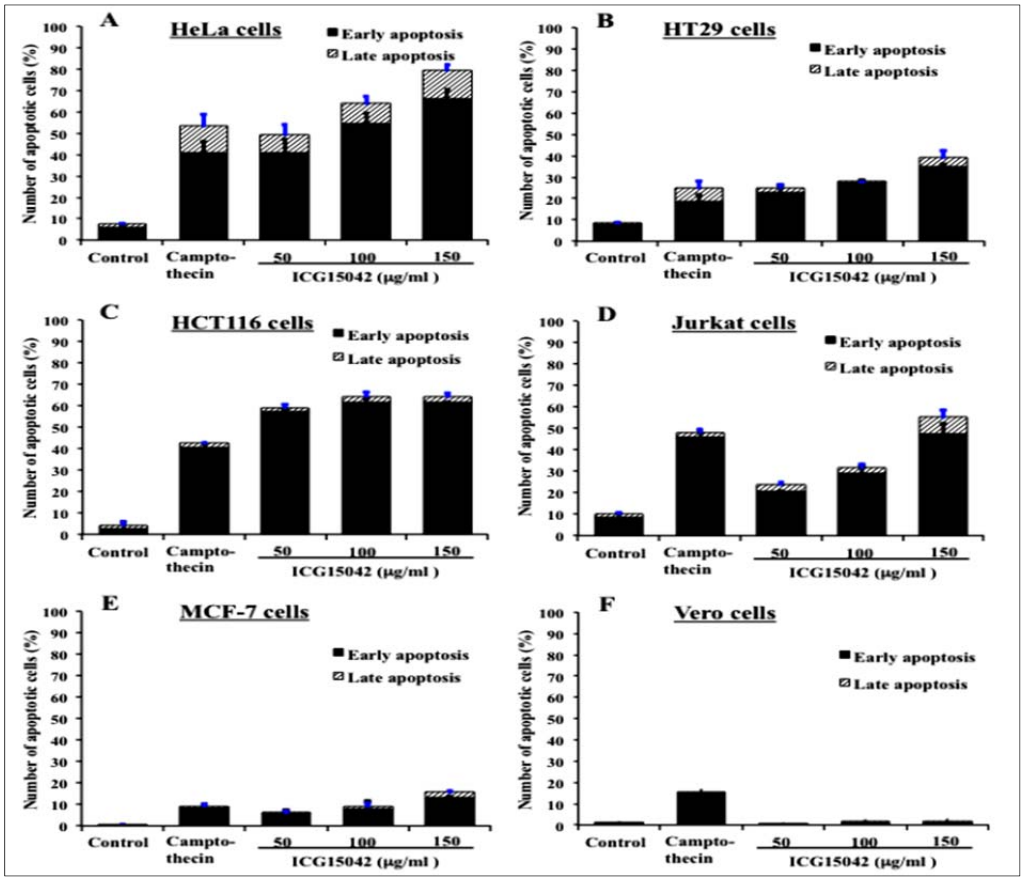


Fig. 2. Apoptotis induction activity of ICG15042 testa extract on human cancer cell lines; (A) Human Cervical Adenocarcinoma Cell Line (HeLa cells), (B) Human Colon Adenocarcinoma Cell Line (HT29 Cells), (C) Human Colorectal Carcinoma Cell Line (HCT116 Cells), (D) Human T-cell Leukemia Cell Line (Jurkat Cells), (E) Human Breast Adenocarcinoma Cell Line (MCF-7 Cells), and (F) Non cancer cell line (Vero cells). Bar graph shows the summarized data from two independent experiments performed in duplicate. Cells treated with DMSO (solvent control; 0.37%) and Camptothecin (10 µg/mL) were used as negative and positive controls, respectively.

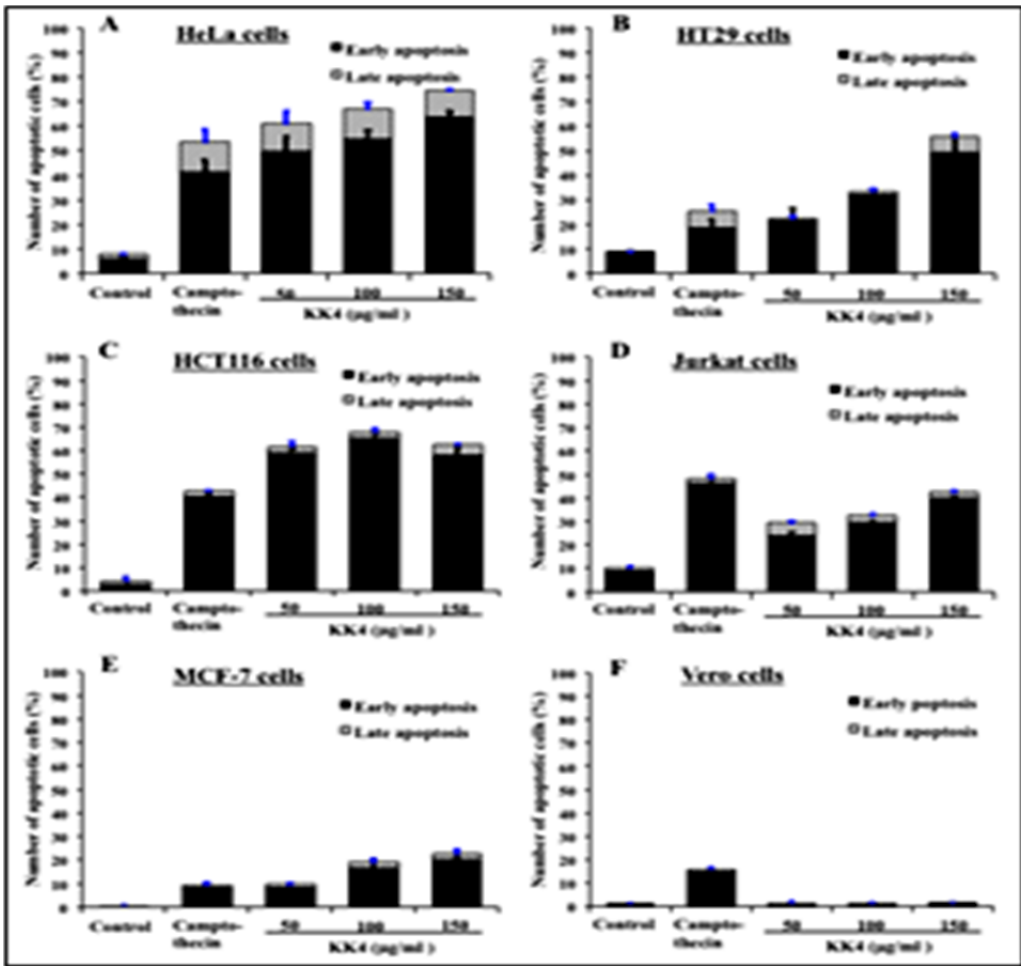


Fig. 3. Apoptotis induction activity of KK4 testa extract on human cancer cell lines; (A) Human Cervical Adenocarcinoma Cell Line (HeLa cells), (B) Human Colon Adenocarcinoma Cell Line (HT29 Cells), (C) Human Colorectal Carcinoma Cell Line (HCT116 Cells), (D) Human T-cell Leukemia Cell Line (Jurkat Cells), (E) Human Breast Adenocarcinoma Cell Line (MCF-7 Cells), and (F) Non cancer cell line (Vero cells). Bar graph shows the summarized data from two independent experiments performed in duplicate. Cells treated with DMSO (solvent control; 0.37%) and Camptothecin (10 □g/mL) were used as negative and positive controls, respectively.

To further confirm that induction of apoptosis underlies cancer cell growth inhibition of the two phenolic-rich extracts, their capacity to induce apoptosis in cancer cell lines was examined**.** Cancer and non cancer cell lines were incubated with different concentrations (50, 100, 150 µg/ml) of ICG15042 and KK4 testa extracts for 24 h, and then the cells were stained with Alexa Fluor 488-Annexin V and PI, followed by quantitative flow cytometry analysis. As shown in Figure 2 and 3, the results confirmed that both ICG15042 and KK4 testa extracts induced apoptosis of all cancer cell lines tested in a dose-dependent manner. Both testa extracts showed the most effective on induction of HeLa and HCT116 cells (Fig. 2 and 3). In contrast, the treatment of a non

cancer cell line with both ICG15042 and KK4 testa extracts at the highest concentration tested (150 μg/ml) resulted in the increase of early apoptotic cells only 1.8 ± 0.57 % and 1.3 ± 0.14 %, respectively, which were not significantly different from the control cells (Fig. 2F and 3F). This indicates that the non cancer cell line appeared to be resistant to an induction of apoptosis by both testa extracts. These results suggest that both ICG15042 and KK4 testa extracts suppress the growth of human cancer cells at least in part through induction of apoptosis.

Phenolic-rich extracts of ICG15042 and KK4 testae exhibited HDAC inhibitory and anticancer activities as presented above, indicating that phenolic compounds in the extracts may underpin such activities. To identify and quantify phenolic components that contribute to HDAC inhibitory and anticancer activities, phenolic acid composition of both extracts was investigated. Phenolic acid profiles of both ICG15042 and KK4 phenolic acid extracts were presented in Fig. 4, which were consistent with our previous results (Khaopha *et al.*, 2012). Fig. 4 demonstrated typical chromatograms of phenolic acid standards and the two phenolic acid extracts containing free phenolic acids prepared for HPLC analyses, in which similar phenolic acid profiles were observed (Fig. 4B and 4C).

Seven phenolic acids including protocatechuic, *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, ferulic and sinapinic acids, were identified in both ICG15042 and KK4 testa extracts whose predominant phenolic acid in both extracts was protocatechuic acid followed by *p*-coumaric acid (Table 2). The confirmation of sample peak was obtained by LC-MS analysis (data not shown). According to HPLC results, the amount of protocatechuic, syringic, and *p*-coumaric acids in ICG15042 testa extract appeared to be slightly greater than that of KK4 testa extract, whereas the amount of vanillic acid in KK4 testa extract was greater than that of ICG15042 testa extract (Table 2).

According to previous studies, protocatechuic acid exhibited antiproliferative activity against HL-60 leukemia cells through induction of apoptosis (Anter *et al.*, 2011). Syringic acid possessed proteasome inhibitory activity and inhibited the growth of human malignant melanoma cells (Orabi *et al.*, 2013). Ferulic acid showed antiproliferative and apoptosis induction activities toward a bladder cancer cell line (T24 cells). *P*-coumaric acid has been shown to inhibit the growth of HCT15 and HT29 cells by inducing apoptosis through ROS-mitochondrial pathway (Jaganathan *et al.*, 2013), and inhibit the growth of Caco-2 cells by inducing a G2/M phase cell cycle arrest (Janicke *et al.*, 2011). Recently, sinapinic acid has been shown to possess histone deacetylase (HDAC) inhibitory activity and inhibit the growth of HeLa, HT29 and HCT116 cells (Senawong *et al.*, 2013). Nonetheless, the possibility that other unidentified compounds in the extracts might underpin the antiproliferative activity of both extracts cannot be excluded. Based on the observed HPLC profiles and data from previous studies, the presence of protocatechuic, syringic, *p*-coumaric, ferulic, and sinapinic acids in both ICG15042 and KK4 testa extracts may at least in part underpin their antiproliferative and apoptosis induction activities.

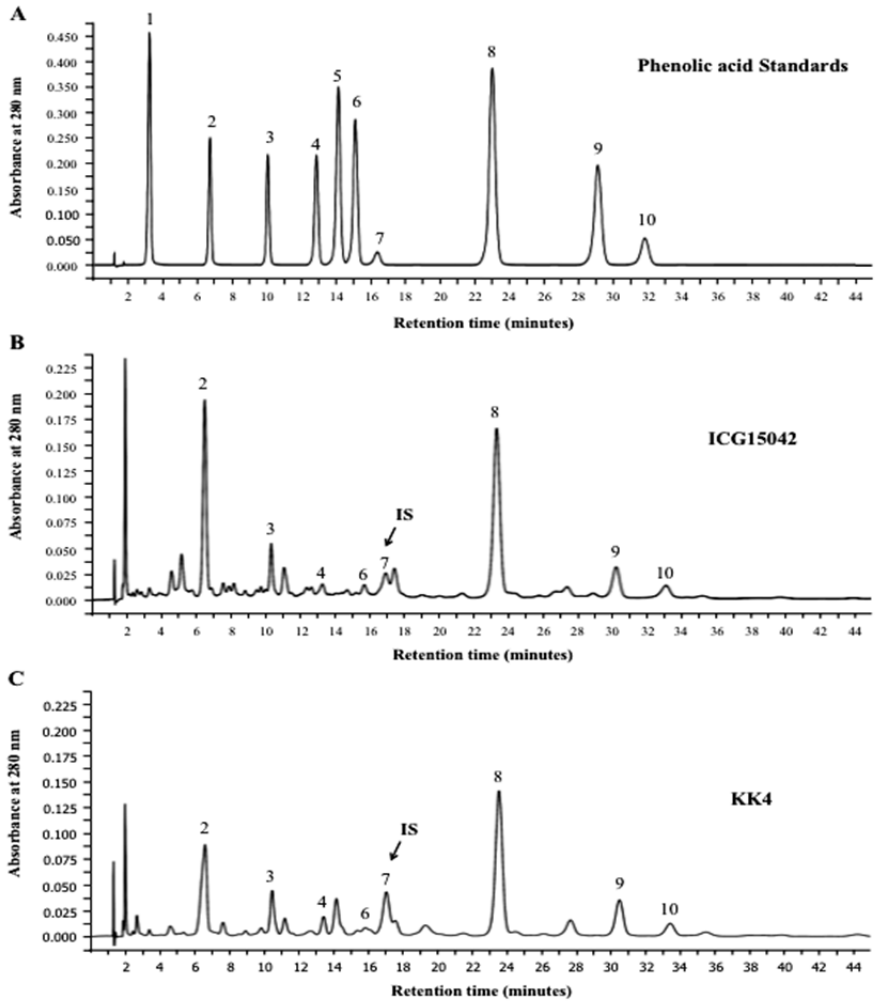
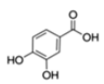
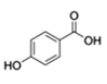
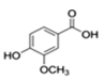
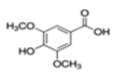
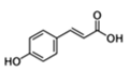
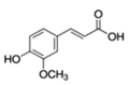
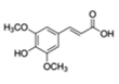


Fig.4. HPLC chromatograms of phenolic acid standards and ICG15042 and KK4 testa extracts; (A) HPLC profiles of standard phenolic acids: 1, gallic acid; 2, protocatechuic acid; 3, *p*-hydroxybenzoic acid; 4, vanillic acid; 5, caffeic acid; 6, syringic acid; 7, *m*-hydroxybenzaldehyde; 8, *p*-coumaric acid; 9, ferulic acid and 10, sinapinic acid, and (B, C) HPLC profiles of ICG15042 and KK4 testa extracts added with the internal standard (IS), *m*-hydroxybenzaldehyde, are displayed comparatively. The data shown are representative of two independent experiments performed in duplicate.

Table 2 Phenolic acid composition of phenolic-rich extracts of two peanut testae analyzed by reversed phase HPLC.

Peanut testae	Phenolics acids* (μg/g dye weight)						
	Protocatechuic acid 	*p*-Hydroxybenzoic acid 	Vanillic acid 	Syringic acid 	*p*–Coumaric acid 	Ferulic acid 	Sinapinic acid 
ICG15042	121.07 ± 7.03[a]	24.63 ± 0.28[a]	10.57 ± 0.13[a]	5.00 ± 0.17[a]	49.20 ± 0.50[a]	19.79 ± 0.39[a]	22.99 ± 1.47[a]
KK4	79.63 ± 9.23[b]	24.44 ± 0.17[a]	14.28 ± 0.21[b]	3.98 ± 0.01[b]	35.00 ± 1.41[b]	20.54 ± 0.63[a]	21.24 ± 0.04[a]

*Values are means ± standard deviation of three replicates. Different superscript letters within the same column indicate significant differences (p<0.05).

Conclusion

The results in this report demonstrated that nine out of fifteen phenolic-rich testa extracts of Valencia-type peanut genotypes possessed HDAC inhibitory activity in mammalian cell model. The growth inhibitory effect on five human cancer cell lines (HeLa, HT29, HCT116, Jurkat and MCF-7 cells) of both ICG15042 and KK4 testa extracts is in accordance with their capability to induce cancerous cell apoptosis. Both ICG15042 and KK4 testa extracts contained similar type and amount of phenolic acids and their antiproliferative activity seemed to correspond with the phenolic acid profiles. Further investigation, with details about HDAC inhibitory activity, anticancer activity of individual phenolic compounds found in peanut testae and their combination with other highly effective anticancer drugs, is of interest. Theoretically, our findings may validate the use of peanut testae as alternative medicines for cancer treatment or dietary prevention of cancer.

References

Anter, J., M. Romero-Jiménez, Z. Fernández-Bedmar, M. Villatoro-Pulido, M. Analla, A., Alonso-Moraga and A. Muñoz-Serrano. 2011. Antigenotoxicity, cytotoxicity, and apoptosis induction by apigenin, bisabolol, and protocatechuic acid. J. Med. Food. 14: 276-283.

Bora-Tatar, G., D. Dayangac-Erden, A.S. Demir, S. Dalkara, K. Yelekci and H. Erdem-Yurter. 2009. Molecular modifications on carboxylic acid derivatives as potent histone deacetylase inhibitors: Activity and docking studies. Bioorg. Med. Chem. 17: 5219-5228.

Carew, J.S., F.J. Giles and S.T. Nawrocki. 2008. Histone deacetylase inhibitors: Mechanisms of cell death and promise in combination cancer therapy. Cancer Letters. 269: 7-17.

Jaganathan, S.K., E. Supriyanto and M. Mandal. 2013. Events associated with apoptotic effect of *p*-Coumaric acid in HCT-15 colon cancer cells. World J. Gastroenterol. 19: 7726-7734.

Janicke, B., C. Hegardt, M. Krogh, G. Onning, B. Akesson ,H.M., Cirenajwis and S.M. Oredsson. 2011. The antiproliferative effect of dietary fiber phenolic compounds ferulic acid and *p*-coumaric acid on the cell cycle of Caco-2 cells. Nutr. Cancer. 63: 611-622.

Khan, N., M. Jeffers, S. Kumar, C. Hackett, F. Boldog, N. Khramtsov, X.Z. Qian, E. Mills, S.C. Berghs, N. Carey, P.W.Finn, L.S. Collins, A. Tumber, J.W. Ritchie, P.B. Jensen, H.S. Lichenstein and M. Sehested. 2008.Determination of the class and isoform selectivity of small-molecule histone deacetylase inhibitors.Biochem. J. 409: 581-589.

Khaopha, S., T. Senawong, S. Jogloy and A. Patanothai. 2012. Comparison of total phenolic content and composition of individual phenolic acids in testae and testa-removed kernels of 15 Valencia-type peanut (*Arachis hypogaea* L.) genotypes. Afr. J. Biotechnol. 11: 15923-15930.

Kim, H.J. and S.C. Bae. 2011. Histone deacetylase inhibitors: molecular mechanisms of action and clinical trials as anti-cancer drugs. Am. J. Transl. Res. 3: 166-179.

Kouzarides, T. 2007. Chromatin modifications and their function.Cell. 128: 693-705.

Marks, P.A. and W.S. Xu. 2009. Histone Deacetylase Inhibitors: Potential in cancer therapy. J. Cell. Biochem. 107: 600-608.

Orabi, K.Y., M.S. Abaza, K.A. El Sayed, A.Y. Elnagar, R. Al-Attiyah and R.P. Guleri. 2013.Selective growth inhibition of human malignant melanoma cells by syringic acid-derived proteasome inhibitors. Cancer Cell International. http://dx.doi.org/10.1186/1475-2867-13-82.

Rikiishi, H., 2011.Autophagic and apoptotic effects of HDAC inhibitors on cancer cells. J. Biomed. Biotechnol. doi: 10.1155/2011/830260

Senawong, T., S. Misuna, S. Khaopha, S. Nuchadomrong, P. Sawatsitang, C. Phaosiri, A. Surapaitoon and B. Sripa. 2013. Histone deacetylase (HDAC) inhibitory and antiproliferative activities of phenolic-rich extracts derived from the rhizome of *Hydnophytum formicarum* Jack.: sinapinic acid acts as HDAC inhibitor. BMC Complement. Altern. Med. doi:10.1186/1472-6882-13-232.

Publication

Khaopha, S., S. Jogloy, A. Patanothai and T. Senawong. 2014. HDAC inhibitory activity of peanut testa extracts against human cancer cell lines. J. Food Biochem. (*Major revision*). (Impact factor = 0.853).

Sub-project 2

Basic research for supporting varietal improvement of Jerusalem artichoke for drought tolerance, stem rot resistance and high inulin yield

Associated Prof. Dr. Sanun Jogloy

Sub-project leader

Jerusalem artichoke or kaentawan is mainly grown for its edible tubers. The crop produces tubers which contain a high amount of inulin (Suzuki, 1993). This compound is used in various ways and is high in economic value. It is a dietary fiber (Orafti, 2005) that is not soluble in the digestive system, but it is a carbon source for useful bacteria such as lactobacillus and bifidobacteria in colon that synthesize vitamin B. Inulin is well recognized as a prebiotic food, and 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).

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 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.

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 these 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 the gas station, the production systems and downstream industry should be established in Thailand to produce value-added products from kaentawan for reducing the imports and increasing the exports.

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, and most importantly low tuber yield and low inulin content. From our initial breeding work, we have released three cultivars for commercial production. However, further improvements are still needed, particularly in high inulin content, stem rot resistance and drought tolerance, and these are the current priority areas of our kaentawan breeding program.

This sub-project consists of ten studies:

2.1 Variations in morphological and agronomic traits among Jerusalem artichoke (*Helianthus tuberosus* L.) accessions

2.2 Genetic diversity of water use efficiency in Jerusalem artichoke (*Helianthus tuberosus* L.) Germplasm

2.3 Genotypic variability for tuber yield, biomass and drought tolerance in Jerusalem artichoke germplasm

2.4 Photoperiod and growing degree days effect on dry matter partitioning in Jerusalem artichoke

2.5 Spectrophotometric method as an alternative method for the determination of inulin in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers

2.6 Molecular investigation on *tuberization* of Jerusalem artichoke

2.7 Levels of *Sclerotium rolfsii* inoculum influence identification of resistant genotypes in Jerusalem artichoke

2.8 Effects of host growth stage, re-isolation and culture medium on screening for resistance to stem rot disease caused by *Sclerotium rolfsii* Sacc. in Jerusalem artichoke

2.9 Evaluation of seedling and adult plant stages resistance to *Sclerotium rolfsii* in Jerusalem artichoke (*Helianthus tuberosus* L.)

2.10 Biological control of southern stem rot caused by *Sclerotium rolfsii* using *Trichoderma harzianum* and arbuscular mycorrhizal fungi onJerusalem artichoke (*Helianthus tuberosus* L.)

These studies are reported in this section.

References

Farnworth, E.R. 1993. Fructans in human and animal diets. *In:* M. Suzuki and N.J. Chatterton, Eds. Science and Technology of Fructans. CRC press, London.

Orafti. 2005. Active food scientific monitor. An orafti Newsletter, 12.

Suzuki, M. 1993. Fructans in crop production and presearvation. *In:* M Suxuki and N.J. Chatterton, Eds. Science and Technology of Fructans. CRC Press. London.

2.1 Variations in morphological and agronomic traits among Jerusalem artichoke (*Helianthus tuberosus* L.) accessions

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

Jerusalem artichoke (*Helianthus tuberosus* L.) is a hexaploid plant species (2n = 102) in the Asteraceae family, and is native to temperate regions of North America (Encheva *et al.*, 2003; Tassoni *et al.*, 2010; Gao *et al.*, 2011). The crop is currently grown in other parts of the world for edible tubers and inulin production (Ma *et al.*, 2011). Jerusalem artichoke is a versatile crop that can be used to produce a variety of value-added products such as health products, sweeteners, pharmaceuticals, cosmetics (Baldini *et al.*, 2011), animal feed, fodder (Seiler and Campbell, 2006) and bioethanol (Curt *et al.*, 2006).

The crop is rather wild with minimum breeding efforts compared to other well utilized crop, thus, genetic improvement of this is needed. However, genetic information on important traits of this crop is rare. Attempts have been made to understand genetic variations in morphological traits in Jerusalem artichoke germplasm to enable breeders to selected parents for their breeding programs. In addition, some morphological characteristics correlate well with the characteristics that are difficult to evaluate and therefore may be useful as markers in the breeding program (Karimi *et al.*, 2009; Mansyah *et al.*, 2010). Moreover, Grouping of germplasm into different clusters using qualitative traits and quantitative traits is a means to identify genetic differences of crop germplasm (Wati *et al.*, 2010). The clusters should primarily reveal genetic distance and possible heterotic groups of the germplasm before extensive progeny evaluation is made, and this information is scantily available in the literature for Jerusalem artichoke.

Objectives

The objectives of the present study were to evaluate genetic variability in qualitative traits and quantitative traits, and to identify different groups of accessions using morphological and agronomic traits.

Materials and Methods

A field experiment was conducted at the agronomy experimental farm of the Faculty of Agriculture, Khon Kaen University, for two late rainy seasons (September-December 2008 and 2009) and one early rainy season (March-July 2009). Seventy-nine accessions of Jerusalem artichoke (Table 1) were planted in a randomized complete block design with two replications. Nine qualitative traits (Table 2) and 15 quantitative traits (Table 3) were recorded based on the International Board for Plant Genetic Resources (IBPGR) descriptors for cultivated and wild sunflower and biology (IBPGR ,1985) and chemistry of Jerusalem artichoke (*Helianthus tuberosus* L.) (Kays and Nottingham, 2008) with a slight modification. Frequency distributions of the nine qualitative characters were constructed. Mean, minimum (min), maximum (max), standard error (SE), standard deviation (SD), coefficient of variation (CV) and combined analysis of variance of three-season data of the 15 quantitative characters were calculated. The relationships among traits were calculated by the Pearson's correlation analysis. A data matrix of the 79 Jerusalem artichoke accessions was constructed using means of the 29 morphological and agronomic characteristics. The cluster analysis based on Ward's method and squared Euclidian distance was performed and the dendrogram was constructed in SAS 6.12 software (SAS, 2001).

Table 1. Jerusalem artichoke accessions, country of originandsource of material**.**

Entry no.	Accession no.	Name of accession	Origin	Genetic resources
1	JA 1	7305	Canada	PGRC
2	JA 2	7306	Canada	PGRC
3	JA 3	7307	Canada	PGRC
4	JA 4	7308	Canada	PGRC
5	JA 5	7309	Canada	PGRC
6	JA 6	7310	Canada	PGRC
7	JA 7	7312	Canada	PGRC
8	JA 8	7512	Canada	PGRC
9	JA 9	7513	Canada	PGRC
10	JA 10	HM Hybrid A	Canada	PGRC
11	JA 11	HM Hybrid B	Canada	PGRC
12	JA 12	HM Hybrid C	Canada	PGRC
13	JA 13	HM–2	Canada	PGRC
14	JA 14	HM–3	Canada	PGRC
15	JA 15	HM–5	Canada	PGRC
16	JA 16	HM–7	Canada	PGRC
17	JA 18	HM–9	Canada	PGRC
18	JA 20	HM–11	Canada	PGRC
19	JA 21	HM–12	Canada	PGRC
20	JA 23	DHM–3	Canada	PGRC
21	JA 25	DHM–5	Canada	PGRC
22	JA 30	DHM–16	Canada	PGRC
23	JA 35	W–97	Canada	PGRC
24	JA 36	W–106	Canada	PGRC
25	JA 46	DHM–14–3	Canada	PGRC
26	JA 47	DHM–14–6	Canada	PGRC
27	JA 55	–	USA	PGRC
28	JA 58	Intress	USSR	PGRC
29	JA 59	Volzskij–2	USSR	PGRC
30	JA 60	Jamcovskij krashyj	USSR	PGRC
31	JA 61	Vadim	USSR	PGRC
32	JA 69	TUB–346 USD–ARS–SR	USA	PGRC
33	JA 70	TUB–365 USD–ARS–SR	USA	PGRC
34	JA 71	TUB–675 USD–ARS–SR	USA	PGRC
35	JA 72	TUB–676 USD–ARS–SR	USA	PGRC
36	JA 75	#2	Canada	PGRC
37	JA 76	#4	Canada	PGRC
38	JA 77	#5	Canada	PGRC
39	JA 92	Industrie	USSR	PGRC
40	JA 93	Leningradskii (NC10–65)	USSR	PGRC
41	JA 97	D19–63–340	France	PGRC
42	JA 108	83–001–3 (37 × 6)	Canada	PGRC
43	JA 109	83–001–4 (37 × 6)	Canada	PGRC
44	JA 114	83–001–9 (37 × 6)	Canada	PGRC
45	JA 120	83–003–1 (6 × 20)	Canada	PGRC
46	JA 122	83–004–2 (6 × 20)	Canada	PGRC
47	JA 132	83–007–2 (69 × 3)	Canada	PGRC
48	CN 52867	PGR–2367	USSR	PGRC
49	JA 37	Comber	Canada	PGRC
50	JA 38	B.C. #1	Canada	PGRC
51	JA 67	Oregon White	USA	PGRC
52	JA 89	Waldspindel	France	PGRC
53	JA 102	073–87	Germany	PGRC
54	HEL 53	–	Germany	IPK
55	HEL 61	Tambovskij Krasnyi	Russian Federation	IPK
56	HEL 62	Sachalinskij Krasnyi	Russian Federation	IPK
57	HEL 65	Sejanec 19	Russian Federation	IPK
58	HEL 66	Kievskij Belyj	Ukraine	IPK
59	HEL 68	–	Unknown	IPK
60	HEL 69	–	Unknown	IPK
61	HEL 231	–	Germany	IPK
62	HEL 335	–	Unknown	IPK
63	Ames 2729	TUB–49	South Dakota	NCRPRIS
64	HEL 243	Bianka	Germany	IPK
65	HEL 246	–	Unknown	IPK
66	HEL 248	Rote Zonenkugel	Germany	IPK
67	HEL 250	Medius	France	IPK
68	HEL 253	–	Unknown	IPK
69	HEL 256	–	Unknown	IPK
70	HEL 265	BT4	Hungry	IPK
71	HEL 272	D19–63–340	France	IPK
72	HEL 278	Voelkenroder Spindel	Unknown	IPK
73	HEL 280	BS–83–22	Unknown	IPK
74	HEL 288	RA1	Poland	IPK
75	HEL 293	RA9	Poland	IPK
76	HEL 308	–	Unknown	IPK
77	HEL 315	–	Unknown	IPK
78	HEL 316	–	Unknown	IPK
79	HEL 317	–	Unknown	IPK

Kays and Nottingham (2008); *NCRPIS*: the North Central Regional Plant Introduction; *IPK*: the Leibniz Institute of Plant Genetics and Crop Plant Research of Germany; *PGRC*: the Plant Gene Resources of Canada.

Results

Our results showed high variations among the Jerusalem artichoke accessions for qualitative (Table 2) and quantitative characters (Table 4). High variations were also observed for tuber width (CV = 31.5%), number of tubers per plant (CV = 34.1%) and tuber size (CV = 50.3%) (Table 3). Correlation coefficient between fresh tuber yield and tuber size was positive and significant (r = 0.58**) (Table 5), indicating that selection for improvement of these traits are possible. Improvement of tuber size is a means to improve yield and tuber quality. The dendrogram was able to classify 79 Jerusalem artichoke accessions into four distinct groups (R-square = 0.82) (Fig. 1 and Table 6).

Table 2. Frequency distribution of 9 qualitative characters of 79 Jerusalem artichoke accessions across three seasons.

Characteristics	Frequency	(%)	Characteristics	Frequency	(%)
Leaf shape			Stem color		
Lanceolate	31	39.2	Light-green	12	15.2
Lanceolate-ovate	48	60.8	Green-violet	67	84.8
Leaf margin			Tuber color		
Smooth	18	22.8	Light-brown	1	1.3
Serrated	39	49.4	Brown	74	93.7
Strongly serrated	22	27.8	Violate-brown	4	5.1
Branch color			Tuber shape		
Ligth-green	17	21.5	Spindle	2	2.5
Green-violet	62	78.5	Oblong	30	38.0
Branching distribution			Slender	38	48.1
Top-basal branching	1	1.3	Long slender	9	11.4
Fully branching	78	98.7	Surface topography of tuber		
Branching orientation			Shallow	25	31.6
Opposite	24	30.4	Medium	38	48.1
Opposite and alternate	55	69.6	Deep	16	20.3

Table 3. Means, standard errors (SE), minnmum (min), maximum (max), standard deviation (SD), coefficient of variation (CV) and least significant difference (LSD) of 15 quantitative characters of 79 Jerusalem artichoke accessions across three seasons.

Characters	Mean ± SE	Min	Max	SD	CV (%)	LSD (5%)
Plant height (cm)	70.1 ± 2.0	41.1	112.7	17.9	25.6	7.56
Leaf petiole (cm)	2.8 ± 0.1	1.6	4.9	0.7	25.4	0.38
Leaf width (cm)	6.3 ± 0.1	4.6	9.3	1.0	16.5	0.70
Leaf length (cm)	13.3 ± 0.2	10.6	17.3	1.4	10.8	1.22
Branch length (cm)	34.0 ± 0.6	21.6	46.8	5.0	14.7	4.22
Branch angle (degree)	57.8 ± 0.6	43.2	71.8	5.2	9.0	4.59
Internode length (cm)	5.4 ± 0.1	3.9	7.1	0.7	13.8	0.77
Basal stem diameter (cm)	0.91 ± 0.03	0.51	1.50	0.26	28.4	0.13
Mid stem diameter (cm)	0.66 ± 0.02	0.41	1.11	0.19	28.9	0.08
Top stem diameter (cm)	0.20 ± 0.00	0.13	0.31	0.04	17.4	0.04
Number of tuber per plant	36 ± 1.4	16	74	12.3	34.1	10.88
Tuber width (cm)	1.9 ± 0.1	1.1	3.3	0.6	31.5	0.27
Tuber length (cm)	8.4 ± 0.1	5.5	11.6	1.3	15.8	1.24
Tuber size (g/tuber)	9.8 ± 0.6	2.0	21.1	4.9	50.3	2.14
Total soluble solids(°Brix)	21.0 ± 0.2	18.2	25.6	1.5	7.2	2.49

Table 4. Proportion of sum of squares to tatal sum of squares for 15 quantitative characters of 79 Jerusaelm artichoke accessions across three seasons.

Source of variation	df	Plant height	Leaf petiole	Leaf width	Leaf length	Branch length	Branch angle	Internode length	Basal stem diameter
Environment (E)	2	3.1**	6.4ns	3.9ns	1.7ns	15.5ns	14.0ns	9.2ns	15.4ns
Rep/E	3	0.1	2.0	12.0	5.2	6.2	1.3	14.6	2.5
Genotypes (G)	78	84.5**	74.1**	63.0**	62.8**	43.5**	48.5**	40.2**	69.5**
G × E	156	6.4**	9.1**	10.1*	12.8ns	22.7**	21.7**	19.3**	6.0*
Error	234	5.8	8.4	11.1	17.5	12.1	14.6	16.8	6.6
CV (%)		9.5	12.1	9.8	8.0	10.9	7.0	12.6	12.4

Source of variation	df	Mid stem diameter	Top stem diameter	No. of tubers per plant	Tuber width	Tuber length	Tuber size	Total soluble solids
Environment (E)	2	15.1ns	2.5*	3.9**	20.0**	44.4**	10.0**	5.1ns
Rep/E	3	2.5	0.3	0.0	0.1	0.4	0.1	1.0
Genotypes (G)	78	71.1**	60.0**	48.1**	54.0**	25.4**	64.2**	29.2**
G × E	156	6.2**	16.0ns	33.5**	21.8**	21.4**	21.0**	34.0**
Error	234	5.2	21.2	14.5	4.1	8.5	4.6	30.7
CV (%)		11.1	14.7	26.5	12.2	12.9	19.2	10.4

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

Table 5. Correlation coefficients among 20 quantitative characters of 79 Jerusalem artichoke accessions across three seasons.

Characters	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15
Plant high (Q1)															
Leaf petiole (Q2)	0.49**														
Leaf width (Q3)	0.75**	0.46**													
Leaf length (Q4)	0.60**	0.75**	0.63**												
Branch length (Q5)	0.46**	0.15ns	0.20ns	0.20ns											
Branch angle (Q6)	0.18ns	0.38**	0.35**	0.41**	-0.06ns										
Internode length (Q7)	0.54**	-0.02ns	0.36**	0.25*	0.58**	-0.17ns									
Basal stem diameter (Q8)	0.87**	0.66**	0.73**	0.63**	0.37**	0.41**	0.25*								
Mid stem diameter (Q9)	0.87**	0.68**	0.75**	0.64**	0.30**	0.44**	0.22ns	0.98**							
Top stem diameter (Q10)	0.59**	0.48**	0.68**	0.53**	0.05ns	0.47**	0.12ns	0.67**	0.71**						
No. of tubers per plant	-0.34**	-0.40**	-0.24*	-0.34**	-0.02ns	-0.27*	0.03ns	-0.50**	-0.54**	-0.42**					
Tuber width (Q12)	0.79**	0.58**	0.74**	0.59**	0.27*	0.32**	0.27*	0.88**	0.88**	0.72**	-0.49**				
Tuber length (Q13)	-0.46**	-0.16ns	-0.39**	-0.12ns	-0.20ns	-0.02ns	-0.13ns	-0.46**	-0.48**	-0.39**	0.28*	-0.50**			
Tuber size (Q14)	0.75**	0.56**	0.68**	0.56**	0.32**	0.29*	0.29**	0.86**	0.85**	0.67**	-0.56**	0.88**	-0.41**		
Total soluble solids (Q15)	0.13ns	-0.01ns	-0.01ns	-0.13ns	0.17ns	-0.31**	0.30**	0.01ns	-0.03ns	-0.06ns	0.14ns	-0.01ns	-0.07ns	0.01ns	
Days to maturity (Q16)	0.58**	0.61**	0.60**	0.62**	0.00ns	0.40**	0.09ns	0.66**	0.69**	0.59**	-0.38**	0.66**	-0.33**	0.55**	0.04ns
Fresh tuber yield (Q17)	0.60**	0.25*	0.54**	0.32**	0.51**	0.17ns	0.51**	0.58**	0.53**	0.41**	0.10**	0.58**	-0.23**	0.58**	0.14ns
Biomass (Q18)	0.82**	0.48**	0.67**	0.50**	0.47**	0.30**	0.44**	0.83**	0.80**	0.55**	-0.12ns	0.79**	-0.34**	0.74**	0.13ns
Harvest index (Q19)	-0.25*	-0.42**	-0.17ns	-0.33**	0.10ns	-0.35**	0.28*	-0.35**	-0.37**	-0.18ns	0.42**	-0.19ns	0.19ns	-0.10ns	0.12ns
Inulin content (Q20)	0.11ns	-0.09ns	0.08ns	-0.07ns	0.19ns	-0.08ns	0.40ns	0.01ns	-0.01ns	-0.10ns	0.17ns	-0.003ns	-0.05ns	0.03ns	0.27*

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

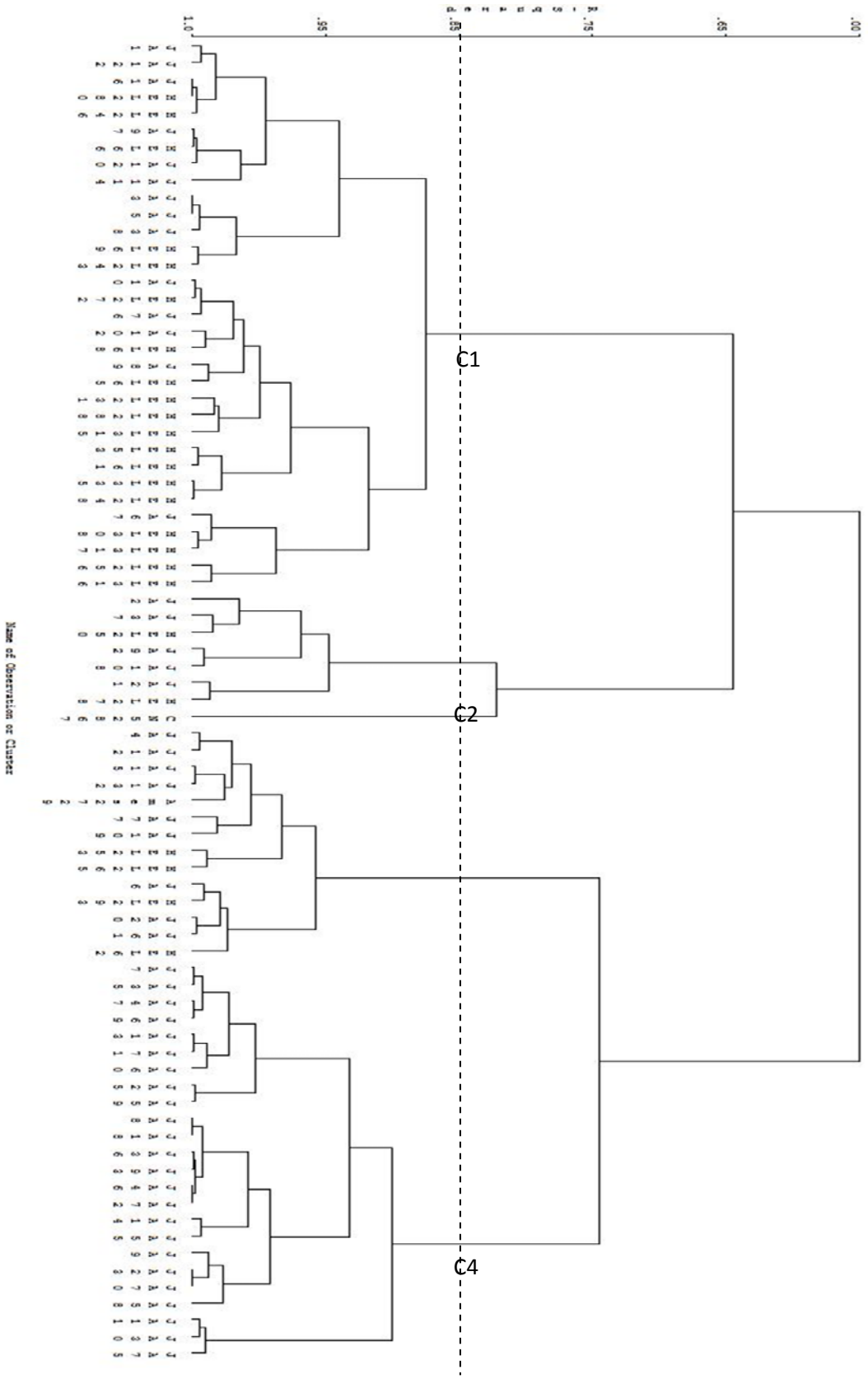


Fig. 1. Dendrogram of 79 Jerusalem artichoke accessions generated by Ward's minimum variance cluster analysis based on 9 qualitative and 20 quantitative characteristics across three seasons.

Table 6. Nine qulitative characters and means ± SE of 20 quantitative characters in 4 clusters grouped by cluster analysis of 79 Jerusalem artichoke accessions across three seasons.

Characters	Cluster1	Cluster 2	Cluster 3	Cluster 4
Leaf shape	Lanceolate-ovate	Lanceolate-ovate	Lanceolate-ovate	Lanceolate
Leaf margin	Serrated	Serrated	Serrated	Smooth
Branch color	Green-violate	Green-violate	Green-violate	Green-violate
Branching distribution	Fully branching	Fully branching	Fully branching	Fully branching
Branching orientation	Opposite	Opposite and	Opposite and	Opposite and
Stem color	Green-violate	Green-violate	Green-violate	Green-violate
Tuber color	Brown	Brown	Brown	Brown
Tuber shape	Slender	Oblong	Oblong	Slender
Surface topography of tuber	Medium	Medium	Medium	Shallow
Plant height (cm)	81.7± 2.55	76.2± 4.76	74.9± 3.18	51.8± 2.35
Leaf petiole (cm)	3.0± 0.15	2.6± 0.21	2.8± 0.11	2.5± 0.07
Leaf width (cm)	6.7± 0.13	6.8± 0.28	6.9± 0.24	5.4± 0.16
Leaf length (cm)	13.8± 0.27	13.3± 0.50	13.6± 0.28	12.7± 0.23
Branch length (cm)	35.6± 0.84	37.8± 1.57	33.9± 0.97	31.2± 1.03
Branch angle (degree)	59.1± 0.86	57.7± 1.63	55.9± 1.09	57.0± 1.45
Internode length (cm)	5.5± 0.12	6.2± 0.27	5.5± 0.15	5.0± 0.15
Basal stem diameter (cm)	1.11± 0.04	0.95± 0.06	0.91± 0.04	0.66± 0.03
Mid stem diameter (cm)	0.80± 0.03	0.67± 0.05	0.67± 0.03	0.49± 0.02
Top stem diameter (cm)	0.22± 0.00	0.21± 0.00	0.22± 0.01	0.17± 0.01
Number of tubers per plant	32± 1.80	46± 4.11	37± 3.37	39± 2.50
Tuber width (cm)	2.4± 0.07	2.1± 0.09	2.0± 0.13	1.3± 0.08
Tuber length (cm)	7.8± 0.15	8.6± 0.22	8.0± 0.39	9.5± 0.29
Tuber size (g/tuber)	13.0± 0.64	12.0± 1.35	10.3± 1.05	5.3± 0.76
Total soluble solids(°Brix)	20.8± 0.18	21.2± 0.82	21.7± 0.41	21.0± 0.42
Days to maturity (days)	108± 0.68	106± 0.73	109± 0.89	105± 0.60
Fresh tuber yield (g)	338.2± 5.39	497.9± 35.91	242.7± 6.22	177.5± 19.48
Biomass (g)	173.1± 6.16	198.0± 12.95	135.7± 6.05	85.1± 8.26
Harvest index	0.5± 0.02	0.7± 0.03	0.5± 0.02	0.6± 0.02
Inulin content (% tuber dry wt)	65.8± 0.56	67.2± 1.48	65.9± 0.99	64.1± 0.98

Conclusion

The Jerusalem artichoke accessions investigated have high variations for both qualitative and quantitative characters and selection for these characters is possible among these accessions. Variations were high for number of tubers per plant, tuber width and tuber size. The accessions were clearly grouped into 4 clusters by using 9 qualitative traits and 20 quantitative traits. These results will enable breeders to make informed decisions about possible heterotic groups for their breeding programs and germplasm conservation.

References

Baldini, M., F. Danuso, A. Rocca, E. Bulfoni, A. Monti and G. De Mastro. 2011. Jerusalem artichoke (*Helianthus tuberosus* L.) productivity in different Italian growing areas: a modeling approach. Ital. J. Agron. 2: 291-307.

Curt, M.D., P. Aguadro, M. Sanz, G. Sánchez and J. Fernández. 2006: Clone precocity and the use of *Helianthus tuberosus* L. stems for bioethanol. Ind. Crop Prod. 24: 314-320.

Encheva, J., M. Christov and P. Ivanov. 2003. Characterization of interspecific hybrids between cultivated sunflower *H. annuus* L. (cv. ALBENA) and wild species *Helianthus tuberosus*. Helia. 26: 43-50.

Gao, K., T. Zhu and G. Han. 2011. Water and nitrogen interactively increased the biomass production of Jerusalem artichoke (*Helianthus tubersus* L.) in semi-arid area. African. J. Biot. 10: 6466-6472.

IBPGR, 1985. Descriptors for cultivated and wild sunflower. IBPGR Secretariat, Rome.

Karimi, H.R., Z. Zamani, A. Ebadi, and M.R. Fatahi 2009. Morphological diversity of *Pistacia* species in Iran. Genet. Resour. Crop. Evol. 56: 561-571.

Kays, S.J. and S.F. Nottingham. 2008. Biology and Chemistry of Jerusalem Artichoke (*Helianthus tuberosus* L.) CRC press, Florida.

Ma, X.Y., L.H. Zhang, H.B. Shao, G. Xu, F. Zhang, F.T. Ni and M. Brestic. 2011. Jerusalem artichoke (*Helianthus tuberosus*), a medicinal salt-resistant plant has high adaptability and multiple-use values. J. Med. Plants. Res. 5: 1275-1282.

Mansyah, E., I. Muas, A.S.M. Jawal and Sobir. 2010. Morphological variability of apomictic mangosteen (*Gurcinia mangostana* L.) in Indonesia: morphological evidence of natural populations from Sumatra and Java. SABRAO J. Breed. Genet. 42: 1-8.

SAS, Institute. 2001: SAS/STAT user's guide. 2nd ed. SAS Institute Inc., Cary, NC, USA.

Seiler, G.J. and L.G. Campbell. 2006. Genetic variability for mineral concentration in the forage of Jerusalem artichoke cultivars. Euphytica. 150: 281-288.

Tassoni, A., N. Bagni, M. Ferri, M. Franceschetti, A. Khomutov, M.P. Marques, S.M. Fiuza, A.R. Simonian and D. Serafini-Fracassini. 2010. *Helianthus tuberosus* and polymine research: past and recent applications of a classical growth model. Plant. Physiol. Biochem. 48: 496-505.

Wati, F., A. Hartana and B.S. Purwoko. 2010. Diversity of Indonesian mango (*Mangifera indica*) cultivars based on morphological and RAPD markers. SABRAO J. Breed. Genet. 42: 84-95.

Publication

Puttha R, S. Jogloy, B. Suriharn, P.P. Wangsomnuk, T. Kesmala and A. Patanothai. 2013. Variations in morphological and agronomic traits among Jerusalem artichoke (*Helianthus tuberosus* L.) accessions. Genet. Resour. Crop Evol.60: 731–746. (Impact factor = 1.482).

2.2 Genetic diversity of water use efficiency in Jerusalem artichoke (*Helianthus tuberosus* L.) Germplasm

A. Janket, S. Jogloy, N. Vorasoot, T. Kesmala, C.C. Holbrook and A. Patanothai

Jerusalem artichoke has been grown in many parts of the world and production conditions range from rainfed to fully irrigated. The crop can be grown in all seasons in a wide range of climates, although the productivity varies greatly across regions (Baldini *et al.*, 2006; Rodrigues *et al.*, 2007). Drought is a recurring problem for crops including Jerusalem artichoke grown in most growing conditions. When only 50% of the water requirement was available, tuber yield of Jerusalem artichoke was reduced by 20% (Conde *et al.*, 1991) and 22.8% (Losavio *et al.*, 1997).

Among inulin containing and sugar containing crops, Jerusalem artichoke is more susceptible to water stress than sugar beet and root chicory (Schittenhelm, 1999). Previous studies indicated that the crop requires adequate soil moisture for optimum yield. The questions arising from these studies are "1) what is the optimal amount of water to be applied to Jerusalem artichoke with supplemental irrigation or full irrigation under rainfed conditions, and 2) is there variation in water use efficiency among Jerusalem artichoke accessions under different water gradients?" These questions are important for water management of the crop and for further improvement of water use efficiency by the crop. Jerusalem artichoke varieties with high water use efficiency should be more productive under water limited conditions. The trait can be used as a selection criterion for drought resistance which is more complex and difficult to access (Teare *et al.*, 1982).

Variation in water use efficiency among genotypes has been reported in other crops such as peanut (Jongrungklang *et al.*, 2008; Puangbut *et al.*, 2009), isabgol (*Plantago ovata*), French phyllium (*Plantago psyllium*) (Rahimi *et al.*, 2011) and cotton (Tennakoon and Milroy, 2002). Previous investigations on water use and water use efficiency conducted so far have been limited to six Jerusalem artichoke genotypes (Yang *et al.*, 2010). Studies on a wide range of diverse genotypes are required to fully exploit genetic variations in these characters.

Objectives

The objectives of this study were to compare water use efficiency among Jerusalem artichoke genotypes under different water gradients and to identify Jerusalem artichoke genotypes with high water use efficiency. The information obtained in this study will be useful for irrigation management of Jerusalem artichoke and breeding of Jerusalem artichoke for high water use efficiency.

Materials and Methods

A field experiment was conducted at the Field Crop Research Station of Khon Kaen University during October 2010 to January 2011 and repeated during October 2011 to January 2012. Forty Jerusalem artichoke accessions were evaluated in a strip plot design with four replications. Three strip plots represented three water regimes (W1= 100% ET crop, W2 = 75% ET crop and W3 = 45% ET crop), and Jerusalem artichoke

genotypes were randomly arranged in the strips. Drought mimicking was accomplished by line source sprinkler irrigation from 14 days after transplanting (DAT) to harvest.

Soil moisture content was measured with neutron probe at the depths of 30, 60 and 90 cm at 7-day intervals throughout the course of the experiment. Plant data were recorded for relative water content at 40, 60 and 70 DAT. Tuber dry weight and biomass were recorded at harvest in the area of 2.1 m^2 and amounts of water that were supplied to the crop at all moisture levels were monitored by catch cans. Water use efficiency was estimated using the formula proposed by Teare *et al.* (1982):

WUE for biomass (WUEb) = dry matter yield/water used in evapotranspiration and

WUE for tubers (WUEt) = tuber dry weight/water used in evapotranspiration.

Results

Combined analysis of variance showed significant differences between water regimes and genotypes for water use efficiency (WUE) of biomass and tuber (data not shown). Year × genotype interaction and genotype × water regime interaction effect were significant for both WUE of biomass and tubers. Thus, data of each year and water regime were analyzed separately.

The genotypes with high or low WUEb and WUEt could be identified. HEL 53, JA 89, KKUAc001, JA102×JA89(8), HEL 253, HEL 231, HEL 65 and HEL 61 had consistently high WUEb and WUEt across water regimes in 2010/11 (Table 1). HEL 335 had consistently high WUEb and WUEt under W1 and W2, whereas HEL 256 had high WUEb across water regimes but WUEt exhibited high water use efficiency under W1 only.

JA 61, JA 70, JA 1, JA 77, JA 97, JA 46, JA 109, JA 60, JA 36 and JA 125 had low WUEb under W1 in 2010/11, whereas JA 61, JA 70, JA 1, JA 77, JA 60 and JA 36 had consistently low WUEb across water regimes. The genotypes with low WUEb also had low WUEt except for HEL 62 showing low WUEt only and JA 46 showing low WUEb only. JA 70, JA 1, JA 77, JA 61 and JA 36 showed consistently low WUEb and WUEt across water regimes.

In the experiment in 2011/12, HEL 256, JA 89, JA 6, HEL 231, HEL 65, CN 52867, KKUAc001, HEL 324, JA102×JA89(8) and JA 16 had high WUEb under W1 (Table 2), and, among these genotypes, JA 6, HEL 231, HEL 65 and JA102×JA89(8) had high water use efficiency across water regimes. HEL 256, JA 89, JA 6, HEL 65, HEL 257, CN 52867, JA 122, JA 16, HEL 324 and JA102×JA89(8) had high WUEt under W1. Among these accessions, there were 3 genotypes (JA 6, HEL 65 and CN 52867) with high water use efficiency across water regimes. The genotypes with low WUEb under W1 were JA 1, JA 70, JA 36, JA 109, HEL 62, JA 60, JA 46, JA 61, JA 125, JA 92, and the genotypes showing consistently WUEb across water regimes were JA 1, JA 92, JA 70, JA 36, JA 109, JA 60, JA 46 and HEL62.

Most genotypes showing low WUEb also had low WUEt. However, JA 125 and JA 61 had low WUEb but their WUEt was relatively high under W1. In contrast, JA 67 and JA 77 had low WUEt but WUEb was relatively high. JA 70, JA 109, HEL 62 and JA 36 showed consistently low WUEt across water regimes. JA 89, KKUAc001, [JA102×JA89]-8, HEL 231 and HEL 65 had high WUEb across years under W1, whereas JA 89, [JA102×JA89]-8 and HEL 65 had high WUEt.

Table 1. Ten selected genotypes showing the highest water use (WU), water use efficiency for biomass (WUEb) and water use efficiency for tubers (WUEt) and 10 selected genotypes showing the lowest performances for these traits and drought tolerance index (DTI) in the dry seasons 2010/11.

Group No.		Water use (WU) (mm)				Water use efficiency for biomass (WUEb) (kg mm^{-1} ha^{-1})				Water use efficiency for tubers (WUEt) (kg mm^{-1} ha^{-1})			
		Genotypes	W1	W2	W3	Genotypes	W1	W2	W3	Genotypes	W1	W2	W3
High	1	HEL 62	217.5 a	161.8 a	93.3 b	HEL 53	32.7 a	36.6 a	31.4 a	HEL 53	24.8 a	27.2 a	32.0 a
	2	HEL 246	211.2 ab	157.7 ab	114.7 a	HEL 253	32.0 a	28.5 b	31.9 a	HEL 335	24.3 a	18.9 ab	13.2 g-k
	3	KKUAc001	210.3 abc	158.3 ab	93.1 b	HEL 335	30.7 ab	20.4 e-h	19.2 e-i	HEL 65	22.5 ab	15.9 c-f	22.8 c
	4	HEL 256	209.6 a-d	158.7 ab	93.3 b	HEL 256	30.7 ab	24.0 cde	26.9 a-d	HEL 256	22.1 abc	12.9 e-i	14.5 e-j
	5	JA 125	209.3 a-d	155.8 a-e	92.4 b	HEL 61	28.7 bc	22.1 bc	22.8 c-f	HEL 61	22.1 abc	15.7 c-f	17.9 def
	6	HEL 257	209.0 a-d	155.4 a-f	92.6 b	HEL 65	28.3 bc	21.1 efg	28.5 ab	HEL 253	22.0 abc	16.1 cde	20.1 cd
	7	JA 77	208.9 a-d	158.0 ab	92.3 b	JA102xJA89(8)	28.0 bc	21.5 ef	28.4 ab	JA 89	21.4 a-d	20.9 b	26.7 b
	8	JA 67	208.9 a-d	157.1 abc	92.3 b	JA 89	25.7 cd	27.5 bc	27.7 ab	JA102xJA89(8)	20.4 a-e	16.1 cde	17.9 def
	9	HEL 53	208.7 a-d	157.8 ab	93.1 b	HEL 231	25.7 cd	26.1 bcd	24.3 b-e	HEL 231	19.7 a-f	16.0 c-f	19.5 cd
	10	HEL 335	208.1 a-d	158.1 ab	93.4 b	KKUAc001	24.0 d	22.7 de	27.2 abc	KKUAc001	17.3 a-g	20.5 b	19.7 cd
Low	1	HEL 253	194.7 b-f	157.9 ab	93.4 b	JA 125	10.2 m-p	13.7 j-o	13.3 jp	JA 36	8.4 g-j	5.1 pq	7.1 n
	2	JA 21	194.5 b-f	146.6 i-m	86.8 b	JA 36	9.9 n-q	9.2 p-s	8.5 p	HEL 62	8.3 a-j	8.4 k-p	8.4 lmn
	3	HEL 65	194.5 b-f	156.5 a-d	91.1 b	JA 60	9.4 n-r	10.7 n-r	10.1 nop	JA 125	8.3 g-j	10.1 g-n	11.3 j-n
	4	HEL 324	193.7 b-f	145.6 j-m	86.5 b	JA 109	9.3 n-r	13.7 j-o	20.3 e-h	JA 60	7.8 g-j	9.1 i-o	10.7 j-n
	5	JA 3	193.3 b-f	144.7 klm	86.4 b	JA 46	8.8 n-r	10.9 m-r	12.2 k-p	JA 109	6.8 g-j	9.6 h-o	17.2 d-h
	6	JA 76	192.5 c-f	156.6 a-d	93.1 b	JA 97	8.6 o-r	8.7 qrs	15.5 h-n	JA 61	6.5 a-j	6.1 opq	9.5 k-n
	7	JA 36	191.7 c-f	143.4 lm	85.5 b	JA 77	7.5 pqr	8.5 qrs	8.5 p	JA 77	6.3 hij	7.1 l-q	7.1 ln
	8	JA 6	191.5 def	146.6 i-m	86.6 b	JA 1	6.9 qr	5.6 s	9.1 p	JA 97	6.0 ij	6.8 m-q	11.5 j-n
	9	JA 16	188.9 ef	141.8 m	84.7 b	JA 70	6.8 qr	7.9 rs	10.3 m-p	JA 1	5.8 ij	4.7 q	7.9 mn
	10	JA 15	181.8 f	148.6 e-m	85.2 b	JA 61	6.3 r	7.1 rs	9.4 op	JA 70	5.5 j	6.6 n-q	8.4 lmn
Mean			201.8 A	152.5 B	90.5 C		16.9 AB	15.7 B	17.7 A		13.45 A	12.15 B	14.27 A
Min			181.8	141.8	84.7		6.3	5.6	8.5		5.5	4.7	7.1
Max			217.5	161.8	114.7		32.7	36.6	31.9		24.8	27.2	32.0

Maximum, minimum and mean values were calculated from 40 genotypes; for comparison among Jerusalem artichoke genotypes and for comparison among water regimes, means in the same column followed by the same letter(s) are not significantly different at $P< 0.05$ probability levels by Duncan's multiple range test (DMRT).

[a]DTI = Drought tolerance index was calculated by the ratio of stressed conditions / non stressed conditions. W1= 100%ET, W2= 75%ET and W3=45%ET.

Three genotypes (HEL 231, HEL 65 and [JA102×JA89]-8 had consistently high WUEb across water regimes and years, and HEL 65 had high WUEt across water regimes and years. There were 6 genotypes (JA61, JA 70, JA 1, JA 109, JA 60 and JA 36) showing consistently low WUEb across years under W1 and 7 genotypes (JA 70, JA 1, JA 109, HEL 62, JA 36, JA 60 and JA 77) showing consistently low WUEt under W1. However, there were only four genotypes (JA 70, JA 1, JA 60 and JA 36) with consistently low WUEb across water regimes and years and three genotypes (JA 70, HEL 62 and JA 36) with consistently low WUEt across water regimes and years.

Table 2. Ten selected genotypes showing the highest water use (WU), water use efficiency for biomass (WUEb) and water use efficiency for tubers (WUEt) and 10 selected genotypes showing the lowest performance for these traits and drought tolerance index (DTI) in the dry seasons 2011/12

Group No.		Water use (WU) (mm)				Water use efficiency for biomass (WUEb) (kg mm^{-1} ha^{-1})				Water use efficiency for tubers (WUEt) (kg mm^{-1} ha^{-1})			
		Genotypes	W1	W2	W3	Genotypes	W1	W2	W3	Genotypes	W1	W2	W3
High	1	HEL 62	215.9 a	163.5 abc	103.3 abc	HEL 256	35.6 a	20.7 j-p	28.1 a-h	HEL 256	27.6 a	14.8 g-m	17.5 f-m
	2	HEL 65	214.8 ab	164.4 ab	104.5 ab	JA 89	32.5 ab	23.9 d-k	26.7 c-j	JA 89	23.5 b	16.3 d-j	18.9 d-h
	3	HEL 256	212.6 abc	166.4 a	105.6 a	JA 6	31.7 bc	31.9 a	31.2 a-d	JA 6	23.1 bc	21.1 ab	23.8 abc
	4	HEL 253	210.0 a-d	162.9 abc	103.2 abc	HEL 231	31.2 bcd	26.7 b-f	31.7 abc	HEL 65	22.5 bcd	20.3 ab	21.9 a-e
	5	HEL 335	208.6 a-e	163.4 abc	100.8 a-e	HEL 65	30.3 b-e	30.3 abc	29.7 a-e	HEL 257	21.3 b-e	18.7 b-f	20.7 b-f
	6	JA 132	207.3 a-f	158.5 b-e	101.1 a-e	CN 52867	29.9 b-e	27.3 b-e	27.3 a-i	CN 52867	21.0 b-f	20.3 ab	25.1 a-d
	7	JA102XJA89(8)	207.3 a-f	153.7 d-i	99.6 b-g	KKUAc001	28.9 b-f	28.0 a-d	26.9 c-i	JA 122	20.2 c-g	19.6 abc	17.9 f-k
	8	JA 76	206.5 a-g	158.4 b-e	99.9 b-f	HEL 324	28.0 c-g	24.1 d-j	29.5 a-e	JA 16	19.7 g-h	16.6 c-i	14.4 j-o
	9	JA 37	206.0 a-g	158.3 b-e	100.3 b-e	JA102XJA89(8)	27.9 d-g	30.1 abc	28.7 a-f	HEL 324	19.5 d-i	15.5 f-k	17.7 f-l
	10	JA 67	205.8 a-g	160.1 a-d	101.9 a-d	JA 16	27.7 d-g	23.9 d-k	20.1 l-m	JA102XJA89(8)	19.5 d-i	19.9 abc	18.2 e-j
Low	1	JA 114	187.2 l-q	144.6 k-n	93.0 j-o	JA 92	18.0 o-u	21.3 h-o	22.7 h-o	JA 77	13.3 n-t	15.0 g-l	16.7 f-n
	2	CN 52867	187.0 l-q	147.6 h-m	95.0 f-n	JA 125	17.5 p-u	16.7 p-s	17.5 o-r	JA 46	13.0 o-t	13.1 j-o	14.7 i-o
	3	JA 3	186.2 m-q	141.4 lmn	91.8 l-o	JA 61	17.4 p-u	19.2 l-q	23.1 h-n	JA 92	12.9 o-t	15.6 f-k	16.9 f-n
	4	JA 38	186.0 m-q	145.1 j-n	93.8 h-o	JA 46	16.9 p-u	17.8 n-s	18.7 n-r	JA 67	12.8 o-t	12.9 k-o	18.5 e-i
	5	JA 5	185.2 n-q	143.8 k-n	92.7 k-o	JA 60	16.5 r-u	14.9 rs	18.9 m-r	JA 60	12.5 p-t	11.7 mno	15.0 h-o
	6	HEL 324	184.7 n-q	138.7 n	90.6 no	HEL 62	16.3 r-u	15.7 qrs	20.3 l-p	JA 36	11.6 q-t	11.9 l-o	9.6 q
	7	JA 36	181.2 opq	142.1 lmn	91.7 mno	JA 109	16.1 stu	16.0 qrs	14.9 qr	HEL 62	11.5 rst	11.0 no	13.4 nop
	8	JA 122	180.9 opq	141.2 mn	91.9 l-o	JA 36	15.9 tu	15.8 qrs	16.1 pqr	JA 109	11.3 rst	10.8 o	9.7 q
	9	JA 16	179.4 pq	140.1 mn	90.7 no	JA 70	14.5 u	14.0 s	13.9 r	JA 1	10.9 st	14.1 h-n	8.7 q
	10	JA 6	176.6 q	139.0 n	89.9 o	JA 1	14.5 u	17.2 o-s	14.3 r	JA 70	10.2 t	10.3 o	10.1 pq
Mean			196.9 A	152.0 B	97.1 C		23.2 AB	22.5 B	24.4 A		16.6 AB	16.2 B	17.3 A
Min			176.6	138.7	89.9		14.5	14.0	13.9		10.2	10.3	8.7
Max			215.9	166.4	105.6		35.6	31.9	32.5		27.6	22.4	25.1

Maximum, minimum and mean values were calculated from 40 genotypes; for comparison among Jerusalem artichoke genotypes and forcomparison among water regimes, means in the same column followed by the same letter(s) are not significantly different at $P< 0.05$ probability levels by Duncan's multiple range test (DMRT).

[a]DTI = Drought tolerance index was calculated by the ratio of stressed conditions / non stressed conditions. W1= 100%ET, W2= 75%ET and W3=45%ET.

Conclusion

Variations in water use efficiency for biomass and tubers were observed among Jerusalem artichoke genotypes, and the use of water use efficiency as a surrogate trait for drought tolerance in Jerusalem artichoke is promising. In this germplasm, the identified genotypes with high water use efficiency in all of drought levels were HEL 231, HEL 65 and [JA102×JA89]-8 had high water use efficiency for biomass, whereas HEL 65 were identified as the accessions with high water use efficiency for tubers. The genotypes identified might be useful in future breeding programs for high water use efficiency.

References

Baldini, M., F. Danuso, A. Monti, T. Amaducci, P. Stevanato and G.D. Mastro. 2006. Chicory and Jerusalem artichoke productivity in different areas of Italy, in relation to water availability and time of harvest. Ital. J. Agron. 2: 291-307.

Conde, J.R., J.L. Tenorio, B. Rodriguez-Maribona and L. Ayerbe, 1991: Tuber yield of Jerusalem artichoke (*Helianthus tuberosus* L.) in relation to water stress. Biomass Bioenerg. 3: 131-142.

Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, T. Kesmala and A. Patanothai, 2008. Identification of peanut genotypes with high water use efficiency under drought stress conditions from peanut germplasm of diverse origins. Asian J. Plant Sci. 7: 628–638.

Losavio, N., N. Lamascese and A.V. Vonella, 1997: Water requirements and nitrogen fertilization in Jerusalem artichoke (*Helianthus tuberosus* L.) grown under Mediterranean conditions. Acta Hort. 449, 205–209.

Rahimi, A., M.R. Jahansoz, S. Madah Hoseini, A.R. Sajjadinia, H.R. Roosta and E. Fateh, 2011: Water use and water-use efficiency of Isabgol (*Plantago ovata*) and French psyllium (*Plantago psyllium*) in different irrigation regimes. Aust. J. Crop Sci. 5: 71–77

Rodrigues M.A, L. Sousa, J.E. Cabanas and M. Arrobas, 2007: Tuber yield and leaf mineral composition of Jerusalem artichoke (*Helianthus tuberosus* L.) grown under different cropping practices. Span. J. Agric. Res. 5: 545-553.

Puangbut, D., S. Jogloy, N. Vorasoot, C. Akkasaeng, T. Kesmala, C.N. Rachaputi Rao, 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.

Schittenhelm, M.S. 1999. Agronomic performance of root chicory, Jerusalem artichoke and sugarbeet in stress and non-stress environment. Crop Sci. 39: 1815-1823.

Teare, I.D., N. Sionit and P.J. Kramer.1982. Change in water status during water stress at different stages of development in wheat. Physiol. Plantarum. 55: 296-300.

Tennakoon, S.B and S.P. Milroy. 2002. Crop water use and water use efficiency on irrigated cotton farms in Australia. Agr. Water Manage. 61: 179-194.

Yang, B., H.J. Zhang, L. You-Xian and Y.L. Xue. 2010. Effect of different irrigation on development and water use efficiency of Jerusalem artichoke. Irrig. Drain. 29: 168-175.

Publication

Janket, A., S. Jogloy, N. Vorasoot, T. Kesmala, C.C. Holbrook and A. Patanothai. 2013. Genetic diversity of water use efficiency in Jerusalem artichoke (*Helianthus tuberosus* L.) Germplasm. Aust. J. Crop Sci. 7: 1670-1681. (Impact factor = 1.632).

2.3 Genotypic variability for tuber yield, biomass and drought tolerance in Jerusalem artichoke germplasm

R. Ruttanaprasert, P. Banterng,S. Jogloy, N. Vorasoot, T. Kesmala, R. S. Kanwar, C. C. Holbrook and A. Patanothai

High oil price stimulated interest in bio-ethanol as a potential liquid fuel for transportation (Margaritis and Pratima, 1983). Bio-ethanol is currently produced using carbohydrate sources in many countries. Common feedstocks for ethanol production are sugarcane, cassava, corn grain, and many other agricultural raw materials rich in fermentable carbohydrates. These feedstocks are then chemically converted to yield fermentable sugars (Lin and Tanaka, 2006). Corn, sorghum, Jerusalem artichoke, potato and lignocellulosic biomass are sources of feedstock with great potential for ethanol production (Azhar and Hamdy, 2003).

Mainstream raw materials such as sugarcane, cassava and corn grain have limited harvest times and are rarely available for year round production. Non-conventional feedstocks such as sweet sorghum and Jerusalem artichoke can diversify raw materials and extend production times for the bio-ethanol industry (Walker, 2010). Of these non-conventional raw materials, Jerusalem artichoke is of great interest (Szambelan *et al.*, 2005). The ethanol yield from Jerusalem artichoke tubers is equivalent to that obtained from sugar beets and two folds that of corn (Azhar and Hamdy, 2003). The crop has high carbohydrate yields ranging between 5 and 14 ton ha^{-1} (Stephen *et al.*, 2006), and, because of this, it has been evaluated as a potential crop for ethanol production (Denoroy, 1996). Bio-ethanol of about 4.0 to 4.7 tons per hectare was produced from this crop (Walker, 2010). Jerusalem artichoke is also a promising candidate for inulin production in the tropics. Although Jerusalem artichoke grown in the tropics is not as productive as when it is grown in temperate regions, it can be grown successfully and profitably, and agronomic studies and breeding efforts are ongoing at Khon Kaen University.

Drought stress is a major limitation to the production of the crop in Northeast Thailand. Breeding and for drought resistance genotypes is required for a long-term solution. This would be possible if diverse germplasm sources are screened and drought resistant genotypes are identified. Previous studies on drought resistance in Jerusalem artichoke have been conducted only in temperate regions with few genotypes and water regimes (Conde *et al.*, 1991; Losavio *et al.*, 1997; Schittenhelm, 1999; Monti *et al.*, 2005; Lium *et al.*, 2012). Screening diverse genotypes for these traits is needed.

Objectives

The objectives of this study were to determine the effects of drought stress on tuber dry weight and biomass under tropical conditions, and to evaluate the genotypic variability in Jerusalem artichoke germplasm. The information will be useful for both breeding and production of Jerusalem artichoke to increase its productivity under drought conditions.

Materials and Methods

Forty genotypes of Jerusalem artichoke with differences in biomass, plant height and harvest date were screened for drought tolerance using a line-source sprinkler system (Hank et al 1976). The experiment was set up in a strip plot design with four replications in the dry periods during October 2010 to January 2011 and October 2011 to January 2012 at the Field Crop Research Station of Khon Kaen University. Three water gradients (W1, W2 and W3) were assigned in horizontal plot and 40 Jerusalem artichoke genotypes were randomly assigned in vertical plot. W1 was the control treatment with a full crop water requirement (ETcrop), W2 was the mild drought and W3 was the most severe drought conditions. Plot size was 2 x 4 m with the spacing was 50 x 30 cm of 4 rows per plot. Data were recorded for tuber dry weight and biomass at harvest.

Analysis of variance was performed for each character followed a strip plot design. Simple linear response in tuber dry weight and biomass to the three water regimes was also performed to determine average reduction for tuber dry weight and biomass of each genotype to water stress.

Results

The interactions between genotype and year and genotype and water regime were significant for tuber dry weight and biomass (Table 1), therefore, the data for the two years were analyzed separately (Table 2 and 3).

Drought reduced tuber dry weight and biomass in both years, and the reductions in tuber dry weight and biomass were more severe under W3 than W2. Significant differences among Jerusalem artichoke genotypes were found for tuber dry weight and biomass under W1, W2 and W3 in both years (Table 2 and 3). The slopes for all Jerusalem artichoke genotypes had negative and high determination coefficients.

Table 1. Mean squares for tuber fresh weight, shoot dry weight, tuber dry weight and biomass of 40 Jerusalem artichoke genotypes grown under three water regimes (W1 = 100% ET, W2 = 75% ET and W3 = 45% ET) during the dry seasons 2010/11 and 2011/12.

Source	df	Tuber fresh weight (tons ha^{-1})			Tuber dry weight (tons ha^{-1})			Biomass (tons ha^{-1})		
Year	1	1181.3	(7.7)	**	78.0	(7.3)	**	243.3	(11.6)	**
Rep within year	6	187.8	(7.4)		13.5	(7.5)		22.5	(6.4)	
Water	2	3328.1	(43.6)	**	192.5	(35.9)	**	352.1	(33.5)	**
Year × Water	2	4.8	(0.1)	ns	0.5	(0.1)	ns	1.9	(0.2)	ns
Error (a)	12	10.5	(0.8)		0.6	(0.7)		0.8	(0.5)	
Genotypes	39	70.6	(18.0)	**	5.9	(21.4)	**	14.1	(26.3)	**
Year × Genotypes	39	18.2	(4.6)	**	1.5	(5.4)	**	2.1	(3.9)	**
Error (b)	234	5.2	(7.9)		0.5	(10.5)		0.8	(8.5)	
Water × Genotypes	78	6.9	(3.5)	**	0.6	(4.0)	**	1.1	(3.9)	**
Year × Water x Genotypes	78	3.0	(1.6)	**	0.2	(1.7)	**	0.3	(1.2)	**
Error (c)	468	1.6	(4.7)		0.1	(5.4)		0.2	(3.9)	
Total	959									
CV%(a)		40			36			30		
CV%(b)		28			32			30		
CV%(c)		15			16			14		

Numbers within the parentheses are percent (%) of sum squares to total sum of squares.
ns, *,** Non significant, significant and highly significant at $P \leq 0.05$ and ≤ 0.01 probability levels, respectively.

In the year 2010/11, CN 52867, KKU Ac 001, HEL 53, HEL 61, HEL 231, HEL 335, JA 76, HEL 246, JA 15, JA 89, HEL 65, HEL 253, HEL 256 and JA 102 x JA 89 (8) had consistently high tuber dry weight across water regimes, and KKU Ac001, HEL 53, HEL 61, HEL 231, HEL 335, JA 76, JA 15, JA 89, HEL 65, HEL 253, HEL 256 and JA 102 x JA 89 (8) had consistently high biomass across water regimes (Table 1).

In the year 2011/12, JA 6, JA 21, JA 38, JA 97, JA 132, JA 122, CN 52867, HEL 53, HEL 231, HEL 335, JA 76, HEL 257, HEL 65 and JA 102 x JA 89(8) had consistently high tuber dry weight across water regimes. JA 6, JA 97, JA 132, HEL 324,

CN 52867, KKU Ac001, HEL 53, HEL 61, HEL 231, HEL 335, JA 76, HEL 257, JA 15, JA 89, HEL 65, HEL 256 and JA 102 X JA 89 (8) had consistently high biomass across water regimes (Table 3).

The genotypes with high tuber dry weight and biomass under drought conditions were those with high tuber dry weight and biomass under fully-irrigated conditions. The genotypes with high potential for tuber yield and biomass in general had more reductions than did the genotypes with low potential. Therefore, the main criterion for selection of drought tolerant genotypes in this study was yield under drought stress.

Table 2. Mean tuber dry weight and biomass, b-values and coefficient of determinations (R^2) for 40 Jerusalem artichoke genotypes grown under three water regimes (W1 = 100% ET, W2 = 75% ET and W3 = 45% ET) during the dry season 2010/11.

Entry No.	Genotypes	Tuber dry weight (ton ha^{-1})						b value	R^2	Biomass (ton ha^{-1})						b value	R^2
		W1		W2		W3				W1		W2		W3			
1	JA 1	1.2	l	0.7	l	0.7	fg	-0.26	0.82	1.4	m	0.8	h	0.8	h	-0.31	0.84
2	JA 4	2.8	c-i	1.9	b-k	1.0	c-g	-0.88	1.00	3.4	e-k	2.2	d-g	1.3	e-h	-1.05	1.00
3	JA 6	2.8	c-i	1.5	f-l	0.9	d-g	-0.95	0.96	3.5	d-j	2.0	e-h	1.2	f-h	-1.15	0.97
4	JA 36	1.6	j-l	0.9	kl	0.7	fg	-0.42	0.90	1.9	k-m	1.1	gh	0.9	h	-0.50	0.92
5	JA 70	1.1	l	1.0	j-l	0.6	g	-0.22	0.80	1.3	m	1.2	f-h	0.8	h	-0.28	0.88
6	JA 92	2.8	d-j	1.5	f-l	0.9	d-g	-0.92	0.96	3.5	d-j	1.9	e-h	1.1	gh	-1.18	0.97
7	JA 114	2.1	g-l	1.3	i-l	1.0	c-g	-0.56	0.90	2.6	g-m	1.6	e-h	1.2	f-h	-0.70	0.93
8	JA 3	2.0	h-l	1.2	i-l	0.8	e-g	-0.59	0.94	2.3	i-m	1.4	f-h	1.0	h	-0.68	0.96
9	JA 16	2.1	g-l	1.4	g-l	1.0	d-g	-0.59	0.99	2.5	h-m	1.7	e-h	1.1	f-h	-0.68	0.98
10	JA 21	2.1	g-l	1.2	i-l	0.9	d-g	-0.59	0.92	2.5	h-m	1.5	f-h	1.1	gh	-0.70	0.93
11	JA 37	4.0	a-c	2.2	b-i	1.1	c-g	-1.45	0.98	4.7	b-f	2.5	c-f	1.3	e-h	-1.69	0.98
12	JA 38	2.5	e-k	1.5	g-l	0.8	fg	-0.86	0.98	2.9	g-l	1.7	e-h	1.0	h	-0.98	0.98
13	JA 97	2.0	h-l	1.4	g-l	1.0	d-g	-0.50	1.00	2.5	h-m	1.7	e-h	1.2	f-h	-0.66	0.99
14	JA 132	2.6	d-j	2.1	b-j	1.3	a-g	-0.68	0.99	3.3	f-k	2.5	c-f	1.5	c-h	-0.89	1.00
15	JA 5	2.7	d-j	1.7	e-l	0.9	d-g	-0.89	0.99	3.3	f-k	2.1	e-h	1.2	f-h	-1.05	0.99
16	JA 122	2.5	e-k	1.9	b-k	1.2	b-g	-0.63	1.00	3.0	g-l	2.3	d-g	1.5	c-h	-0.78	1.00
17	HEL 324	2.2	f-l	1.7	e-l	1.3	a-g	-0.46	0.99	2.9	g-m	2.1	e-h	1.7	b-h	-0.60	0.98
18	JA 61	2.4	f-k	1.7	e-l	1.2	a-g	-0.57	0.99	2.6	g-m	1.8	e-h	1.4	d-h	-0.63	0.98
19	CN 52867	2.9	c-i	1.8	d-k	1.4	a-f	-0.74	0.96	3.6	d-j	2.2	d-g	1.6	b-h	-0.96	0.95
20	KKUAc001	3.4	a-f	2.4	a-g	1.6	a-e	-0.90	1.00	4.7	b-f	3.4	a-d	2.1	a-e	-1.26	1.00
21	HEL 53	4.5	a	3.2	a	1.9	a	-1.27	1.00	6.2	a	4.4	a	2.7	a	-1.73	1.00
22	HEL 61	4.0	a-c	2.6	a-f	1.7	a-c	-1.11	0.98	5.2	a-c	3.4	a-d	2.3	a-c	-1.48	0.98
23	HEL 231	3.6	a-e	2.5	a-f	1.6	a-d	-0.98	1.00	4.8	a-e	3.5	a-c	2.2	a-d	-1.31	1.00
24	HEL 335	4.1	ab	2.6	a-f	1.3	a-g	-1.37	1.00	5.8	ab	3.7	a-c	2.0	a-g	-1.91	0.99
25	JA 46	1.7	i-l	1.3	h-l	0.7	fg	-0.53	0.99	2.2	j-m	1.6	e-h	0.9	h	-0.64	1.00
26	JA 60	1.7	i-l	1.2	i-l	0.8	fg	-0.46	1.00	2.0	j-m	1.5	e-h	0.9	h	-0.55	1.00
27	JA 109	2.0	h-l	1.4	g-l	0.8	e-g	-0.60	1.00	2.5	h-m	1.7	e-h	1.0	h	-0.75	0.99
28	JA 76	3.4	a-f	2.3	a-h	1.6	a-d	-0.87	0.99	4.1	c-g	2.8	b-e	2.0	a-f	-1.04	0.98
29	JA 77	1.4	kl	1.1	j-l	0.7	fg	-0.34	0.99	1.6	lm	1.3	f-h	0.8	h	-0.43	1.00
30	HEL 62	1.8	i-l	1.1	i-l	0.7	fg	-0.52	0.97	2.5	h-m	1.5	f-h	1.0	h	-0.78	0.96
31	HEL 246	3.2	b-g	1.9	b-k	1.4	a-f	-0.91	0.93	3.9	c-h	2.2	d-g	1.7	b-h	-1.10	0.93
32	HEL 257	2.8	d-j	1.9	b-k	1.0	c-g	-0.87	1.00	3.4	d-j	2.3	d-g	1.3	e-h	-1.09	1.00
33	JA 15	3.1	b-h	1.9	c-k	1.3	a-g	-0.86	0.95	3.8	c-i	2.3	d-g	1.6	b-h	-1.06	0.95
34	JA 67	2.1	g-l	1.3	i-l	1.0	d-g	-0.57	0.91	3.2	f-k	1.8	e-h	1.4	d-h	-0.91	0.92
35	JA 89	3.7	a-d	2.9	ab	1.9	ab	-0.93	0.99	4.9	a-d	3.9	ab	2.5	ab	-1.21	0.99
36	JA 125	1.9	h-l	1.3	h-l	1.0	c-g	-0.43	0.98	2.3	i-m	1.7	e-h	1.2	f-h	-0.54	0.99
37	HEL 65	4.1	ab	2.7	a-e	1.9	ab	-1.11	0.97	5.2	a-c	3.5	a-d	2.4	ab	-1.40	0.98
38	HEL 253	4.1	ab	2.9	a-c	1.9	ab	-1.12	1.00	6.1	ab	3.9	ab	2.7	a	-1.69	0.97
39	HEL 256	4.0	a-c	2.5	a-f	1.7	a-c	-1.12	0.97	5.8	ab	3.7	a-c	2.5	ab	-1.66	0.98
40	JA102 × JA 89 (8)	3.7	a-d	2.8	a-d	1.9	ab	-0.92	1.00	5.0	a-c	3.8	ab	2.6	a	-1.23	1.00
	Means	2.7		1.8		1.2		-0.76	0.97	3.5		2.3		1.5		-0.98	0.97

Means in the same column followed by the same letter (s) are not different at$P \leq 0.01$ probability levels by Duncan's multiple range test (DMRT).
W1 = 100% ET, W2 = 75% ET and W3 = 45% ET

Table 3. Mean tuber dry weight and biomass, b-values and coefficient of determinations (R^2) for 40 Jerusalem artichoke genotypes grown under three water regimes (WI = 100% ET, W2 = 75% ET and W3 = 45% ET) during the dry season 2011/12.

Entry No.	Genotypes	Tuber dry weight (ton ha^{-1})						b value	R^2	Biomass (ton ha^{-1})						b value	R^2
		W1		W2		W3				W1		W2		W3			
1	JA 1	2.2	j-l	2.0	g-k	1.2	f-h	-0.49	0.93	3.0	m-o	2.5	j-m	1.6	j-n	-0.69	0.98
2	JA 4	3.6	b-i	2.4	c-j	1.9	b-e	-0.83	0.95	4.9	c-h	3.3	b-k	2.6	b-h	-1.19	0.96
3	JA 6	3.9	a-f	3.2	a-c	2.0	a-e	-0.96	0.98	5.6	a-e	4.5	a	2.8	b-e	-1.40	0.99
4	JA 36	2.1	kl	1.7	i-k	1.1	gh	-0.49	0.99	2.9	no	2.3	lm	1.5	l-n	-0.71	0.99
5	JA 70	2.0	l	1.5	k	1.0	h	-0.50	0.99	2.8	o	2.1	m	1.3	n	-0.73	1.00
6	JA 92	3.4	b-i	2.4	c-j	1.6	c-g	-0.89	0.99	4.4	d-l	3.3	b-l	2.2	d-m	-1.13	1.00
7	JA 114	2.5	i-l	2.3	e-k	1.6	d-h	-0.46	0.93	3.8	g-o	3.1	e-l	2.1	e-m	-0.83	0.99
8	JA 3	3.0	c-l	2.5	b-i	1.9	b-e	-0.54	1.00	4.2	e-n	3.2	b-l	2.6	b-h	-0.84	0.99
9	JA 16	3.2	c-k	2.3	d-j	1.6	d-h	-0.81	1.00	4.4	d-l	3.1	e-l	2.1	e-n	-1.19	1.00
10	JA 21	3.5	b-i	2.9	a-e	2.1	a-d	-0.68	0.99	4.5	d-l	3.6	a-h	2.6	b-g	-0.95	1.00
11	JA 37	3.2	c-k	2.5	b-i	1.4	e-h	-0.93	0.98	4.1	f-o	3.1	d-l	1.8	h-n	-1.11	0.99
12	JA 38	3.3	c-j	2.6	a-g	2.1	a-d	-0.64	1.00	4.5	d-l	3.6	a-i	2.8	b-e	-0.84	1.00
13	JA 97	3.6	b-i	3.1	a-d	1.8	b-f	-0.89	0.93	4.7	d-l	3.9	a-g	2.3	c-k	-1.18	0.96
14	JA 132	3.3	c-j	2.7	a-g	1.8	b-g	-0.79	0.99	4.8	c-i	3.5	a-i	2.4	c-i	-1.23	1.00
15	JA 5	3.2	c-k	2.5	b-i	1.5	d-h	-0.85	0.99	4.3	e-m	3.3	b-j	2.1	e-n	-1.13	0.99
16	JA 122	3.7	a-i	3.0	a-e	1.7	c-g	-1.00	0.96	4.9	c-i	3.9	a-g	2.1	e-n	-1.39	0.97
17	HEL 324	4.1	a-d	2.4	c-j	1.6	d-h	-1.24	0.96	5.6	a-de	3.6	a-i	2.5	c-i	-1.59	0.97
18	JA 61	2.7	f-l	2.3	d-j	1.5	d-h	-0.64	0.96	3.5	h-o	2.9	f-m	1.8	h-n	-0.85	0.97
19	CN 52867	4.1	a-c	2.7	a-g	1.7	c-g	-1.20	0.99	5.3	b-f	3.6	a-i	2.4	c-j	-1.49	0.99
20	KKUAc001	3.2	c-k	2.6	a-h	1.5	d-h	-0.84	0.98	5.1	c-g	4.0	a-f	2.4	c-i	-1.31	0.99
21	HEL 53	3.3	c-j	2.7	a-g	2.0	a-e	-0.69	0.99	5.6	a-e	4.2	a-c	3.0	a-c	-1.26	1.00
22	HEL 61	2.9	d-l	2.5	b-i	1.9	a-e	-0.49	0.99	4.6	d-l	3.7	a-g	2.9	a-e	-0.89	1.00
23	HEL 231	3.9	a-f	2.9	a-f	2.3	a-c	-0.83	0.98	6.1	a-c	4.2	a-c	3.3	ab	-1.43	0.96
24	HEL 335	4.5	ab	3.0	a-e	1.7	c-g	-1.39	1.00	6.6	ab	4.2	a-d	2.5	b-h	-2.01	0.99
25	JA 46	2.5	i-l	2.0	g-k	1.2	f-h	-0.65	1.00	3.3	k-o	2.4	j-m	1.6	k-n	-0.88	1.00
26	JA 60	2.5	i-l	1.8	h-k	1.5	d-h	-0.51	0.96	3.3	l-o	2.3	k-m	1.9	g-n	-0.71	0.95
27	JA 109	2.1	kl	1.6	jk	1.0	h	-0.59	1.00	3.0	m-o	2.4	j-m	1.4	mn	-0.81	0.99
28	JA 76	3.9	a-e	3.3	ab	2.0	a-e	-0.95	0.97	5.6	a-de	4.2	a-d	2.7	b-f	-1.46	1.00
29	JA 77	2.7	g-l	2.3	d-j	1.9	b-e	-0.39	0.99	3.4	j-o	2.9	g-m	2.3	c-k	-0.54	1.00
30	HEL 62	2.5	i-l	1.8	h-k	1.2	f-h	-0.65	1.00	3.5	i-o	2.6	i-m	1.7	i-n	-0.89	1.00
31	HEL 246	3.8	a-h	3.0	a-e	1.5	d-h	-1.14	0.97	5.0	c-g	3.9	a-g	2.2	d-l	-1.38	0.99
32	HEL 257	3.8	a-g	2.9	a-e	2.4	ab	-0.73	0.97	4.8	c-j	3.6	a-i	3.0	a-d	-0.91	0.97
33	JA 15	3.2	c-k	2.9	a-f	2.5	a	-0.35	1.00	4.6	d-l	4.1	a-e	3.6	a	-0.51	1.00
34	JA 67	2.6	h-l	2.1	f-k	1.6	d-h	-0.53	1.00	4.1	f-o	3.2	c-l	2.3	c-k	-0.90	1.00
35	JA 89	4.2	a-c	2.4	c-i	1.9	b-e	-1.13	0.91	5.9	a-d	3.6	a-i	2.8	b-e	-1.53	0.92
36	JA 125	2.8	e-l	2.1	f-k	1.6	d-h	-0.61	0.98	3.5	i-o	2.6	h-m	2.0	f-n	-0.78	0.99
37	HEL 65	4.0	a-d	3.4	a	2.0	a-e	-0.99	0.96	5.6	a-e	4.5	a	2.8	a-e	-1.40	0.99
38	HEL 253	3.0	c-l	2.0	g-k	1.7	c-g	-0.69	0.90	4.7	d-k	3.3	b-k	2.7	b-f	-0.99	0.95
39	HEL 256	4.8	a	2.5	b-i	1.6	d-h	-1.61	0.93	6.8	a	3.7	a-g	2.5	b-i	-2.16	0.94
40	JA102 × JA 89 (8)	3.6	b-i	2.8	a-f	1.8	b-f	-0.91	0.99	5.5	b-f	4.3	ab	2.7	b-f	-1.38	0.99
	Means	3.3		2.5		1.7		-0.79	0.98	4.6		3.4		2.3		-0.78	0.98

Means in the same column followed by the same letter (s) are not different at$P \leq 0.01$ probability levels by Duncan's multiple range test (DMRT).
W1 = 100% ET, W2 = 75%ET and W3 = 45%

The genotypes with high potential for tuber yield and biomass across water regimes of both years could be identified. CN 52867, HEL 53, HEL 231, HEL 335, JA 76, HEL 65 and JA 102 x JA 89 (8) had consistently high tuber dry weight (1.3 to 4.5 tons/ha) and HEL 53, HEL 61, HEL 231, HEL 335, JA 76, JA 15, JA 89, HEL 65, HEL 256 and JA 102 x JA 89 (8) had consistently high biomass (2.0 to 6.8 tons/ha) across water regimes of both years.

Conclusion

Drought reduced tuber dry weight and biomass, and the reductions in these traits were greater under severe drought than under moderate drought conditions. Genotypic variations in tuber dry weight and biomass were observed. Over both seasons, CN 52867, HEL 53, HEL 231, HEL 335, JA 76, HEL 65 and JA 102 x JA 89 (8) had consistently high tuber dry weight (1.3 to 4.5 tons ha^{-1}) and HEL 53, HEL 61, HEL 231, HEL 335, JA 76, JA 15, JA 89, HEL 65, HEL 256 and JA 102 × JA 89 (8) had consistently high biomass (2.0 to 6.8 tons ha^{-1}). These genotypes could be used to develop high yielding cultivars with improved drought tolerance.

References

Azhar, A. and M.K. Hamdy. 2003. Alcohol fermentation of sweet potato. I. Acid hydrolysis and factors involved. Biotechnol. Bioeng. 23:79-886.

Denoroy.P. 1996. The crop physiology of *Helianthus tuberosus* L.: A model orienter view. Biomass Bioenerg. 11: 11-32.

Hank, R.J., J. Keller, V.P. Rasmussen and G.G. Wilson. 1976. Line source sprinkler for continuous variable-crop production studies. Soil Sci. Soc. Am. J. 40: 426-429.

Lin, Y. and S. Tanaka. 2006. Ethanol fermentation from biomass resources: current state and prospects. Appl. Microbiol. Biotechnol. 69: 627-642.

Lium Z.X., J.H.J. Spiertz, S. Jing, X. Shuai and HX. Guang. 2012. Growth and yield performance of Jerusalem artichoke clones in a semiarid region of China. Agron. J. 104: 1538-1546.

Margaritis, A. and B. Pratima.1983. Effect of sugar concentration in Jerusalem artichoke extract on *Kluyveromyces marxianus* growth and ethanol production. Appl. Environ. Microbiol. 45: 723-725.

Monti, A., M.T. Amaducci and G. Venturi.2005. Growth response, leaf gas exchange and fructans accumulation of Jerusalem artichoke (*Helianthus tuberosus* L.) as affected by different water regimes. Eur. J. Agron. 23: 136-145.

Schittenhelm, S. 1999. Agronomic performance of root chicory, Jerusalem artichoke and sugar beet in stress and non-stress environment. Crop Sci. 39: 1815-1823.

Stephen, A.M., G.O. Phillips and P.A. Willians. 2006. Food Polysaccharides and Their Applications. CRC Press, Boca Raton.

Szambelan, K., J. Nowak and H. Jelen.2005. The composition of Jerusalem artichoke (*Helianthus tuberosus* L.) spirits obtained from fermentation with bacteria and yeasts. Eng. Life Sci. 5: 68-71.

Walker, G.M. 2010. Bioethanol: Science and Technology of Fuel Alcohol. Ventus Publishing. ISBN 978-87-7681-681-0 pp: 59-73.

Publication

Rattanaprasert, R., P. Banterng, S. Jogloy, N. Vorasoot, T. Kesmala, R.S. Kanwar C.C. Holbrook and A. Patanothai. 2014. Genotypic variability for tuber yield, biomass and drought tolerance in Jerusalem artichoke germplasm. Tur. J. Agr. Fores. 38:1-11. (Impact factor = 0.914)

2.4 Photoperiod and growing degree days effect on dry matter partitioning in Jerusalem artichoke

R. Ruttanaprasert, S. Jogloy, N. Vorasoot, T. Kesmala, R.S. Kanwar,
C.C. Holbrook and A. Patanothai

In the tropics, Jerusalem artichoke (*Helianthus tuberosus* L.) is not as productive as in temperate regions. Plants are much smaller and mature earlier, yielding smaller and fewer tubers than in temperate regions (Kay and Nottingham, 2008). In regions near the Equator, the crop grows fast and matures early, and production of two or more crops per year is possible (Kay and Nottingham, 2008). The environmental conditions that affect off-season production of Jerusalem artichoke in the tropics, however, are not well understood. Observations in yield trials in Thailand indicated that low temperature associated with short photoperiod during the dry season greatly reduced growth and tuber yield (Pimsaen *et al.*, 2010) and total soluble solids (Puangbut *et al.*, 2011) of the crop.

In temperate regions, the sum of temperature (equivalent to growing degree days) during the growing season is a main factor affecting growth and yield of Jerusalem artichoke (Kocsis *et al.*, 2007; Kocsis *et al.*, 2008). Day length can affect the above and below ground growth of the crop (Soja and Dersch, 1993; Kocsis *et al.*, 2007). The effects of growing environments during the dry season on Jerusalem artichoke growth and tuber yield in the tropics have not been investigated. The responses of different Jerusalem artichoke genotypes to planting date also have not been sufficiently evaluated. This information is very important for appropriate planting date management to improve productivity of this crop under tropical environments.

Objective

The objective of this study was to determine the effects of growing degree days (GDD) and photoperiod on dry matter production and partitioning of different Jerusalem artichoke genotypes.

Materials and Methods

Three Jerusalem artichoke genotypes (CN 52867, JA 89 and HEL 65) with differences in maturity and yield performance were planted in containers (31 cm in diameter and 28 cm in height) under an open environment with natural sunlight at Khon Kaen University agronomy farm (latitude 16 ◦26´N, longtitude102◦ 48´E, 200 masl). There was one plant in each container, and each experimental unit consisted of two containers. The three Jerusalem artichoke genotypes were arranged in a completely randomized design with four replications for 13 planting dates. The experiment was conducted for two years in 2008/09 and 2009/10. The planting dates in both years were exactly the same with 15-day intervals during the growing period of September 20 to March 20.

Leaf area (cm^2), shoot dry weight, roots dry weight, tubers dry weight, total biomass, tuber size (weight of individual tuber) and harvest index (HI) were recorded. Growing degree day (GDD) for each genotype in each planting date was calculated as the summation of daily mean temperatures above the base temperature (0 ^oC) from the day of transplanting to harvest time.

Photoperiod was calculated as: $\Sigma P_i/DAT$

where P_i ($_i = 1 – n$) is the photoperiod for each genotype in the i^{th} planting date, DAT is the day after transplanting, and n is the number of photoperiods from transplanting to harvest.

Results

Significant differences in the GDD, photoperiod, biomass, shoot dry weight, leaf area, HI, tuber dry weight, number of tubers per plant and weight of individual tubers (tuber size) were observed between planting dates (PD) and between Jerusalem artichoke genotypes (G) (Table 1). The interactions between year and planting date (Y x PD), between year and genotype (Y x G) and between planting date and genotype (PD x G) were also significant for all traits. The secondary levels of interactions (Y x PD x G) were also significant.

Table 1. Mean square from the combine analysis of variance for growing degree days (GDD), biomass, shoot dry weight, leaf area, harvest index, number of tuber per plant and weight of individual tuber of three Jerusalem artichoke genotypes in two years (2008-09 to 2009-10) and thirteen planting dates spaced at 15 day-intervals from September, 20 to March, 20.

SOV	df	Growing Degree day (^oC)[a]		Photoperiod (h)		Biomass (g/plant)		Shoot dry weight (g/plant)		Leaf area (cm^2)	
Year (Y)	1	330070	**	1.88	**	49610	**	19591	**	166306	ns
Planting date (PD)	12	5015395	**	81.90	**	14211	**	9790	**	2915296	**
Y*PD	12	715161	**	1.65	**	13740	**	2677	**	2211533	**
Genotype (G)	2	1214568	**	0.04	**	22159	**	14110	**	4784774	**
Y*G	2	18742	**	0.06	**	788	**	272	**	384355	**
PD*G	24	78011	**	0.59	**	2545	**	1319	**	543327	**
Y*PD*G	24	30061	**	0.53	**	988	**	331	**	377490	**
Pooled error	228	1E-26		4E-29		125		23		29766	
Total	311										

SOV	df	Harvest index		Tuber dry weight (g/plant)		Number of tuber per plant)		Individual of tuber (g)	
Year (Y)	1	0.051	**	2940	**	2326	**	98	**
Planting date (PD)	12	0.541	**	3544	**	1426	**	93	**
Y*PD	12	0.019	**	2734	**	347	**	21	**
Genotype (G)	2	0.658	**	3825	**	2116	**	97	**
Y*G	2	0.028	**	1474	**	61	**	36	**
PD*G	24	0.033	**	1107	**	100	**	17	**
Y*PD*G	24	0.016	**	651	**	173	**	8	**
Pooled error	228	0.002		58		18		2	
Total	311								

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

[a]Growing degree days (GDD) for each genotype for each planting date was calculated as the summation of daily mean temperatures above base temperature 0 ^oC from day of transplanting to harvest time.

GDD was positively associated with shoot dry weight (r = 0.40^{ns} to 0.71**), leaf area (r = 0.50^{ns} to 0.82**) and number of tubers (r = 0.52^{ns} to 0.86**), but it was negatively associated with harvest index (r = -0.56* to -0.81**) and tuber size (r = -0.53** to -0.89**) (Table 2). However, the relationships were slightly different between years, showing genotype x environment interactions for these traits. When the relationships across genotypes were considered, they were still similar to those for the individual genotypes.

The relationships between photoperiod and plant parameters were rather similar to those for GDD (r = 0.14^{ns} to 0.90**) (Table 3). The results indicated that GDD and photoperiod were closely related especially for the range of planting dates in this study.

Fig. 1a, b showed the patterns of shoot dry weight of the three Jerusalem artichoke genotypes at 13 planting dates for two years. Shoot dry weights were high at the start and at the end of the experiment but low at the middle.

Plant grown in long photoperiod with a higher number of GDD produced shoot dry weight rather than the number of harvestable tubers, whereas short photoperiod induced high partitioning of assimilates to harvestable tubers. Jerusalem artichoke plants grown during the short photoperiod were smaller and produced larger tubers than those grown during the long photoperiod.

Table 2. Simplec orrelation coefficients between growing degree days (GDD) and agronomic traits of three Jerusalem artichoke genotypes in two years at thirteen planting dates.

Genotypes	Shoot dry weight (g/plant)		Leaf area (cm^2)		Harvest index		Number of tuber per plant		Weight of individual tuber (g)	
	2008/09	2009/10	2008/09	2009/10	2008/09	2009/10	2008/09	2009/10	2008/09	2009/10
CN 52867	0.40^{ns}	0.61*	0.62*	0.82**	−0.60*	−0.57*	0.56*	0.57*	−0.77**	−0.53^{ns}
JA 89	0.47^{ns}	0.58*	0.57*	0.79**	−0.81**	−0.56*	0.52^{ns}	0.63**	−0.89**	−0.60*
HEL 65	0.59*	0.71**	0.50^{ns}	0.60*	−0.78**	−0.77**	0.86**	0.72**	−0.74**	−0.70**

^{ns}, *, ** Non significant and significant at P≤ 0.05 and 0.01 probability levels, respectively.
Harvest index (HI) was calculated as tuber dry weight divided by the total biomass of the plants.

Table 3. Simple correlation coefficients between photoperiod and agronomic traits of three Jerusalem artichoke genotypes in two years at thirteen planting dates.

Genotypes	Shoot dry weight (g/plant)		Leaf area (cm^2)		Harvest index		Number of tuber per plant		Weight of individual tuber (g)	
	2008/09	2009/10	2008/09	2009/10	2008/09	2009/10	2008/09	2009/10	2008/09	2009/10
CN 52867	0.48^{ns}	0.82**	0.57*	0.84**	−0.70**	−0.77**	0.61*	0.75*	−0.78**	−0.59*
JA 89	0.55*	0.83**	0.14^{ns}	0.83**	−0.90**	−0.90**	0.61*	0.67**	−0.89**	−0.72**
HEL 65	0.66**	0.77**	0.27^{ns}	0.65**	−0.85**	−0.85**	0.75**	0.77**	−0.65**	−0.81**

^{ns}, *, ** Non significant and significant at P≤ 0.05 and 0.01 probability levels, respectively.
Harvest index (HI) was calculated as tuber dry weight divided by the total biomass of the plants.

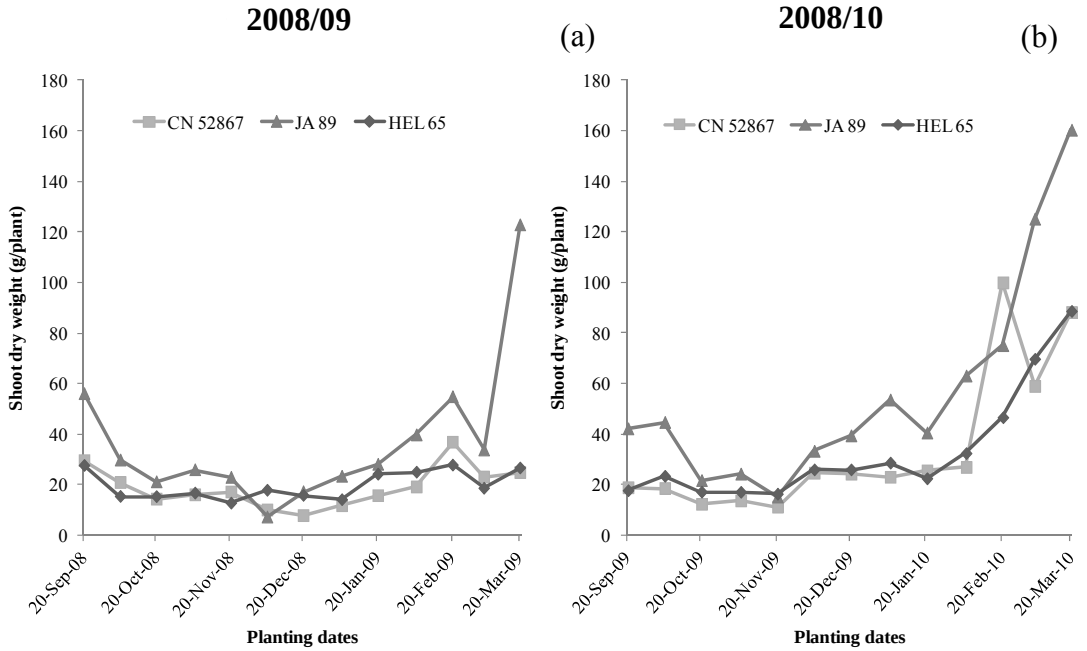


Fig. 1. Patterns of shoot dry weight of three Jerusalem artichoke genotypes across planting dates (representing different growing degree days).

Conclusion

Jerusalem artichoke genotypes responded differently to varying GDD and photoperiod, as induced by different planting dates and years, for HI, shoot dry weight, leaf area, number of tubers and tuber size. Therefore, the most productive planting dates were difficult to determine. However, CN-52867 and JA-89 were the most productive genotypes at the 20 September planting date, and performed well at other planting dates. High GDD was positively associated with high shoot dry weight, high leaf area and a high number of tubers, but it had negative correlations with HI and tuber size. However, the experiment was limited to growing Jerusalem artichoke in containers and further studies in the fields are necessary.

References

Kays, S. J. and S.F. Nottingham. 2008. Biology and Chemistry of Jerusalem Artichoke (*Helianthus tuberosus* L.). CRC press, Florida.

Kocsis, L., H-P Kaul, W. Praznik and P. Liebhard. 2007. Influence of harvest date on shoot and tuber yield of different Jerusalem artichoke (*Helianthus tuberosus* L.) cultivars in the semiarid production area of Austria. Ger. J. Agron. 11: 67-76.

Kocsis, L., H-P Kaul, W. Praznik and P. Liebhard. 2008. Influence of harvest date on tuber growth, tuber dry matter content, inulin and sugar yield of different Jerusalem artichoke (*Helianthus tuberosus* L.) cultivars in the semiarid production area of Austria. Ger. J. Agron. 12: 8-21.

Pimsaen, W., S. Jogloy, B. Suriharn, T. Kesmala, V. Pensuk and A. Patanothai. 2010. Genotype by environment (G x E) interaction for yield components of Jerusalem artichock (*Helianthus tuberosus* L.). Asian J. Plant Sci. 9: 11-19.

Puangbut, D., S. Jogloy, S. Srijaranai, N. Vorasoot, T. Kesmala and A. Patanothai. 2011. Rapid assessment of inulin content in *Helianthus tuberosus* L. tubers. SABRAO J. Breed. Genet. 43: 188-200.

Soja, G. and G. Dersch. 1993. Plant development and hormonal status in the Jerusalem artichoke *(Helianthus tuberosus* L.). Ind. Crops Prod. 1: 219-228.

Publication

Rattanaprasert, R., S. Jogloy, N. Vorasoot, R.S. Kanwar, T. Kesmala, C.C. Holbrook and A. Patanothai. 2013. Photoperiod and growing degree days effect on dry matter partitioning in Jerusalem artichoke. Int. J. Plant Prod. 7(3):393-416. (Impact factor 2012 = 1.028).

2.5 Spectrophotometric method as an alternative method for the determination of inulin in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers

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

Inulin is a linear polymer of D-fructose joined by β(2→1) linkages and terminated with a D-glucose, which is linked to fructose by an α(1→2) bond (Modler, 1994; Bornet, 2001). Inulin has a degree of polymerization (DP) in the range of 2–60 (Prosky and Hoebregs, 1999; Saengthongpinit and Sajjaanantakul, 2005). It is a natural carbohydrate source found mainly in roots and tubers of many plants, such as chicory artichoke (*Cynara scolymnus* L.), viper's grass (*Scorzonera hispanica* L.), and Jerusalem artichoke (*Helianthus tuberosus* L.) (Lopez-Molina *et al.*, 2005; Saengthongpinit and Sajjaanantakul, 2005; Kay and Nottingham, 2007; Wei, 2007). Nowadays, inulin has gained enormous attention because of its various applications in many fields (Fuchs, 1987; Modler, 1994; Van Loo, 1995; Prosky and Hoebregs, 1999; Bornet, 2001; Lopez-Molina *et al.*, 2005; Saengthongpinit and Sajjaanantakul, 2005; Kay and Nottingham, 2007; Wei, 2007) such as nutrition, medicine and alternative energy. Different degrees of inulin quality, which is determined by its chain length or the degree of polymerization are used in various applications of inulin. Therefore, the development of analytical methods for extraction, quantification, and determination of DP is of great importance for the characterization of inulin and the derivatives in plant samples.

Determination of inulin can be performed by either the direct or indirect approach. High-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) has been accepted as the most powerful method for direct determination of inulin (Hogarth, 2000; Marsilio *et al.*, 2000; Stober *et al.*, 2004; Bruggink, 2005; Cuany *et al.*, 2010). It provides not only the content of inulin but also the DP profiles. However, this method has some limitations on the difficulty in interpreting the elution order of sugar oligomers since there are limited of standards available. In addition, the instrument and analytical anion exchange columns are expensive. Indirect determination methods are based on hydrolysis of inulin followed by measurement of the released fructose and glucose by different techniques including HPAEC-PAD (Timmermans *et al.*,

1994; Hogarth, 2000; Ku *et al.*, 2003; Stober *et al.*, 2004; Saengthongpinit and Sajjaanantakul, 2005) and spectrophotometry. Several reagents have been used as derivatizing reagents before the detection by spectroptotometer such as dinitrosalicylic acid (DNS) (Rocha *et al.*, 2006) and p-hydroxybenzoic acid hydrazide (PAHBAH) (Blakeney and Mutton, 1980; Toran-Diaz *et al.*, 1985)

In addition, many works using analytical methods based on enzymatic hydrolysis and detection have been reported (Toran-Diaz *et al.*, 1985; Stober *et al.*, 2004; Rocha *et al.*, 2006). However, the procedures involve several types of expensive enzymes and/or time-consuming reactions. Therefore, development of a simple analytical method using common chemicals available in laboratories for the determination of inulin is a challenging task. A simple reaction using iodine has been used for the determination of sugars (Cajori, 1922), and a later report (Carneiro *et al.*, 2005) describes the use of spectrophotometry based on the periodate reagent to measure the amount of glucose and fructose in syrup samples.

Objective

The aim of this study was to develop a simple method for the determination of inulin in Jerusalem artichoke tuber.

Materials and Methods

Sample preparation

Eleven varieties of Jerusalem artichoke (*H. tuberosus* L.) were selected, including CN52867, KKU AC001, HEL 61, HEL 65, HEL 66, HEL 69, HEL 231, HEL 335, JA 37, JA 38 and JA 102. They were planted in July 2008 at the Field Crop Research Station of Khon Kaen University, and harvested in October 2008. The representative samples used for the optimization and method validation experiments are Beneo HP and Jerusalem artichoke (CN 52867). Tubers of five sampled plants for each variety were randomly collected, washed with tap water and longitudinally sliced at the middle to get approximately 2-mm thick pieces. The samples were dried at 60 ^{o}C for 10 h in an oven, milled and sieved through an 850-μm sieve. The powdered samples were kept for short periods of time at an ambient atmosphere in desiccators, until extraction.

Extraction of inulin

The Jerusalem artichoke powder samples (2.0 g) were extracted by accelerated solvent extractor (ASE) and using water as the extraction solvent for 20 min at 80 ^{o}C and 1,500 Psi.

Determination of inulin content

1. Determination of free fructose (F_f)

The extract (150 μL) was mixed with 10 mmol L^{-1} sodium periodate reagent (100 μL), 20 mmol L^{-1} citrate buffer pH 6.0 (5.00 mL) and water (4.60 mL). After 5 min, 100 mmol L^{-1} potassium iodide (150 μL) was added, and the mixture was left for an additional 5 min. The absorbance was subsequently measured at 350 nm using a UV–Vis spectrophotometer. The concentration of free fructose was deduced from calibration curve of standard fructose.

2. Determination of total fructose (F_{tot})

The extract (1.00 mL) was acidified with 0.2 mol L^{-1} HCl in a final volume of 50 mL, and subjected to acid hydrolysis at 97 ± 2 ^{o}C for 45 min. The solution was then adjusted to pH 7.0 with NaOH before diluting with water to 50.00 mL. The neutral hydrolysate (150 μL) was analyzed spectrophotometrically by the same procedure as described above for free fructose (F_f) analysis.

Results

In this study, ASE was used for the extraction of inulin from Jerusalem artichoke samples. The highest extraction yield (of 93% on average) was obtained by heating at 80 ^{o}C for 20 min. The results demonstrated that ASE has advantages of automated operation, fast extraction which the extraction time could be reduced by at least 3 times in comparison to the conventional method.

Acid hydrolysis was chosen in this study because it is a powerful and simple method for hydrolyzing inulin to release glucose and fructose components. In addition, it is much less expensive than the enzymatic hydrolysis (Toran-Diaz *et al.*, 1985). The optimum conditions for hydrolysis of inulin were 0.2 mol L^{-1} HCl and 45-min incubation at 97 ^{o}C.

The released fructose from the hydrolysis of inulin was spectrophotometrically determined by periodate reaction (Cajori, 1922). The method is based on the oxidation of fructose by periodate (IO_4^-) and evaluation of the remaining periodate by monitoring the triiodide (I_3-) complex formed (at 350 nm) after the addition of potassium iodide (I^-).

The optimum conditions for the determination of fructose were sodium periodate 0.10 mmol L^{-1} and potassium iodide 1.50 mmol L^{-1}, at pH 6.0,

Quantification of inulin in Jerusalem artichoke tubers

To simplify the quantification of inulin, the content of inulin can be calculated based only on the fructose content (Simonovska, 2000; Steegmans *et al.*, 2004). The following equation was used (Steegmans *et al*., 2004):

$$I = k(F_{tot} - F_f)$$

where I is the inulin content, F_{tot} is total fructose content, F_f is free fructose, and k is a correction factor for the glucose part of the inulin and for the water loss during hydrolysis. In this study, k = 0.995 is adopted, this value is recommended for the unknown DP inulin ((Steegmans *et al.*, 2004).

The DP was also evaluated using the following equation (Simonovska, 2000):

$$DP = \text{number of F units per G unit} + 1G \text{ unit}$$

where F and G are fructose and glucose, respectively.

Table 1 summarizes the inulin contents along with the DP from artichoke tuber samples. The results showed the contents in the range 62.96–74.90% dry weight, along with free fructose concentration in the range of 1.18–1.60% dry weight. In addition, to verify the reliability of the proposed method for the analysis of inulin in the artichoke samples, the results obtained using spectrophotometry were compared to that using HPAEC-PAD (Table 1), where both glucose and fructose were quantified.

Table 1. Content and average DP of inulin from Jerusalem artichoke samples obtained from spectrophotometry and HPAEC-PAD.

Jerusalem artichoke	Content of sugar (dry wt. %)							
	spectrophotometer			Anion exchange chromatography				
	F_f	F	I	F_i	F	G	I	Ave. DP
HEL 61	1.25±0.05	66.17±0.54	64.59	1.12±0.05	66.10±0.65	4.93±0.21	64.65	14.4
HEL 65	1.50±0.06	75.95±0.54	74.07	1.44±0.06	75.94±0.79	4.97±0.19	74.12	16.3
HEL 66	1.30±0.03	67.04±0.66	65.41	1.15±0.03	66.66±0.31	4.62±0.24	65.18	15.4
HEL 69	1.50±0.04	74.80±0.73	72.93	1.61±0.04	73.95±0.53	4.89±0.70	71.97	16.1
HEL 231	1.48±0.04	76.76±0.67	74.90	1.33±0.05	76.52±0.80	4.10±0.28	74.81	19.7
HEL 335	1.60±0.05	65.66±0.20	63.73	1.52±0.05	64.29±0.37	3.88±0.49	62.45	17.6
JA 37	1.40±0.06	74.67±0.67	72.90	1.29±0.07	74.03±0.61	5.03±0.41	72.37	15.7
JA 38	1.22±0.04	73.21±0.20	71.63	1.13±0.05	74.70±0.25	4.28±0.18	73.20	18.5
JA 102	1.18±0.03	71.44±0.27	69.90	1.10±0.05	72.63±0.11	4.58±0.12	71.17	16.9
KKUAC 001	1.23±0.02	64.51±0.48	62.96	1.17±0.02	64.09±0.69	3.58±0.45	62.60	18.9

F is total content fructose, *G* is glucose, *I* is inulin, DP is degree of polymerization of inulin

Conclusion

The indirect method for quantitative determination of inulin in Jerusalem artichoke tubers was developed. The extracted inulin from Jerusalem artichoke tubers was hydrolyzed with acid. The amount of inulin was evaluated from fructose in the hydrolysate by spectrophotometric detection via periodate reaction. The inulin contents detected in 10 varieties of Jerusalem artichoke grown in Thailand were in the range 63-75 % dry weight and the DP ranged from 14-19. The proposed method is rapid, simple, and reliable for the determination of inulin in Jerusalem artichoke samples. Moreover, it is less cost than the enzymatic hydrolysis method. The method is suitable for routine analysis of Jerusalem artichoke, especially for plant breeding purposes.

References

Bornet, F.R.J. 2001. Fructo-oligosaccharides and other fructans: chemistry structure and nutritional effects. Advanced Dietary Fiber Technology. Blackwell, Oxford.

Blakeney, A.B. and L.L. Mutton. 1980. A simple calorimetric method for the determination of sugars in fruit and vegetables. J. Sci. Food Agric. 31: 889–897.

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

Cajori, F.A. 1922. The use of iodine in the determination of glucose, fructose and maltose. J. Biol. Chem. 54: 617-627.

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. Chem. Acta. 531: 279-284.

Cuany, D., T. Be´net and S. Austin. 2010. Development and single laboratory validation of a method for the determination of total fructans in infant formula. J. AOAC Int. 93: 202–212.

Fuchs, A. 1987. Potentials for non-food utilization of fructose and inulin. Stärke 39: 335–343.

Hogarth, A.J.C.L., D.E. Hunter, W.A. Jacobs, K.A. Garleb and B.W. Wolf. 2000. Ion chromatographic determination of three fructooligosaccharide oligomers in prepared and preserved foods. J. Agric. Food Chem. 48: 5326–5330.

Kay, S.J. and S.F. Nottingham. 2007. Biology and Chemistry of Jerusalem Artichoke: *Helianthus tuberosus* L. CRC Press LLC, New York.

Ku, Y., O. Jansen, C.J. Oles, E.Z. Lazar and J.I. Rader. 2003. Precipitation of inulins and oligoglucoses by ethanol and other solvents. Food Chem. 81: 125–132.

Lopez-Molina, D., M.D. Navarro-Martinez, F.R.Melgarejo, A.N.P. Hiner, S. Chazarra and J.N. Rodriguez-Lopez. 2005. Molecular properties and prebiotic effect of inulin obtained from artichoke (*Cynara scolymus* L.). Phytochemistry 66: 1476-1484.

Marsilio, R., M. Naturale, P. Manghi, G. Montini, L. Murer, M. Ros, G. Bisogno, B. Andretta, N. Dussini, G. Giordano, G. Zacchello and R. Dall'Amico. 2000. Rapid and simple determination of inulin in biological fluids by high-performance liquid chromatography with light-scattering detection. J. Chromatogr. B. 744: 241-247.

Modler, H.W. 1994. Bifidogenic factors-sources, metabolism and applications. Int. Dairy J. 4: 383–407.

Prosky, L. and H. Hoebregs. 1999. Methods to determine food inulin and oligofructose. J. Nutr. 129: 1418s–1423s.

Rocha, J.R., R. Catana, B.S. Ferreira, J.M.S. Cabral and P. Fernandes. 2006. Design and characterisation of an enzyme system for inulin hydrolysis. Food Chem. 95:77–82

Saengthongpinit, W. and T. Sajjaanantakul. 2005. Influence of harvest time and storage temperature on characteristics of inulin from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. Postharvest Biol. Tec. 37:93–100.

Simonovska, B. 2000.Determination of inulin in foods. J. AOAC int. 83: 675–678.

Steegmans, M., S. Iliaens and H. Hoebregs. 2004. Enzymatic, spectrophotometric determination of glucose, fructose and inulin/oligofructose in food. J. AOAC. Int. 87: 1200–1207.

Stober, P., S. Benet and C. Hischenhuber. 2004. Simplified enzymatic high-performance anion exchange chromatographic determination of total fructans in food and pet food-limitations and measurement uncertainty. J. Agric. Food Chem. 52: 2137–2146.

Timmermans, J.W., M.B. van Leeuwen, H. Tournois, D. de Wit and J.F.G. Vliegenthart. 1994. Quantitative analysis of the molecular weight distribution of inulin by means of anion exchange HPLC with pulsed amperometric detection. J. Carbohydr. Chem. 13: 881-888.

Toran-Diaz, I., V.K. Jain, J.J. Allais and J. Baratti. 1985. Effect of acid or enzymatic hydrolysis on ethanol production by *Zymomonas mobilis* growing on Jerusalem artichoke juice. Biotechnol.Lett. 7: 527-530.

Van Loo J, Coussement P, L. De Leenheer, H. Hoebregs and G. Smits. 1995. On the presence of inulin and oligofructose as natural ingredients in the western diet. CRC Crit. Rev. Food Sci. Nutr. 35: 525–552.

Wei, L., J. Wang, X. Zheng, D. Ten, Y. Yang, C. Cai, T. Feng and F. Zhang. 2007. Studies on the extracting technical conditions of inulin from Jerusalem artichoke tubers. J. Food Eng. 79: 1087–1093.

Publication

Saengkanuk, A., S. Nuchadomrong, S. Jogloy, A. Patanothai and S. Srijaranai. 2012. A simplified spectrophotometric method for the determination of inulin in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. Eur. Food Res. Technol. 233: 609-616. (Impact factor = 1.436).

2.6 Molecular investigation on tuberization of Jerusalem artichoke

P.P. Wangsomnuk, S. Jogloy, P. Wangsomnuk, Y-B Fu, Su. Khampa, B. Ruttawat, T. Mornkham, W. Rittithum and A. Patanothai

Jerusalem artichoke (*Helianthus tuberosus* L.) has recently received renewed interest in genetic improvement for multiple purposes (Breton *et al.*, 2010). However, little attention has been paid to characterizing and conserving its genetic resources (Mandel *et al.*, 2011). Conserved Jerusalem artichoke genetic resources are relatively limited due to insufficient conservation efforts (Volk and Richards, 2006; Kays and Nottingham, 2008). Currently, only several hundred Jerusalem artichoke accessions are maintained in plant germplasm collections worldwide. These accessions represent germplasm only from a dozen or so countries and include wild and weedy accessions, landraces, or traditional and obsolete cultivars, and advanced or improved cultivars. Some efforts have been made to characterize existing Jerusalem artichoke germplasm. However, these characterizations were mainly focused on phenotypic and genotypic data and would be more informative with supplementary applications of informative molecular markers.

Many molecular markers have been developed for plant genetic research over the recent decades (Arif *et al.*, 2010). The random amplified polymorphism DNA (RAPD) (Williams *et al.*, 1990) and inter simple sequence repeats (ISSR) (Zietkiewic *et al.*, 1994) were among the earliest developed molecular tools used to assess plant genetic diversity due to technical simplicity and practical feasibility. The sequence-related amplified polymorphism (SRAP) (Li and Quiros, 2001) represents another simple and reliable PCR-based marker tool for genetic diversity analysis. However, these molecular markers have rarely been applied to assess genetic variation of Jerusalem artichoke. To facilitate the molecular analyses of Jerusalem artichoke germplasm and the development of effective molecular tools for Jerusalem artichoke breeding programs, we performed a comparative assessment of five commonly used methods of extracting genomic DNA from the leaf and seed of four Jerusalem artichoke genotypes. We assessed the efficiency of RAPD, ISSR and SRAP markers for characterization of genetic structure and genetic relatedness of 47 diverse Jerusalem artichoke accessions. RAPD markers were also used to characterize 147 diverse Jerusalem artichoke accessions that originated from nine countries.

So far, there are no genome sequences of Jerusalem artichoke available in public database. There are 633 nucleotide sequences and 40,362 ESTs with /without transcribed

information (http://compgenomics.ucdavis.edu/index.php). In this study *CONSTANS*, *GA20-oxidase* and *GA2-oxidase* homologues in Jerusalem artichoke were cloned and used for the investigation on their roles in the initiation of tuber formation *in vitro*.

Objectives

The objectives of this study were to (a) assess five commonly used methods of extracting genomic DNA from leaf and seed of four Jerusalem artichoke genotypes, (b) assess the genetic distinctiveness of the Jerusalem artichoke accessions from the world germplasm collection, (c) improve existing methods for extracting total RNA from Jerusalem artichoke tissue, and (d) clone and analyse *CONSTAN*S, *GA20-oxidase* and *GA2-oxidase* expression levels in Jerusalem artichoke at initiation stage in tuber development *in vitro*.

Materials and Methods

Plants used in this study were kindly donated from the Plant Genetic Resources of Canada at Saskatoon Research Center (PGRC) and Gatersleben Genebank Department at the Foundation Leibniz, Institute of Plant Genetics and Crop Plant Research (IPK) of German. Jerusalem artichoke cv. JA102, JA7, JA37, CN52867, HEL65 and HEL335 were used for DNA isolation while the cultivar JA37 and HEL53 were used for total RNA extraction. For expression studies, Jerusalem artichoke cv. JA102 was grown *in vitro* on MS (Murashige and Skoog, 1962) or modified MS media containing 8% sucrose and 1 mg/L 6-Benzylaminopurine (BA) which was used as tuber-inducing media. The single cutting nodes were grown on induction media under SD condition, 8-h light and 16-h dark, using OSRAM L36W/10 light bulb, at 25 $^{\circ}$C.

In order to investigate the candidate genes expression by RT-PCR technique, specific primers were designed. Five DNA extraction methods from young leaf and seed were compared to obtain the superior method which gave the best DNA quality and quantity for molecular cloning.

Total RNA were extracted with five independent replications following the five extraction methods. The quantity and quality of extracted total RNA were analyzed based on the 260 nm/280 nm absorbance ratios obtained from a spectrophotometer. An aliquot of 2 µl of total RNA were used in the spectrophotometer NanoDrop^{TM}(Thermo Scientific, USA) according to manufacturer's instructions.The variation in the efficiency of RNA extractions was analyzed using *Statistix 8* (*Analytical Software*, 2003). RNA integrity was evaluated from the 28s and 18s rRNA bands on 1.0% agarose gel electhrophoresis, staining with ethidium bromide and visualization with UV light *(Vilber Lourmat*, France).

A mixture of 1.25 µg total RNA, 2 µl of 5X VILO™ reaction mix and 1 µl of 10X Superscript® enzyme mix was made up to 10 µl with DNase-free water and incubated at 25 $^{\circ}$C for 10 min and then at 42 $^{\circ}$C for 60 min. The reaction was incubated at 85 $^{\circ}$C for 5 min to stop reaction. 1st cDNA was diluted 5 fold in DNase-free water and subsequently used for PCR amplification.

For optimization of the amplification efficiency of specific targeted, the appropriate cycles and primer concentration were tested. 20 µl of PCR were performed in 0.2 ml tube. 2 µl of diluted 1st-stranded cDNA from section 3.3.1 was mixed with 2 µl of 2.0 mM dNTPs (Fermantas), 2 µl of 2 mM $MgCl_2$ (Fermantas), 0.4 unit Taq DNA polymerase (Vivantis), 2 µl of 10X PCR buffer (750 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$ and 0.1% Tween 20: Fermantas), primer concentration was varied from indicated: 0.05 µM, 0.1 µM, 0.15 µM to 0.2 µM (for primer sequences please refer to table 3.2). Then the

PCR reaction was performed on Thermal cycler (Agilent,USA.). PCR cycling conditions were as follow: an initial denaturation at 95 °C for 3 min, then the gradient PCR from 48°C to 60°C was performed by denaturation at 94°C for 30 s, annealing step for 50 s and elongation at 72°C for 30 s and final extension at 72°C for 5 min. The optimum of PCR cycles was determined to avoiding plateau phase effect by varied cycles from 26 to 34. PCR products were stored at 4°C for short term use.

Jerusalem artichoke using for gene expression study were grown under the control condition. Total RNA was extracted from different tissues. The qRT-PCR was performed using the most appropriate condition on the Light Cycler 480® (Roche, France), using the SYBR® Green PCR MasterMix (Roche). The PCR was run in tripicated using the following program: one cycle of 10 min at 95°C; 35 cycles of 20 s at 95°C for DNA denaturing, 50 s at annealing temperature of 50 °C and 30 s at 72 °C for extension. The relative quantifications were performed by the $2^{-\Delta\Delta ct}$ method, the threshold cycle value (Ct) for candidate genes were normalized against the Ct values of *actin* gene. The target/*actin* gene ratios during time-course of experiments were normalized to the target/*actin* ratio at time zero. All calculations, including error estimation were performed using the LightCycler480® package (Roche).

Results

DNA extraction is an essential step for a molecular analysis of an organism, but it is difficult to acquire a sufficient amount of pure DNAs from a plant tissue with substantial levels of phenolic compounds, carbohydrates, proteins and second metabolites. We performed a comparative assessment of five commonly used methods of extracting genomic DNAs from the leaf and seed of four Jerusalem artichoke (*Helianthus tuberosus* L.) genotypes. The modified method of Tai and Tanksley (1990) was found to be superior for both young leaf and seed seed (Fig.1, Table 1). The quality of the extracted DNAs was also confirmed with the analysis of sequence related amplified polymorphism. The results presented here are useful for molecular analyses of Jerusalem artichoke and other related *Helianthus* species.

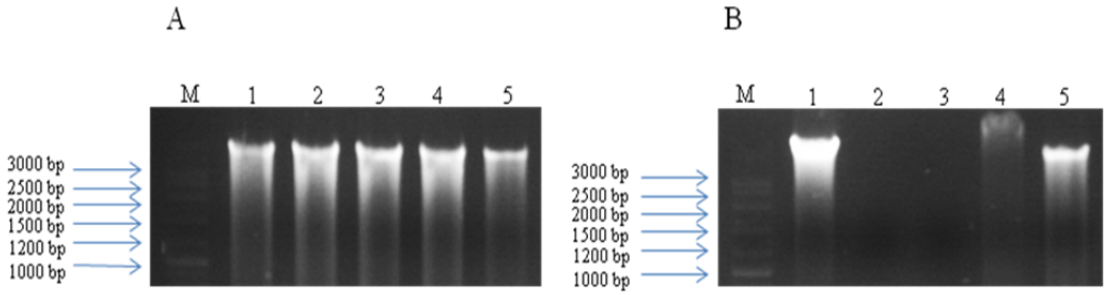


Fig.1. Electrophoresis on agarose gel for the DNA extracted from young leaves and seeds of Jerusalem artichoke using five extraction protocols. AandBare for Jerusalem artichoke DNA extracted from young leaves of JA102 and from seeds of CN52867 x HEL65, respectively. M is 100 bp DNA ladder plus. The numbers from left to right indicate the extracted protocols: 1. Modified method of Tai and Tanksley (1990); 2. CTAB DNA extraction method; 3. Modified CTAB with high NaCl concentration; 4. Modified method of Štorchová*et al.* (2000); and 5. E.N.Z.A. Plant DNA Kit.

Table 1. Comparision in quality and quantity of DNA extracted from young leaves of Jerusalem artichoke among five DNA extraction methods.

Extraction methods	JA102		HEL335 x JA37	
	DNA Conc. (µg/1g tissue)[(1)]	Absorption ratio (260nm/280nm) [(1)]	DNA Conc. (µg/1g tissue)[(1)]	Absorption ratio (260nm/280nm)[(1)]
1. Modified method of Tai and Tanksley (1990)	63.44^{ab}	1.98^{a}	58.28^{ab}	1.98^{a}
2. CTAB	61.27^{ab}	1.96^{ab}	62.19^{ab}	1.96^{a}
3. CTAB with NaCl	20.84^{c}	1.90^{b}	24.46^{c}	1.87^{b}
4. Modified method of Štrorchová (2000)	73.37^{a}	1.83^{c}	74.59^{a}	1.84^{b}
5 E.N.Z A. Plant DNA kit	53.70^{b}	1.79^{c}	53.21^{b}	1.77^{c}
LSD[(2)]	*	*	*	*

[(1)] Values with different letters within column are significantly different at $p \leq 0.05$ by LSD.
[(2)] * Significant at the $p \leq 0.05$ probability level.

The preliminary assessment of genetic structure and genetic relatedness of 47 diverse Jerusalem artichoke accessions using RAPD, ISSR and SRAP markers gave a total of 296 (87.1%) polymorphic bands from 13 RAPD markers; 92 (80%) from six ISSR primers; and 194 (88.6%) for nine combinations of SRAP primers. Five optimal clusters were inferred by the STRUCTURE program from the RAPD or ISSR data, while six optimal clusters were found from the SRAP data or combined marker data (Fig. 2). Significant linear relationships between the distance matrices for all pairs of individual accessions were detected for all marker pairs and the estimated correlation coefficient was 0.40 for RAPD-ISSR, 0.53 for RAPD-SRAP, and 0.43 for ISSR-SRAP. Based on the combined data, the neighbor-joining clustering of the 47 accessions matched closely with those inferred from the STRUCTURE program. Three ancestral groups were observed for the Canadian germplasm. Most diverse germplasm harbored in the USA collection.

We further characterized 147 Jerusalem artichoke accessions from nine countries using random amplified polymorphic DNA (RAPD) markers (Table 2, Fig. 3). Thirty RAPD primers were screened and 13 of them detected a total of 357 reproducible RAPD bands, of which 337 were polymorphic. More than 93% RAPD variation resided within accessions of a country. Weak genetic differentiation was observed between wild and cultivated accessions. Six optimal groups were detected in this germplasm set. Four ancestral groups were found for the Canadian germplasm. The most genetically distinct accessions were identified. These findings provided the first set of useful diversity information for understanding the Jerusalem artichoke gene pool, conserving Jerusalem artichoke germplasm, and utilizing distinctive germplasm for genetic improvement.

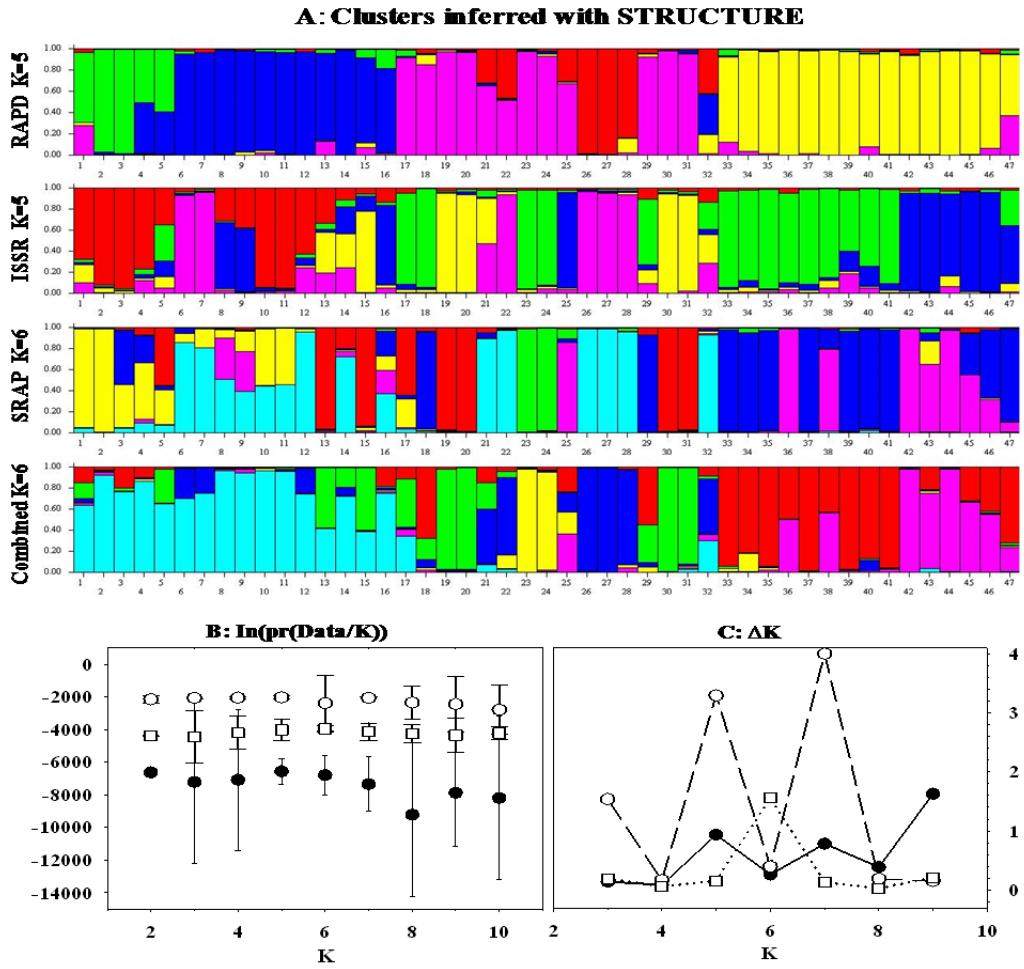


Fig. 2. The genetic structure of the 47 Jerusalem artichoke accessions inferred with STRUCTURE and the sensitivity assessment of inference with STRUCTURE with respect to marker type. A: the most likelihood genetic structures inferred with STRUCTURE for three marker data and the combined marker data. Each column represents an accession from 1 to 47. B and C: the log-likelihood profiles and the rates of change in log-likelihood for models with K=2 to 10 for RAPD, ISSR and SRAP markers labeled with filled circle, open circle, and open square, respectively. Note that the standard deviations of the log-likelihoods for RAPD markers when K=8 and 10 were reduced in half for ease of illustration.

Table 2. List of 147 Jerusalem artichoke accessions with country origin, average dissimilarity based on RAPD markers and the cluster inferred from the STRUCTURE program.

Acc	Orig/Sour	AD	StC	Acc	Orig/Sour	AD	StC	Acc	Orig/Sour	AD	StC
KKU001	UNK/U	0.360	2	JA55	USA/P	0.333	3	JA131	CAN/P	0.345	6
CN52867	RUS/P	0.363	2	JA58	RUS/P	0.344	3	JA132	CAN/P	0.343	6
JA1	CAN/P	0.338	1	JA59	RUS/P	0.313	3	JA133	CAN/P	0.344	6
JA2	CAN/P	0.339	1	JA60	RUS/P	0.339	3	JA134	CAN/P	0.331	6
JA3	CAN/P	0.347	1	JA61	RUS/P	0.319	3	JA135	CAN/P	0.344	6
JA4	CAN/P	0.329	1	JA66	USA/P	0.335	3	AMES2714*	USA/N	0.384	1
JA5	CAN/P	0.345	1	JA67	USA/P	0.352	2	AMES2722*	USA/N	0.377	1
JA6	CAN/P	0.335	1	JA69*	USA/P	0.310	3	AMES2723*	USA/N	0.355	1
JA7	CAN/P	0.338	1	JA70*	USA/P	0.313	3	AMES2729*	USA/N	0.369	2
JA8	CAN/P	0.335	1	JA71*	USA/P	0.316	3	AMES2730*	USA/N	0.363	1
JA9	CAN/P	0.328	1	JA72*	USA/P	0.354	3	AMES2736*	USA/N	0.370	1
JA10	CAN/P	0.339	1	JA73*	USA/P	0.326	3	AMES2746*	USA/N	0.356	1
JA11	CAN/P	0.349	1	JA74*	USA/P	0.337	3	AMES2747*	USA/N	0.356	1
JA12	CAN/P	0.341	4	JA75	CAN/P	0.347	3	AMES8380	USA/N	0.382	1
JA13	CAN/P	0.322	4	JA78	FRA/P	0.323	3	AMES22229	CAN/N	0.358	1
JA14	CAN/P	0.338	4	JA81	FRA/P	0.354	2	PI451980*	USA/N	0.382	1
JA15	CAN/P	0.364	4	JA86	FRA/P	0.356	6	PI503262*	USA/N	0.369	1
JA16	CAN/P	0.336	4	JA87	FRA/P	0.341	6	PI547230*	USA/N	0.359	1
JA17	CAN/P	0.335	4	JA88	RUS/P	0.343	6	PI547232*	USA/N	0.367	1
JA18	CAN/P	0.344	4	JA89	FRA/P	0.359	2	PI547233*	USA/N	0.384	1
JA19	CAN/P	0.363	4	JA91	RUS/P	0.329	6	PI547237*	USA/N	0.369	1
JA20	CAN/P	0.348	4	JA92	RUS/P	0.334	6	PI547241*	USA/N	0.402	1
JA21	CAN/P	0.353	4	JA93	RUS/P	0.317	6	HEL53	DEU/I	0.357	2
JA22	CAN/P	0.352	4	JA95	RUS/P	0.329	6	HEL61	RUS/I	0.340	2
JA23	CAN/P	0.336	4	JA97	FRA/P	0.332	6	HEL62	RUS/I	0.350	2
JA24	CAN/P	0.351	4	JA98	FRA/P	0.332	6	HEL65	RUS/I	0.383	2
JA25	CAN/P	0.343	4	JA100	FRA/P	0.322	6	HEL66	O-U/I	0.356	2
JA26	CAN/P	0.341	4	JA102	DEU/P	0.367	2	HEL68	UNK/I	0.364	2
JA27	CAN/P	0.340	4	JA105	RUS/P	0.309	6	HEL69	UNK/I	0.334	2
JA28	CAN/P	0.314	5	JA106	CAN/P	0.325	6	HEL231	DEU/I	0.325	2
JA29	CAN/P	0.325	5	JA107	CAN/P	0.313	6	HEL243	DEU/I	0.350	2
JA30	CAN/P	0.336	5	JA108	CAN/P	0.318	6	HEL246	UNK/I	0.357	2
JA31	CAN/P	0.334	5	JA109	CAN/P	0.313	6	HEL248	DEU/I	0.360	2
JA32	CAN/P	0.318	5	JA110	CAN/P	0.324	6	HEL250	FRA/I	0.339	2
JA33	CAN/P	0.318	5	JA111	CAN/P	0.323	6	HEL253	UNK/I	0.350	2
JA34	CAN/P	0.326	5	JA112	CAN/P	0.329	6	HEL256	UNK/I	0.326	2
JA35	CAN/P	0.331	5	JA113	CAN/P	0.330	6	HEL257	UNK/I	0.346	2
JA36	CAN/P	0.324	5	JA114	CAN/P	0.330	6	HEL265	O-H/I	0.350	2
JA37	CAN/P	0.352	2	JA116	CAN/P	0.332	6	HEL267	O-Y/I	0.325	2
JA38	CAN/P	0.352	2	JA117	CAN/P	0.334	6	HEL272	FRA/I	0.336	2
JA42	CAN/P	0.321	5	JA118	CAN/P	0.326	6	HEL278	UNK/I	0.343	1
JA43	CAN/P	0.337	5	JA119	CAN/P	0.332	6	HEL280	UNK/I	0.346	1
JA44	CAN/P	0.323	5	JA120	CAN/P	0.332	6	HEL293	O-P/I	0.371	1
JA45	CAN/P	0.329	5	JA122	CAN/P	0.336	6	HEL308	UNK/I	0.362	1
JA46	CAN/P	0.324	5	JA123	CAN/P	0.324	6	HEL324	UNK/I	0.347	2
JA47	CAN/P	0.326	5	JA125	CAN/P	0.336	6	HEL327	UNK/I	0.349	6
JA48	CAN/P	0.322	5	JA126	CAN/P	0.334	6	HEL335	UNK/I	0.363	2
JA49	CAN/P	0.331	3	JA127	CAN/P	0.340	6				
JA50	CAN/P	0.331	3	JA128	CAN/P	0.333	6				
JA54	USA/P	0.347	3	JA130	CAN/P	0.336	6				

Acc=accession label described in Kays and Nottingham (2008), *=an accession collected from a wild population in the USA; Orig/Sour= country origin and germplasm source, country code following ISO 3166-1 alpha-3 country code, RUS=the former Union of Soviet Socialist Republics (USSR), O-H=other-Hungry, O-Y=other-the former Yugoslavia, O-P=other-Poland, O-U=other-Ukraine, UNK=unknown origin, Source I=The Leibniz Institute of Plant Genetics and Crop Plant Research (*IPK*) in Gatersleben, Germany, P=Plant Gene Resources of Canada (PGRC), N=The North Central Regional Plant Introduction Station (NPRPIS), USA, U=unknown; AD=average dissimilarity; StC=clusters obtained from the STRUCTURE program.

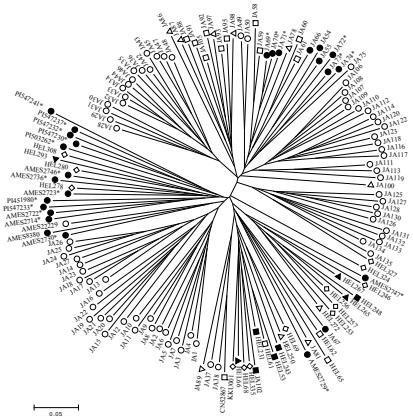


Fig. 3. The Neighbor-Joining (NJ) tree displaying the genetic associations based on RAPD markers of the 147 Jerusalem artichoke accessions representing nine countries. Each accession is labeled with its country origin: open circle for Canada; filled circle for the USA; open square for the former Union of Soviet Socialist Republics (USSR); filled square for Germany; open triangle for France; filled triangle for other four countries (Hungry, Poland, Ukraine or the former Yugoslavia); and open diamond for unknown origin. The accession with a star was collected from a wild population in the USA.

RNA extraction from Jerusalem artichoke

We improved the original protocol of Ma and Yang (2011) with several considerations (Mornkham *et al.*, 2013). This has helped to overcome the problem of viscous pellets in the original method of Trizol® and produced significant higher yields of total RNA (35 µg RNA/30 mg) than those using the original method of Ma and Yang. The modified Ma and Yang method also made the RNA extraction effort simpler and more reproducible, and the overall effort required approximately one and a half hours. The quality of RNA prepared by this method was demonstrated by intact sharp 28S and 18S rRNA bands and no sign of RNA degradation on agarose gel (Fig. 4). The extracted RNA was of high quality indicated by the A260/A280 and the A260/A230 ratios.

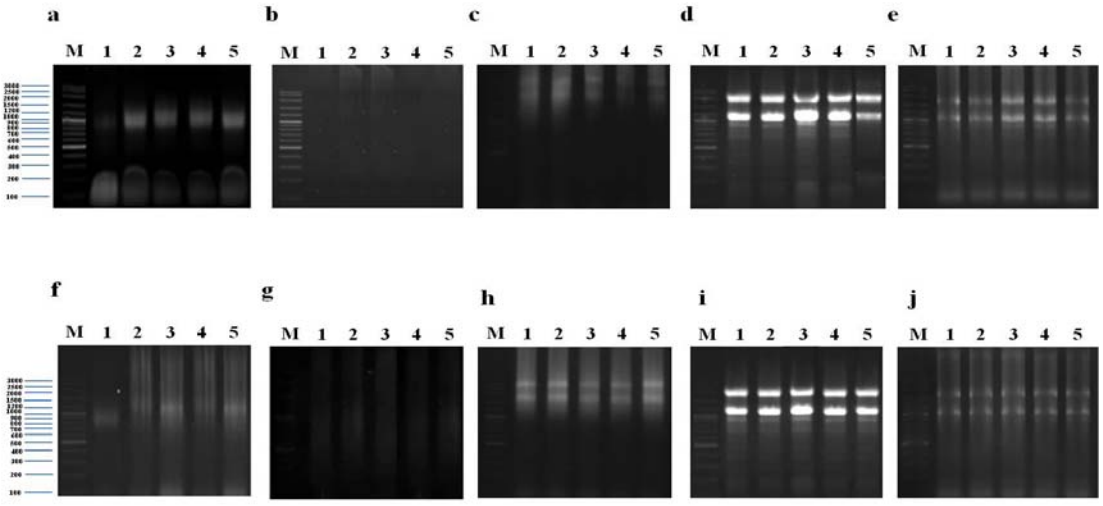


Fig. 4. Agarose gel electrophoresis of RNA extracted from dry seeds of Jerusalem artichoke cv. HEL53 (a, b, c, d and e) and JA37 (f, g, h, i and j) using five extraction protocols.

Isolation of candidate genes involving in tuberization of Jerusalem artichoke and searching for appropriate reference genes

The plants were grown on MS media (Murashige and Skoog, 1962) and tuber-inducing media (adding 8% sucrose and 1 mg/l 6-Benzylaminopurine) under SD condition (8-h light and 16-h dark cycles). Total RNA were extracted from internodes of Jerusalem artichoke cv. JA 102 every seven days until 3 weeks using Trizol® reagent (Invitrogen) as described previously. The tubers from single cutting nodes grown on either MS or tuber-inducing media under condition describe above were also used to extract total RNA. The extracted RNA was analyzed on 1% agarose gel electrophoresis. Spectrophotometer was used to quantitate RNA concentration by measuring the transmittance at 260 nm. 1.25 µg of each total RNA were subsequently used for 1st-stranded cDNA synthesis (SuperScript® VILO™, Invitrogen). The morphological of Jerusalem artichoke cv. JA102 grown on tuber-inducing media under SD condition appeared a visible swelling within the 1st week after growth (Fig. 5). While the single cutting nodes which growing on MS media did not show any sign of swelling within 3 weeks. The visible swelling of internodes was observed within 5 days and stem length is decreased in response to sucrose and cytokinin which presented in tuber-inducing media compared to MS media. Consistent with previously proposed by Gamburg *et al.* (1999), which showed that the high concentration of sucrose (6% - 10%) and cytokinin were necessary for microtuber formation in Jeresalem artichoke. The cytokinin has proved to be able to activatecell division and involved in the initial tuber formation of potato (Riou-Khamlichi *et al.*, 1999) and required during tuberization as accumulation.

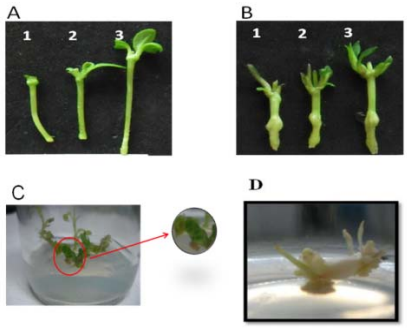


Fig. 5. Morphology of Jerusalem artichoke cv. JA102: A: grown on MS media, B: grown on MS media containing 80 g/l sucrose and 1 mg/l BA. C: the 8 weeks old tuber grown on MS media, and D; The 6 weeks old tuber grown on tuber-inducing media. The number indicates weeks after culture. The number indicates weeks after grown.

In order to isolate Jerusalem artichokegene homologs of *Arabidopsis CO*, *GA20-oxidase* and *GA2-oxidase*, genomic DNA extracted using the modified method of Tai and Tanksley (1990) or the 1st-stranded cDNAs were amplified by polymerase chain reaction using the primers which were designed based on the conserved regions of each gene described in section 3.2.3. The amino acid and nucleotide sequences were retrieved from NCBI database (National center for biotechnology information, http://www.ncbi.nlm.nih.gov) and the Compositae genome project data base (CGPDB, http://compgenomics.ucdavis.edu/.compositae_ index.php). The expected amplified sizes from Expressed Sequenced Tags sequences are 221, 373 and 535 bp for *CO*, *GA20-oxidasse* and *GA2-*

oxidase, respectively. The PCR products from the first-stranded cDNA as a template were subsequently sequenced (Fig 6). This confirmation ensured the specification of designed primers to analyze the expression of candidate genes.

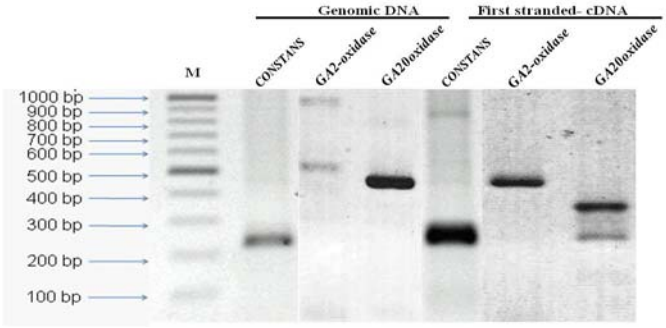


Fig. 6. PCR amplification of candidate genes from genomic DNA and first-stranded cDNAs.

Cloning of CONSTANS gene homologs from Jerusalem artichoke.

The *Arabidopsis CO* gene comprises the highly conserved regions encoding two zinc finger domains (B-box 1 and B-box 2) and a coiled-coil domain (CCT domain). These conserved regions are separated by the variable middle sequence. Primers designed from the middle region and CCT domain sequences in *Arabidopsis* were used to obtained the 221 bp fragment of *CONSTANS* (*HtCO*) from Jerusalem artichoke cv. JA102. BLAST search for similar sequences deposited in the GenBank (http://www.ncbi.nlm.nih.gov/) showed several previously characterized *CONSTANS* homologs of dicot and monocot plants. The partial nucleotide sequences of the Jerusalem artichoke fragment was 84% similar to that of Arabidopsis *CONSTANS* (AY114006), 82% to *CONSTANS* of *Solanum lycoopersicum* (AK325127.1) and 98% to the putative *CONSTANS* of *Helianthus tuberosus* from CGPDB database (Heli_tube.EL463197). The phylogenetic analyses from predicted amino acid level revealed that the Jerusalem artichoke fragment is found to resemble much wider range of CONSTANS homologs (Fig. 7). It is 63% identical to *S. lycoopersicum* and *Glycine max* (ACC95379.1 and ACX42572.1), 61% to *Arabidopsis* (BAB09583.1) and 59% to *Pisum sativum* (AAX20015.1). Such expansion of homology range in the case of amino acid sequences due to manifest codon bias in plant taxa belonging to asterids and rosids (Christianson, 2005).

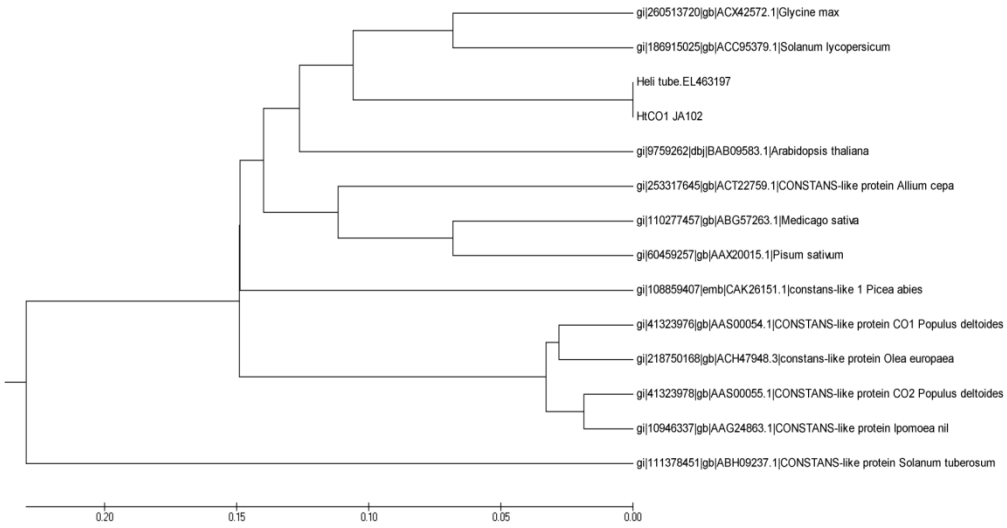


Fig. 7. The phylogenetic analysis at nucleotide level of the *HtCO* gene isolated from Jerusalem artichoke cv. JA102**.**

```
CO_HEL.EL463197     GAAATTGAAGTAGGTGTTGTACCGGATCACAAGGCAGCAATGACTGATGTATCGAACAAC 60
CO_JA102            GAAATTGAAGTAGGTGTTGTACCGGATCACAAGGCAGCAATGACTGATGTATCGAACAAC 60
                    ************************************************************

CO_HEL.EL463197     AACAACAACACCACCTCATCAGCGGATGTTTATCCAACTCCGTTAGTCGGATACGATCGA 120
CO_JA102            A---------CCACCTCATCAGCGGATGTTTATCCAACTCCGTTAGTCGGATACGATCGA 111
                    *         *************************************************

CO_HEL.EL463197     GAAGCTAGGGTTTTAAGATACAGAGAGAAGAAGAAGAACAGGAAGTTTGAGAAGATGATT 180
CO_JA102            GAAGCTAGGGTTTTAAGATACAGAGAGAAGAAGAAGAACAGGAAGTTTGAGAAGATGATT 171
                    ************************************************************

CO_HEL.EL463197     CGATATGCTTCGAGGAAGGCATATGCAGAGACGAGGCCGAGAATC 225
CO_JA102            CGATATGCTTCGAGGAAGGCATATGCAGAGACGAGGCCGAGAATC 216
                    *********************************************
```

Fig. 8. The alignment of *HtCO* and the HEL.EL463197 available from CGPDB represents the different between the $(ACA)_3$ repeat**.**

The partial *HtCO* sequence encoded the polar uncharged side chain, asparagine towards the C-end of the middle part before the CCT domain, and contained trinucleotide repeat $(ACA)_3$. The putative *CO* sequence from CGPDB database and the *HtCO* from Jerusalem artichoke cv. JA102 definitely differ in the number and length of this motif (Fig. 8). The detailed studies of CONSTANS-LIKE 1 polymorphism in *Brassica nigra* demonstrated that the number of asparagine residues varied from two to four and was related to plant adaptation to long and short days (Oesterberg *et al.*, 2002). Further characterization of several Jerusalem artichoke accessions reveals three HtCO homologs. Each homolog has several informative alleles (Fig. 9).

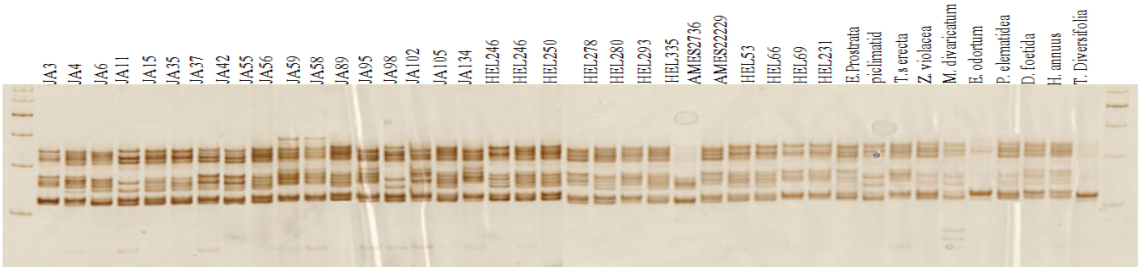


Fig. 9. *CONSTANS* homologs in Jerusalem artichoke and related species in Asteraceae.

The expression trend of both *HtCO* and *HtGA20-oxidase* genes was similar, with maximum expression level in the internode before cultured in the medium at t_0. The level of expression was decreased within the 1st week after grown on either MS or tuber-inducing media showed a particular tendency to increase later in the 2nd week. While expression trend of *GA2-oxidase* was different. The level of expression increased either on MS media or induction media (Fig. 10).

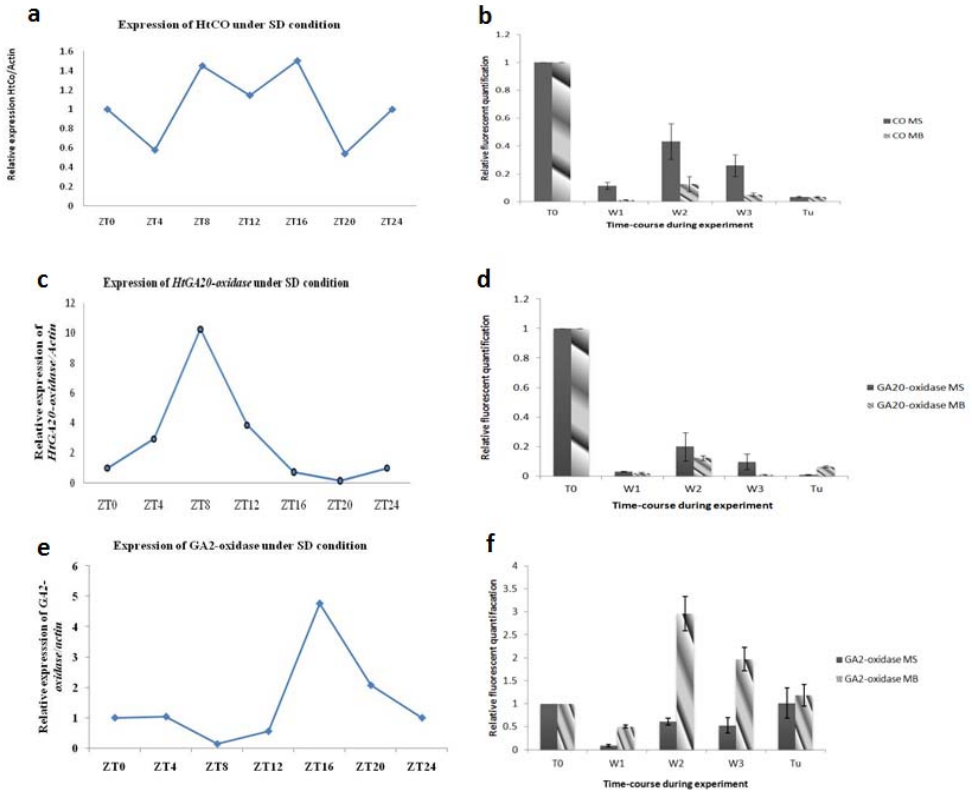


Fig. 10. The oscillation of *HtCO* (a), *HtGA20-oxidase* (b) *and HtGA2-oxidase* (c) under SD (8h/ 16h: light/dark) condition. The ZT (zeitgeber time) number indicates the experimental time on hour and the relative abundant of *HtCO* (d), *HtGA20-oxidase* (e) *and HtGA2-oxidase* (f) mRNA from the single cutting node grown on MS or modified MS (MB) media under SD condition. T0 indicates the time before cultured, W1: 1st week after cultured, W2: 2nd week after cultured, W3: 3rd week after cultured and Tu: tuber stage**.**

Conclusion

The efficient DNA and RNA extraction protocols for Jerusalem artichoke tissue were developed and proved to be useful for other plant species. Genetic relatedness among 147 Jerusalem artichoke from nine countries of origin was elucidated. The molecular information on *in vitro* tuberization of Jerusalem artichoke was reported.

References

Arif, I.A., M. A. Bakir, H. A. Khan, A. H. A. Farhan, A. A. A. Homaidan, A. H. Bahkali, M. A. Sadoon and M. Shobrak. 2010. A brief review of molecular techniques to assess plant diversity. Int. J. Mol. Sci. 11: 2079-2096.

Breton, C., H. Serieys and A. Bervill. Gene transfer from wild *Helianthus* to sunflower: topicalities and limits. 2010. OCL. 17: 104-114.

Christianson, M.L. 2005. Codon usage patterns distort phylogenies from or of DNA sequences. Am. J. of Bot. 92: 1221-1233.

Gamburge, K.Z., E.F. Vysotskaya and L.V. Gamanets. 1999. Microtuber formation in micropropagated Jerusalem artichoke *(Helianthus tuberosus* L.). Plant Cell Tiss. Organ Cult. 55: 115-118.

Kays, S.J., Nottingham, S.F., 2008. Biology and Chemistry of Jerusalem Artichoke *Helianthus tuberosus* L. CRC Press, Florida.

Li, G. and C. F. Quiros. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica.* Theor. Appl. Genet.103: 455-461.

Ma, X.B. and J. Yang. An optimized preparation method to obtain high-quality RNA from dry sunflower seeds. 2011. Genet. Mol. Res. 10: 160-168.

Mandel, J.R., J.M. Dechaine, L.F. Marek and J.M. Burke. 2011. Genetic diversity and population structure in cultivated sunflower and a comparison to its wild progenitor, *Helianthus annuus* L.Theor. Appl. Genet. 123: 693-704.

Mornkham, T., P.P. Wangsomnuk, S. Jogloy, P. Wangsomnuk, A. Patanothai and Y.B. Fu.2012. Comparison of five DNA extraction methods for molecular analysis of Jerusalem artichoke (*Helianthus tuberosus*). Genet. Mol Res. 11: 572-581.

Murashige, T. and G. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum15: 72-497.

Oesterberg, M.K., O. Shavorskaya, M. Lascoux and U. Lagercrantz. 2002. Naturally Occurring indel variation in the *Brassica nigra* COL1 gene is associated with variation in flowering time.Genetics 161: 299-309.

Riou-Khamlichi, C., R. Huntley, A. Jacqmard and J.A.H. Murray. 1999. Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. Sci. 283: 1541-1544.

Seiler, G.J. 2003. The potential of wild sunflower species for industrial uses. Helia 30: 175–198.

Štorchová, H., R. Hrdličková, J. Chrtek and M. Tetera. 2000. An improved method of DNA isolation from plants collected in the field and conserved in saturated NaCl/CTAB solution.Taxon. 49: 79-84.

Tai, T. H. and S. D. Tanksley. 1990. A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. Plant Mol. Biol. Rep. 8: 297-303.

Volk G.M. and K. Richards. 2006. Preservation methods for Jerusalem artichoke cultivars. Hort. Sci. 41: 80-83.

Williams, J.G K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18: 6531-6535.

Zietkiewicz, E., A. Rafalski and D. Labuda. 1994. Genome fingerprinting by simple sequence repeats (SSR)-anchored PCR amplification.Genom. 20: 176-183.

Publication

Wangsomnuk, P.P., S. Khampa, S. Jogloy, A. Patanothai and Y-B.Fu. Assessing genetic structure and relatedness of Jerusalem artichoke (*Helianthus tuberosus* L.) germplasm with RAPD, ISSR and SRAP markers. 2011. Am. J. Plant Sci. 2: 753-764. (No impact factor)

Wangsomnuk, P.P., S. Khampa, P. Wangsomnuk, S. Jogloy, T. Mornkham, B. Ruttawat, A. Patanothai, Y-B.Fu. 2011. Genetic diversity of worldwide Jerusalem artichoke (*Helianthus tuberosus*) germplasm as revealed by RAPD markers. Genetics and Mol. Res. 10:4012-4025. (Impact factor = 1.593)

Mornkham, T., P.P. Wangsomnuk, Y-B.Fu, P. Wangsomnuk, S. Jogloy and A. Patanothai. 2013. Extractions of high quality RNA from seeds of Jerusalem Artichoke and other plant species with high levels of starch and lipid. Plants 2: 302-316. (No impact factor)

2.7 Levels of *Sclerotium rolfsii* inoculum influence identification of resistant genotypes in Jerusalem artichoke

R. Sennoi, S. Jogloy, W. Saksirirat, T. Kesmala, N. Singkham and A. Patanothai

Jerusalem artichoke (*Helianthus tuberosus* L.) is currently an important crop for production of healthy food (Danilčenko *et al.*, 2008), as the crop produces substantial amounts of the carbohydrate inulin rather than starch in its tubers. Stem rot disease caused by *S. rolfsii* is a major disease of the crop. Breeding Jerusalem artichoke for resistance to *S. rolfsii* may provide a sustainable means of disease management. Many attempts have been made to find resistant genotypes against *S. rolfsii* (Gorbet *et al.*, 2004; Infantino *et al.*, 2006; Akram *et al.*, 2008). As Jerusalem artichoke is rather new to breeders and plant pathologists, an optimal screening procedure for resistance is not available. Previous work on disease resistance in Jerusalem artichoke was only on *Sclerotinia sclerotiorum* (Cassells and Walsh, 1995). In a recent study at Khon Kaen University in Thailand showed that inoculum grown on sorghum seed was more effective than on agar (Shokes *et al.*, 1996). However, the question still remains on how many *S. rolfsii*-infested seeds should be used per inoculated plant.

Objective

The objective of this study was to determine the levels of sorghum seed inoculum that provide the most reliable and effective results for evaluation *S. rolfsii* resistance of Jerusalem artichoke.

Materials and methods

Five Jerusalem artichoke genotypes (HEL 278, HEL 246, HEL 280, JA 1 and HEL 65) that exhibited a high level of resistance to *S. rolfsii* in our previous work and five genotypes (CN 52867, HEL 62, JA 102, JA 37 and JA 122) that were most susceptible were used in this study. Four levels of inoculum density (1, 2, 3 or 4 *S. rolfsii*-infested sorghum seeds/plant) were tested with the 10 Jerusalem artichoke genotypes in a factorial design in a randomized complete block (RCBD) with four replications. There were four plants in each treatment unit.

Isolate 1 of *S. rolfsii*, which was obtained from a KKU field in Khon Kaen and was very aggressive on Jerusalem artichoke as determined by previous screening assays (Sennoi *et al.* 2010), was used in the trials. The isolate was transferred to potato dextrose agar (PDA) medium in petri dishes and incubated at room temperature (25 ± 2°C) for 3 days. After incubation, mycelium plugs were transferred to steamed sorghum seeds and incubated at room temperature for 10 days; the inoculum was then ready to use. At the 6- to 8-leaf stage, plants were inoculated by placing infested sorghum seeds at the crowns of stems at determined levels (1, 2, 3 and 4 sorghum seeds/plant). Cotton wool was used to cover infested sorghum seeds in order to maintain moisture.

The numbers of infected plants and lesion length (cm) were recorded every two days after inoculation. Days to permanent wilting was observed daily after inoculation. Number of symptomatic plants was later converted to disease incidence (% plants exhibiting symptoms). Area under disease progress curve (AUDPC) was calculated from disease incidence according to the formula suggested by Marcel *et al.* (2008)

Results

Base on high F-ratio values for genotypes and a low coefficient of variation, data collected for disease incidence and lesion length were reported at 3 and 5 days after inoculation. Significant differences (P<0.01) between the two runs of the experiment (E), genotype (G) and G × E interaction were found for disease incidence, lesion length, days to permanent wilting and AUDPC (Table 1).

From the regression analysis, the plants inoculated with one sorghum seed had the lowest disease incidence (62.5 %), whereas the plants inoculated with four sorghum seeds had the highest disease incidence (92.5 %) (Figure 1a). Lesion length ranged from 1.1 to 1.5 cm (Figure 1b). Plants inoculated with one sorghum seed had the shortest lesions (1.1 cm), but lesion length did not differ significantly from the plants inoculated with three sorghum seeds (1.2 cm) nor did two seeds differ significantly from four seeds (1.4 cm and 1.5 cm, respectively). Days to permanent wilting ranged from 2.2 to 3.2 (Figure 1c). The treatment with one sorghum seed required 3.2 days to permanent wilting. The other inoculum levels of two, three and four sorghum seeds required 2.8, 2.6 and 2.2 days to permanent wilting, respectively. The 1-seed treatment gave the lowest AUDPC, whereas the highest AUDPC was observed for the 4-seeds treatment (Fig. 1d). The correlation coefficient between seed treatment and AUDPC was positive and significant ($R^2 = 0.98$).

Table 1. Mean squares from combined ANOVA for disease incidence (at 3 days after inoculation), lesion length (at 5 days after inoculation), days to permanent wilting and area under disease progress curve (AUDPC).

SOV	df	Disease incidence	Lesion length	Days to permanent wilting	AUDPC
Experiment (E)	1	34031.3**	2.3**	37.8**	303195*
Rep/experiment	6	134.1	0.1	2.1	9945
Genotype (G)	9	4397.6**	4.2**	12.7**	79010**
G × E	9	5094.6**	0.8**	2.4**	18699**
Inoculum level (I)	3	12085.9**	2.0**	12.3**	129607**
I × E	3	2536.5**	0.1	0.4**	22617**
G × I	27	1366.9**	1.1**	1.2**	7810**
G × I × E	27	1979.5**	0.7**	0.9**	5841**
Pooled error	234	110.1	0.1	0.4	2135
C.V. (%)		13.5	22.8	20.3	9.9

** Significant at $P<0.01$.

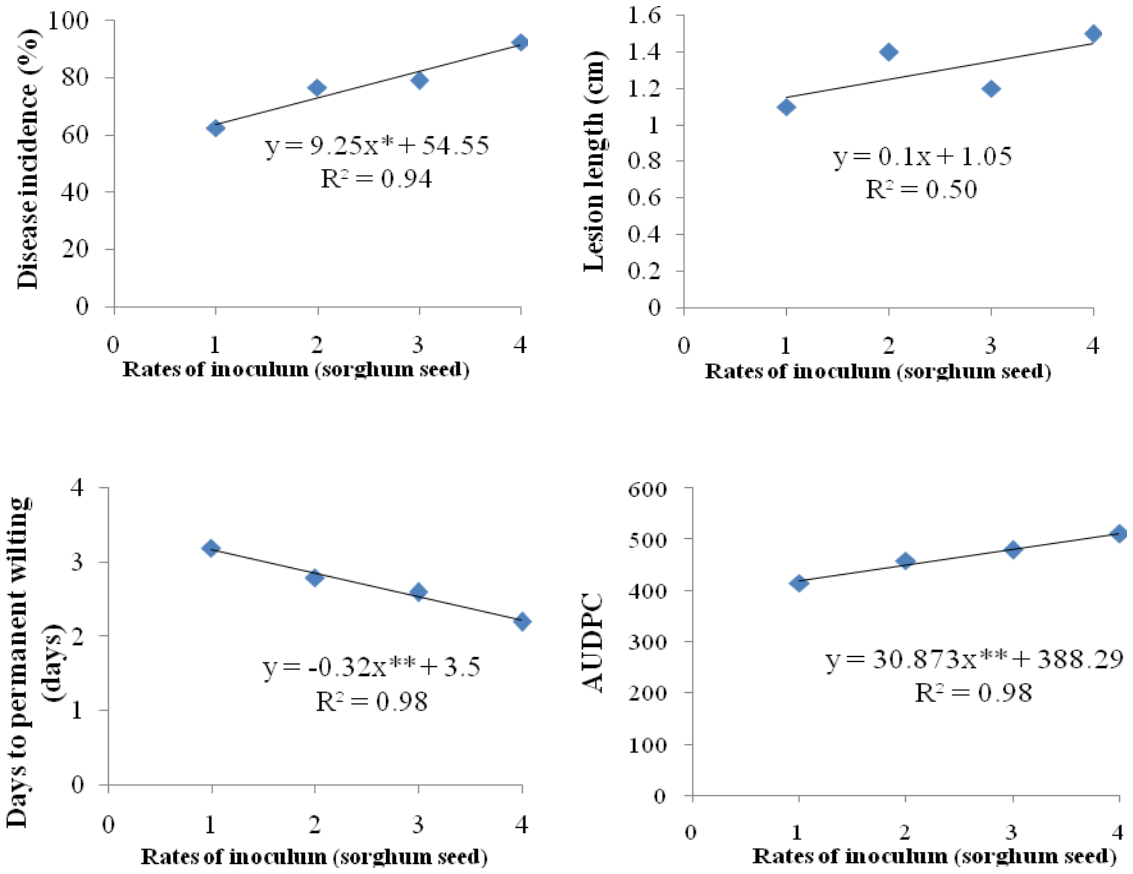


Fig. 1. Disease incidence (**a**), lesion length (**b**), days to permanent wilting (**c**) and area under disease progress curve (AUDPC) of Jerusalem artichoke at four different levels of *Sclerotium rolfsii*-infested seeds per plant.
*, ** Significant at $P< 0.05$ and $P< 0.01$, respectively.

The highest variation among Jerusalem artichoke genotypes was observed for days to permanent wilting in the plants inoculated with three sorghum seeds as indicated by a high F-ratio (21.4) (Table 2).

Table 2. Mean squares from combined ANOVA for days to permanent wilting at different level of *Sclerotium rolfsii* inoculum in Jerusalem artichoke.

SOV	df	1 seed	2 seeds	3 seeds	4 seeds
Experiment (E)	1	16.2**	7.2*	9.1*	6.6**
Rep/experiment	6	0.9	0.7	0.8	0.4
Genotype (G)	9	4.5**	4.4**	4.5**	3.0**
G × E	9	2.0**	1.5**	1.4**	0.2
Pooled error	54	0.4	0.4	0.2	0.3
F-ratio for genotypes		11.6	12.3	21.4	11.1
C.V. (%)		19.7	21.1	17.7	23.2

*, ** Significant at $P<0.05$ and $P<0.01$, respectively.

Inoculation with three sorghum seeds resulted in the greatest range of variation in disease response among Jerusalem artichoke genotypes. Using three sorghum seeds per plant, disease incidence ranged from 50 to 100 % among genotypes (Figure 2a). Genotypes HEL 278, JA 1, HEL 65, CN 52867 and JA 37 showed the lower of disease incidence (50 to 68.8 %), whereas genotypes HEL 246, HEL 280, HEL 62, JA 102 and JA 122 had the higher disease incidence (90.6 to 100 %).

The genotypes that required the longer time (2.8 to 3.3 days) to reach the permanent wilting were HEL 278, HEL 246, HEL 280, JA 1, HEL 65, CN 52867 and JA 37. In contrast, genotypes HEL 62, JA 102 and JA 122 required only 1.4 to 2.3 days to reach the permanent wilting (Figure 2b). Higher AUDPC were observed for HEL 62, JA 102 and JA 122 (540.6 to 565.6), and lower AUDPC were observed for HEL 278, HEL 246, HEL 280, JA 1, HEL 65, CN 52867 and JA 37 (403.1 to 487.5) (Figure 2c).

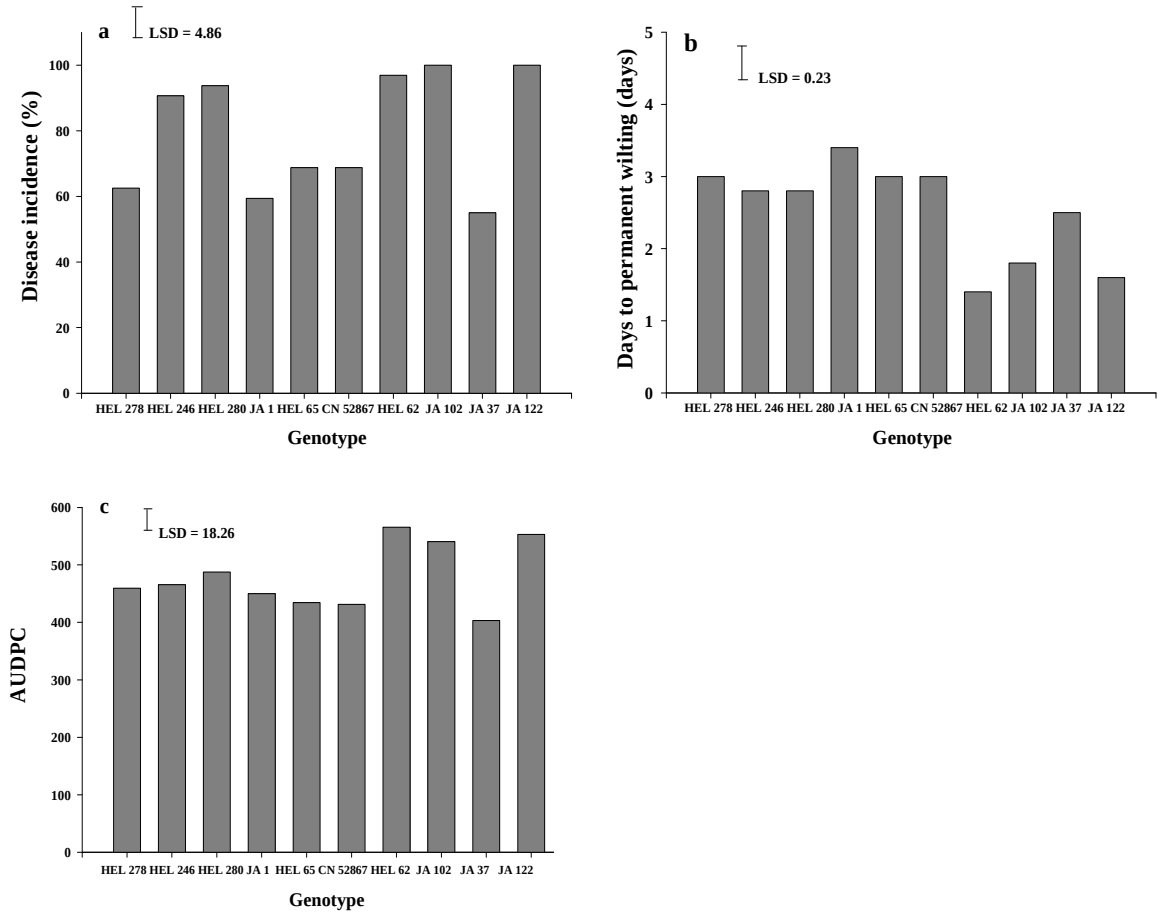


Fig. 2. Disease incidence (a), days to permanent wilting (b) and area under disease progress curve (c) of Jerusalem artichoke genotypes after inoculation with three *Sclerotium rolfsii*-infested seeds per plant.

Conclusion

Inoculation with three sorghum seeds obtained the highest variations in Jerusalem artichoke genotypes and provided replicable results for most genotypes. This method will be further used to evaluate Jerusalem artichoke genotypes for resistance to stem rot disease caused by *S. rolfsii*.

References

Akram, A., S.M. Iqbal, C.A. Rauf and R. Aleem. 2008. Detection of resistant sources for collar rot disease in chickpea germplasm. Pak. J. Bot. 40(5): 2211-2215.

Cassells, A.C. and M. Walsh. 1995. Screening for *Sclerotinia* resistance in *Helianthus tuberosus* L. (Jerusalem artichoke) varieties, lines and somaclones, in the field and in vitro. Plant Pathol. 44: 428-437.

Danilčenko, H., E. Jarienė, P. Aleknavičienė and M. Gajewski. 2008. Quality of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers in relation to storage conditions. Not. Bot. Hort. Agrobot. Cluj. 36: 23-27.

Gorbet, D.W., T.A. Kucharek, E.M. Shokes and T.B. Brenneman. 2004. Field evaluations of peanut germplasm for resistance to stem rot. Peanut Sci. 31: 91-95.

Infantino, A., M. Kharrat, L. Riccioni, C.J. Coyne, K.E. McPhee and N.J. Grünwald. 2006. Screening techniques and sources of resistance to root diseases in cool season food legumes. Euphytica 147: 201-221.

Marcel, T.C., B. Gorguet, M.T. Ta, Z. Kohutova, A. Vels and R.E. Niks. 2008. Isolate specificity of quantitative trait loci for partial resistance of barley to *Puccinia hordei* confirmed in mapping populations and near-isogenic lines. New Phytol. 177(3): 743-755.

Sennoi, R., S. Jogloy, W. Saksirirat and A. Patanothai. 2010. Pathogenicity test of Sclerotium rolfsii, a causal agent of Jerusalem artichoke (*Helianthus tuberosus* L.) stem rot. Asian J. Plant Sci. 9(5): 281-284.

Shokes, F.M., K. Rozalski, D.W. Gorbet, T.B. Brenneman and D.A Berger. 1996. Techniques for inoculation of peanut with *Sclerotium rolfsii* in the greenhouse and field. Peanut Sci. 23: 124-128.

Publication

Sennoi, R., S. Jogloy, W. Saksirirat, T. Kesmala, N. Singkham and A. Patanothai. 2012. Levels of *Sclerotium rolfsii* inoculum influence identification of resistant genotypes in Jerusalem artichoke. Afr. J. Microbiol. Res. 6(38): 6755-6760. (Impact factor = 0.539)

2.8 Effects of host growth stage, re-isolation and culture medium on screening for resistance to stem rot disease caused by *Sclerotium rolfsii* Sacc. in Jerusalem artichoke

R. Sennoi, S. Jogloy, W. Saksirirat, T. Kesmala and A. Patanothai

Jerusalem artichoke (*Helianthus tuberosus* L.) has a high potential for uses as healthy food for human and animal. It can be grown commercially in the tropics (Pimsaen *et al*., 2010; Puangbut *et al*., 2011). However, stem rot disease caused by *Sclerotium rolfsii* poses a threat to the crop (Sennoi *et al*., 2010). A breeding program for resistance to this fungus is needed, and reliable and effective inoculation methods are required for screening of Jerusalem artichoke genotypes for resistance to *S. rolfsii*. However, a screening method for resistance to this fungus in Jerusalem artichoke has not been reported. The first step toward this goal would be to clarify host, pathogen, and/or environmental influences on this disease during resistance screening trials. Although young chickpea plants are more susceptible to the disease than older ones (Hussain *et al*., 2006; Sconyers *et al*., 2007), possible impacts of plant age on stem rot caused by *S. rolfsii* in Jerusalem artichoke have not been evaluated. A primary cause of culture collection loss has been the inability to store and maintain individual isolates in the state in which they were originally collected (Day and Stacey, 2008). It is also unclear whether repeated serial passage through *in vitro* subculturing would affect pathogenicity of *S. rolfsii*, and this factor needs to be assessed in order to develop reliable screening techniques. The type of culture medium often affects the mycelial growth rate of fungi (Hubballi *et al*.,

2010), thus, types of media should be compared to determine the most effective medium for inoculation.

Objective

The objectives of this work were to investigate the effects of seedling stage, cultural status of *S. rolfsii* inoculum (serial *in vitro* subculture *vs.* re-isolation of *S. rolfsii* from infected plants) and medium used to produce inoculum on the incidence and severity of stem rot disease caused by *S. rolfsii* in Jerusalem artichoke.

Materials and Methods

The experiment was conducted in an open-sided greenhouse at Khon Kaen University (KKU) Agronomy Farm, Khon Kaen, Thailand, in July 2011 and repeated in September 2011. The treatments were arranged in 3 × 2 × 2 factorial combinations in a randomized complete block design with six replications. Three developmental stages of seedlings (6-, 8-, and 10-leaf stages) were assigned as factor A, two sources of *S. rolfsii* (serial *in vitro* subculture and re-isolated from a symptomatic host) were assigned as factor B, and two types of inoculum (PDA and sterilized sorghum seeds) were assigned as factor C. A recommended genotype by the Jerusalem artichoke Improvement Project of Khon Kaen University for commercial cultivation (Kaen Tawan # 2) was used. The three sets of seedling stage treatments were prepared at four-day intervals. The soil of the experiment belongs to Roi-et series (Re**;** fine-loamy, mixed, subactive, isohyperthermic Aeric Kandiaquults).

The data were recorded daily from 1 to 30 days after inoculation for the number of infected plants and then converted to percent infected plants, and the number of days to permanent wilting. Data for each experiment were analyzed statistically for each parameter, and a combined analysis of variance for the two experiments was done according to a factorial in RCBD experiment (Hoshmand, 2006).

Results

Inoculum preparation methods (serial subculture *vs.* re-isolated from a host) were significantly different for days to permanent wilting ($p < 0.0$). In addition, medium (PDA vs. sorghum grain) for inoculum preparation differed significantly for disease incidence ($p < 0.01$), but not for days to permanent wilting. The interactions between inoculum preparation methods and medium types were found for both disease incidence ($p < 0.01$) and days to permanent wilting ($p < 0.01$) (data not shown).

Plants inoculated at the 6-leaf stage had a much higher disease incidence than those inoculated at 8- and 10-leaf stages (Fig. 1). Plants inoculated at the 6-leaf stage took the shortest time (3 days) to permanent wilting followed by plants inoculated at the 8-leaf stage (5 days), whereas plants inoculated at the 10-leaf stage took the longest time (8 days) (Fig. 2). Inoculum preparation methods (serial subculture vs. re-isolation from the host) were not significantly different for disease incidence, but plants inoculated with the sub-cultured inoculum required less time (4 days) to permanent wilting than those inoculated with the re-isolated fungus (6 days) (Fig. 3). Inoculum prepared on sorghum seeds generally had a higher disease incidence than did when prepared on PDA, especially at 5 days after inoculation (Fig 4).

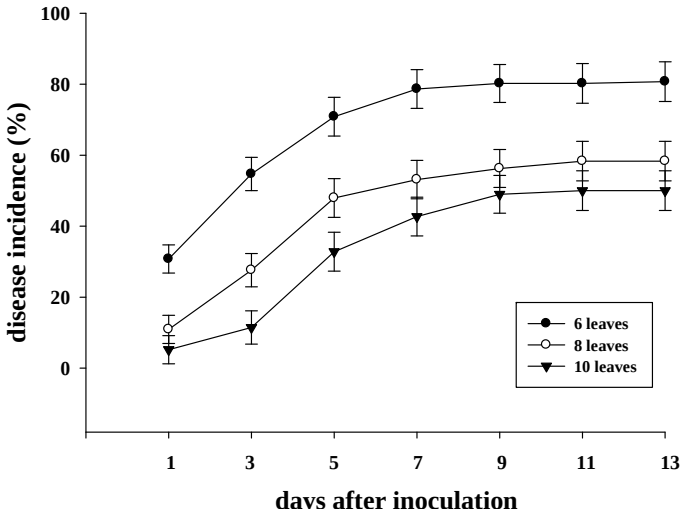


Fig. 1. Effects of plant stages at inoculation on the incidence of *Sclerotium rolfsii* stem rot disease in Jerusalem artichoke.

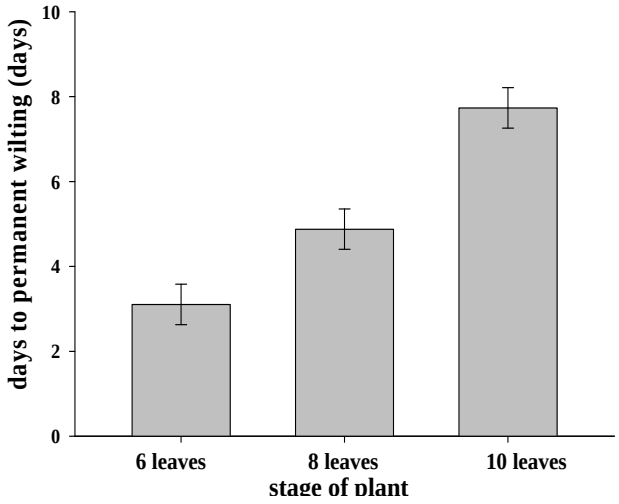


Fig. 2. Effects of plant stages at inoculation of *Sclerotium rolfsii* on time to permanent wilting of Jerusalem artichoke plants.

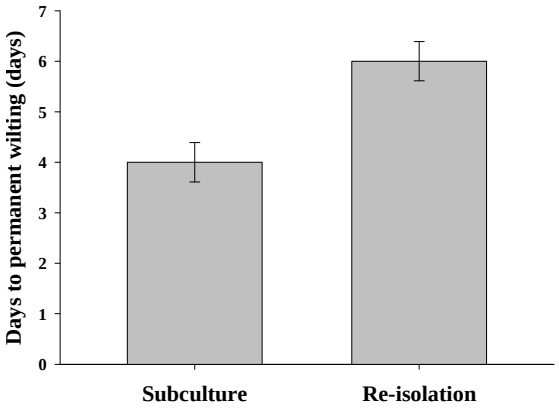


Fig. 3. Effect of inoculums of *Sclerotium rolfsii* from serial subculture and from re-isolation on days to permanent wilting of Jerusalem artichoke plants.

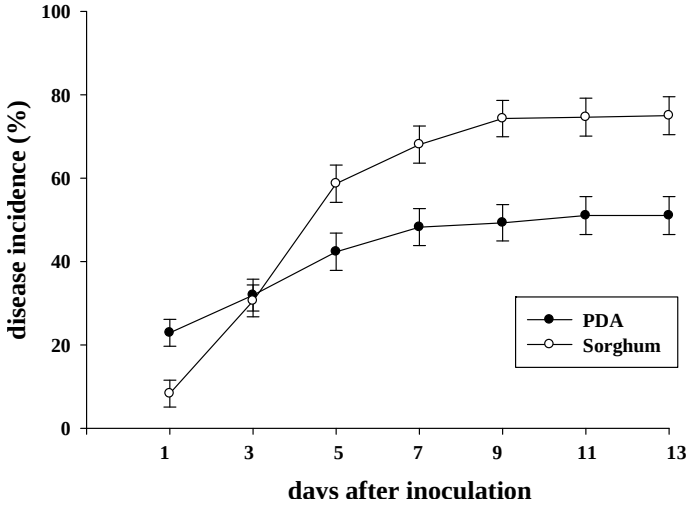


Fig. 4. Effects of media types for preparing *Sclerotium rolfsii* inoculation on disease incidence in Jerusalem artichoke.

Conclusion

Plants at the 6-leaf stage were more susceptible than those at the 8- or 10-leaf stages. The *S. rolfsii* inoculum derived from serial *in vitro* subculture caused more severe stem rot symptoms than the inoculum derived by re-isolated from symptomatic host plants, indicating that serial *in vitro* subculture did not reduce pathogenicity of *S. rolfsii*. The inoculum culture on the sorghum-based medium resulted in a higher incidence of stem rot than PDA. The highest incidence of disease was observed with the inoculum grown on PDA from serial *in vitro* subculture and on the sorghum-based medium that was re-isolated from symptomatic host tissue.

References

Day, J.G. and G.N. Stacey. 2008. Biobanking. Mol. Biotechnol. 40: 202–213.

Hoshmand, A.R. 2006. Design of Experiments for Agriculture and the Natural Sciences. 2nd ed. Chapman & Hall/CRC, Boca Raton, Florida.

Hubballi, M., S. Nakkeeran, T. Raguchander, T. Anand and R. Samiyappan. 2010. Effect of environmental conditions on growth of *Alternaria alternata* causing leaf blight of Noni. World J. Agric. Sci. 6(2): 171–177.

Hussain, A., SH.M. Iqbal, N. Ayub and M.A. Zahid. 2006. Factors affecting development of collar rot disease in chickpea. Pak. J. Bot. 38(1): 211–216.

Pimsaen, W., S. Jogloy, B. Suriharn, T. Kesmala, V. Pensuk and A. Patanothai. 2010. Genotype by environment (G × E) interactions for yield components of Jerusalem artichoke (*Helianthus tuberosus* L.). Asian J. Plant Sci. 9(1): 11–19.

Puangbut, D., S. Jogloy, S. Srijaranai, N. Vorasoot, T. Kesmala and A. Patanothai. 2011. Rapid assessment of inulin content in *Helianthus tuberosus* L. tubers. SABRAO J. Breed. Genet. 43(2): 188–200.

Sconyers, L.E., T.B. Brenneman and K.L. Stevenson. 2007. Effects of row pattern, seeding rate, and inoculation date on fungicide efficacy and development of peanut stem rot. Plant Dis. 91: 273–278.

Sennoi, R., S. Jogloy, W. Saksirirat and A. Patanothai. 2010. Pathogenicity test of *Sclerotium rolfsii*, a causal agent of Jerusalem artichoke (*Helianthus tuberosus* L.) stem rot. Asian Journal of Plant Sci. 95: 281–284.

Publication

Sennoi, R., S. Jogloy, W. Saksirirat, T. Kesmala and A. Patanothai. 2013. Effects of host growth stage, re-isolation and culture medium on screening for resistance to stem rot disease caused by *Sclerotium rolfsii* Sacc. in Jerusalem artichoke. Pak. J. Bot. 45(5): 1825-1829. (Impact factor = 0.907)

2.9 Evaluation of seedling and adult plant stages resistance to *Sclerotium rolfsii* in Jerusalem artichoke (*Helianthus tuberosus* L.)

R. Sennoi, S. Jogloy, W. Saksirirat, P. Banterng, T. Kesmala and A. Patanothai

Southern stem rot caused by *Sclerotium rolfsii* is a serious disease worldwide. The fungus pathogen has a wide range of hosts, including vegetables, flowers, legumes, cereals, forage plants and weeds (Agrios, 2005). Screening for *S. rolfsii* resistance has been reported in many plant species, including crops and ornamental plants (Shew *et al.*, 1987; Fery and Dukes, 2002; Akram *et al.*, 2008; Xu *et al.*, 2009). To the best of our knowledge, screening for *S. rolfsii* resistance in Jerusalem artichoke has not been reported. Relationships between seedling and adult plant resistance to other pathogen have been shown in *Brassica napus* (Li *et al.*, 2006), wheat (Wang *et al.* 2005; Hovmøller, 2007) and barley (Prashant *et al.*, 2009). However, the relationship between the resistance to *S. rolfsii* at seedling stages and at mature plant stages has not been evaluated in Jerusalem artichoke. The question is whether the Jerusalem artichoke genotypes with resistance to *S. rolfsii* at seedling stages are also resistant at mature stages. If the resistant genotypes can be readily identified at seedling stages and the resistance persists at mature stages, the screening of Jerusalem artichoke for resistance to *S. rolfsii* will be much easier and effective.

Objective

The objective of this work was to evaluate the relationship between seedling and adult plant resistance to *S. rolfsii* in Jerusalem artichoke.

Materials and methods

Two pot experiments were conducted at Khon Kaen University Agronomy Farm, Khon Kaen, Thailand. A split-plot design with four replications was used in both experiments. Two plant ages, one at a seedling stage (20 days after transplanting (DAT)) and the other at a mature stage (85 DAT), were assigned as main-plots, and ten genotypes of Jerusalem artichoke (HEL 280, HEL 278, HEL 246, JA 98, HEL 65, JA 102, JA 6, HEL 62, JA 2 and JA 102) were assigned as sub-plots.

Seedling and adult plants were inoculated at the same time. Days to permanent wilting of the plants (defined as all leaves wilted) were recorded every day. Plants were evaluated every other day after inoculation for lesion length (cm) and the number of plants with permanent wilting symptom. The number of plants with permanent wilting symptom was later converted to disease incidence. Area under disease progress curve (AUDPC) was calculated from the disease incidence according to the formula suggested by Marcel *et al.* (2008)

Results

Seedling and adult plant stages were statistically different for lesion length, days to permanent wilting and the area under disease progress curve (AUDPC) but were not different for disease incidence (Table 1).

Table 1. Mean squares from combined analysis of variance for lesion length, disease incidence (%), days to permanent wilting and area under disease progress curve (AUDPC) of Jerusalem artichoke.

Source of variation	df	Lesion length	% Disease incidence	Days to permanent wilting	AUDPC
Experiment (E)	1	2.2*	180.6^{ns}	75.4**	14497.0^{ns}
Rep within experiment	6	0.2	98.9	2.0	6441.6
Stage of plant (S)	1	44.8**	600.6^{ns}	5535.4**	25560000.0**
E × S	1	0.7*	1050.6*	5.7^{ns}	20407.8^{ns}
Rep within stage of plant and experiment	6	0.1	152.3	3.7	36564.7
Genotypes (G)	9	1.7**	2361.2**	48.4**	581801.0**
E × G	9	0.3*	89.0^{ns}	2.4**	8009.8^{ns}
S × G	9	0.2^{ns}	947.9**	29.9**	319940.0**
E × S × G	9	0.1	120.1^{ns}	1.8*	23191.3^{ns}
Pooled error	108	0.1	134.0	0.9	14186.2
C.V. (%)		30.1	15.7	8.6	13.8

*Significant at 5%; ** Significant at 1%.

The correlations between two plant ages for lesion length, disease incidence, days to permanent wilting and AUDPC were low, although they were positive and significant, especially for disease incidence (r = 0.24) and days to permanent wilting (r = 0.28) (Table 2).

Lesion lengths of ten Jerusalem artichoke genotypes in the seedling stage ranged from 1.1 to 2.6 cm, and genotypes JA 6, JA 122 and JA 102 had longer lesion lengths than the others. For the adult plant stage, lesion lengths ranged from 0.2 to 1.3 cm, and longer lesion lengths were observed for HEL 62, JA 102 and JA 2 (Table 3).

Disease incidences ranged from 56.2 to 87.5 % in the seedling stage, while severer disease incidences were observed for HEL 62 and JA 102 (Table 3). Disease incidences of adult plants ranged from 50.0 to 97.5 %, and JA 6, HEL 246 and JA 98 had lower disease incidences than the others. The differences between the two plant ages and among the Jerusalem artichoke genotypes were observed for days to permanent wilting (Table 3), which ranged from 3 to 7 days for the seedling stage and from 13.6 to 19.9 days for the adult plant stage.

The data indicated that adult plants were more resistant than young plants. HEL 62, JA 6, HEL 278 and JA 122 took fewer days to reach permanent wilting, whereas HEL

65, HEL 280 and JA 98 took more days to reach the permanent wilting date, indicating that they were more resistant to the disease at seedling stage. For adult plant resistance, HEL 62, HEL 280 and 102 spent fewer days than the others to reach the permanent wilting date, whereas JA 98, JA 6 and HEL 246 took more days to reach the permanent wilting date, indicating that they were more resistant at the mature plant stage.

Area under disease progress curve ranged from 1132.9 to 1562.5 for the seeding stage, and from 31.3 to 850.0 for the adult plant stage. The genotypes that had higher AUDPC at the seedling stage were HEL 62, HEL 278 and JA 102, and those that had lower AUDPC included HEL 280, JA 122 and JA 6. However, there were not much different between genotypes for the lower AUDPC group. For the adult plant stage, higher AUDPC were observed for JA 102, HEL 62, JA 122 and HEL 280, and lower AUDPC were observed for JA 6, HEL 246 and JA 98 (Table 3).

Table 2. Correlations between *Sclerotium rolfsii* resistance traits of 10 Jerusalem artichoke genotypes at the seedling stage and at the adult plant stage.

Seedling plant	Adult plant			
	Lesion length (cm)	Disease incidence (%)	Days to permanent wilting (days)	AUDPC
Lesion length (cm)	0.16^{ns}			
Disease incidence (%)		0.24^{*}		
Days to permanent wilting (days)			0.28^{*}	
AUDPC				0.16^{ns}

* Significant at P 5%

Table 3. Ranks of Jerusalem artichoke genotypes for traits indicating resistance to *Sclerotium rolfsii* at seedling and adult plant stages**.**

Genotype	Lesion length (cm)		Disease incidence (%)		Days to permanent wilting (days)		AUDPC	
	Seedling	Adult	Seedling	Adult	Seedling	Adult	Seedling	Adult
HEL 280	1.4 ± 0.2	0.7±0.3	56.3 ± 7.4	67.5 ± 10.4	6.4 ± 0.5	13.7 ± 1.6	1132.9 ±84.7	680.0 ± 104.4
HEL 278	1.9 ± 0.2	0.5±0.1	67.5 ± 14.9	77.5 ± 12.8	3.9 ± 0.7	16.3 ± 1.8	1478.1 ± 113.5	437.5 ± 151.0
HEL 246	1.1 ± 0.2	0.4 ± 0.5	77.5 ± 12.8	57.5 ± 12.8	5.0 ± 1.0	18.5 ± 1.1	1218.8 ± 173.4	103.1 ± 62.7
JA 98	1.7 ± 0.3	0.2 ± 0.1	70.0 ± 10.7	52.5 ± 14.9	5.7 ± 1.0	19.9 ± 1.5	1265.6 ± 161.9	143.8 ± 119.4
HEL 65	1.2 ± 0.4	1.1 ± 0.6	62.5 ± 12.8	80.0 ± 10.7	6.9 ± 2.0	17.9 ± 2.0	1171.9 ± 141.2	431.3 ± 187.5
JA 122	2.4 ± 0.6	0.6 ± 0.4	70.0 ± 10.7	92.5 ± 10.4	4.1 ± 0.7	17.8 ± 1.2	1143.8 ± 125.8	706.3 ± 94.3
JA 6	2.6 ± 0.5	0.7 ± 0.6	62.5 ± 12.8	50.0 ± 10.7	3.5 ± 0.9	18.9 ± 1.3	1148.1 ± 77.8	31.3 ± 9.4
HEL 62	1.6 ± 0.3	1.3 ± 0.5	87.5 ± 10.4	87.5 ± 14.9	3.0 ± 1.0	10.4 ± 1.1	1562.5 ± 45.3	825.0 ± 138.2
JA 2	1.8 ± 0.6	0.9 ± 0.5	77.5 ± 12.8	92.5 ± 10.4	5.5 ± 0.5	17.7 ± 1.7	1164.4 ± 99.8	425.0 ± 139.7
JA 102	2.3 ± 0.5	1.2 ± 0.5	85.0 ± 9.3	97.5 ± 7.1	4.3 ± 0.4	15.0 ± 1.7	1341.3 ± 152.8	850.0 ± 69.0
Max.	3.5	2.3	100.0	100.0	9.0	22.5	1600.0	950.0
Min.	0.5	0.1	40.0	40.0	2.0	7.3	1000.0	25.0
Mean	1.8	0.8	71.6	75.5	4.8	16.6	1262.7	463.3

Conclusion

Some Jerusalem artichoke genotypes that exhibited resistance to *S. rolfsii* at the seedling stage were not resistant at the adult stage. The correlations between the seedling stage and the adult plant stage were low for all traits. The results pointed out that the mechanisms controlling resistance to *S. rolfsii* at seedling and adult growth stages might be different. Therefore, selection only at the seedling stage will not be effective.

References

Agrios, G.N. 2005. Plant Pathology 5th ed. Academic Press, London.

Akram, A., S.M. Iqbal, C.A. Rauf and R. Aleem. 2008. Detection of resistant sources for collar rot disease in chickpea germplasm. Pak. J. Bot. 40: 2211-2215.

Fery, R.L. and P.D. Dukes. 2002. Southern blight (*Sclerotium rolfsii* Sacc.) of cowpea: yield-loss estimates and sources of resistance. Crop Prot. 21: 403-408.

Hovmøller, M.S. 2007. Sources of seedling and adult plant resistance to *Puccinia striiformis* f.sp. *tritici* in European wheats. Plant Breed.126: 225-233.

Li, H., F. Smyth, M.J. Barbetti and K. Sivasithamparam. 2006. Relationship between *Brassica napus* seedling and adult plant responses to *Leptosphaeria maculans* is determined by plant growth stage at inoculation and temperature regime. Field Crops Res. 96: 428–437.

Marcel, T.C., B. Gorguet, M.T. Ta, Z. Kohutova, A. Vels and R.E. Niks. 2008. Isolate specificity of quantitative trait loci for partial resistance of barley to *Puccinia hordei* confirmed in mapping populations and near-isogenic lines. New Phytol.177: 743-755.

Prashant, G.G., S. Davinder and F.P. Robert. 2009. Evaluation of seedling and adult plant resistance to *Puccinia hordei* in barley. Euphytica. 166: 183-197.

Shew, B.B., J.C. Wynne and M.K. Beute. 1987. Field, microplot, and greenhouse evaluation of resistance to *Sclerotium rolfsii* in peanut. Plant Dis. 71: 188-191.

Wang, Z.L., L.H. Li, Z.H. He, X.Y. Duan, Y.L. Zhou, X.M. Chen, M. Lillemo, R.P. Singh, H. Wang and X.C. Xia. 2005. Seedling and adult plant resistance to powdery mildew in Chinese bread wheat cultivars and lines. Plant Dis. 89: 457-463.

Xu, Z., M.L. Gleason and D.S. Mueller. 2009. Development of rapid method using oxalic acid to assess resistance among hosta cultivars to petiole rot caused by *Sclerotium rolfsii* var. *delphinii*. Online. Plant Health Progress. doi: 10.1094/PHP-2009-0128-01-RS.

Publication

Sennoi, R., S. Jogloy, W. Saksirirat, P. Banterng, T. Kesmala and A. Patanothai. 2013. Evaluation of seedling and adult plant stages resistance to *Sclerotium rolfsii* in Jerusalem artichoke (*Helianthus tuberosus* L.). SABRAO J. Breed. Genet. 45 (2): 324-331.

2.10 Biological control of southern stem rot caused by *Sclerotium rolfsii* using *Trichoderma harzianum* and arbuscular mycorrhizal fungi on Jerusalem artichoke (*Helianthus tuberosus* L.)

R. Sennoi, N. Singkham, S. Jogloy, S. Boonlue, W. Saksirirat,
T. Kesmala and A. Patanothai

Jerusalem artichoke *(Helianthus tuberosus* L.) has been identified as a crop with a high potential for inulin production in Thailand (Puttha *et al.*, 2012) and for producing food, ethanol, and industrial products in China (Jin *et al.*, 2012). Southern stem rot caused by *Sclerotium rolfsii* is a major disease of the crop, both in the temperate (Koike, 2004) and in the tropical (Sennoi *et al.*, 2012) regions.

Biological control has been investigated for its potential to provide a more viable and sustainable means to control southern stem rot disease in several crops (Ika *et al.*, 2011; Rakh *et al.*, 2011). The use of *Trichoderma* spp. for controlling *S. rolfsii* has been reported (Cilliers *et al.*, 2003). Among them are *Trichoderma harzianum* and *Trichoderma koningii*. Arbuscular mycorrhizal fungi (AMF) have also been applied for biological control of plant pathogens. The fungus also can help increase mineral absorption by host plants and alter the structure and biochemical characteristics of root cells. Combined AMF and *Trichoderma* spp. has been shown to increase plant growth and control plant diseases (Chandanie *et al.*, 2009). However, this approach has not been investigated in Jerusalem artichoke.

Objectives

The objective of this study was to evaluate the efficacy of co-inoculation of *T. harzianum* and Arbuscular mycorrhizal fungi (AMF) in controlling southern stem rot caused by *S. rolfsii* in Jerusalem artichoke.

Materials and Methods

Two greenhouse experiments were conducted during October to November 2011 using a randomized complete block design with four replicated. The treatments were 2 × 2 × 2 factorial combinations of two Jerusalem artichoke genotypes (HEL 246 and JA 37), presence or absence of *T. harzianum* isolate T9 (originally isolated in Thailand) and presence or absence of *Glomus clarum* isolate KKURA0305. The time from inoculation until permanent wilting (defined as wilting of all leaves on a plant) was determined by observing the plants daily until 30 days after inoculation with *S. rolfsii*. The number of plants with permanent wilting was later converted to disease incidence (% symptomatic plants). Plant height was measured every other day, and plant weight was determined as soon as the shoot had wilted permanently. Shoots were excised at the stem base, and the roots were removed from the soil by washing them with tap water. Shoots and roots were oven-dried at 80 °C for 72 h, and their dry weights were measured.

Results

Combined analysis of variance for the two trials showed significant main effects and interactions for disease incidence and days to permanent wilting (Table 1). Significant differences between Jerusalem artichoke genotypes were observed for percent disease incidence and days to permanent wilting. The interactions of genotype × *T. harzianum* (G × T), *T. harzianum* × *G. clarum* (T × M), and genotype × *T. harzianum* ×

G. clarum (G × T × M) were also highly significant for these traits. Jerusalem artichoke genotypes were not statistically different for plant height, shoot dry weight and root dry weight. Plants inoculated with *G. clarum* or *T. harzianum* were not statistically different from the non-inoculated control.

Disease incidence in the treatment in which *T. harzianum* was the sole biological control amendment did not differ significantly from the control treatment in which *T. harzianum* was absent (Table 2). In contrast, inoculation with *G. clarum* resulted in a significantly lower disease incidence and a longer period of time to permanent wilting than the control treatment without *G. clarum*. Furthermore, a combination of *T. harzianum* and *G. clarum* was not better than *G. clarum* alone in controlling *S. rolfsii*.

Disease incidence in HEL 246 with *T. harzianum* present was 51% compared to 68% in its absence (Fig. 1). For JA 37, in contrast, inoculation with *T. harzianum* resulted in a higher in disease incidence (85%) than the non-inoculated control (66%). Similarly, HEL 246 plants inoculated with *T. harzianum* required 10 days to permanent wilting compared to 6 days for the non-inoculated control (Fig. 2), whereas *T. harzianum* inoculated JA 37 plants required 5 days to permanent wilting compared to 7 days for non-inoculated plants.

The interactions between genotype and *G. clarum* were significant for the disease incidence, but not for the time to permanent wilting. The incidences for HEL 246 and JA 37 without *G. clarum* were 80% and 86%, respectively (Fig. 3). When the plants were inoculated with *G. clarum,* the disease incidences were 38.8% and 65% for HEL 246 and JA 37, respectively.

Table 1. Mean squares in the combined ANOVA of disease incidence (%), days to permanent wilting, plant height, shoot dry weight and root dry weight for biological-control treatments against *Sclerotium rolfsii* on Jerusalem artichoke.

Source of variation	df	Plant height	Shoot dry weight	Root dry weight	% Disease incidence[a]	Days to permanent wilting[b]
Experiment (E)	1	37.3*	0.4**	0.01*	225.0 ^{ns}	0.4 ^{ns}
Rep within experiment	6	2.2	0.0	0.0	79.2	0.9
Genotype (G)	1	5.7 ^{ns}	0.0 ^{ns}	0.0 ^{ns}	4225.0**	58.1**
Trichoderma harzianum (T)	1	1.4 ^{ns}	0.0 ^{ns}	0.0 ^{ns}	25.0 ^{ns}	2.6*
Glomus clarum (M)	1	1.4 ^{ns}	0.1 ^{ns}	0.0 ^{ns}	156525.0**	70.1**
E × G	1	3.7 ^{ns}	0.1 ^{ns}	0.0 ^{ns}	0.0 ^{ns}	0.0 ^{ns}
E × T	1	4.0 ^{ns}	0.0 ^{ns}	0.0 ^{ns}	400.0 ^{ns}	0.1 ^{ns}
E × M	1	0.0 ^{ns}	0.0 ^{ns}	0.0 ^{ns}	100.0 ^{ns}	0.1 ^{ns}
G × T	1	7.1 ^{ns}	0.0 ^{ns}	0.0 ^{ns}	4900.0 **	112.9**
G × M	1	1.1 ^{ns}	0.1 ^{ns}	0.0 ^{ns}	1600.0 **	0.1 ^{ns}
T × M	1	0.3 ^{ns}	0.0 ^{ns}	0.0 ^{ns}	900.0**	13.1**
G × T × M	1	23.2 ^{ns}	0.1 ^{ns}	0.0 ^{ns}	1225.0**	23.8**
Pooled error	46	6.6	0.1	0.0	93.5	0.4
C.V. (%)		20.4	46.6	49.7	14.3	9.5

*, ** and ns Significant at P = 0.05, P = 0.01 and non significant probability levels, respectively.
[a] Disease incidence 5 days after inoculation with *S. rolfsii.*
[b] Time from date of inoculation with *S. rolfsii* until permanent wilting.

Table 2. Mean disease incidence and days to permanent wilting (averaged over experiments and genotypes) of two Jerusalem artichoke genotypes (HEL 246 and JA 37) for the different treatments.

Treatment	Disease incidence (%)[a]	Time to permanent wilting (days)[b]
-*T. harzianum*	66.9b	7.1b
+*T. harzianum*	68.1b	7.2b
-*G. clarum*	83.1a	5.4c
+*G. clarum*	51.8d	8.0a
+*T. harzianum*+ *G. clarum*	56.3c	7.9a

Means followed by the same letter in the same column do not differ significantly according to Fisher's least significant difference (LSD) test (P = 0.05).
[a] Disease incidence was evaluated at five days after inoculation with *S. rolfsii.*
[b] Time from the date of inoculation with *S. rolfsii* until permanent wilting.

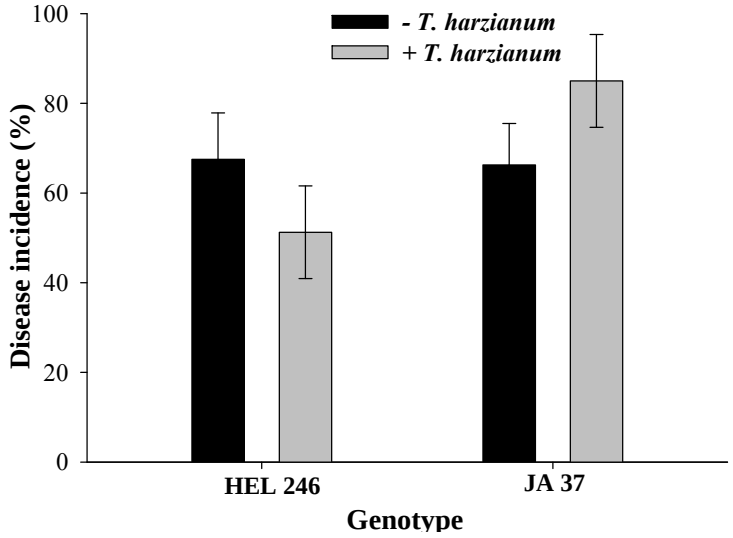


Fig. 1. Influence of *Trichoderma harzianum* isolate T9 on disease incidence of two Jerusalem artichoke genotypes.

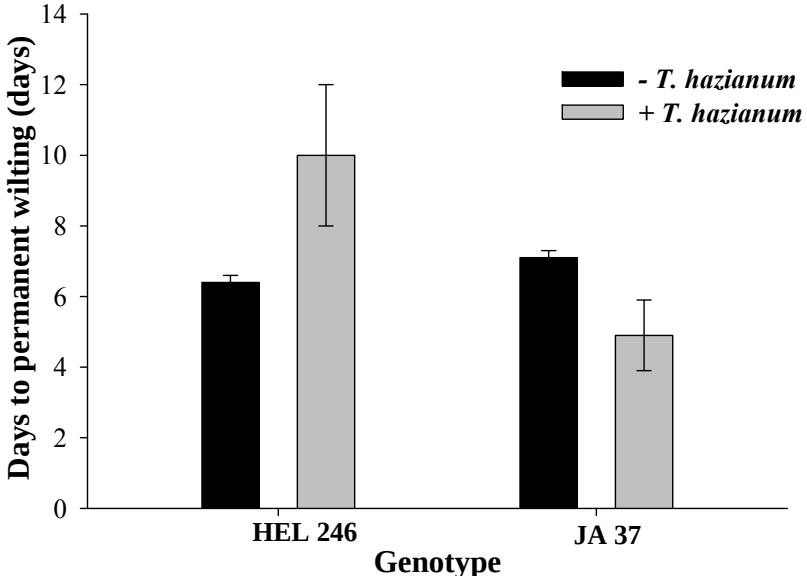


Fig. 2. Influence of *Trichoderma harzianum* T9 on days to permanent wilting of two Jerusalem artichoke genotypes.

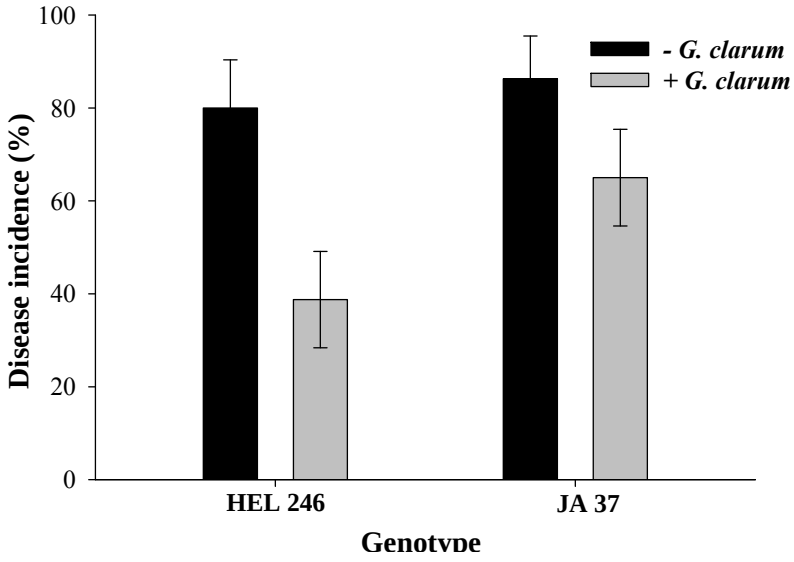


Fig. 3 Influencing of *Glomus clarum* KKURA0305 on disease incidence of two Jerusalem artichoke genotypes.

Comparing all eight treatments, the combination of cultivar HEL 246 with *G. clarum* and *T. harzianum* resulted in the lowest disease incidence (30%) (Fig. 4). For both cultivars, *G. clarum* resulted in a lower disease incidence than T. harzianum. HEL 246 inoculated with *G. clarum* and T. harzianum prior to inoculation with *S. rolfsii* had longer times to permanent wilting than when the same cultivar received no biological control

treatment. This cultivar alone or in combination with one or both biocontrol organisms had longer times to permanent wilting than JA 37, while cv. JA 37 with G. clarum took more time to wilt permanently than HEL 246 with *G. clarum* (Fig. 5).

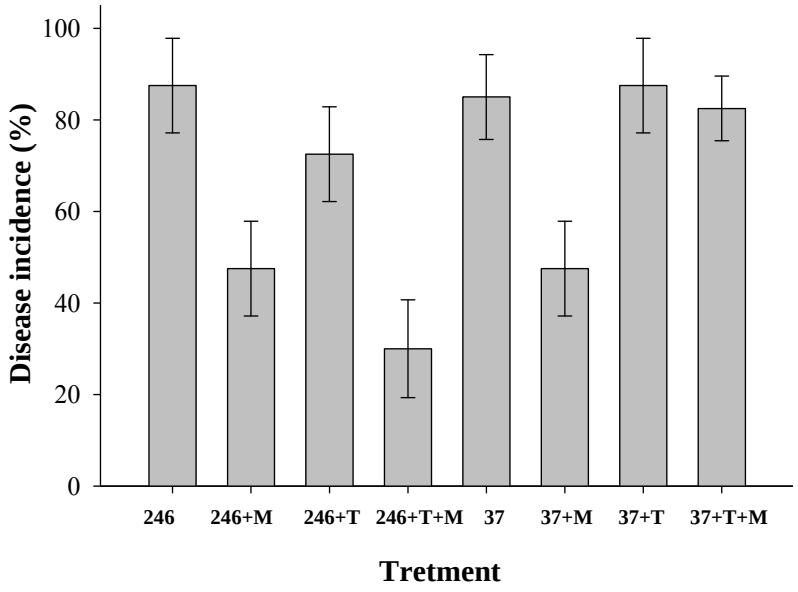


Fig. 4. Comparison of disease incidences caused by *Sclerotium rolfsii* among 8 treatments (HEL 246 and JA 37 (represented above as "246" and "37"); M-*Glomus clarum* and T-*Trichoderma harzianum*). Means are combined for two runs of the experiment.

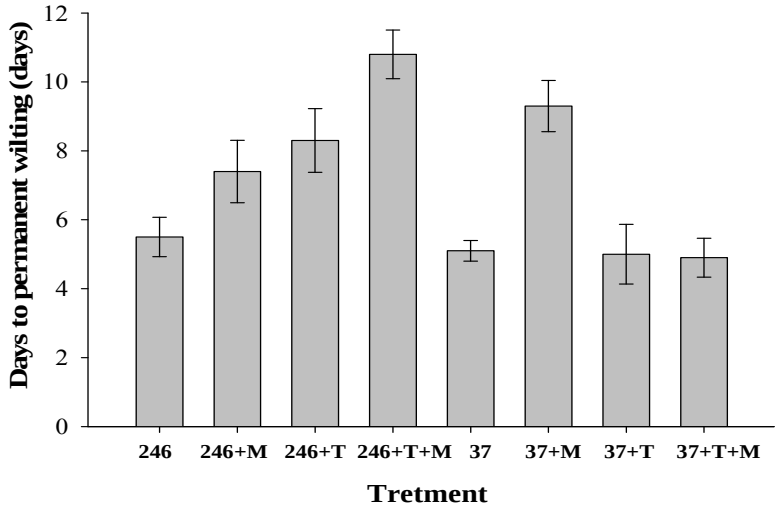


Fig. 5. Comparison of days to permanent wilting caused by *Sclerotium rolfsii* among 8 treatments (HEL 246 and JA 37 (represented above as "246" and "37"); M-*Glomus clarum* and T-*Trichoderma harzianum*). Means are combined for two runs of the experiment.

Conclusion

The combination of cv. HEL 246 with the addition of both *G. clarum* and *T. harzianum* had the lowest disease incidence (30%) and required the longest time to permanent wilting (11 days after inoculation). Inoculation of cv. JA 37 and HEL 246 with G. clarum alone gave better control of the disease than did inoculation with *T. harzianum* alone. The results are the first published report of biological control of *S. rolfsii* on Jerusalem artichoke.

References

Chandanie, W.A., M. Kubota and M. Hyakumachi. 2009. Interactions between the arbuscular mycorrhizal fungus *Glomus mosseae* and plant growth-promoting fungi and their significance for enhancing plant growth and suppressing damping-off of cucumber (*Cucumis sativus* L.). Appl. Soil Ecol. 41: 336–341.

Cilliers, A.J., J.A. Pretorius and P.S. Van Wyk. 2003. Integrated control of *Sclerotium rolfsii* on groundnut in South Africa. J. Phytopathol. 151: 249-258.

Ika, R.S., D. Syamsuddin, S. Nasir and M. Anton. 2011. Control of "damping off" disease caused by *Sclerotium rolfsii* Sacc. using actinomycetes and VAM fungi on soybean

in the dry land based on microorganism diversity of rhizosphere zone. Agrivita 33: 40–46.

Jin, S., L. Liu, Z. Liu, X. Long, H. Shao and J.Chen. 2012. Characterization of marine *Pseudomonas spp*. antagonist towards three tuber-rotting fungi from Jerusalem artichoke, a new industrial crop. Ind. Crop Prod. 43: 556-561.

Koike, S.T. 2004. Southern blight of Jerusalem artichoke caused by *Sclerotium rolfsii* in California. Plant Dis. 88: 769.

Puttha, R., S. Jogloy, P.P. Wangsomnuk, S. Srijaranai, T. Kesmala and A. Patanothai. 2012. Genotypic variability and genotype by environment interactions for inulin content of Jerusalem artichoke germplasm. Euphytica 183: 119-131.

Rakh, R.R., L.S. Raut, S.M. Dalvi1 and A.V. Manwar. 2011.Biological control of *Sclerotium rolfsii*, causing stem rot of groundnut by *Pseudomonas cf. monteilii* 9. Rec. Res. Sci. Tech. 3: 26–34.

Sennoi, R., S. Jogloy, W. Saksirirat, T. Kesmala and A. Patanothai. 2012. Genotypic variation of resistance to southern stem rot of Jerusalem artichoke caused by *Sclerotium rolfsii*. Euphytica 190: 415–424.

Publication

Sennoi, R., N. Singkham, S. Jogloy, S. Boonlue, W. Saksirirat, T. Kesmala and A. Patanothai. 2013. Biological control of southern stem rot caused by *Sclerotium rolfsii* using *Trichoderma harzianum* and *arbuscular mycorrhizal* fungi onJerusalem artichoke (*Helianthus tuberosus* L.). Crop Prot. 54: 148-153. (Impact factor = 1.539).

Sub-project 3

Basic research for supporting varietal improvement of sweet gourd (*Momordica cochinchinensis* Spreng) for high fruit yield and high lycopene and *β*-carotene content

Assistant Prof. Dr. Patcharin Songsri

Sub-project leader

Spiny bitter gourd (*Momordica cochinchinensis* (Lour.) Spreng) Sweet gourd is an indigenous crop of Southeast Asia which has long been used as a food and traditional medicine in East and Southeast Asia (Kubola and Siriamornpun, 2011). It's also called baby jackfruit, Gac fruit, sweet gourd, cochinchin gourd. The crop is known under different names in different countries such as Gac in Vietnam, Fak kao in Thailand, Bhat Kerala in India, Moc Niet Tu in China and Mak kao in Laos. It is a member of perennial dioecious cucurbit family (Sanwal *et al.* 2011). The crop is becoming to be known as a premier source of carotenoids, especially β-carotene and lycopene (Aoki, *et al.*, 2002; Vuong, *et al.*, 2002; Voung and King, 2003; Vong, *et al.*, 2006; Ishida *et al.*, 2009). Sweet gourd aril has over 70 times of lycopene/gram higher than that of tomato and this has important implications for prostate health. Its β-carotene is also more bioavailable than that of the synthetic form (Vuong *et al.*, 2002). Thus, sweet gourd provides a rich source of valuable antioxidants that have good bioavailability (Burke *et al.*, 2005).

As spiny bitter gourd has low variation for morphological characters possibly due to variation in environment, we are interested in using molecular markers to investigate genetic diversity and genetic relatedness of these accessions, and we selected random amplified polymorphic DNA (RAPD) for this purpose as this method is rather simple, rapid and cost-effective compared to other molecular markers (William *et al.*, 1990; Rafalski and Tingey, 1993; Dey *et al.*, 2006). To the best of our knowledge, genetic diversity and genetic relatedness based on molecular markers in spiny bitter gourd have not been reported to date.

This sub-project consists of two studies:

3.1 Molecular diversity among selected *Momordica cochinchinensis* (Lour.) Spreng accessions using RAPD markers

3.2 Genetics diversity based on agricultural traits and phytochemical contents in spiny bitter gourd (*Momordica cochinchinensis* (Lour.) Spreng)

The details of these studies are presented below.

References

Aoki, H., N.T. Kieu, N. Kuze, K. Tomisaka and N. Van Chuyen. 2002. Carotenoid pigments in gacfruit (*Momordica cochinchinensis* Spreng). Biosci. Biotechnol. Biochem. 66: 2479-2482.

Burke, D.S., C.R. Smidt and L.T. Vuong. 2005. *Momordica cochinchinensis*, *Rosa roxburghii*, wolfberry, and sea buckthorn - Highly nutritional fruit supported by tradition and science. Curr. Top. Nutraceut. R. 3(4): 259-266.

Dey, S.S., A.K. Singh, D. Chandel and T.K. Behera. 2006. Genetic diversity of bitter gourd (*Momordica charantia* L.) genotypes revealed by RAPD markers and agronomic traits. Sci. Horti. 109: 21-28.

Ishida B.K. and M.H. Chapman. 2009. Carotenoid extraction from plants using a novel, environmentally friendly solvent. J. Agric. Food Chem. 57: 1051-1059.

Kubola, J. and S. Siriamornpun. 2011. Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng). Food Chem. 127: 1138–1145.

Rafalski, J.A. and S.V. Tingey. 1993. Genetic diagnos is in plant breeding: RAPDs microsatellites and machines. Trends Genet. 9: 275-280.

Vuong, L.T., S.R. Dueker and S.P. Murphy. 2002. Plasma β-carotene and retinol concentrations of children increase after a 30-d supplementation with the fruit *Momordica cochinchinensis* (gac). Clin. Nutr. 75: 872-879.

Vuong, T.L. and J.C. King. 2003. A method of preserving and testing the acceptability of gac fruit oil, a good source of β-carotene and essential fatty acids. Food Nutr. Bull. 24: 224-230.

Vuong, L.T., A.A. Franke, L.J. Custer and S.P. Murphy. 2006. *Momordica cochinchinensis* Spreng. (gac) fruit carotenoids reevaluated. Food Compos. Anal. 19: 664-668.

William, J.G.K., A.R. Kubelik, K.J. Livak, J.A.Rafalski and S.V. Tingery. 1990. DNA polymorphism samplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res. 18: 6531-6535.

3.1. Molecular diversity among selected *Momordica cochinchinensis* (Lour.) Spreng accessions using RAPD markers

N. Bootprom, P. Songsri, B. Suriharn, P. Chareonsap, J. Sanitchon and K. Lertrat

Spiny bitter gourd (*Momordica cochinchinensis* (Lour.) Spreng) or gac fruit is an underutilized tropical vegetable crop in Asia (Kubola and Siriamornpun, 2011). Placenta or aril (seed membrane) and fruit oil are excellent sources of bio-accessible carotenoids (lycopene and beta carotene) (Vuong *et al.*, 2006). The crop, thus, provides an acceptable source of high levels of valuable antioxidants that have good bioavailability (Burke *et al.*, 2005). Spiny bitter gourd aril has lycopene/gram over 70 times higher than that of tomato, and the lycopene has been well recognized as a health beneficial phytochemical. Fatty acids in spiny bitter gourd make β-carotene more bioavailable than that of the synthetic form (Vuong *et al.*, 2002). Conversely, consumption of certain β-carotene-rich foods has been shown to produce little increase in plasma β-carotene or retinol concentrations (Vuong *et al.*, 2002).

In order to improve this crop for industrial uses, several agronomic traits such as high carotenoids and yield should be improved through breeding because of the requirement for high and uniform phyto-chemical content of the industry. The first germplasm collection was carried out in Thailand and Vietnam, and some accessions were obtained. However, genetic diversity and genetic relationships among these landraces are not known. This information is very important for genetic improvement of this crop.

Objective

The objective of this research was to study genetic diversity and genetic relatedness among 25 landraces of spiny bitter gourd collected in Thailand and Vietnam using RAPD markers. This information is very useful for genetic improvement of this crop.

Materials and Methods

Twenty five accessions of spiny bitter gourd were collected from Thailand and Vietnam. They were grown in October 2010 at the Fruit Crops Research Station, Faculty of Agriculture, Khon Kaen University, using a 2 x 6 m plant spacing. The chemical fertilizer was applied at 15, 45, and 90 days after transplanting. Pests and diseases were controlled by weekly applications of insecticide and fungicide.

Genomic DNA was extracted from young, healthy leaves following the procedure of DNA Trap I (DNA Technology Laboratory, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom), and the DNA samples were run in 1% agarose gel to check the quality. Thirty six RAPD primers were used for screening polymorphism in spiny bitter gourd accessions.

The amplification reactions were set at a final volume of 20 µL contained 15 ng genomic DNA, 5x PCR buffer, 25 mM $MgCl_2$, 10 mM dNTPs, 5 µM primer and 0.5 U Tag DNA Polymerase. DNA amplification was carried out in a DNA Thermal Cycler. DNA denaturation was done at 94^{o}C for 5 min, followed by a 43-cycle amplification (94 ^{o}C, 30 sec; 32^{o}C, 1 min; 72 ^{o}C, 2 min) and the final extension step at 72 ^{o}C for 5 min. Finally, the amplified product was brought down at 10 ^{o}C. The amplified products were run in 2% agarose gel in 1X TBE buffer. Gel electrophoresis was performed at a constant voltage of 75V for 2.30 hrs and stained with ethidium bromide for 30 min. The RAPD banding patterns were photographed using the gel documentation system of Biorad.

Reproducible DNA bands, i.e. bands present in both repetitions of individual sample were scored manually. Weak bands with negligible intensity were excluded from final data analysis. Band profiles for each parent were scored in a binary mode with 1 indicating its presence and 0 in dicating its absence. Pairwise comparisons of genotypes employed to calculate Jaccard's similarity coefficient (GS):

$$\frac{a}{n - d'}$$

where a is the number of positive matches, d' is the number of negative matches and n is the total sample size (Jaccard, 1908). Genetic distance (GD) between pairs of the accessions were estimated as GD = 1 - GS. A dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic averages (UPGMA) and the computation for multivariate analysis was done using the computer program NTSYSpc software Version 2.0 (Rohlf, 1998).

Results

Eleven decamer primers from 36 primers gave reproducible DNA polymorphisms, and the numbers of polymorphic bands ranged from 250 bp to 2000 bp. A sample of polymorphic bands for OPW03 primer is shown in Fig. 1. These 11 primers generated a total of 176 reproducible bands. The total numbers of bands ranged from 10 to 23 and the polymorphic bands ranged from 10 to 23. Most primers were 100% polymorphic except for OPF10 (92%). The maximum number of polymorphic bands (23) was obtained with the primers OPW03.

The coefficients of genetic similarity within spiny bitter gourd genotypes were from 0.63 to 0.90. Genetic similarity coefficient (0.79) was the lowest between the accession KKU ac. 09-008(F) from Thailand and the accession KKU ac. 10-094(M) from Vietnam, whereas the similarity coefficient (0.90) was the highest between the accession KKU ac. 10-040(F) and the accession KKU ac. 10-043(F). Both of them were collected in Thailand. Other accessions collected from Thailand also showed high genetic similarity.

The dendrogram constructed based on the RAPD analysis using UPGMA (NTSYS-PC) is shown in Fig. 2. The spiny bitter gourd accessions were grouped into eight major clusters. Cluster 1 had one accession (KKU ac. 10-094(M)) from Vietnam. Cluster 2 had one accession (KKU ac. 10-032(M)) from Thailand. Cluster 3 had one accession (KKU ac. 10-036(M)) from Thailand. Cluster 4 had KKU ac. 10-090(M), KKU ac. 09-087(M), KKU ac. 10-040(M), KKU ac. 09-034(M), KKU ac. 09-033(M), KKU ac. 09-018(M), and KKU ac. 09-003(M) from Thailand. Cluster 5 had one accession (KKU ac. 10-094(F)) from Vietnam. Cluster 6 had one accession (KKU ac. 10-087(F)) from Thailand. Cluster 7 had KKU ac. 10-077(F), KKU ac. 10-049(F), KKU ac. 10-043(F), KKU ac. 10-040(F), KKU ac. 10-038(F), KKU ac. 09-036(F) and KKU ac. 09-034(F)) from Thailand, and Cluster 8 had KKU ac. 09-016(F), KKU ac. 09-013(F), KKU ac. 09-019(F), KKU ac. 09-018(F), KKU ac. 09-030(F) and KKU ac. 09-008(F) from Thailand.

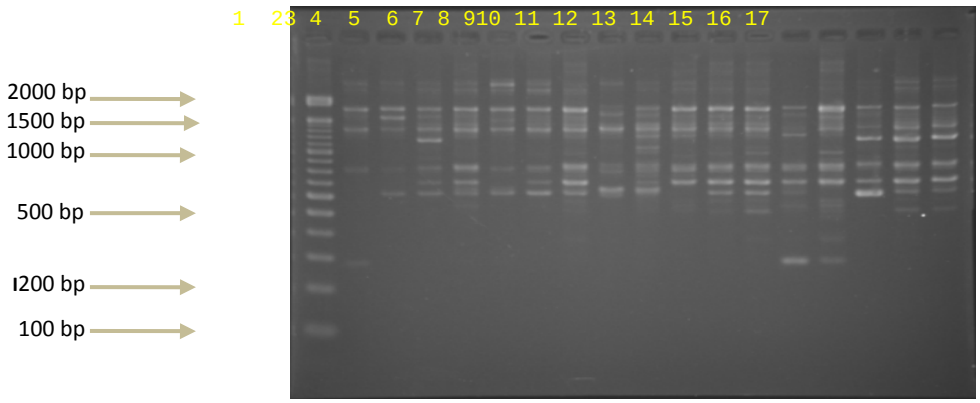


Fig. 1. RAPD polymorphism amongst *Momordicacochinchinensis* (Lour.) Spreng genotypes detected with primer OPW03. M = primer OPW03; 1 = KKU ac.09-008(F); 2 = KKU ac.09-013(F); 3 = KKU ac.09-016(F); 4 = KKU ac.09-018(F); 5 = KKU ac.09-019(F); 6 = KKU ac.09-030(F); 7 = KKU ac.09-034(F); 8 = KKU ac.09-036(F); 9 = KKU ac.10-038(F); 10 = KKU ac.10-040(F); 11 = KKU ac.10-043(F); 12 = KKU ac.10-049(F); 13 = KKU ac.10-077(F); 14 = KKU ac.10-087(F); 15 = KKU ac.10-094(F); 16 = KKU ac.10-094(M); 17 = KKU ac.09-003(M)

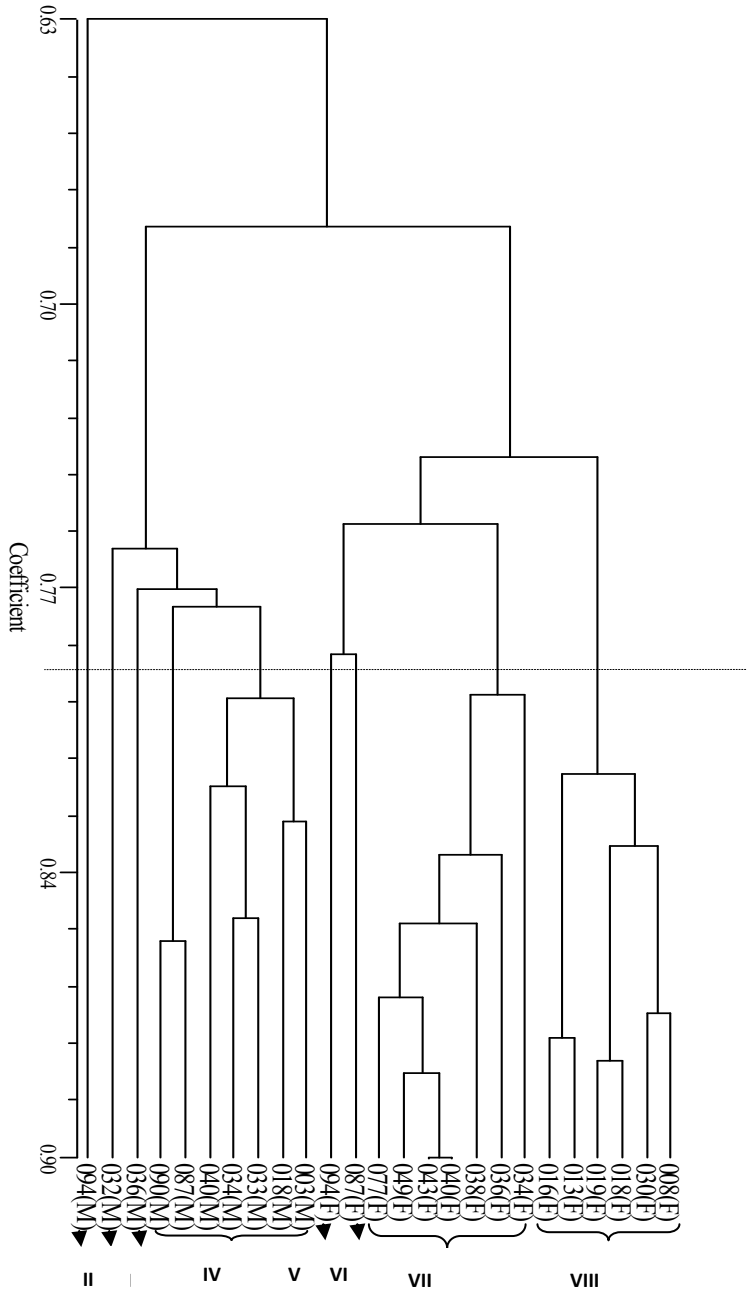


Fig. 2. Dendrogram showing genetic relatedness of 25 spiny bitter gourd accessions collected from Thailand and Vietnam using 11 RAPD primers.

It is interesting to note that the dendrogram clearly separated male and female genotypes into distinct clusters. Male genotypes formed clusters 1, 2, 3 and 4, whereas female genotypes consisted of clusters 5, 6, 7 and 8. These markers are potentially used for distinguishing male and female genotypes. This finding will be of great benefit for crop breeding and crop production because sex of the plant can be identified at the seedling stage.

Conclusion

RAPD markers could clearly separate the source and sex of 25 spiny bitter gourd genotypes. The 25 accessions were divided into eight major groups based on their genetic dissimilarity. RAPD markers were found to be an effective tool for germplasm management (such as finger printing), diversity study, and selection of parents and progenies with desire characters. The markers may also be useful in marker-assisted selection for economically important traits.

References

Burke DS, C.R. Smidt, and L.T. Vuong LT. 2005.*Momordica cochinchinensis*, *Rosa roxburghii*, wolfberry, and sea buckthorn-Highly nutritional fruit supported by tradition and science. Curr. Top Nutraceut. R. 3(4): 259-266.

Jaccard, P.1908. Nouvelles recherches surla distribution florale. Bull Soc. Vaud. Sci. Nat. 44: 223-270.

Kubola, J. and S. Siriamornpun. 2011. Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng). Food Chem. 127: 1138–1145.

Rohlf, F.J. 1998.NTSYS-pc Numerical taxonomy and multivariate analysis system Version 2.01. Exeter Software, NY, USA.

Vuong, L.T., S.R. Dueker and S.P. Murphy. 2002. Plasma β-carotene and retinol concentrations of children increase after a 30-d supplementation with the fruit *Momordica cochinchinensis* (gac). Clin. Nutr. 75: 872-879.

Vuong, L.T., A.A. Franke, L.J. Custer and S.P. Murphy. 2006. *Momordica cochinchinensis* Spreng. (gac) fruit carotenoids reevaluated. Food Compos. Anal. 19: 664–668.

Publication

Bootprom, N., P. Songsri, B. Suriharn, P. Chareonsap, J. Sanitchon and K. Lertrat. 2012. Molecular diversity among selected *Momordica cochinchinensis* (Lour.) Spreng accessions using RAPD markers. SABRAO J. Breed. Genet. 44 (2): 406-417. (impact factor = 0.227)

3.2 Genetics diversity based on agricultural traits and phytochemical contents inspiny bitter gourd (*Momordicacochinchinensis*(Lour.) Spreng)

N. Bootprom, P. Songsri, B. Suriharn, K. Lomthaisong and K. Lertrat

Spiny bitter gourd (*Momordica cochinchinensis* (Lour.) Spreng) is a member of the perennial dioecious cucurbit family (Sanwal *et al.*, 2011), which has long been used as a food and traditional medicine in East and Southeast Asia (Kubola and Siriamornpun 2011). Placenta or aril (seed membrane) is an excellent source of bio-accessible carotenoids (lycopene and beta-carotene) (Vuong *et al.*, 2006). These phytochemicals are beneficial to health as they can reduce the risk of several diseases such as prostate cancer, colon cancer, stomach cancer and cerebral thrombosis (Voung *et al.*, 2006; Ishida *et al.*,

2009). In addition, spiny bitter gourd oil has been readily accepted by women and children of Vietnam, and consumption of the oil can reduce lard intake (Vuong *et al.*, 2002; Vuong and King, 2003; Ishida *et al.*, 2004).

The potential uses of this underutilized crop are as raw materials for natural colorant industry, food additive and functional food products because of its high carotenoid content in placenta. Breeding for high and stable carotenoid content and yield is important for industrial utilization of spiny bitter gourd, and the information on genetic diversity in the germplasm collection is important for an effective breeding program.

Objective

The objective of this study was to determine the genetic diversity and the genetic relatedness between the 26 landraces of spiny bitter gourd accessions collected from Thailand and Vietnam, using horticultural traits and phytochemical contents.

Materials and Methods

The 26 spiny bitter gourd accessions, of which 25 were collected from Thailand and one from Vietnam, were evaluated in this study. These accessions were grown in October 2010 at the fruit orchard section, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand. The accessions were arranged in a randomized complete block design with three replications. The spacing was 6 m between plants and 2 m between rows. NPK fertilizers formula 15–15–15 were applied at 15, 45 and 90 days after transplanting. Pests and diseases were controlled by weekly applications of insecticides and fungicides. Irrigation was applied as necessary by mini-sprinkler to avoid drought stress. Metal supports were also constructed for each plot. The plants that produced male flowers were used as pollinators to pollinate all accessions in the experiment. The receptive flowers were artificially pollinated and the pollinated flowers were tagged and labeled.

Data were recorded on fruit fresh weight, aril weight, days to fruit maturity, number of seeds, total carotenoids, lycopene, beta-carotene and lycopene per fruit. The fruits were harvested at ripening maturity which was determined by red skin of the fruits. One fruit was randomly chosen from three ripening fruits for each of three sampling dates which were considered as three replications in un-replicated plot. The ripening fruits were determined by red fruits and red aril. The fruits were cleaned and separated into pulp, peeled seeds and aril. The aril samples were stored in a freezer (-20 oC) until further analysis.

Lycopene and beta-carotene were analyzed in triplicate using the HPLC by a method modified from Kubola and Siriamornpun (2011). Analysis was performed using Shimadzu LC-20AD pumps, a SPD-20M diode array detector, and inertsil ODS-3 C-18 column reverse phase (4.6 x 250 mm i.d., 5 μM). The mobile phase consisted of Acetonitrile methanol (solvent A)/ Dichloromethane (solvent B) and Methanol (solvent C) at a flow rate of 1 ml/min. The absorbance was read at 450 nm.

The data for the agronomic traits and phytochemical contents were analyzed statistically according to a randomized complete block design. The relationships among traits were calculated by the Pearson's correlation analysis using accession means (Ireland, 2010). A data matrix of the 26 accessions was constructed using means for agronomic traits and phytochemical contents. The cluster analysis based on Ward's method and Squared Euclidian distance was performed and the dendrogram was constructed. All calculations were performed using the SAS 6.12 software.

Results

High variations were observed for all characters under investigation, as indicated by high CV values and high F-ratio (Table 1). KKU ac. 10-094 had the highest fruit fresh weight (1787 g/fruit), aril weight (393 g/fruit) and days to fruit maturity (108 days after flowering), whereas KKU ac. 09-010 had the highest number of seeds (53 seeds/fruit). Other accessions had smaller fruits and lower aril weight and matured earlier than KKU ac. 10-094 (Table 2). Also KKU ac. 10-094 had the highest total carotenoids (4041 µg/g dry weight), beta-carotene (1513 µg/g dry weight) and lycopene per fruit (52608 µg/fruit) and its lycopene content was also high although it was lower than 2487 µg/g dry weight of KKU ac. 09-008. Other interesting accessions included KKU ac. 09-002 because of high total carotenoids, KKU ac. 09-008 because of high total carotenoids and lycopne and KKU ac. 09-016 because of high total carotenoids (Table 3).

Positive and high correlation coefficients between total carotenoids with lycopene, beta-carotene and lycopene per fruit were observed (Table 4). Total carotenoids also had positive correlations with days to fruit maturity, aril weight and fruit fresh weight although it was not significant, but it is not associated with number of seeds.

Lycopene was positively and significantly associated with beta-carotene and lycopene per fruit, but was not significantly associated with fruit fresh weight, aril weight, days to fruit maturity and number of seeds. Beta-carotene was associated with lycopene per fruit, fruit fresh weight, aril weight and days to fruit maturity, but was not associated with number of seeds. Similarly, lycopene per fruit was associated with fruit fresh weight, aril weight and days to fruit maturity, but was not associated with number of seeds.

Fruit characters (fruit fresh weight and aril weight) and days to fruit maturity were inter-related, with correlation coefficients ranging from $P \leq 0.01$, $r = 0.80$ to $P \leq 0.01$, $r = 0.94$. Fruit fresh weight was also associated with number of seeds, however, aril weight and days to fruit maturity were not associated with number of seeds (Table 4).

A dendrogram constructed based on agronomic traits and phytochemical contents grouped 26 accessions of spiny bitter gourd into seven distinct clusters at the coefficient of determination (R2) of 0.99 (Fig. 1).

Cluster 1 consisted of KKU ac.09-002, KKU ac.09-016 and KKU ac.09-010. This cluster had total carotenoids between 2226 to 3551 µg/g dry weight, lycopene between 1274 to 1734 µg/g dry weight, beta-carotene between 384 to 1159 µg/g dry weight, lycopene per fruit between 13374 to 14545 µg/fruit, fruit fresh weight between 355 to 715 g/fruit, aril weight between 65 to 90 g/fruit, days to fruit maturity between 55 to 59 days after flowering, number of seeds between 35 to 53 seeds/fruit and elliptical with pointed end fruit type.

Cluster 2 had one accession (KKU ac. 09-008). This accession had 3412 µg/g dry weight of total carotenoids, 2487 µg/g dry weight of lycopene, 141 µg/g dry weight of beta-carotene, 16024 µg/fruit of lycopene per fruit, 280 g/fruit of fresh fruit weight, 48 g/fruit of aril weight, 60 days after flowering of fruit maturity, 34 seeds/fruit and round fruit type.

Cluster 3 comprised KKU ac.09-003, KKU ac.09-027, KKU ac.09-018, KKU ac.09-019, KKU ac.09-030, Kaenpayorm1, KKU ac.10-049, KKU ac.10-090 and KKU ac.10-077. This cluster had total carotenoids between 2241 to 3447 µg/g dry weight, lycopene between 1513 to 1734 µg/g dry weight, beta-carotene between 384 to 763 µg/g dry weight, lycopene per fruit between 18639 to 23816 µg/fruit, fruit fresh weight between 643 to 715 g/fruit, aril weight between 120 to 142 g/fruit, days to fruit maturity

between 55 to 61 days after flowering, number of seeds between 22 to 48 seeds/fruit and elliptical with pointed end fruit type.

Cluster 4 consisted of KKU ac.09-004, KKU ac.10-087, KKU ac.09-012, KKU ac.09-034, KKU ac.09-033 and KKU ac.10-080. This cluster had total carotenoids between 840 to 2599 µg/g dry weight, lycopene between 364 to 1294 µg/g dry weight, beta-carotene between 100 to 715 µg/g dry weight, lycopene per fruit between 2846 to 9673 µg/fruit, fruit fresh weight between 350 to 725 g/fruit, aril weight between 55 to 138 g/fruit, days to fruit maturity between 52 to 73 days after flowering, number of seeds between 33 to 50 seeds/fruit and elliptical with pointed end fruit type.

Cluster 5 had two accessions (KKU ac.09-011 and KKU ac.09-013). This cluster had total carotenoids of 1550 and 1,934 µg/g dry weight, lycopene of 555 and 827 µg/g dry weight, beta-carotene of 234 and 242 µg/g dry weight, lycopene per fruit of 4,853 and 5033 µg/fruit, fruit fresh weight of 410 and 548 g/fruit, aril weight of 60 and 80 g/fruit, days to fruit maturity of 55 and 58 days after flowering, number of seeds of 30 and 34 seeds/fruit and elliptical with pointed end fruit type.

Cluster 6 consisted of KKU ac.10-020, KKU ac.10-043, KKU ac.10-086 and KKU ac.11-138. This cluster had total carotenoids between 840 to 1285 µg/g dry weight, lycopene between 364 to 490 µg/g dry weight, beta-carotene between 100 to 392 µg/g dry weight, lycopene per fruit between 2500 to 3797 µg/fruit, fruit fresh weight between 350 to 568 g/fruit, aril weight between 58 to 85 g/fruit, days to fruit maturity between 52 to 60 days after flowering, number of seeds between 21 to 44 seeds/fruit and elliptical with pointed end fruit type.

Cluster 7 had one accession (KKU ac. 10-094) collected from Vietnam. This accession had 4041 µg/g dry weight of total carotenoid, 1893 µg/g dry weight of lycopene, 1513 µg/g dry weight of beta-carotene, 52608 µg/fruit of lycopene per fruit, 1787 g/fruit of fresh fruit weight, 393 g/fruit of aril weight, 108 days after flowering to fruit maturity, 43 seeds/fruit and flattened (oblate) fruit type.

Table 1. Means, standard errors (SE), minimum (min), maximum (max), coefficient of variation (CV) and F-ratio of quantitative characters of 26 accessions of spiny bitter gourd.

Characters	Mean ± SE	Min - Max	C.V. (%)	F-ratio
Total carotenoid (µg/g dry weight)	2013 ± 261	840-4041	23	9.8**
Lycopene (µg/g dry weight)	1051 ± 131	364-2487	22	16.0**
Beta-carotene (µg/g dry weight)	417 ± 51	100-1513	21	39.6**
Lycopene per fruit (µg/fruit)	9746 ± 2099	2500-52608	37	20.4**
Fruit fresh weight (g/fruit)	578 ± 23	280-1787	7	152.7**
Aril weight (g/fruit)	87 ± 11	48-393	21	36.0**
Days to fruit maturity (days)	60 ± 2	52-108	5	37.8**
Number of seeds (seeds/fruit)	36 ± 2	21-53	8	22.0**

** significant at $P<0.01$

Table 2. Fruit fresh weight (g/fruit), aril weight (g/fruit), days to fruit maturity (days) and number of seeds (seeds/fruit) of 26 spiny bitter gourd accessions.

Accessions	Fruit fresh weight	Aril weight	Days to fruit maturity	Number of seeds
KKU ac.09-002	355 i	65 def	56 f-i	35 g-k
KKU ac.09-003	450 gh	65 def	63 c	23 n
KKU ac.09-004	725 c	110 bc	59 c-h	39 d-g
KKU ac.09-008	280 j	48 f	60 c-f	34 i-l
KKU ac.09-010	715 c	90 bcd	59 c-h	53 a
KKU ac.09-011	410 hi	60 def	58 d-h	34 h-l
KKU ac.09-012	460 gh	73 def	56 f-i	35 g-k
KKU ac.09-013	548 ef	80 cde	55 hi	30 lm
KKU ac.09-016	643 d	80 cde	55 ghi	42 def
KKU ac.09-018	455 gh	85 cde	58 d-h	36 g-k
KKU ac.10-019	478 g	85 cde	57 d-i	22 n
KKU ac.10-020	350 i	58 ef	52 i	21 n
KKU ac.09-027	495 fg	55 ef	59 c-h	28 m
KKU ac.09-030	553 ef	80 cde	58 d-h	37 f-j
KKU ac.09-033	715 c	82 cde	60 c-f	50 ab
KKU ac.09-034	673 cd	72 def	61 cde	33 j-m
KKU ac.10-043	568 e	67 def	60 c-g	44 cd
KKU ac.10-049	365 i	55 ef	62 cd	38 f-i
KKU ac.10-077	503 fg	65 def	55 ghi	37 f-j
KKU ac.10-080	555 ef	62 def	57 e-i	39 e-h
KKU ac.10-086	507 efg	85 cde	57 e-i	35 j-k
KKU ac.10-087	854 b	117 b	55 f-i	48 bc
KKU ac.10-090	645 d	88 bcd	54 hi	48 bc
KKU ac.10-094	1787 a	393 a	108 a	43 de
KKU ac.11-138	445 gh	63 def	59 c-h	31 klm
Kaenpayorm1	503 fg	83 cde	73 b	41 def
Mean	578	87	60	36

Means in the same column followed with the same letter are not significant at $P< 0.05$ by LSD

Table 3. Total carotenoids (µg/g dry weight), lycopene (µg/g dry weight), beta-carotene (µg/g dry weight), and lycopene per fruit (µg/fruit) of 26 spiny bitter gourd accessions.

Accessions	Total carotenoid	Lycopene	Beta-carotene	Lycopene per fruit
KKU ac.09-002	3551 a	1274 de	1159 b	13374 b-e
KKU ac.09-003	2599 b	1207 d-g	441 def	7169 f-j
KKU ac.09-004	1443 e-j	835 g-j	219 i-l	9764 c-g
KKU ac.09-008	3412 a	2487 a	141 kl	16024 b
KKU ac.09-010	2226 bcd	1513 cd	384 d-h	14545 bc
KKU ac.09-011	1934 b-g	827 hij	234 i-l	5033 g-j
KKU ac.09-012	1669 d-h	1294 de	358 d-i	9673 c-h
KKU ac.09-013	1550 d-j	555 i-m	242 h-l	4853 g-j
KKU ac.09-016	3447 a	1734 bc	763 c	14316 bcd
KKU ac.09-018	1830 c-h	789 h-k	248 g-k	7475 e-j
KKU ac.10-019	1910 b-g	808 hij	272 g-k	6864 f-j
KKU ac.10-020	925 ij	426 klm	100 l	2846 ij
KKU ac.09-027	2037 b-f	1272 def	715 b	7445 e-j
KKU ac.09-030	1572 d-j	805 hij	302 f-j	6776 f-j
KKU ac.09-033	1321 f-j	979 e-h	326 e-i	8560 c-i
KKU ac.09-034	2241 bcd	1515 cd	642 c	9474 d-h
KKU ac.10-043	1152 hij	364 m	235 i-l	2500 j
KKU ac.10-049	1572 d-j	946 e-h	484 d	6137 f-j
KKU ac.10-077	2150 b-e	900 f-i	382 d-h	6225 f-j
KKU ac.10-080	2465 bc	1791 bc	454 de	11449 b-f
KKU ac.10-086	840 j	400 lm	145 kl	3797 hij
KKU ac.10-087	1750 c-h	750 h-l	173 jkl	10246 b-g
KKU ac.10-090	1768 c-h	653 h-m	360 d-i	6158 f-j
KKU ac.10-094	4041 a	1893 b	1513 a	52608 a
KKU ac.11-138	1285 g-j	490 j-m	392 d-g	3269 ij
Kaenpayorm1	1654 d-i	826 hij	163 jkl	6823 f-j
Mean	2013	1051	417	9746

Means in the same column followed with the same letter are not significant at $P< 0.05$ by LSD

Table 4. Correlation coefficients between quantitative characters of 26 accessions of spiny bitter gourd.

Characters	Total carotenoid	Lycopene	Beta-carotene	Lycopene per fruit	Fruit fresh weight	Aril weight	Days to fruit maturity
Lycopene	0.83**						
Beta-carotene	0.74**	0.48*					
Lycopene per fruit	0.73**	0.62**	0.75**				
Fruit fresh weight	0.37^{ns}	0.24^{ns}	0.59**	0.84**			
Aril weight	0.43*	0.24^{ns}	0.62**	0.90**	0.94**		
Days to fruit maturity	0.46*	0.34^{ns}	0.62**	0.86**	0.80**	0.89**	
Number of seeds	0.08^{ns}	0.13^{ns}	0.09^{ns}	0.27^{ns}	0.44*	0.27^{ns}	0.18^{ns}

^{ns}, *, ** non significant and significant at 0.05 and 0.01 probability levels, respectively (n = 26).

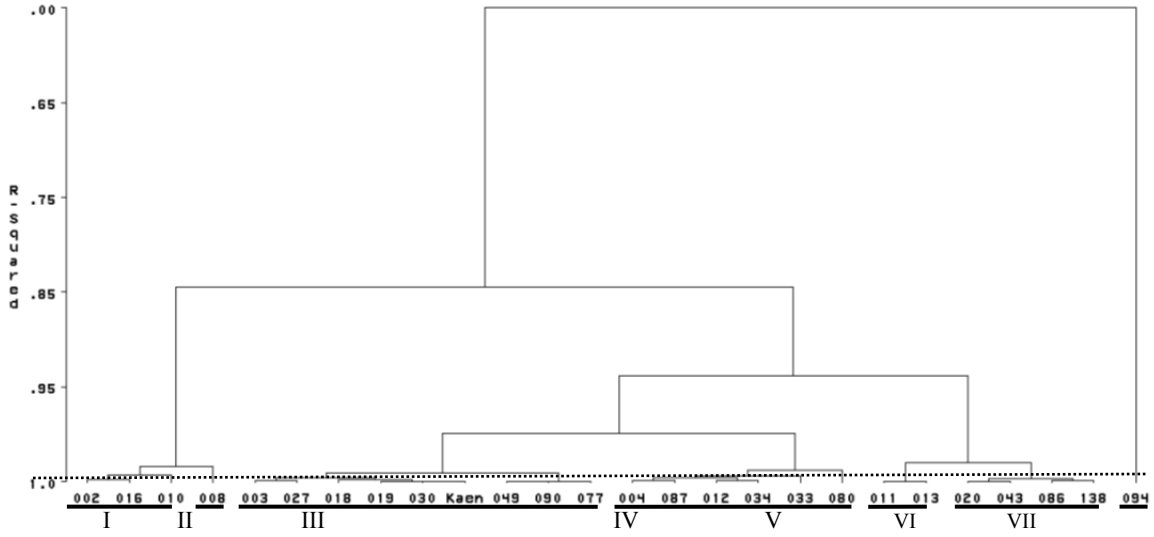


Fig. 1. Dendrogram showing genetic relatedness of 26 spiny bitter gourd accessions collected in Thailand and Vietnam using agronomic traits and phytochemicals content.

Conclusion

The results indicated that agronomic traits and phytochemical contents are useful parameters for evaluation of genetic diversity in spiny bitter gourd. Although newer methods of genetic diversity evaluation in crop plants have been invented and used widely, the conventional methods are still useful and more cost effective. The results will enable breeders to make informed decisions about possible heterotic groups for their breeding programs and germplasm conservation.

References

Ireland, C.R. 2010. Experimental Statistics for Agriculture and Horticulture.Cambridge University Press, Cambridge, UK.

Ishida, B.K., C. Turner, M.H. Chapman and T. McKeon. 2004. Fatty acid and carotenoid composition of gac (*Momordica cochinchinensis* Spreng) fruit. Food Chem. 52: 274–279.

Ishida, B.K. and M.H. Chapman. 2009. Carotenoid extraction from plants using a novel, environmentally friendly solvent. J. Agric. Food Chem. 57: 1051-1059.

Kubola, J. and S. Siriamornpun. 2011. Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng). Food Chem.127: 1138-1145.

Sanwal S.K., S. Kozak, M. Kumar, B. Singh and B.C. Deka. 2011. Yield improvement through female homosexual hybrids and sex genetics of sweet gourd (*Momordica cochinchinensis* Spreng). Acta Physiol. Plant. 33: 1991-1996.

Vuong, L.T., S.R. Dueker and S.P. Murphy. 2002. Plasma β-carotene and retinol concentrations of children increase after a 30-d supplementation with the fruit *Momordica cochinchinensis* (gac). Clin.Nutr. 75: 872-879.

Vuong, L.T. and J.C. King. 2003. A method of preserving and testing the acceptability of gac fruit oil, a good source of β-carotene and essential fatty acids. Food Nutr Bull. 24: 224–230.

Vuong, L.T., A.A. Franke, L.J. Custer and S.P. Murphy. 2006.*Momordica cochinchinensis* Spreng. (gac) fruit carotenoids reevaluated. J. Food Compos. Anal.19: 664-668.

Publication

Bootprom, N., P. Songsri, B. Suriharn, K. Lomthaisong and K. Lertrat. 2014. Genetics diversity based on agricultural traits and phytochemical contents inspiny bitter gourd(*Momordicacochinchinensis*(Lour.) Spreng). SABRAO J. Breed. Genet.(*Submitted*). (No impact factor)

Sub-project 4

Basic research for supporting varietal improvement of purple waxy corn for high anthocyanin content

Assistant Prof. Dr. Bhalang Suriharn

Sub-project leader

Purple corn is a type of waxy corn found in our germplasm collection. Waxy or glutinous corn (*Zea mays* L. var. ceratina) is a type of corn wildly grown as a cash crop in several countries in Eastern Asia, including China, Myanmar, Laos, Vietnam, Cambodia, Taiwan , Korea and Thailand (Thongnarin *et al.*, 2008; Kesornkeaw *et al.*, 2009). Currently, Thailand is the exporter of waxy corn hybrid seeds and frozen waxy corn. Khon Kaen University has long been working on varietal improvement of waxy or glutinous corn.

For breeding purposes, the program has collected germplasm of waxy corn from various sources. These germplasm lines have different kernel colors, ranging from white, yellow, black and purple. The purple corn is of special interest because it is a rich source of anthocyanin colorants and functional ingredients (Jing and Giusti, 2007). Li *et al.* (2008) found that purple corn produced anthocyanin pigment throughout the plant, particularly high in the husk and cob parts. The reports on the positive effects of anthocyanin in normal purple corn on antioxidant and anticarcinogenic properties make the crop attractive for the nutraceutical and functional food market (Cevallos-Casals *et al.*, 2003; 2004). Yang and Zhai (2010) suggested that the seed and cob of purple corn possessed excellent antioxidant activity, which could lead to increased application of these natural food colorants by the food industry. These researches showed performance of purple corn in term of health benefits, product differentiation and value added for agricultural products. This type of corn, thus, has a great potential for the functional food industry in Thailand.

In Thailand, waxy corn cultivar with high anthocyanin has not been released to corn grower. However, there are pre-commercial varieties of waxy corn with high anthocyanin of some seed companies that are being tested in Thailand. Khon Kaen University has just started a breeding program on purple corn for functional food quality.

This sub-project consists of two studies:

4.1 Variability in phytochemicals and antioxidant activity in corn.

4.2 Effect of location, genotype and their interactions for anthocyanins and antioxidant activities of purple waxy corn cob.

The details of these studies are presented below.

References

Cevallos-Casals, B.A. and L. Cisneros-Zevallos. 2003. Stoichiometric and kinetic studies of phenolic antioxidants from andean purple corn and red-fleshed sweet potato. J. Agric. Food Chem. 51: 3313-3319.

Cevallos-Casals, B.A. and L. Cisneros-Zevallos. 2004. Stability of anthocyanin-based aqueous extracts of andean purple corn and redfleshed sweet potato compared to synthetic and natural colorants. Food Chem. 86: 69-77.

Jing, P., V. Noriega, S.J. Schwartz and M.M. Giusti. 2007. Effects of growing conditions on purple corncob (*Zea mays* L.) anthocyanins. J. Agric. Food Chem. 55: 8625-8629.

Kesornkeaw, P., K.Lertrat and B. Suriharn. 2009. Response to four cycles of mass selection for prolificacy at low and high population densities in small ear waxy corn. J. Plant Sci. 8(6): 425-432.

Li, C.Y., H.W. Kim, S.R. Won, H.K. Min, K.J. Park, J.Y. Park, M.S. Ahn and H.I. Rhee. 2008. Corn husk as a potential source of anthocyanins. J. Agric. Food Chem. 56: 11413-11416.

Thongnarin, N., K. Lerteat and S. Techawongstien. 2008. Combining ability study in waxy corn (*Zea mays* var. *ceratina*) inbred lines. Acta Hortic 769: 151-156.

Yang Z. and Zhai, W. 2010. Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (*Zea mays* L.). Innovative Food Sci. Emerging Technol. 11: 169–176.

4.1Variability in phytochemicals and antioxidant activity in corn

S. Khampas, K. Lertrat, K. Lomthaisong, and B. Suriharn

Corn (*Zea mays* L.) is one of the most widely cultivated cereals in the world. It also has a wide range of kernel colors such as white, yellow, orange, purple and black. In addition to the attractive colors, pigmented corn is rich in phytochemical and many secondary metabolites such as phenolic compounds, carotenoids, anthocyanins and flavonoids (Hu and Xu, 2011). These phytochemicals have antioxidant activities and anticarcinogenic properties, and corn products are therefore regarded as functional food that is beneficial to health (Adom and Liu, 2002). However, most studies so far have focused on colored kernels, and limited information is available for other types of corn at both immaturity and physical maturity stages.

Objectives

The objectives of this study were to evaluate the performance of corn genotypes for carotenoids, anthocyanins, phenolic compounds and antioxidant activities at the immaturity and the physiological maturity stages, to estimate the correlation among the studied traits and to identify corn genotypes with high phytochemical and antioxidant activities.

Materials and Methods

Thirteen hybrids/open-pollinated corn varieties, consisting of one field corn (orange), two super sweet corn (yellow and bi-color) and ten waxy corns (white, yellow, purple, bi-color and tri-color), were used in this study. These corn genotypes were grown in the dry season of 2011/2012 at the Vegetable Research Farm, Khon Kaen University, Thailand. Kernels of these varieties were harvested at the immature growth stage and the physiological maturity stage. Samples of the immature corn were immediately frozen in

liquid nitrogen to block the enzymatic activities and stored at -20 ºC until use. All corn kernels were lyophilized and stored for chemical analysis. The corn samples were ground into fine powder and passed through a 64 mesh screen. The flours and milling fractions were mixed and used for extraction. All chemicals and reagents used in the experiments were of analytical grade. The phytochemicals including anthocyanins, carotenoids, phenolics and antioxidant activities (DPPH, FRAP and TEAC assays) were analyzed by spectrophotomettic method. Only anthocyanins were analyzed by HPLC method.

Combined analysis of variance was performed for these traits at the two maturity stages. Mean separation was obtained using a Least Significant Difference (LSD) at $P \leq 0.05$. The correlations between antioxidant compounds and antioxidant activities were determined by Pearson's correlation analysis.

Results

The combined analysis of variance showed that maturity stage (S), genotype (G) and the interaction between genotype by maturity stage (G × S) were highly significant ($P \leq 0.01$) for all traits. Means at the mature stage (dry kernel) were slightly higher than those at the immature stage (fresh kernel) for all traits (Table 1). However, TCC and DPPH at the mature stage were significantly higher than those at the immature stage.

Table 1. Means for total anthocyanin content (TAC), total carotenoid content (TCC), total phenolic content (TPC), DPPH radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), and trolox equivalent antioxidant capacity (TEAC) of immature kernel and mature kernel of 13 corn genotypes.

Stages	TAC (mg of CGE /g DW)	TCC (µg per g DW)	TPC (mg of GAE/g DW)	DPPH (% reduction)	FRAP (µmol Fe(II)/g DW)	TEAC (µmol TE/g DW)
Immature	0.3	6.7	2.1	28.7	0.09	3.1
Mature	0.4	9.7	2.8	34.2	0.10	3.8
LSD 0.05	0.2	4.9	0.3	8.2	0.02	0.7

Anthocyanin content (TAC): Corn genotypes were significantly different for TAC, ranging from 0.00 to 1.52 mg of C-3-G per g DW for fresh kernel stage and 0.05 to 1.65 mg of C-3-G per g DW for dry kernel stage (Table 2). WP (purple waxy corn genotype) had the highest TAC followed by WP2 and WP1, both at the immature kernel stage and at the dry kernel stage.

Carotenoid content (TCC): TCC values ranged from 0.9 to 23.3 µg per g DW at the fresh kernel stage and 1.0 to 35.6 µg per g DW at the dry kernel stage (Table 2). FC or field corn with orange kernel color had the highest TCC followed by SWWY or bi-color sweet corn genotype for the fresh kernel stage. For the dry kernel stage, FC also had the highest TCC followed by SWY and SWWY.

Phenolic content (TPC): TPC values ranged from 1.3 to 3.1 mg of GAE per g DW at the fresh kernel stage and 2.0 to 4.5 mg of GAE per g DW at the dry kernel stage (Table 2). At the fresh kernel stage, SWWY, a sweet corn genotype with yellow and white kernel color, had the highest TPC followed by WP, SWY, WP1 and WP2, which were waxy corn and sweet corn genotypes with purple and yellow kernels colors, respectively. At the dry kernel stage, WP, WP1 and WP2 (the purple waxy corn genotypes) had the highest TPC values.

Antioxidant activity: Corn genotypes can be classified into high, intermediate and low groups for DPPH at both maturity stages (Table 3). The high group consisted of WP, WP1 and WP2 of purple waxy corn, the intermediate group comprised of the two genotypes of sweet corn (SWY and SWWY), and the low group included other waxy corn genotypes and field corn.

For both FRAP and TEAC, WP, WP1 and WP2 (purple waxy corn) formed the high group, and other genotypes, consisting sweet corn, waxy corn and field corn, were designated as the low group.

Table 2. Means for total anthocyanin content (TAC), total carotenoid content (TCC), total phenolic content (TPC) of fresh and dry kernels from 13 corn varieties grown in the dry season 2011/12.

Genotypes	TAC (mg of C-3-G per g DW)		TCC (µg per g DW)		TPC (mg of GAE per g DW)	
	Fresh	Dry	fresh	Dry	Fresh	Dry
sweet corn						
SWY[1/]	0.08	0.05	9.6	33.3	2.5	3.5
SWWY	0.06	0.11	20.2	31.1	3.1	3.3
Waxy corn						
Small earW	0.09	0.05	1.2	1.3	1.9	2.1
Small earY	0.09	0.13	11.1	10.8	1.7	2.2
Small earWY	0.11	0.16	7.5	2.9	1.9	2.1
Small earWYP	0.19	0.16	6.2	2.7	1.8	2.0
WW1[1/]	0.00	0.11	1.1	1.4	1.9	2.2
WW2	0.00	0.35	1.6	2.0	1.3	2.2
WWP[1/]	0.08	0.35	0.9	1.2	1.9	2.1
WP[1/]	1.52	1.65	2.6	1.1	2.6	4.5
WP1	0.54	1.22	1.1	1.0	2.4	4.1
WP2	0.78	1.15	1.1	1.4	2.3	4.1
Field corn						
FC	0.12	0.14	23.3	35.6	1.7	2.4
LSD 0.05	0.04	0.06	0.8	0.4	0.5	0.1

[1/]Commercial variety

The analysis of anthocyanin by HPLC: The corn genotypes of waxy corn with tri-color (white, yellow and purple), bi-color (white and purple) and mono color (purple) could be classified into two classes based on the value of cyanidin-3-glucoside (Table 4). WP (purple waxy corn) had the highest cdyanidin-3-glucoside at the fresh kernel stage, whereas WP2 and WP had high cyanidin-3-glucoside at the dry kernel stage. Other genotypes were rather low for this chemical.

The fresh kernel stage had slightly higher values of pelargonidin-3-glucoside than the dry kernel stage. WP1 and WP2 had the highest pelargonidin-3-glucoside at fresh kernel stage, and WP and WP2 had the highest pelargonidin-3-glucoside at dry kernel stage. Other genotypes showed rather low pelargonidin-3-glucoside.

Correlations: The correlations between TAC with TEAC, DPPH, FRAP and TPC were positive and significant, whereas the correlation between TAC with TCC was not significant (Table 5). The correlations between TPC with TEAC, with FRAP and with

DPPH were also positive and significant. Positive and significant correlations between DPPH with TEAC and FRAP were observed, whereas FRAP was positively and significantly correlated with TEAC.

Table 3. Means for radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP) and trolox equivalents antioxidant capacity (TEAC) of fresh and dry seeds from 13 corn varieties grown in dry season 2011/12.

Genotypes	DPPH (% reduction)		FRAP (µmol Fe(II)/g		TEAC (µmol TE/g DW)	
	Fresh	Dry	Fresh	dry	Fresh	Dry
			sweet corn			
SWY[1/]	34.5	29.9	0.12	0.09	3.6	2.7
SWWY	29.1	33.9	0.12	0.10	3.8	4.3
			Waxy corn			
Small earW	15.0	34.9	0.04	0.07	1.7	2.4
Small earY	17.5	17.2	0.05	0.07	1.6	2.4
Small earWY	17.6	17.4	0.04	0.08	1.8	2.7
Small earWYP	19.6	21.6	0.04	0.08	2.1	2.7
WW1[1/]	19.0	31.2	0.06	0.09	1.9	3.2
WW2	16.1	25.8	0.06	0.01	1.8	2.0
WWP[1/]	25.4	23.9	0.08	0.19	2.1	2.5
WP[1/]	62.8	55.8	0.17	0.18	6.4	7.5
WP1	48.5	68.9	0.13	0.20	5.3	6.8
WP2	54.7	68.0	0.14	0.17	6.4	6.9
			Field corn			
FC	13.2	15.7	0.07	0.07	1.8	2.7
LSD 0.05	5.0	17.6	0.01	0.02	0.1	0.2

[1/]Commercial variety

Table 4. Means for cyanidin-3-glucoside and pelargonidin-3-glucoside determined by HPLC (520 nm) for fresh and dry kernels of 5 waxy corn genotypes.

Variety	Cyanidin-3-glucoside (mg/g seed)		Pelargonidin-3-glucoside (mg/g seed)	
	Fresh	dry	Fresh	Dry
		Waxy corn		
Small earWYP	nf	nf	nf	nf
WWP[1/]	nf	nf	nf	nf
WP[1/]	0.07	0.09	0.65	0.57
WP1	0.06	0.08	0.72	0.29
WP2	0.06	0.10	0.71	0.53
LSD 0.05	0.01	0.02	0.08	0.05

nf, not found.
[1/]Commercial variety

Table 5. Correlations between total anthocyanin content (TAC), total carotenoid content (TCC), total phenolic content (TPC), radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP) and trolox equivalents antioxidant capacity (TEAC) for fresh and dry seeds of 13 corn varieties.

	TAC	TCC	TPC	DPPH	FRAP
TCC	0.15ns				
TPC	0.73**	0.61**			
DPPH	0.85**	0.42**	0.93**		
FRAP	0.80**	0.48**	0.94**	0.94**	
TEAC	0.86**	0.46**	0.95**	0.97**	0.96**

ns, ** not significant and significant at 0.01 level of probability, respectively.

Conclusion

Corn harvested at the mature stage was significantly higher than corn harvest at the immature stage for all parameters investigated. WP and WP1 (purple waxy corn) had the highest TAC at both maturity stages. Field corn (FC) with orange kernel color had the highest TCC followed by the sweet corn genotype at both maturity stages. SWWY (sweet corn) had the highest TPC at the fresh kernel stage. Purple waxy corn genotypes had the highest antioxidant activity (DPPH, FRAP and TEAC) at both maturity stages. HPLC analysis found that purple kernel colors of purple waxy corn genotypes had the highest cyanidin-3-glucoside and pelargonidin-3-glucoside at both maturity stages. TAC was closely related with TEAC, DPPH, FRAP, and TPC, and TPC had positive and significant correlations TEAC, FRAP, and DPPH. Positive and significant correlation between DPPH with TEAC and FRAP were observed, whereas FRAP had a close relationship with TEAC.

References

Adom, K.K. and R.H. Liu. 2002. Antioxidant activity of grains. Agricultural and Food Chemistry 50: 6182-6187.

Hu, Q.P. and J.G. Xu. 2011. Profiles of carotenoids, anthocyanins, phenolics, and antioxidant activity of selected color waxy corn grains during maturation. Agr.Food Chem. 59: 2026-2033.

Publication

Khampas, S., K. Lertrat, K. Lomthaisong, and B. Suriharn. 2013. Variability in phytochemicals and antioxidant activity in corn at immaturity and physiological maturity stages. Int. Food Res. J. 20: 3149 -3157. (No impact factor)

4.2 Effect of location, genotype and their interactions for anthocyanins and antioxidant activities of purple waxy corn cob

S. Khampas, K. Lertrat, K. Lomthaisong, S. Simla and B. Suriharn

High and stable yield across a range of environments is the primary goal of plant breeding programs. Recently, breeding for high anthocyanins was set a priority of the waxy corn breeding program at Khon Kaen University, as this character defines its possible use as colorants and functional foods. Several factors such as pH, temperature, light intensity, oxygen, and metallic ions were found to affect anthocyanin stability (Bordignon-Luiz *et al.*, 2007; de Rosso and Mercadante 2007; Mollov *et al.*, 2007; Veigas *et al.*, 2007). However, the effects of various factors such as genotypes, environments and their interaction for anthocyanins in purple waxy corn have not been clearly understood. The information is important for purple waxy corn breeding programs.

Objectives

The objectives were to evaluate the genotype by environment interaction for anthocyanin content and cyanindin 3-glucoside under growing conditions in Thailand and to identify purple waxy corn genotypes that may be used in breeding programs for high anthocyanins.

Materials and methods

Five purple waxy corn lines/cultivars and one normal waxy corn were used in this study. KGW1, KGW2, KGW3, KNDM4 and Fancy 111 are purple waxy corn with purple seeds and cobs. Fancy 121 is a normal waxy corn cultivar with white cream seeds and cobs. The experiments were carried out at four locations at different elevations in Thailand in the dry season 2012/13 (Table 1). These four locations represent waxy corn growing areas in Thailand. A randomized complete block design with three replications was used. The plot size was four rows, 5 m in length, and the spacing was 0.80 m between rows and 0.25 m between plants within row. The recommended cultural practices were used for all locations. Weather and soil data were collected at each location as shown in Tables 1 and 2.

Table 1. The test locations used in this study.

Locations	Elevations (m)	Temperature (C^o)		Rainfall (mm)	Relative humanity (%)			Solar radiation ($MJ\ m^{-2}day^{-1}$)
		Max.	Min.		Max.	Min.	Ave.	
Saraburi (SR)	120	33.5	23.4	52.0	80.0	42.0	66.0	17.3
Khon Kaen (KK)	200	31.6	19.7	28.5	92.0	55.0	75.4	18.9
Nakhon Ratchasima (NR)	356	31.4	19.8	93.0	94.0	50.0	68.4	18.3
Chiang-Rai (CR)	380	33.8	20.2	145.0	90.0	51.0	68.7	19.8

Purple corn cobs were collected at the harvest maturity stage (R6) or 90 days after planting and stored at room temperature for determination of anthocyanins. Data were recorded for total anthocyanin content (TAC) and cyanindin 3-glucoside as described by Yang and Zhai (2010). Anthocyanins and cyanindin 3-glucoside were analyzed by spectrophotomettic method and HPLC method.

Combined analysis of variance was performed across locations. Stability analysis was done by calculating the linear regression of a cultivar's means on the mean yield of each environment as described by Eberhart and Russell (1966). Means were compared statistically using Least Significant Different at $P \leq 0.05$.

Table 2. Soil physical and chemical properties.

Locations	Soil types	pH (1:1 H_20)	EC (1:5 H_20) (dS/m)	Organic Matter (%)	Total nitrogen (%)	Available phosphorus (mg/kg)	Available potassium (mg/kg)	Available calcium (mg/kg)
Saraburi (SR)	Sandy loam	7.4	0.09	1.22	0.06	234	155	2,432
Khon Kaen (KK)	Sandy	4.9	0.01	0.73	0.03	307	139	54
Nakhon Ratchasima (NR)	Loamy sand	7.1	0.13	2.49	0.13	52	162	3,137
Chiang-Rai (CR)	Loamy sand	5.6	0.08	3.40	0.09	45	149	593

Results

Location (L), genotype (G) and GxL interactions were highly significant for all characters (data not showed). Location means for TAC ranged from 271.0 and 275.4 mg of C3G/100gDW at CR and SR, respectively, to 730.4 mg of C3G/100gDW at NR (Table 3). KNDM4 had the highest TAC of 903.4 mg of C3G/100gDW across locations. For individual locations, KNDM4 also had the highest TAC of 1,165.5 mg of C3G/100gDW followed by FC111 (1,075.8 mg of C3G/100gDW) at NR. For purple waxy corn, KGW1 had the lowest TAC of 67.4 mg of C3G/100gDW at SR.

The means of locations for C3G ranged from 22.9 µg/gDW at CR to 1,294.5 µg/g DW at NR. KNDM4 had the highest C3G of 1,121.0 µg/gDW across locations, and it also had the highest C3G of 3,144.2 µg/gDW for individual locations followed by KGW2 (2,612.9 µg/gDW) at NR.

The genotypes with stable yield should have high yield across locations, regression coefficient close to one (b=1) and deviation from regression (S.D.) close to zero (Eberhart and Russell, 1966). KNDM4 had the highest TAC and regression coefficient close to one, but had high deviation from regression (Table 4). This cultivar ranked first at three locations except at CR (Table 3), indicating that it responded poorly to unfavorable conditions.

Regression coefficients for C3G for all genotypes were significantly different from one, indicating that these genotypes were not stable for this trait. KNDM4 and KGW2 had the highest C3G, but their regression coefficients were larger than one and their deviations from regression were also high, indicating that they performed well under favorable conditions but performed poorly under unfavorable conditions.

Table 3. Means for total anthocyanin content (TAC) and cyanidin 3-glucoside (C3G) of six waxy corn lines/cultivars evaluated at four locations in 2012/13.

Genotypes/Locations	SR	KK	NR	CR	Means
			TAC (mg of C3G/100gDW)		
KGW1	67.4 lm	246.5 j	608.7 e	128.9 kl	262.9 E
KGW2	341.4 h	448.8 g	981.5 c	373.0 h	536.2 B
KGW3	263.2 ij	327.9 hi	547.2 ef	330.1 hi	367.1 D
KNDM4	825.7 d	1,132.1 ab	1,165.5 a	490.3 fg	903.4 A
FC111	148.2 k	346.4 h	1,075.8 b	302.0 hij	468.1 C
FC121	6.4 m	3.9 m	3.4 m	1.6 m	3.8 F
Means	*275.4 c*	*417.6 b*	*730.4 a*	*271.0 c*	
			C3G (µg/gDW)		
KGW1	35.0 ijk	67.9 hijk	386.9 f	8.6 k	124.6 E
KGW2	91.7 hij	266.2 g	2,612.9 b	21.1 ijk	748.0 B
KGW3	60.8 ijk	143.7 h	847.1 d	29.9 ijk	270.4 C
KNDM4	229.9 g	1,052.2 c	3,144.2 a	57.5 ijk	1,121.0 A
FC111	23.9 ijk	97.4 hi	764.7 e	11.6 jk	224.4 D
FC121	7.2 k	10.6 k	10.9 jk	8.8 k	9.4 F
Means	*74.8 c*	*273.0 b*	*1,294.5 a*	*22.9 d*	

Mean in the same column and row followed by a common letter (s) are not significantly different by Least Significant Different at $P \leq 0.05$. Different capital letter (s) indicates significant difference between genotypes.
Abbreviation of locations: SR: Saraburi, KK: Khon Kaen, NR: Nakhon Ratchasima, CR: Chiang Rai.

Table 4. Stability parameters of total anthocyanin content (TAC) and cyanidin 3 glucoside (C3G) of six waxy corn lines/cultivars.

Genotypes	TAC (mg of C3G / 100gDW)			C3G (µg / g DW)		
	Mean	*b*	S.D.	Mean	*b*	S.D.
Purple waxy corn						
KGW1	262.9 e	1.1	34.1	124.6 e	0.3**	13.1
KGW2	536.2 b	1.4	67.3	748.0 b	2.1*	152.4
KGW3	367.1 d	0.5	45.4	270.4 c	0.6**	27.8
KNDM4	903.4 a	1.1	253.4	1,121.0 a	2.4*	224.2
FC111	468.1 c	1.9	121.3	224.4 d	0.6**	34.5
White waxy corn						
FC121	3.8 f	0.0**	2.4	9.4 f	0.0**	1.5

*, ** Significant different at $P \leq 0.05$ and $P \leq 0.01$, respectively

Conclusion

Location (L), genotype (G) and G × L interaction were highly significant for all characters. NR was the highest location for TAC and C3G. KNDM4 gave the highest TAC and its regression coefficient (b) was close to one. However, it had the highest S^2D, indicating that it had specific adaptation to favorable environments. KNDM4 genotype performed well under unfavorable environments for all traits. This information is useful for breeding programs and production of anthocyanins from purple waxy corn.

References

Bordignon-Luiz, M.T., C. Gauche, E.F. Gris and L.D. Falcao. 2007. Colour stability of anthocyanins from Isabel grapes (*Vitis labrusca* L.) in model systems. LWT-Food Sci. Technol. 40: 594-599.

de Rosso, V.V. and A.Z. Mercadante. 2007. Evaluation of colour and stability of anthocyanins from tropical fruits in an isotonic soft drink system. Innovative Food Sci. Emerging Technol. 8: 347-352.

Eberhart, S.A. and W.A. Russel. 1966. Stability parameters for controlling variation. Crop Science 6: 36-40.

Mollov, P., K. Mihalev, V. Shikov, N. Yoncheva and V. Karagyozov. 2007. Colour stability improvement of strawberry beverage by fortification with polyphenolic copigments naturally occurring in rose petals. Innovative Food Sci. Emerging Technol. 8: 318-321.

Veigas, J.M., M.S. Narayan, P.M. Laxman and B. Neelwarne. 2007. Chemical nature, stability and bioefficacies of anthocyanins from fruit peel of Syzygium cumini Skeels. Food Chem. 105: 619-627.

Yang, Z. and W. Zhai.2010. Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (*Zea mays* L.). Innovative Food Sci. Emerging Technol. 11: 169–176.

Publication

Khampas,S., K. Lertrat, K. Lomthaisong, S. Simla and B. Suriharn. 2014. Effect of location, genotype and their interactions for anthocyanins and antioxidant activities of purple waxy corn cob. Turk. J. Field Crops. (*Accepted*). (Impact factor 0.641)

Project outputs

1) Capacity building of researchers

Research team:

Number of researchers: 13
Number of research resistants: 2
Number of post-doctoral: 4
Number of Ph.D. Students: 13
Number of M.S. Students: 7

Improved research capacity of researchers and post-docteral researchers

Reseachers 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 capapcity of seeking their own research fund, and have their own projects in which they are the project leaders. All of team have research projects that received supports from the the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Food and Functional Food Research Cluster of Khon Kaen University. In addition, Dr. Sanun Jogloy, Dr. Kamol Lertrat and Dr. Supalax Srijaranai are grantees of the RGJ-PhD program of the TRF, and Dr. Kamol Lertrat and Dr. Jirawat Snitchon has a project supported by the National Science and Technology Development Agency (NSTDA).

Training of graduate students

Thirteen Ph.D. and 7 M.S. students were trained under the project. Seven Ph.D. students had completed their program. Name of student and their status are listed below:

No.	Degree	Name of sudent	Major advisor	Expected date of completion
1.	Ph.D.	Mr. Surasak Boontang	Dr. Sanun Jogloy	Graduated
2.	Ph.D.	Mr. Wunna Htoon	Dr. Sanun Jogloy	Graduated
3.	Ph.D.	Miss Rattikarn Sennoi	Dr. Sanun Jogloy	Graduated
4.	Ph.D.	Miss Ratchanee Puttha	Dr. Sanun Jogloy	Graduated
5.	Ph.D.	Miss Rattanaporn Koolachart	Dr. Sanun Jogloy	Graduated
6.	Ph.D.	Miss Waraluck Senakoon	Dr. Suporn Nuchadomrong	Graduated
7.	Ph.D.	Miss Rattanajira Ruttanaprasert	Dr. Sanun Jogloy	Graduated
8.	Ph.D.	Miss Chokaew Aninbon	Dr. Sanun Jogloy	June 2015
9.	Ph.D.	Miss Nuengsap Thangthong	Dr. Sanun Jogloy	June 2016
10.	Ph.D.	Miss Chatsuda Junsopa	Dr. Sanun Jogloy	June 2017
11.	Ph.D.	Miss Wanalai Viriyasuthee	Dr. Sanun Jogloy	June 2018
12.	Ph.D.	Miss Wanwipa Pinta	Dr. Jirawat Snitchon	June 2015
13.	Ph.D.	Mr. Somprasong Khaopha	Dr. Thanaset Senawong	June 2015

No.	Degree	Name of sudent	Major advisor	Expected date of completion
1.	M.S.	Mr. Anon Janket	Dr. Sanun Jogloy	Graduated
2.	M.S.	Mr. Dinh Thai Hoang	Dr. Sanun Jogloy	Graduated
3.	M.S.	Miss Sujittra Khampas	Dr. Bhalang Suriharn	Graduated
4.	M.S.	Mr. Tanupat Mornkham	Dr. Preeya Wangsomnuk	Graduated
5.	M.S.	Miss Supattra Mahakoosee	Dr. Sanun Jogloy	June 2015
6.	M.S.	Miss CholadaAduldecha	Dr. Wanwipa Kaewpradit Polpinit	June 2015
7.	M.S.	Miss Natthayaporn Nanta	Dr. Patcharin Songsri	June 2015

2) Published papers in accredited journals

2.1 Number of published or accepted papers: 28

(1) Singkham, N., S. Jogloy, T. Kesmala, P. Swatsitang, P. Jaisil, N. Puppala and A. Patanothai. 2011. Combining ability for oleic acid in peanut (*Arachis hypogaea* L.). SABRAO J. Breed. Genet. 43(1): 59-72. (Impact factor = 0.227).

(2) Wangsomnuk, P.P., S. Khampa, S. Jogloy, A. Patanothai and Y-B. Fu.Assessing genetic structure and relatedness of Jerusalem artichoke (*Helianthus tuberosus* L.) germplasm with RAPD, ISSR and SRAP markers. 2011. Am. J. Plant Sci. 2: 753-764. (No impact factor).

(3) Wangsomnuk, P.P., S. Khampa, P. Wangsomnuk, S. Jogloy, T. Mornkham, B. Ruttawat, A. Patanothai and Y-B. Fu. 2011. Genetic diversity of worldwide Jerusalem artichoke (*Helianthus tuberosus*) germplasm as revealed by RAPD markers. Genetics and Mol. Res. 10:4012-4025. (Impact factor = 1.593).

(4) Arunyanark, A, S. Pimratch, S. Jogloy, S. Wongkaew, N. Vorasoot ,G. Akkasang, A. Patanothai and C.C. Holbrook. 2012. Association between aflatoxin contamination and N_2 fixation in peanut under drought conditions. Int. J. Plant Prod. 6 (2):161-172. (Impact factor 1.028).

(5) Bootprom, N., P. Songsri, B. Suriharn, P. Chareonsap, J. Sanitchon and K. Lertrat. 2012. Molecular diversity among selected *Momordica cochinchinensis* (Lour.) Spreng) accessions using RAPD markers. SABRAO J. Breed. Genet. 44 (2): 406-417. (Impact factor = 0.227).

(6) Girdthai, T., S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew, **A.** Patanothaiand C.C. Holbrook. 2012. Inheritance of physiological traits for drought resistance under terminal drought condition and genotypic correlations with agronomic traits in peanut. SABRAO J. Breed. Genet. 44: 240-262. (Impact factor = 0.227).

(7) Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, K.J. Boote, G. Hoogenboom and A. Patanothai. 2012. Classification of root distribution patterns and their contributions to yield in peanut genotypes under mid-season drought stress. Field Crops Res. 127:.181-190. (Impact factor = 2.474).

(8) Saengkanuk, A., S. Nuchadomrong, S. Jogloy, A. Patanothai and S. Srijaranai. 2012.A simplified spectrophotometric method for the determination of inulin in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. Eur. Food Res. Technol. 233: 609-616. (Impact factor = 1.436).

(9) Sennoi, R., S. Jogloy, W. Saksirirat, T. Kesmala, N. Singkham and A. Patanothai. 2012. Levels of *Sclerotium rolfsii* inoculum influence identification of resistant genotypes in Jerusalem artichoke. Afr. J. Microbiol. Res. 6(38): 6755–6760. (Impact factor = 0.539).

(10) Htoon, W., W. Kaewpradit, S. Jogloy, N. Vorasoot, B. Toomsan, C. Akkasaeng, N. Puppala and A. Patanothai. 2013. Responses of peanut (*Arachis hypogaea* L.) genotypes to N_2 fixation under terminal drought and their contributions to peanut yield. SABRAO J. Breed. Genet. 45(2): 296-310. (Impact factor 2010 = 0.227).

(11) Htoon, W., W. Kaewpradit, S. Jogloy, N. Vorasoot, B. Toomsan, C. Akkasaeng, N. Puppala and A. Patanothai. 2013. Relationship between root traits and nutrient uptake and nitrogen fixation in peanut under terminal drought. SABRAO J. Breed. Genet. 45(2): 311-323. (Impact factor = 0.227).

(12) Janket, A., S. Jogloy, N. Vorasoot, T. Kesmala, C.C. Holbrook and A. Patanothai. 2013. Genetic diversity of water use efficiency in Jerusalem artichoke (*Helianthus tuberosus* L.) germplasm. Aust. J. Crop Sci. 7: 1670-1681. (Impact factor = 1.632).

(13) Jongrungklang, N., B. Toomsan, N. Vorasoot, S. Jogloy, K.J. Boote, G. Hoogenboom and A. Patanothai. 2013. Drought tolerance mechanisms for yield responses to pre-flowering drought stress of peanut genotypes with different drought tolerant levels. Field Crops Res. 144: 34-42. (Impact factor = 2.608).

(14) Junjittakarn, J., S. Pimratch, S. Jogloy, W. Htoon, N. Singkham,N. Vorasoot, B. Toomsan, C.C. Holbrook and A. Patanothai. 2013. Nutrient uptake of peanut genotypes under different water regimes. Int. J. Plant Prod. 7(4): 691-706. (Impact factor = 1.028).

(15) Khampas, S., K. Lertrat, K. Lomthaisong, and B. Suriharn. 2013. Variability in phytochemicals and antioxidant activity in corn at immaturity and physiological maturity stages. Int. Food Res. J. 20: 3149 -3157. (No impact factor).

(16) Koolachart R., S. Jogloy, N. Vorasoot, S. Wongkaew, C.C. Holbrook, N. Jongrungklang, T. Kesmala and A.Patanothai. 2013. Rooting traits of peanut genotypes with different yield responses to terminal drought. Field Crops Res. 149: 366–378. (Impact factor = 2.608).

(17) Koolachart R., B. Suriharn, S. Jogloy, N. Vorasoot, S. Wongkaew, C.C. Holbrook, N. Jongrungklang, T. Kesmala, and A.Patanothai. 2013. Relationships between physiological traits and yield components of peanut genotypes with different levels of terminal drought resistance. SABRAO J. Breed.Genet. 45 (3): 422–446. (Impact factor 2013 = 0.227).

(18) Mornkham, T., P.P. Wangsomnuk, Y-B. Fu, P. Wangsomnuk, S. Jogloy and A. Patanothai. 2013. Extractions of high quality RNA from seeds of Jerusalem artichoke and other plant species with high levels of starch and lipid. Plants 2: 302-316. (No impact factor).

(19) Puangbut, D., S. Jogloy, N. Vorasoot, T. Kesmala, C.C. Holbrook and **A.** Patanothai. 2013. Response of reproductive parts and their contributions to yield of peanut under pre-flowering drought. Aust. J. Crop Sci. 7(11): 1627-1633. (Impact factor = 1.632).

(20) Puttha R, S. Jogloy, B. Suriharn, P.P. Wangsomnuk, T. Kesmala and A.Patanothai. 2013. Variations in morphological and agronomic traits among Jerusalem artichoke (*Helianthus tuberosus* L.) accessions. Genet. Resour. Crop Evol.60: 731–746. (Impact factor = 1.482).

(21) Rattanaprasert, R., S. Jogloy, N. Vorasoot, R.S. Kanwar, T. Kesmala, C.C. Holbrook and A. Patanothai. 2013. Photoperiod and growing degree days effect on dry matte partitioning in Jerusalem artichoke. Int. J. Plant Prod. 7(3):393-416. (Impact factor = 1.145).

(22) Sennoi, R., N. Singkham, S. Jogloy, S. Boonlue, W. Saksirirat, T. Kesmala and A. Patanothai. 2013. Biological control of southern stem rot caused by *Sclerotium rolfsii* using *Trichoderma harzianum* and arbuscular mycorrhizal fungi onJerusalem artichoke (*Helianthus tuberosus* L.). Crop Prot. 54: 148-153. (Impact factor = 1.303).

(23) Sennoi, R., S. Jogloy, W. Saksirirat, P. Banterng, T. Kesmala and A. Patanothai. 2013. Evaluation of seedling and adult plant stages resistance to *Sclerotium rolfsii* in Jerusalem artichoke (*Helianthus tuberosus* L.). SABRAO J. Breed. Genet. 45 (2): 324-331. (No impact factor).

(24) Sennoi, R., S. Jogloy, W. Saksirirat, T. Kesmala and A. Patanothai. 2013.Effects of host growth stage, re-isolation and culture medium on screening for resistance to stem rot disease caused by *Sclerotium rolfsii* Sacc. in Jerusalem artichoke. Pak. J. Bot. 45(5): 1825-1829. (Impact factor = 0.907)

(25) Dinh, H.T., W. Kaewpradit, S. Jogloy, N. Vorasoot and A. Patanothai. 2014. Nutrient uptake of peanut genotypes with different levels of drought tolerance under midseason drought. Turk. J. Agric. For. 38: 495-505. (Impact factor = 0.914).

(26) Htoon,W., S. Jogloy, N. Vorasoot, B. Toomsan, W. Kaewpradit,N. Puppala, and A. Patanothai. 2014. Nutrient uptakes and their contributions to yield in peanut genotypes with different levels of terminal drought resistance. Turk. J. Aric. For. 38: 781-791. (Impact factor 0.914).

(27) Khampas, S., K. Lertrat, K. Lomthaisong, S. Simla and B. Suriharn. 2014. Effect of location, genotype and their interactions for anthocyanins and antioxidant activities of purple waxy corn cob. Turk. J. Field Crop. *Accepted.* (Impact factor = 0.641).

(28) Rattanaprasert, R., P. Banterng, S. Jogloy, N. Vorasoot, T. Kesmala, R.S. Kanwar C.C. Holbrook and A. Patanothai. 2014. Genotypic variability for tuber yield, biomass and drought tolerance in Jerusalem artichoke germplasm. Turk. J. Agr.Fores. 38:1-11. (Impact factor = 0.731).

2.2) Number of first-submitted papers: 3

(1) Bootprom, N., P. Songsri, B. Suriharn, K. Lomthaisong and K. Lertrat. 2014. Genetics diversity based on agricultural traits and phytochemical contents inspiny bitter gourd (*Momordica cochinchinensis* (Lour.) Spreng). *Submitted* to SABRAO J. Breed.Genet. (No impact factor)

(2) Khaopha, S., S. Jogloy, A. Patanothai and T. Senawong. 2014. HDAC inhibitory activity of peanut testa extracts against human cancer cell lines. J. Food Biochem. (*Major revision*). (Impact factor = 0.853).

(3)Senakoon, W., S. Nuchadomrong, P. Jeeranaipepame, G. Senawong, S. Jogloy, P. Songsri and A. Patanothai. 2014. *Aspergillus flavus* invasion and relevant activity in aflatoxin synthesis in seeds of peanuts of different drought tolerant genotypes. *Submitted* to Turk. J. Bot. (No impact factor)

3) New crop cultivar

A new cultivar of Jerusalem artichoke, KT 50-4, was recommended for commercial production. KT 50-4 has higher tuber yied than those of Kaentawan #1, Kaentawan #2 and Kaentawan #3. It has large tubers with less branched. Therefore, it is recommended for grown as fresh vegetable.

4) Annual technical seminars

Three annual seminars had been hold during the period of the project.

The first was held at the Sirindhorn Dam, Ubon Ratchathani, during 26-27 May 2012.

The second was held at the Imperial Phukaew Hill Resort, phetchabun, during 20-22 March 2013.

The third was held at the Lam Nam Oon Irrigation Project, Sakon Nakhon, during 27-29 March 2014.

5) Linkages with Thai and foreign institutes

5.1 Linkages with Thai institutes

Linkages have been established and collaborative research on food crops has been undertaken with the following Thai researchers at different institutes:

1) Prof. Dr. Bung-orn Sripanidkulchai (Plant medicinal chemistry)
Institute: Khon Kaen University
Address: Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, Khon Kaen Universit, Khon Kaen,Thailand
E-mail: bungorn@kku ac.th

2) Assist. Prof. Dr. Kasem Nuntachai (Food product development)
Institute: Khon Kaen University
Address: Department of Food Technology, Faculty of Technology, Khon Kaen Universit, Khon Kaen, Thailand
E-mail: kasem@kku ac.th

3) Assist. Prof. Dr. Juntanee Uriyapongson(Food product development)
Institute: Khon Kaen University
Address: Department of Food Technology, Faculty of Technology, Khon Kaen Universit, Khon Kaen, Thailand
E-mail: juntanee@kku.ac.th

4) Chanchana Siripanwattana, (Food technology and product development)
Institute: Suan Dusit Rajabhat University
Address: Faculty of Science and Technology, Suan Dusit Rajabhat University, Bangkok, Thailand
E-mail: chtnuch@hotmail.com

5) Assoc. Prof. Dr. Jintanaporn Wattanathorn (Neuroscience, Physiology)
Institute: Khon Kaen University
Address: Department ofPhysiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
E-mail: jintan_w@kku.ac.th

6) Sukrichaya Hemathulin(Food product development)
Institute: Rajamungala University of Technology ISAN Sakon Nakhon Campus
Address: Faculty of Natural Resources, Rajamungala University of Technology ISAN Sakon Nakhon Campus, Sakon Nakhon, Thailand
E-mail: sukrichaya@hotmail.com

5.2 Linkages with foreign institutes

Linkages have been established and collaborative research on food crops has been undertaken with the following foreing researchers at different institutes:

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, USA.
   E-mail: Holbrook@tifton.usda.gov

2) Dr. G. Hoogenboom (Professor)
   Institute: Washington State University
   Address: Weather Net, Washington State University, Prosser, WA,USA.
   E-mail: gerrit.hoogenboom@wsu.edu

3) Dr. K.J. Boote (Professor)
   Institute: University of Florida
   Address: Agronomy Department, University of Florida, Gainesville, Florida, USA.
   E-mail: kjb@mail.ifas.ufl.edu

4) Dr. R.K. Varshney: Senior Researcher
   Institute: International Crop Research Insitute for the Semi-arid Tropic (ICRISAT)
   Address: Applied genomic lab, International Crops Research Institute For the Semi-arid Tropics (ICRISAT), Patancheru, Andra Pradesh, India.
   E-mail: r.k.varshney@cgiar.org, varshney.raj@gmail.com

5) Dr. Ramesh S. Kanwar (Environment and Water Resource Engineering)
   Institute: Iowa State University
   Address: Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, USA.
   E-mail: rskanwar@iastate.edu

6) Dr. Bill Davies (Environmental Physiology)
   Institute: Lancaster University
   Address: The Lancaster Environment Centre, Department of Biological Sciences, Lancaster University, Lancaster, UK.
   E-mail: w.davies@lancater.ac.uk

7) Dr. Ian Dodd (Root Systems)
   Institute: Lancaster University
   Address: The Lancaster Environment Centre, Department of Biological Sciences, Lancaster University, Lancaster, UK.
   E-mail: i.dodd@lancater.ac.uk

8) Dr. Naveen Puppala (Plant breeding)
Institute: New Mexico State University
Address: Agricultural Experiment Station College of Agriculture and Home Economics, New Mexico State University, New Mexico, USA
E-mail: npuppala@nmsu.edu

9) Dr. Mark Gleason (Plant pathologist)
Institute: Iowa State University
Address: Department of Pathology, Iowa State University, Iowa, USA.
E-mail: mgleason@iastate.ed

10) Dr. Susana Goggi (Seed Science)
Institute: Iowa State University
Address: Department of Agronomy, Seed Science Center, Iowa State University, Ames, Iowa, USA.
E-mail: Susana@iastate.edu

11) Dr. Thomas Sinclair (Plant Physiologist)
Institute: North Carolina State University
Address: Department of Crop Science, North Carolina State University, North Carolina State, USA.
E-mail: trsincla@ncsu.edu

12) Dr. Ratchaputi Nageswara Rao (Plant Physiologist)
Institute: The University of Queensland
Address: Centre for Plant Science, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Australia
E-mail: rao.rachaputi@uq.edu.au

13) Dr. Marvin Scott (Research Geneticist (Plants))
Institute: Iowa State University
Address: Corn Insects and Crop Genetics Research, Iowa State University, Ames, Iowa, USA
E-mail: paul.scott@ars.usda.gov

14) Dr. Jay-lin Jane (Food Science)
Institute: Iowa State University
Address: Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa, USA
E-mail: jjane@iastate.edu

6) Linkages with private enterprises

Linkages have been established with the following enterprises:

1) **The Lily Industry Co., TTD**: Cooperated with the project in testing the quality of promising peanut lines for making various industrial products.

2) **Tipco Asphalt Public Company Limited** located in Pran buri, Prachuap Khiri Khan, has a strong co-operation with the project. The co-operation includes the test of Jerusalem artichoke varieties, multiplication of seed tubers, and research in inulin extraction for commercial production of inulin

3) **Chokechai-Farm** located in Nong Nam Daeng, Pak Chong, Nakhon Ratchasima, has a strong co-operation with project. The farm uses Jerusalem artichoke for improving landscape for tourist attractions and sells seed tubers and fresh vegetable tubers.

4) **Phumarn-mek Resort** located in Pak Chong, Nakhon Ratchasima, also uses Jerusalem artichoke for improving landscape for tourist attractions and sells tubers.

5) **Rai Piriya** located in Pak Chong, Nakhon Ratchasima, uses Jerusalem artichoke and sells seed tubers and fresh vegetable tubers.

6) **Rai Kaentawan@Wangnamkeaw** located in Wangnamkeaw, Nakhon Ratchasima, uses Jerusalem artichoke for improving landscape for tourist attractions and sells seed tubers and fresh vegetable tubers.

7) **Nopphawan Khanom Thai Company Limited** located in Ladyuo, Jatujack, Bangkok, uses Jerusalem artichoke as an ingredient of bakery products.

8) **Jim Thompson Farm** located in Pak Thong Chai, Nakhon Ratchasima, produces fresh vegetable tubers and markets the tubers.

9) **Chia Tai Co., Ltd. (Choncharoen Farm)** located in Kanchanaburi-Saiyok Rd, Wangdong, Muang, Kanchanaburi, produces the highest quality seed tubers and fresh vegetable tubers.