Physiological and Metabolic Responses to Water-deficit and Heat Stress of Virginia-type Peanut Cultivars and Breeding Lines

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ABSTRACT

The Virginia-Carolina (VC) region including Virginia, North Carolina, and South Carolina, is the most important peanut production region for the large seeded, virginia-type peanut in the United States. In recent years, an increased frequency of heat and drought episodes with significant effects on peanut yield was observed in the VC region. Because limited information is available on the mechanisms virginia-type peanut develops in response to heat and water stress, the present study evaluated several physiological and metabolic characteristics and their relationship with yield for eight cultivars and breeding lines. Experiments were conducted under rainfed and irrigated field trials in 2011 and 2012, and in a growth chamber under optimum (30/25 ºC) and high temperature (40/35 ºC) conditions. The long term goal of this study is to help development of more tolerant peanut cultivars to heat and drought in the VC region. Visible symptoms of water-deficit stress were observed in peanut during the field experiments in both years. Significant ($p \leq 0.05$) variations for yield, membrane injury, chlorophyll fluorescence (F_v/F_m ratio), specific leaf area, SPAD chlorophyll content, and relative levels of polar and non-polar metabolites were observed in response to water regime, growth stage, and genotype in both years during the field studies. Similarly each year, the F_v/F_m ratio, organic acids, and saturated fatty acids decreased in rainfed *vs*. irrigated plants, while the sugar and sugar alcohol relative levels increased. Regardless the water regime, lower levels of saturated fatty acids and sugars, and higher levels of unsaturated fatty acids and sugar alcohols were associated ($p < 0.05$) with

higher pod yield in field conditions. Genotypes Phillips, SPT06-07, and N05006 showed potential tolerance and N04074FCT, CHAMPS, and Bailey susceptibility to water deficit in field studies. Significant physiological and metabolic changes were also observed in response to heat stress under controlled conditions in peanut seedlings. A general decrease in organic acid and saturated fatty acid levels and an increase in membrane injury, sugar, and unsaturated fatty acid levels were observed under both water deficit and heat stress conditions. Overall, results from both experiments were suggestive of natural stress responses rather than adaptive mechanisms to water deficit and heat stress of the virginia-type genotypes used in this study. Among all genotypes, SPT 06-07 showed improved tolerance to both stresses. Our results suggest that monitoring chlorophyll fluorescence and changes in the levels of selected metabolites can be used to screen new peanut lines for drought and heat stress tolerance.

Dedication

This thesis is dedicated to my grandmother, Gurbachan Kaur. Thanks for your childhood stories that instilled in me the seed of curiosity. I would also like to dedicate this thesis to my parents S. Harjinder Singh and Karamjit Kaur. Thanks for your selfless love and endless faith, without which I would not be a person I am today. Lastly, to my brother and best friend Taranjit, he has always been a source of inspiration and motivation to me.

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Table of Contents

List of Tables

Chapter 2.

Appendix: Supplemental Tables

Chapter 2.

List of Figures

Chapter 2.

Chapter 3.

Chapter 1 - Literature Review

Peanut Crop. Cultivated peanut (*Arachis hypogaea* L.) is a self-pollinated, allotetraploid (2n = 4X = AABB = 40) plant with genomic size of 2891 Mbp (ICRISAT.ORG). Hybridization of diploids *A. duranensis* Krapov. & W.C. Gregory and *A. ipaensis* Krapov. & W.C. Gregory gave rise to the tetraploid *A. monticola* Krapov. & Rigoni which is considered the wild relative of cultivated peanut (Kochert et al., 1996). Peanut is probably originated in South America near Peru around 3000 BC (Seijo et al., 2007; Simpson and Faries, 2001). Earliest evidence of peanut cultivation dates back to 1500-1800 BC in Northwest Peru.

Peanut is the fourth major oilseed crop grown worldwide after soybean [*Glycine max* (L) Merr.], rapeseed (*Brassica napus* L.) and cottonseed (*Gossypium hirsutum* L.) (USDA FAS 2012) and represents a major source of protein in the diet of people in developing countries. For example in 2011, peanut was grown on 21.7 million ha with a total production of 38.61 MMT worldwide (FAOSTAT 2013). With 444,190 ha and 1.65 MMT of production in 2011 (FAOSTAT 2013), the U.S.A. was the fourth largest producer of peanut after China, India, and Nigeria. The major peanut producing states in the U.S.A. are Georgia, Texas, Florida, and North Carolina. It is important to note that most of the peanut producing areas in the world have arid or semi-arid climates and are rainfed.

There are four major market types of peanut classified in the U.S.A.: virginia, runner, spanish, and valencia. Runner market type is primarily grown in the southwest and southeastern part of the country. Virginia market type is mainly grown in the Virginia-Carolina (VC) region including Virginia, North Carolina, and South Carolina. Virginia-type peanut is characterized by its large pods and kernels, and it is often used in confectionary and in-shell peanut trade. In the

VC-region, peanut is annually grown on 50,000-76,000 ha with over 85% of this area rainfed (Balota et al., 2012). Rainfed crops are at high risk of water stress during the growing season. Recent hot dry growing seasons have underscored the importance of drought and heat as major limiting factors for peanut production in the VC-region. The prognosis of a further temperature increase by 5˚C by 2080 suggests that limitation of crop productivity by water and heat stress may become more severe in the mid-Atlantic region.

Abiotic stresses and peanut production. Plants of subtropical origins such as peanut (*Arachis hypogea* L.) are sensitive to drought and heat (Rao and Wright, 1994; Reddy et al., 2003; Vara Prasad et al., 2003). For example, water deficit has been shown to reduce peanut growth rate by 30% and weight per seed by 23% (Sexton et al., 1997). Heat also affected peanut yield and quality. A study by Vara Prasad et al. (2003) has reported a significant decrease in yield and harvest index of peanut as temperatures increased from 32 to 44°C during the day and from 22 to 34 C during the night. In this study, yield decrease was attributed to lower seed set due to poor pollen viability at high temperature. Quality traits were also significantly affected and it has been speculated that peanut yield will decrease under future warmer climates, particularly in regions where present temperatures are near or above optimum (Vara Prasad et al., 2003).

It has been estimated that over two thirds of the peanut crop worldwide is rainfed where water deficit stress often poses a threat to crop productivity (Nageswara Rao et al., 2001). Abiotic stress plays a major role in crop growth, development, and productivity. In particular, drought and heat influence an array of processes at the biochemical and physiological levels as well as whole plant level through plant growth and development. Effects on the crop level reduce yield and quality. Abiotic stresses interfere with the normal functioning of the cells by

alteration of vital physiological processes. Plants experience increased levels of detrimental reactive oxygen species (ROS) production during abiotic stresses (Apel and Hirt, 2004; Bartels, 2001; Gill and Tuteja, 2010). Excessive ROS production mainly occurs in the chloroplasts and mitochondria, and leads to membrane damage and loss of photosynthetic efficiency (Asada, 2006; Foyer and Noctor, 2005; Rhoads et al., 2006). As protective measures, plants develop stress tolerance and recovery mechanisms that involve an intricate network of molecular, biochemical, and physiological changes that act together to achieve a specific adaptive response at the whole plant and crop levels (Prasad et al., 2011; Rizhsky et al., 2004). These mechanisms include steps to minimize water loss and the accumulation of osmoprotectant solutes during stress (Kaiser, 1987; Medrano et al., 2002). However, stress adaptation and protective mechanisms can use plant energy resources, leading to reduced plant growth (Sibly and Calow, 1989; Tong et al., 2004). For example, as an adaptive response to prolonged drought, plants preferentially allocate more photosynthates to the roots rather than to the above-ground plant organs to facilitate active root growth (Pelleschi et al., 1997; Westgate and Boyer, 1985). This would improve plant survival during stressful periods but may reduce above-ground biomass and yield. Therefore, identifying the mechanisms that are conducive to higher yields and quality in a target environment as opposed to just simply survival seems to be very important for increasing production under drought and heat.

Even though some general reactions occur in all plants, some genotypes may better tolerate and recover from abiotic stress compared to others. Therefore, development of stress-tolerant cultivars is important. But in order to do so, it is imperative to identify the metabolic and physiological mechanisms that allow both stress tolerance and high yields in a target environment. For example, identification of metabolites produced under stressful conditions

may provide new insights into the development of stress-tolerant cultivars through either conventional breeding and/or metabolic engineering (Fernie and Schauer, 2009; Valliyodan and Nguyen, 2006).

Physiological adaptations to stress. Reproductive stages of peanut are sensitive to temperatures over 35^{o}C and yield losses were reported when temperatures exceeded this threshold value (Ketring, 1984; Vara Prasad et al., 2000). Apart from heat, drought significantly affects physiological processes in all plants. Drought occurs when the plant leaf water content is below the optimum turgor of 95 to 98% relative water content depending on leaf age. Because peanut is primarily a rainfed crop, it is often hypothesized that improving water use efficiency would be the best strategy to cope with the intermittent drought spells in various peanut growing areas of the world (Krishnamurthy et al., 2007). The biological model developed by Passioura (1986) describes grain yield as a function of water transpired, water use efficiency, and harvest index. Water use efficiency used in Passioura's model is the total biomass produced per unit of water transpired, and greater water use efficiency in crops is often associated with a good adaptation to water stress. Most research related to enhancement of drought tolerance in peanut has highlighted the importance of transpiration efficiency as an important component of water use efficiency (Rao and Wright, 1994). In addition to transpiration and water use efficiency, several other physiological characteristics were reported in peanut in response to drought, and many of them were highly correlated with agronomic traits such as yield. For example, Nigam et al. (2005) reported that water use efficiency, specific leaf area, and leaf chlorophyll content measured by soil plant analysis development (SPAD) chlorophyll meter can be successfully used for selection of drought-tolerant and high-yielding peanut varieties.

Other plant characteristics can also be used as substitutes for transpiration and water use efficiency. For example, specific leaf area and SPAD chlorophyll content were reported as good surrogates to select peanut genotypes with improved transpiration and water use efficiency. The correlation between specific leaf area and transpiration efficiency is negative while that between transpiration efficiency and SPAD chlorophyll reading is positive (Madhava et al., 2003; Sheshshayee et al., 2006). Significant correlations were also reported among SPAD chlorophyll reading, specific leaf area and specific leaf nitrogen in peanut (Nageswara Rao et al., 2001). Sheshshayee et al. (2006) reported a strong and positive relationship between SPAD chlorophyll reading and WUE. Recent studies have shown that genetic variation for SPAD chlorophyll reading exists in peanut (Upadhyaya, 2005). Because of the good correlation between chlorophyll and N content, SPAD chlorophyll readings alone are used to estimate nitrogen content in several economically important crops (Chapman and Barreto, 1997, Rorie et al., 2011). As such, SPAD chlorophyll reading was recommended as an appropriate selection criterion for screening for improvement in drought tolerance in peanut because of its high heritability and simplicity (Songsri et al., 2009; Upadhyaya, 2005).

Maintenance of cell membrane integrity under various abiotic stresses is critical for optimum plant growth and development. For example, drought and heat stress may alter membrane lipid bilayer structure and cause membrane protein displacement, which together with solute leakage is believed to contribute to the loss of membrane selectivity (Blum and Ebercon, 1981; Du et al., 2011). The higher concentration of cellular electrolytes that accompany water stress may exacerbate loss of membrane integrity and protein stability. Increased concentrations of certain metabolites in the cells may increase membrane integrity during water deficit stress (Matos et al., 2010). The rate of injury to cell membranes by water deficit and heat may be

estimated through measurements of electrolyte leakage from the cells (Blum and Ebercon, 1981; Leopold et al., 1981). A membrane injury test such as electrolyte conductivity test has been widely used to differentiate stress tolerant and susceptible cultivars in wheat (Bajji et al., 2002; Blum and Ebercon, 1981; Leopold et al., 1981), turf grass (Du et al., 2011) and peanut (Celikkol Akcay et al., 2010; Ketring, 1985; Lauriano et al., 2000). Stress-induced membrane injury reduced grain yield and quality traits (Saadalla et al., 1990; Fokar et al., 1998). Tripathy et al. (2000) also reported quantitative trait loci associated with membrane injury under water stress conditions in rice (*Oryza sativa* L.). The relationship between membrane injury and other physiological traits such as specific leaf area and chlorophyll content was also demonstrated in peanut (Nautiyal et al., 2008). Thus, membrane injury tests offer a promising tool for assessing the stress tolerance ability in peanut.

Plants employ various mechanisms to avoid stress. Drought-tolerant cultivars tend to maintain cooler canopies during stress periods (Ayeneh et al., 2002). Canopy temperature measured by infrared thermometers above the crop canopies is gaining wide acceptance as a potential selection tool for improved drought and heat tolerance and yield of crops (Balota et al., 2008; Blum et al., 1988; O'Toole et al., 1984). The canopy temperature depression (CTD) method was described as one of the fastest, non-destructive, and non-disruptive methods of screening for drought stress and yield (Balota et al., 2012; O'Toole et al., 1984). Speed and precision of measurement and relatively low cost are key considerations in applying a method of selection in plant breeding. Ayeneh et al. (2002), recommended the use of CTD as a selection tool for heat tolerance in wheat.

Leaf chlorophyll fluorescence, measured as the ratio of variable (F_v) to maximum (F_m) fluorescence (F_v/F_m), is extensively used in abiotic stress studies by physiologists and

ecophysiologists (Burke, 2007; Maxwell, 2000). During the course of photosynthesis, photosystem II emits excessive light in the form of chlorophyll fluorescence, which represents the basic principle behind the F_v/F_m measurement [Read, Maxwell (2000) for review]. Relative decline in the F_v/F_m ratio in leaves is often used as an indication of abiotic stress, including drought. Burke (2007) demonstrated the link between F_v/F_m and sucrose levels in drought-stressed leaves of cotton. Kottapalli et al. (2009) further used this assay to screen drought-tolerant peanut genotypes. In general, lower F_v/F_m values were associated with higher stress levels (Du et al., 2011). Qin et al. (2011) reported a similar decline in the F_v/F_m ratio with increasing salt and chilling stress in peanut. The relationship between chlorophyll fluorescence parameters (including F_v/F_m) and pod yield in field-grown peanuts was also reported (Clavel et al., 2006).

Biochemical adaptations to stress. Various analytical techniques are being used for identification of metabolites involved in plant adaptation to environmental changes (Fiehn, 2002; Kaplan et al., 2004; Guy et al., 2008). Analysis of stress-induced metabolic profiles can provide new insights into the mechanisms of stress tolerance in crops and the resulting information can further assist in developing stress-tolerant cultivars through conventional breeding and metabolic engineering techniques (Fernie and Schauer, 2009; Valliyodan and Nguyen, 2006). In response to abiotic stresses, plants produce metabolites that are involved in primary and secondary metabolism (Rizhsky et al., 2004; Shulaev et al., 2008). In plants, these metabolites act as osmoprotectants, compatible solutes, reactive oxygen species scavengers, and signal transduction molecules (Farooq et al., 2009; Hare et al., 1998). For example, McManus et al. 2000) found that pinitol was the major soluble carbohydrate present in leaf tissue of white clover (*Trifolium repens* L.) after a long period of water stress. In soybean, high-temperature stress significantly

increased leaf pinitol content (Guo and Oosterhuis, 1995). A study conducted by Matos et al. (2010) suggested the role of pinitol as an osmolyte in chickpea (*Cicer arietinum* L.) under drought stress. Sugars serve as a carbon source and osmoprotectants, and their levels change during periods of stress. Significant decreases in sugar content were observed in potato (*Solanum tuberosum* L.) and chickpea exposed to drought stress (Matos et al., 2010; Vasquez-Robinet et al., 2008). It has been shown that the tolerant cultivars of barley (*Hordeum vulgare* L.) (Widodo et al., 2009) and turf grass (Du et al., 2011) accumulate higher levels of specific sugars, sugar alcohols, and organic acids in response to salt and heat stresses. In their comparative metabolomic study of Andean potatoes, Vasquez-Robinet et al. (2008) reported that the drought-tolerant variety showed higher accumulation of organic acids (citric and caffeic acid) during drought than the sensitive variety. Kaplan et al. (2004) reported accumulation of galactinol and raffinose in response to heat stress in *Arabidopsis thaliana* (L.) Heynh. A recent study by Xu et al. (2011) suggested that the ability to maintain high levels of unsaturated fatty acids contributed to improved drought tolerance in Kentucky bluegrass (*Poa pratensis* L.). Their results support the hypothesis that metabolite accumulation induces physiological adaptations to drought, heat, and cold, and thus their further use as tools for varietal selection should be considered.

Changes in levels of specific metabolites are not the only molecular changes accompanying stress responses in plants. Kottapalli et al. (2009) reported differential regulation of proteins involved in various stress adaptive physiological and metabolic functions in drough-stressed peanuts. However, in some cases the accumulation of metabolites, e.g. amino acids, seemed to be merely a consequence of growth reduction under stress and not a stress adaptive response (Munns, 2002; Widodo et al., 2009). By comparing changes in metabolite levels of different

genotypes with differential stress tolerance potential, metabolites can be easily associated with the stress tolerance levels. To our knowledge, no significant work has been done on peanuts in relation to changes of metabolite levels during drought and heat stress, their role in stress tolerance and yield under stress, and their potential role as markers for varietal selection.

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Chapter 2 - Physiological and Metabolic Responses to Water Deficit Stress of Virginia-type Peanut (*Arachis hypogaea* L.) Cultivars and Breeding Lines

ABSTRACT

Water deficit is a major limiting factor for peanut production in the Virginia-Carolina region. We evaluated several agronomic, physiological, and metabolic characteristics of eight virgnia-type peanut cultivars and advanced breeding lines at three growth stages (R1, beginning flower; R3, beginning pod, and R5, beginning seed) in rainfed and irrigated field trials for two consecutive years at Suffolk, VA. Visible symptoms of water-deficit stress were observed at Stages R1 and R3 in 2011 and at Stage R1 in 2012. Inconsistencies in these observations can be attributed to temperature and humidity differences between the both years. Significant ($p \leq 0.05$) variations in yield, grading factors, physiological characteristics, and the levels of polar and non-polar metabolites were observed in response to different water regimes, growth stages, and genotypes in both years. In 2011, rainfed peanut plants exhibited greater membrane injury than irrigated plants at the R1 stage. Rainfed plants had greater specific leaf area compared to the irrigated plants in 2011 (135 *vs*. 131 $cm^2\ g^{-1}$). In contrast, rainfed plants exhibited lesser specific leaf area than irrigated plants in 2012 (133 *vs*. 144 $cm^2\ g^{-1}$). In general, rainfed plants showed greater decline in chlorophyll fluorescence measured as the F_v/F_m ratio compared with the irrigated plants at Stages R1 and R3, while breeding lines N04074FCT and N05024J consistently showed a greater decline in F_v/F_m than other lines. Principal component analysis on correlations of metabolite levels revealed that sucrose, succinate, maleate, citrate, quinate, and saturated fatty acids were the best correlated. This correlation explained the largest proportion of the variance between growth stages in the leaves of rainfed plants. With a few exceptions, fatty acid content

decreased or was unchanged in rainfed plants. Regardless of the water regime, lower levels of saturated fatty acids and sugars ($p < 0.05$) and higher levels of unsaturated fatty acids and sugar alcohols in peanut leaves were associated with higher pod yield.

INTRODUCTION

Virginia-type peanut is an important crop for the Virginia-Carolina (VC) region comprising Virginia, North Carolina, and South Carolina. It is a "cash" crop grown annually on over 50,000-76,000 ha in all three states bringing important revenues to the farmers in these states. Historically, precipitation in this region has been abundant and peanut production of over 250,000 tons per year easily achieved without supplemental irrigation. This is also why over 85% of the current peanut farming is rainfed cultivation rather than irrigated. However, depending on soil type and geography, parts of the VC region have experienced water shortage during peanut growth and development in some years. This was due to inadequate rainfall during the growing season, as well as to high frequency of temperature extremes. In addition, the fine-loamy sandy soils that are predominantly used for peanut production do not have good water holding capacity, exacerbating water deficit stress. For example, in 2009, in general a rainy year, reduced precipitation during most part of June reduced peanut growth and resulted in decreased yields (Balota et al., 2012a). Similarly, due to high temperatures and drought in 2010, yield and yield quality were drastically reduced for peanut grown in the VC region (Balota et al., 2012a). Adoption of irrigation is unlikely due to already high peanut production costs in Virginia and Carolinas. Acreage increase is also improbable, as reflected by the decline in the past decade. Therefore, increasing yield under drought and heat in the VC region can only be achieved by development of drought and heat tolerant cultivars.

Peanut crop can be susceptible to drought and high temperature stress (Bell et al., 1994). Temperature extremes and drought stress both individually or combined negatively influence the physiological fitness of peanut plants, pod and seed quality, and yield. As such, there is a good incentive for development of peanut cultivars with superior tolerance to drought and high

temperature. This goal can be achieved by enhancing the adaptive metabolic and physiological mechanisms of stress responses in peanut through genetic improvement.

In recent years, several physiology-based tools were developed to measure the relative levels of stress experienced by plants under drought and high temperature. These tools are valuable for screening germplasm for stress tolerance. Some of the most commonly used screening tools include measuring chlorophyll content, specific leaf area (SLA), the F_v/F_m chlorophyll fluorescence ratio, membrane injury, and canopy temperature depression (Du et al., 2011; Reynolds et al., 2009; Upadhyaya, 2005). Gas chromatography- and mass spectrometry-based metabolite profiling is also gaining popularity in crop improvement research as it offers a high throughput screening platform to identify metabolites underlying specific stress responses (Fernie and Schauer, 2009; Fiehn, 2002). Integrated use of metabolic, physiological, and genetic tools is likely to enhance the process of cultivar development for various stresses and agronomic traits in the near future (Varshney and Dubey, 2009).

Unquestionably, more challenges for peanut production in terms of peanut yield and quality are expected to emerge over the next years in the VC region. Therefore, it is imperative to develop new strategies to increase crop productivity in parallel with water conservation and reduced production costs. A potential way to increase peanut yield and quality with no additional production costs is through improving inherent stress tolerance of the plants themselves, through breeding for physiological and biochemical traits that increase yield under reduced precipitation and temperature extremes. This strategy has been successfully used for many field crops including peanuts in other states and countries (Kumar et al., 2008; Morgan, 2000; Nigam et al., 2005). However, limited research in relation to drought and heat tolerance of the virginia-type peanut is available in the VC-region. The objectives of this research were to

understand the mechanisms of water deficit and high temperature stress tolerance in the virginia-type peanut genotypes grown in the VC region by identifying potential adaptive traits and metabolites that affect pod yield and quality.

MATERIALS AND METHODS

Plant materials and growth conditions. Eight peanut lines including commercial cultivars Bailey, CHAMPS, and Phillips and and advanced breeding lines N04074FCT, N05006, N05008, N05024J, and SPT 06-07 were selected from the Peanut Variety and Quality Evaluation (PVQE) program based on their phenotypic differences in terms of yield and ability to sustain extreme temperatures and drought (Balota et al., 2012, a and b). This variability could be a result of potentially diverse metabolic and physiological responses to drought and heat stresses. CHAMPS is an early maturing virginia-type peanut developed at Virginia Tech (Mozingo et al., 2006). Bailey and Phillips are relatively new cultivars released by the North Carolina State University (NCSU) (Isleib at al., 2006, 2011). Bailey has higher yielding potential and matures earlier than Phillips. Bailey also has higher transpiration efficiency (TE) than Phillips, but Phillips has more stomata on both sides of leaf and maintains high CO_2 assimilation during drought spells (Balota et al., 2012a). Genotypes N04074FCT, N05006, N05008, N05024J, and SPT 06-07 are advanced breeding lines from the NCSU. Among them, N04074FCT has the highest TE but low yield, N05024J has low TE but high yield, and N05006 and N05008 have high TE and high yields. SPT 06-07 maintains the "stay green" leaf color under drought, suggesting active photosynthesis under adverse stress conditions.

Field experiments were conducted at the Tidewater Agricultural Research and Extension Center, Suffolk, VA (36°68′ N, 76°77′ W, 25 m elevation) in 2011 and 2012 to evaluate the

stress tolerance potential of these genotypes under rainfed and irrigated conditions. The experiment was a split-split plot design with whole plots arranged in randomized complete blocks (RCB). The water regime was the whole plot factor, genotype was the sub-plot factor, and growth stage was the repeating measure factor. Physiological and metabolic measurements were taken on a fully expanded third youngest leaf arising from the main stem at three peanut growth stages, beginning flower (R1), beginning pod (R3), and beginning seed (R5) (Boote, 1982). Membrane injury was not measured at Stage R3 in 2011, and canopy temperature depression was not measured at Stage R1 in 2011 and R3 in 2012. Also, data collected for metabolite profiling on leaf samples taken at R5 in 2011 were dropped due to poor preservation during shipment from Suffolk to Blacksburg, where analysis was conducted. Individual plots of 22 m^2 (12.2 m each row and 0.91 m between rows) and with approximately nine seeds per meter of row were treated as the experimental unit. Cultural practices were performed according to the Virginia Peanut Production Guide (http://www.ext.vt.edu). Air temperature, RH, rainfall, and photosynthetically active radiation (PAR) were continuously monitored next to the plots with a weather station (WatchDog Temperature/RH Station, Model 2450, Spectrum Technologies Inc., Plainfield, IL), in both years. In 2011, 25.4 mm of irrigation was supplied to the irrigated plots on 24 June, 31.8 mm on 1 July, and 22.9 mm on 19 July using a lateral pull boom cart sprinkler irrigation system (E1025 Reel Rain, Amadas Ind., Suffolk, VA). In 2012, plots in the irrigated water regime received 16.5 mm irrigation on 28 June and 15.8 mm on 18 July. Yield data and grade characteristics, *i.e.*, the content of extra-large kernels (ELK) and sound-mature kernels (SMK), were collected at physiological maturity.

Membrane injury. Cell membrane stability of four leaflets per plot from the third youngest fully expanded leaf arising from the main stem was used to measure membrane injury

(MI) based on a modified method by Blum and Ebercon (1981). Leaf discs were sampled with an 11.1 mm diameter disc sampler. After being rinsed twice with distilled water, leaf segments were placed in 20 ml plastic vials containing 15 ml distilled water. Each sample vial contained four leaf discs. After shaking for 24 h, initial conductivity (Ci) of the bathing solution was measured with a conductivity meter (Seven-multi conductivity module and InLab® 741 electrode, Mettler-Toledo Inc., Columbus, OH). Leaf discs were later killed in an autoclave at 120°C for 45 minutes, and placed on a shaker for 24 h before recording the final conductivity (Cf) of the bathing solution. The percent MI was calculated using the formula:

$$MI = Ci/Cf*100$$

Canopy temperature depression. The canopy temperature depression (CTD) of each plot was measured with a hand-held infra-red thermometer (IRT) (Agri-Therm Model 6110L, Everest Interscience Inc., Tucson, AZ) twice at the center of each row in two rows, a total of four readings per plot, at approximately 50 cm above the canopy. The CTD was measured as the difference between canopy temperature (T_c) and air temperature (T_a) and converted to positive values when canopies were cooler than the air by multiplying the Agri-Therm readings with -1:

$$CTD = -1\ (T_c - T_a)$$

Leaf characteristics. Four fully expanded third youngest leaves per plot from the main stem were harvested in the field and transported to laboratory in moistened paper towels for measurements of leaf area using a LI-3000 leaf area meter (LICOR Inc., Lincoln, NE) and for relative chlorophyll content by the SPAD (Soil Plant Analysis Development) chlorophyll reading (SPAD-502, Konica Minolta Optics Inc., Japan). Specific leaf area (SLA) was calculated after weighing the dried leaves in an oven at 65ºC for 24 hours using the formula:

$$SLA = \text{Leaf area } (cm^2) / \text{leaf dry weight } (g)$$

Chlorophyll florescence. Chlorophyll florescence was measured as the Fv/Fm, the ratio of variable (Fv) to maximum (Fm) chlorophyll fluorescence as described (Burke, 2007) with modifications. Five fully expanded third from the top leaves per plot were harvested at peanut growth Stages R1, R3, and R5. The Fv/Fm ratio was measured by modulated chloropyll fluorometry (OS1p, OptiSciences Inc., Hudson, NH). Five leaf discs were prepared using an 11.1mm diameter disc sampler. Leaf discs were then arranged on a moist tissue paper in a Pyrex® glass dish, covered with transparent film (Cling Wrap, GLAD® Products Company), and carefully pressed flat to remove air bubbles, and to ensure good contact between the leaf discs and film. Initial Fv/Fm values were recorded prior to incubation. The samples were incubated at 45oC for 3 h in dark. Subsequently, hourly Fv/Fm measurements were taken from the dark-incubated samples. A total of five Fv/Fm readings were taken per plot to evaluate the relative changes in the chlorophyll fluorescence during incubation.

Metabolite sampling and extraction. At R1, R3, R5 growth stages, indiaviducal leaflets representing the third youngest leaf from ten different plants were pooled together and immediately frozen in liquid nitrogen in the field. Samples were then stored at -80ºC until shipped overnight on dry ice to Blacksburg, VA for metabolite profiling analysis. The leaves were freeze dried under vacuum for 48 hours on a benchtop FreeZone lyophilizer (LABCONCO, Kansas City, MO). The dried leaf tissue was then disrupted to a fine powder with steel beads on a commercial paint shaker. Internal standards (2 µl each of 7.4 mM C17:0 and 10 mM ribitol) were added to each sample prior to the extractions to account for losses during extractions and metabolite derivatization for quantification purposes. Dry leaf tissue powder (4.00 ± 0.05 mg) was weighed and extracted with chloroform and water (400 µl each). After the addition of chloroform, test tubes were vortexed and briefly centrifuged to bring liquid down from the lids.

Subsequently, water was added and tubes vortexed to partition polar and non-polar metabolites. After the final centrifugation for 5 minutes, the polar and non-polar extraction phases were physically separated by a completely white solid interphase containing insoluble material such as cell walls, starch, and proteins. The white color indicated a complete breakage of the membranes and extraction of metabolites, as all chlorophyll from the thylakoid membranes was extracted by chloroform. The top (clear) aqueous phase contained polar metabolites (sugars, sugar-alcohols, organic acids, and amino and carboxylic acids, organic amines, and major phenolics), while the bottom (green) organic phase contained the non-polar metabolites (lipids, chlorophyll, carotenoids, and other non-polar compounds such as sterols). These phases were used for metabolite profiling (MP) and fatty acid (FA) analysis. For polar metabolite profiling, 50 µl of the aqueous phase was transferred to 250 µl-glass inserts placed in auto-sampler vials. A volume of 200 µl of non-polar extracts was transferred into glass derivatization vials for fatty acid analysis. Both water and chloroform were evaporated under a stream of N2 gas at 50oC.

Polar metabolites. After complete drying, polar metabolites were derivatized with 25 µl of methoxyamine.HCl in pyridine. The vials were rigorously vortexed and incubated at 50^{o}C for 2 hours. Subsequently, 25 µl of N-methyl-N-(trimetylsilyl) trifluoroacetamide containing 1% (v/v) trimethylchlorosilane was added. After thorough vortexing, the samples were incubated at 50^{o}C for 30 minutes.

Derivatized extracts (1 µl volume) were injected in a pulsed, 5:1 split mode on an Agilent 7890A series gas chromatograph (GC) (Agilent Technologies, Santa Clara, CA) equipped with a DB-5MS-DG capillary column (30 m x 0.25 mm x 0.25 µm; Agilent Technologies). The injection temperature was set at 280^{o}C. Helium was used as a carrier gas at a constant flow rate of 1.2 ml min^{-1}. The temperature program was: 60^{o}C for 1 min, 10^{o}C min^{-1} to 325^{o}C, and final

hold at 320°C for 5 min. Electron impact was used for molecule ionization and the analysis was carried out in a positive scan mode using an Agilent 5975C series single quadrupole mass spectrometer (MS) (Agilent Technologies).

Individual peaks were characterized using both spectral and retention time information of a given compound against the commercially available reference databases such as spectral NIST library (National Institute of Standards and Technology, Gaithersburg, MD), FiehnLib (Kind et al., 2009), and the custom-built spectral and retention time library generated in Čolláková laboratory. Signals from coeluting compounds were deconvoluted using Automated Mass Spectrometry Deconvolution and Identification System (AMDIS, NIST). Individual metabolite identity and integration quality was checked using ChemStation software (Agilent Technologies). Individual metabolite quantitation recovery was optimized using relative peak areas of internal standards. Relative metabolite levels were determined after normalizing with internal standard ribitol and dry tissue weight.

Fatty acids. After drying with a stream of N2, 500 µl of 1N methanolic HCl was added to each vial. After vortexing, the samples were incubated at 75oC for 2 hours to hydrolyze membrane lipids into glycerol and fatty acids and generate volatile fatty acid methyl esters (FAME). Methanolic HCl was evaporated under a stream of N2 and FAME were dissolved in 100 µl of heptane. After vortexing, the heptane containing FAME was transferred to 250-µl glass inserts placed in GC-MS vials and 1 µl was injected in a pulsed splitless mode on GC coupled to an Agilent flame ionization detector. The injection temperature was set at 250oC. Helium was used as a carrier gas at a flow rate of 1 ml min-1. Air and hydrogen flow rate was 350 and 30 ml min-1, respectively. The temperature program was: 100oC for 0.5 min, 20oC min-1 to 250oC, and final hold at 250oC for 6 min. The individual fatty acids were identified based on the

retention times. Relative peak areas of the internal were used to correct for recovery in quantitation of individual fatty acids. The absolute fatty acid levels were computed after dividing with the dry tissue weight.

Statistical analysis. Data from MI, F_v/F_m, CTD, SLA, SPAD chlorophyll reading, and metabolite profiling were analyzed as repeated measures design in a split-split plot arrangement, with growth stage as the repeated measure factor. Individual plots were treated as the experimental unit and measurements were taken at three peanut growth stages at beginning flower (R1), beginning pod (R3), and beginning seed (R5). The univariate model for the repeated measures data was:

$$y_{jkl} = \mu + w_j + g_k + s_l + (wg)_{jk} + (ws)_{jl} + (gs)_{kl} + (wgs)_{jkl} + e_{jkl}$$

where μ is the overall mean effect, w_j the main effect of the j^{th} water regime (j = 1, 2), g_k the main effect of the k^{th} genotype (k = 1 to 8), s_l the growth stage effect (l = 1 to 3), $(wg)_{jk}$ the interaction of the j^{th} water regime and k^{th} genotype, $(ws)_{jl}$ the interaction of the j^{th} water regime and l^{th} growth stage, $(gs)_{kl}$ the interaction of the k^{th} genotype and l^{th} growth stage, $(wgs)_{jkl}$ the interaction of the j^{th} water regime, k^{th} genotype, and l^{th} growth stage, and e_{jkl} the random error associated with the $ijkl^{th}$ experimental unit. Genotypes and water regimes were treated as fixed effects while the effects of repeated measures, i.e., growth stage were considered to be random. The sphericity requirements of a univariate repeated measures analysis were tested and then adjusted accordingly using Greenhouse-Geisser (G-G) and Huynh-Feldt (H-F) corrections (Collaku and Harrison, 2002). The data were analyzed using MANOVA option for repeated measures on JMP software (SAS Institute Inc., Version 9.0).

Correlations between the physiological, metabolic, and agronomic traits were calculated. For regression analysis, pod yield was treated as the dependent variable and physiological and

metabolic traits as the independent variables. Step-wise regression selection analysis was carried out to identify the most significant variables that explained the largest proportion of variation in pod yield.

Principal component analysis (PCA) was performed on the metabolite data from each year separately using JMP software. Variance component estimation was based on a restricted maximum likelihood method. Loading and score plots were used to reveal the correlations and degree of variation present in the metabolite data. The first two principal components explained the highest percentage of variation between samples, hence further analysis were based on these two principal components.

Data on pod yield and grading factors (ELK and SMK) was taken at the end of the season and analyzed as a split plot design. All the treatments were kept fixed in the ANOVA model:

$$y_{jk} = \mu + w_j + g_k + (wg)_{jk} + e_{jk}$$

where μ is the overall mean effect, w_j the main effect of the j^{th} water regime ($j = 1, 2$), g_k the main effect of the k^{th} genotype ($k = 1$ to 8), $(wg)_{jk}$ the interaction of the j^{th} water regime and k^{th} genotype, and e_{jk} the random error associated with the experimental unit.

For all traits, mean differences for water regimes and genotypes within an year and growth stage were calculated by Student's t-test ($p < 0.05$) and Tukey's HSD test ($p < 0.05$).

RESULTS

Years 2011 and 2012 in general, and the leaf sampling times (June throughout August each year) in particular, were different from each other in terms of temperature, rainfall, relative humidity (RH), and photosynthetically active radiation (Fig. 2.1). Overall, the growing season of 2011 was hot, dry with high maximum temperatures (34.1^{o}C) and relatively low precipitation

(271.0 mm), respectively, from June to August. Later in the growing season, a record of 346 mm rainfall occurred on 27 August, 2011 due to Hurricane Irene, and with repeated rainfall occurring in subsequent days. In 2011, the leaf rolling, discoloration and reduced biomass were symptomatic of drought stress in rainfed but not irrigated plants (Fig. 2.2). The growing season of 2012, was relatively warm and humid with mean maximum temperatures around 32^{o}C, total rainfall 312.4 mm, and mean RH of 79.3% during the June-through-August interval. During this time, and June in particularly, rainfed plants produced less biomass than irrigated plants based on visual comparison, but were not wilted. From July through October, plots received abundant weekly precipitation, and the total rainfall received from May to October was 965 mm, approximately 60% more than usually. Due to persistent cloud cover throughout the entire growing season, photsynthetically active radiation was 30% less in 2012 than in 2011.

Pod yield and quality factors. ANOVA for pod yield showed significant main effects of water regime and genotype in both years, and a significant water regime-by-genotype interaction effect in 2012 (Supplemental Table 2.1). In 2011, rainfed plots yielded less than those in irrigated plots, which received an additional 80-mm water to rainfall during June and July (5783 vs. 6128 kg ha^{-1}, $p < 0.05$). In 2012, average rainfed plot yields were higher than for irrigated plots (4359 vs. 4185 kg ha^{-1}, $p < 0.05$) probably due to reduced radiation and excessive moisture on the irrigated plots during the latter part of the 2012 season. Genotype N04074FCT had the lowest average pod yield and N05008, N05024J, and Phillips the highest among the genotypes tested under rainfed conditions during both years (Table 2.1). Genotype N05006 ranked third greatest for yield in 2012, but performed relatively poorly in 2011, which was the drier year. Low yields under rainfed conditions were recorded for CHAMPS, Bailey, and SPT 06 07 in both years. Genotype N05008 had the highest rainfed yield in 2011, but its yield reduction was 8%,

one of the highest, when compared with its irrigated yield (6360 vs. 7147 kg ha-1). Genotypes Bailey, CHAMPS, N04074FCT and SPT 06 07 showed the greatest percent reduction of average yield (from 6.7 to 7.9%), and Phillips and N05006 the lowest (3.9 and 5.5%, respectively) due to water deficit in 2011. Genotype N05024J was the only genotype which showed a slight increase (1.7%) in pod yield under water deficit stress in 2011, but this was not significantly different from its irrigated yield (Table 2.1). In 2012, irrigated SPT 06 07 had a reduction of 11.2% in pod yield compared with its rainfed yield. For all other genotypes yield was higher in rainfed than in irrigated plots in 2012 (Table 2.1).

Genotype had an effect on ELK and SMK contents in both years ($p < 0.001$), while water regime showed an effect only in 2011 ($p < 0.001$). Water regime-by-genotype interaction was not significant for these traits in any year (Supplemental Table 2.1). Averaging both years, Phillips had 52% and N05024J 50% ELK content, significantly higher than SPT 06-07 (29%), N05006 (38%), and N04074FCT (39%) ($p < 0.05$). SPT 06-07 had high average SMK (67%) content, in particular in the more humid 2012 (71% SMK content) (Table 2.2).

Membrane injury. Repeated measures ANOVA showed significant genotype, growth stage, and growth stage-by-water regime interaction effects on membrane injury (MI), in 2011 (Supplemental Table 2.2). In 2012, growth stage and growth stage-by-genotype interaction were statistically significant. In 2011, membrane injury was almost 46% higher in rainfed than in irrigated plants at R1 growth stage (Table 2.3). At R5, all genotypes in irrigated plots had higher MI than in rainfed plots, i.e., 83 vs. 72% ($p < 0.05$), which may reflect adaptive mechanisms developed by water-stressed plants at earlier stages (Table 2.3). In 2012, only plants at R1 growth stage showed significant water regime effects on MI ($p < 0.05$), with irrigated plants exhibiting slightly higher membrane injury than rainfed plants. In general, Bailey had the least

membrane damage among all genotypes in rainfed and irrigated plots at R1 and R5 in 2011 and irrigated plots at R5 in 2012, but it was significantly different only when compared to N05006 in both years and SPT 06-07 in 2011 at stage R5 ($p < 0.05$). For the rest of the genotypes, MI differences were absent for all growth stages in both years.

Canopy Temperature Depression. Univariate repeated measures ANOVA of canopy temperature depression (CTD) showed significant water regime and growth stage-by-water regime interaction effects in 2011 and 2012 (Supplemental Table 2.2). Growth stage effects were highly significant in 2011 ($p < 0.001$) and were absent in 2012. In 2011, irrigated and rainfed plants were similarly cooler relative to air temperature (2.4°C) at R3 stage, but irrigated plants became significantly cooler than rainfed plants later in the season, at R5 ($p < 0.05$) (Table 2.4). In 2012, rainfed plots were on average 1.7˚C cooler than irrigated canopies during R1 and R5 growth stages. Within each growth stage and water regime, there were no significant differences for CTD among genotypes in both years.

Specific leaf area and SPAD chlorophyll reading. Genotype, water regime, and growth stage affected ($p < 0.05$) specific leaf area (SLA) and SPAD chlorophyll reading in both years (Supplemental Table 2.2). In both years, the smallest SLA values were recorded at R3 (beginning pod), and the greenest leaves were at R5 (beginning seed) (Table 2.5). During both years, there was growth stage-by-water regime interaction ($p < 0.05$) for SLA and SPAD chlorophyll reading.

Overall, leaves of rainfed plants had higher SLA than the irrigated ones in 2011 (135 *vs*. 131 $cm^2\ g^{-1}$) and smaller (133 *vs*. 144 $cm^2\ g^{-1}$) in 2012. A similar pattern was observed for the SPAD chlorophyll readings (Tables 2.6). In 2011, average SPAD chlorophyll readings were

47.4 and 54.3 for the rainfed and irrigated plants, respectively. In 2012, these numbers corresponded to 42.7 and 44.1, respectively.

With a few exceptions, Bailey and CHAMPS had smaller SLA and Phillips higher SLA than other genotypes in both growing seasons (Tables 2.5). For N04074FCT, SLA was relatively high at early stages and small at Stage R5 in 2011 and 2012. N05006 and SPT 06-07 had smaller SLA early than late in the season. Among all genotypes and based on the SPAD chlorophyll readings, SPT 06-07 was the greenest and N04074FCT the least green in both years and at all growth stages (Table 2.6).

Chlorophyll fluorescence. Leaf F_v/F_m ratio decreased with hours of incubation at 45 ˚C. Percent decline of the F_v/F_m ratio from 0 to 3 h of incubation was calculated and univariate repeated measures ANOVA test was performed on the data (Supplemental Table 2.2). Effects of water regime ($p < 0.01$), growth stage ($p < 0.001$), and growth stage-by-water regime interaction ($p < 0.001$) were observed for the F_v/F_m decline during both years. Genotype and genotype-by-water regime interaction effects were detected only in 2012 ($p < 0.05$).

In both years, rainfed plants showed higher or similar F_v/F_m decline than irrigated plants at R1 and R3 growth stages. At R5, F_v/F_m decline was higher in rainfed (25%) than irrigated (0%) plants in 2011 with an opposite trend in 2012 (44% in rainfed *vs*. 56% decline in irrigated plants) (Fig. 2.3). SPT 06-07 and CHAMPS showed the smallest F_v/F_m decline among the genotypes at R1 and R3 stages in both years (Fig. 2.4). Bailey also had less F_v/F_m decline in rainfed and irrigated plots than other genotypes in 2011. N04074FCT and N05024J consistently showed more decline of the F_v/F_m ratio than other genotypes under rainfed conditions during all growth stages and in both years (Fig. 2.4). In 2012, rainfed N05006 and N05008 had the largest decline of the F_v/F_m ratio (51%) (Fig. 2.4).

Polar metabolites. Metabolite profiling analysis revealed a total of 15 metabolites whose levels were changed under the different water regimes (rainfed and irrigated) and growth stages (R1, R3, and R5) (Table 2.7). They belong to four major groups: organic acids, sugars, sugar alcohols, and amino acids, and included eight organic acids (citrate, glycerate, malate, maleate, succinate, shikimate, tartrate, and quinate), three sugars (fructose, glucose and sucrose), two cyclic polyols (myo-inositol and pinitol), and two amino acids (alanine and glycine).

Score plots from the principal component analysis (PCA) on the correlations of metabolite and fatty acid levels are presented (Figs. 2.5 and 2.6). The first two principal components (PC's) explained 48.8 and 43.6 % of the total variance present in the metabolite data during 2011 and 2012, respectively. PC 1, which alone accounted for 34.7% of the total variance in the metabolite data in 2011, separated R1 and R2 growth stages fairly well (Fig. 2.5). The effect of water regime was also apparent and explained 14.1% of the total variance based on PC2. Clusters representing rainfed and irrigated plants within a growth stage were closer to each other than the growth stages itself, indicating that the effect of developmental stage was greater than the water regime effect. Similarly, growth stage effect (25.2% of the total variance) was stronger than the water regime effect (18.4% of total variance) in 2012 based on the PCA score plots (Fig. 2.6). During both years, major metabolites belonging to the same metabolite group, *i.e.* organic acid group (citrate, malate, and maleate), fatty acid group (unsaturated and saturated fatty acid), and sugar group (glucose and fructose), were positively correlated to each other (Figs. 2.7 and 2.8).

Water regime had a significant effect on the total organic acid content in both years (Supplemental Tables 2.3 and 2.4), as the levels of organic acids were lower in rainfed than in irrigated leaves at all stages in both years (Fig. 2.9). Growth stage, water regime and, with a few

exceptions, genotype had significant effects on the levels of the individual organic acids in both years (Supplemental Table 2.3). No genotype-by-water regime interaction was observed, and the genotype-by-growth stage interaction was significant only for some, but not all organic acids.

In particular, citrate content was lower in rainfed than in irrigated plants ($p < 0.05$) (Fig. 2.10) and rainfed CHAMPS, N05008, and N04074FCT had lower levels of citrate than other genotypes at R1 during both years (Fig. 2.11 and Supplemental Table 2.5). Succinate levels also decreased under rainfed condition during R1 and R3 growth stages in 2011 and at R3 in 2012 (data not shown). In both years, tartrate content was unaffected by the water regime but significant differences among genotypes were observed. For example, N05024J consistently had the highest tartrate levels among all genotypes (data not shown). Bailey, CHAMPS, and SPT 06-07 were among the genotypes with the lowest tartrate content in both years.

Genotype, water regime, and growth stage had significant effects on the total relative levels of the cyclic polyols, *i.e.*, myo-inositol and pinitol in both years (Supplemental Tables 2.6 and 2.7). Overall, total cyclic polyol levels were significantly higher in rainfed plants ($p < 0.05$) than in irrigated plants, and it almost doubled in 2011 compared to 2012 (Fig. 2.12). Pinitol and myo-inositol levels were significantly affected on an individual basis by genotype ($p < 0.01$) and growth stage ($p < 0.001$) in both years. Water regime had an effect on myo-inositol levels in both years ($p < 0.001$), while on pinitol levels only in 2011 ($p < 0.001$) (Supplemental Table 2.6). Unlike for most metabolites in this study, the genotype-by-water regime interaction was significant for pinitol in 2011. In 2011, rainfed plants accumulated 56% and 67% more pinitol than irrigated plants at stages R1 and R3, respectively (Fig. 2.13). In 2011, N05006 and N05008 at Stages R1 and R3, and Bailey, N05024J, Phillips, and SPT 06-07 at Stage R3 had over a 70%

increase in pinitol content in water-stressed relative to irrigated plants (Fig. 2.14 and Supplemental Table 2.8).

Total sugar content was significantly affected by growth stage in both years, water regime in 2011, and genotype in 2012 (Supplemental Table 2.6). Genotype-by-water regime and genotype-by-growth stage interactions were absent or small in both years. Overall, total sugar content increased in rainfed plants in both years with the exception of Stage R3 in 2012 when their levels were higher in irrigated than rainfed plants (Fig. 2.15). Across years and stages, rainfed N04074FCT accumulated more sugars compared to rainfed N05006 and Phillips (Supplemental Table 2.9). Among sugars, only glucose and fructose production was significantly different among genotypes ($p < 0.05$) in both years and water regimes ($p < 0.01$) in 2011 (Supplemental Table 2.6). In general, rainfed plants accumulated up to 38% more sucrose than the irrigated plants, but the genotypic differences were not statistically significant (Supplemental Table 2.10).

Growth stage ($p < 0.001$) in both years and water regime ($p < 0.001$) in 2011 affected the relative steady-state levels of alanine and glycine (Supplemental Table 2.11). Together, alanine and glycine content was higher in 2011 than in 2012 (Fig. 2.17). Genotypic differences were small and statistically significant ($p < 0.05$) only in 2011 at Stage R1 (Supplemental Table 2.12). In 2011, glycine content increased and that of alanine decreased under rainfed conditions during R1 and R3 reproductive growth stages (data not shown). In 2012, glycine levels were unaffected by the water regime and alanine levels increased in rainfed plants at R1 and R3.

Fatty acids. Growth stage effect on saturated and unsaturated fatty acids was significant in both years ($p < 0.01$) (Supplemental Table 13). Genotype ($p < 0.001$) and water regime ($p < 0.001$) effects were detected for Palmitic acid (16:0), Palmitoleic acid (16:1), Linoleic acid

(18:2), and Linolenic acid (18:3) only in 2012. In general, both saturated and unsaturated fatty acids decreased in rainfed relative to irrigated plants, but the decrease in the content of saturated fatty acids was more prominent than in the case of the unsaturated fatty acids (Supplemental Table 2.14 and 2.15).

Relationship of agronomic, physiological, and metabolic characteristics. Correlations among physiological, metabolic and agronomic characteristics for individual years and growth stages are presented in Supplemental Tables 2.16 and 2.17. Based on our data, a few physiological characteristics and metabolites explained the relatively large variation for pod yield in rainfed compared to irrigated plots in both years. For example, citrate and the total organic acid levels at Stage R3 were significantly correlated with the pod yield in both years, and explained from 36 to 50% of variation in pod yield. For other individual metabolites and physiological characteristics, their relationship with the pod yield was inconsistent. However, a combination of several physiological characteristics and metabolites could be associated with a yield change from 44 to 59% in rainfed and from 22 to 59% in irrigated environments (Table 2.8). Overall, the yield of rainfed plants was negatively and positively correlated with total sugar and cyclic polyamine and organic acid levels, respectively. More importantly, yield was negatively and positively correlated with saturated and unsaturated fatty acids, respectively, in six out of ten environments representing combinations of years, growth stages, and water regimes (Table 2.8).

DISCUSSION

Yield and quality factors. Although soil moisture was adequate in 2011, i.e., total rainfall from May through October was 783 mm excluding the rainfall received from the tropical storm

Irene, which mostly ran off, rainfed plots yielded in average 6% less than irrigated plots with a range of reduction due to water-deficit from less than 5% for Phillips and N05006 to approximately 7% for N05008, Bailey, CHAMPS, SPT 06-07, and N04074FCT (Table 2.1). Year 2012 was wetter than 2011 and hot based on the historical data (Fig. 1.1). In addition to the 965 mm rainfall from May to October for all plots, irrigated plots received additional 32.3 mm irrigation from end of June to mid-July in 2012. Reduced radiation and excessive moisture, which was exacerbated in the case of the irrigated plots than for rainfed plots, could have contributed to lower yields in irrigated than in rainfed plots in 2012 as well as a 28% decrease in yield in 2012 compared to 2011 (Table 1.1). The grade factors and ELK and SMK contents were not affected by the water regime in 2012. The average pod yields varied significantly for different genotypes under rainfed conditions in both years (Table 1.1). N05008, Phillips, and Bailey were the top yielding genotypes under rainfed conditions in 2011, while Phillips, N05024J, N05006, and N05008 in 2012. In 2012, all genotypes showed increases in pod yield under rainfed conditions excepting only SPT 06-07, for which the pod yield was significantly reduced (11.2%) under rainfed conditions. The lowest yielding genotypes in rainfed plots in both years were N04074FCT, CHAMPS, and SPT 06-07. Greenhouse drought recovery studies demonstrated that SPT 06-07 and N05006 recovered well from severe drought, while N04074FCT and Bailey showed a poor recovery from drought stress (Rosas-Anderson, 2012, personal communication). Our field results are in an agreement with the greenhouse findings for N04074FCT and N05006, but not for Bailey and STP 06-07. The discrepancies for Bailey and STP 06-07 could be the result of their different yield potentials. Bailey has high yield potential that could have allowed this genotype to yield high under rainfed even if the decrease from its irrigated yield was substantial, 7.9%. On the other hand, SPT 06-07 has inherently low yield

potential, for which a 7.5% rainfed yield reduction could have made it the second lowest for yield after N04074FCT. Based on the pod yield results in this study, it is suggested that Phillips, N05008, and N05006 could be the best yielding genotypes under water deficit conditions in the sub-humid mid-Atlantic region.

Membrane injury. The elevated levels of reactive oxygen species under abiotic stresses may lead to increased membrane lipid peroxidation, rendering cellular membranes vulnerable to injury. Cellular membranes are sensitive to stress and the extent of cellular damage reflects stress intensity experienced by the peanut plants. Therefore, membrane injury test has been reported as a useful method for selection of heat and drought tolerant plants including peanut (Celikkol Akcay et al., 2010; Nautiyal et al., 2008). Our results showed relatively high membrane injury in 2011 (dry year), but low in 2012 (wet year), which is in an agreement with previous studies (Bajji et al., 2002; Ketring, 1985). In 2011, rainfed plants had significantly more membrane injury than irrigated plants at Stage R1 (Table 2.3). At Stage R5, irrigated plants had significantly more membrane damage than the rainfed plants. Low membrane injury of the rainfed plants at R5 could have been an adaptive response to the early season (R1) water deficit as suggested by work of Peng et al. (2012). In 2012, membrane injury was relatively low compared to 2011 and similar across water regimes. In both years, Bailey appeared to have the least membrane damage in rainfed and irrigated plots being significantly different from SPT 06-07 and N05006, which had extensive membrane injury (Tables 2.3). Even though membrane injury was useful for drought tolerance selection in other studies (Bajji et al., 2002; Lauriano et al., 2000; Clavel et al., 2006), here the relationship of membrane injury with pod yield was weak and inconsistent. In addition, the method for measuring membrane injury was

time consuming. As such, we think that membrane injury has little value for screening large breeding populations for water deficit in our environment.

Canopy temperature depression. Canopy temperature depression (CTD) has been widely used as a selection tool to screen heat and drought tolerant germplasm in several crops (Amani et al., 1996; Balota et al., 2012a; Turner et al., 1986). The working principle of CTD is that under water stress, transpiration decreases due to stomata closure, but the tolerant genotypes tend to maintain higher transpiration rates, and therefore cooler canopies, than the sensitive genotypes (Jackson, 1982; Turner et al., 1986). In the current study, rainfed plants had in average reduced CTD compared to irrigated plants (2.1 vs. 3.1 ˚C) in 2011, which was a dry year. However, rainfed plants were cooler than the irrigated plants in 2012 (wet year). This was probably due to excessive soil moisture in 2012 that could have negatively affected the roots (Bradford and Hsiao, 1982; Jackson and Hall, 1987) resulting in reduced transpiration and therefore warmer plant canopies (reduced CTD). Within each year, growth stage, and water regime there were no significant differences for CTD among the genotypes in this study (Tables 2.4). In 2011, CTD measured later in the season (Stage R5) showed positive correlations with pod yield ($r = 0.33$, $p<0.01$) and the SMK content ($r = 0.41$, $p<0.01$) (Supplemental Table 2.16). This is in an agreement with the results by Pinter Jr. et al. (1990) in wheat, demonstrating that cool, transpiring canopies can have higher yields than warm canopies. Unlike the other physiological measurements in this research, CTD measurements were easy, fast, and could potentially cover a relatively large number of plots in a relatively short period of time. As such, we see a potential for the CTD method to be used for screening large populations of peanut germplasm for increased yields under water deficit conditions in our environment. In both years, CTD was

significantly related to SLA showing that cool canopies have small and/or thick leaves (Supplemental Tables 2.16 and 2.17).

Specific leaf area and SPAD chlorophyll reading..In both years, growth stage had a significant effect on the SLA and SPAD chlorophyll readings with the smallest SLA values being recorded at R3 (beginning pod), and the greenest leaves at R5 (beginning seed) (Tables 2.5 and 2.6). Overall, leaves from rainfed plants had higher SLA than irrigated plants leaves in 2011, but in 2012, irrigated had higher SLA than rainfed plants leaves (Table 2.5). Also, SPAD chlorophyll reading was higher in rainfed than irrigated plants in 2011, and higher in irrigated than in rainfed plants in 2012 (Tables 2.6). Many studies report a decrease of the SLA in response to water deficit (Arunyanark et al., 2008; Upadhyaya, 2005), but others show an opposite trend (Wu et al., 2008). In our study, the observed increase of SLA can only be related to response to stress in general, if we can consider that excessive moisture in 2012 induced stress in irrigated plants rather than an adaptive mechanism to water deficit. The observation that rainfed plants in 2011 had high SLA and low CTD could be associated with better heat dissipation from thinner leaves (high SLA) when transpiration during the stress was low (Craufurd et al., 1999). There was no relationship between SLA and the SPAD chlorophyll reading in 2011, but a significant correlation was observed at Stages R3 ($r = 0.43$, $p<0.01$) and R5 ($r = 0.48$, $p<0.01$) in 2012. This can be explained by considerable cloudiness in 2012, specifically during which there was approximately 30% less incident radiation than in 2011, for which parallel increase of leaf area (high SLA) and chlorophyll content (better light capture) could have been necessary for increased photosynthesis and yield. In 2012, SPAD chlorophyll readings at early stages (Stages R1 and R3) were positively related to yield. In general, greener leaves did not show an advantage for yield in 2011, when significant negative relationships of

SPAD chlorophyll readings with yield and quality were observed at all stages (Supplemental Tables 2.16 and 2.17).

Chlorophyll fluorescence. Chlorophyll fluorescence, specifically the F_v/F_m ratio have been used for decades to show how abiotic stresses affect electron transport and thylakoid membrane photoinhibition, therefore reducing photosynthesis and production (Baker, 2008; Balota and Lichtenthaler, 1999; Lichtenthaler et al., 1998; Maxwell, 2000; Schreiber et al., 1986). Recently, chlorophyll fluorescence was used to detect genotypic differences to drought and heat in various crops including peanut (Burke, 2007; Kottapalli et al., 2009; Shangguan et al., 2000; Souza et al., 2004). Burke (2007) developed a novel bioassay using the F_v/F_m ratio decrease after incubation of leaf samples at high temperature (39˚C) to detect water stress in field grown cotton. Later, the methodology was used by Kottapalli et al. (2009) to detect water stress and genotypic differences in peanut. In this study, we used Burke's (2007) method, but with increased incubation temperature from 39˚C to 45˚C. In contrast with observations by Burke (2007) and Kottapalli et al. (2009) that F_v/F_m ratio declined in the leaves of stressed than well-watered plants, our results show a decline in the leaves of rainfed than in irrigated plants. This result fits better with the general agreement that the F_v/F_m ratio decline is associated with high stress in plants (Lichtenthaler et al., 1998; Souza et al., 2004; Srinivasan et al., 1996). In 2011 dataset, at Stage R5 there was a significant relationship between the F_v/F_m ratio decline and pod yield ($r = -0.26$, $p < 0.05$) and SMK content ($r = -0.33$, $p < 0.01$) showing that a moderate F_v/F_m decline was conducive to high yield and quality in peanut (Supplemental Table 2.16).

In Burke's (2007) study, slow F_v/F_m ratio decline was explained by sucrose accumulation in water stressed plants that provided cell protection when plants were exposed to prolonged respiratory demand, *i.e.*, incubation at high temperature. In our study, sucrose and other sugars

accumulated more in leaves of rainfed than in. irrigated plants in both years, but this accumulation cannot explain our contradictory result. It may be speculated that metabolites other than sucrose could have had a greater role in affecting the F_v/F_m levels for our set of genotypes. Unfortunately, we cannot identify clearly which of these metabolites could have been involved in the F_v/F_m change based on this set of data.

SPT 06-07, Phillips, CHAMPS, and Bailey exhibited the smallest, and N04074FCT and N05024J the largest F_v/F_m ratio decline in response to water deficit in leaves of rainfed plants in 2011(Fig. 2.4). In 2012, SPT 06-07 and CHAMPS also showed the smallest decline in comparison with the other genotypes in rainfed plants. Although both years were different for precipitation, the F_v/F_m ratio had similar patterns after 3 h of incubation at 45˚C (F_v/F_{m3}).). In both years at growth Stages R1 and R3, the F_v/F_{m3} ratio was approximately 20% lower for the leaves of rainfed than for irrigated plants (45 *vs*. 55 relative units). At Stage R5 in both years, the F_v/F_{m3} was higher for rainfed than irrigated plants (55 *vs*. 50 relative units in 2011 and 45 *vs*. 35 in 2012). This could be due to growth stage effects that need to be considered in future studies involving chlorophyll florescence in peanut, and can also explain our results in contrast to those of Burke (2007) and Kottapalli et al. (2009).

Polar metabolites. Although years were different in terms of precipitation, temperature, relative humidity, and radiation, there were commonalities among metabolite profiling for the two water regimes. With the exception of Stage R3 in 2012, total the levels of organic acids were lower in rainfed than in irrigated plants in both years and at all other growth stages (Fig. 2.9). Among them, citrate levels were distinct in rainfed compared to irrigated plants in both years and at all growth stages, with leaves of rainfed plants having significantly less citrate than those of irrigated plants (Fig. 2.10). Similar decreases in organic acid levels were associated

with drought stress in wheat (Bowne et al., 2012). In order to reduce the negative impacts of water stress, plants may slow down their metabolic processes to maintain a low energy demand. The TCA cycle metabolites are often positively related to cell growth and respiration (Glassop et al., 2007; Vasquez-Robinet et al., 2008). Therefore, reduced organic acid production in the present study could be an indication of slow growth in response to reduced moisture in the rainfed plots (Widodo et al., 2009), which was confirmed by visual observations. However, this could be a counterproductive adaptation to water deficit in our environment as it is suggested by positive relationships between citrate and the total organic acid content at R3 stage with pod yield in 2011 ($r = 0.50$, $p < 0.01$) and 2012 ($r = 0.38$, $p < 0.01$).

Sugar alcohols and cyclic polyols such as pinitol were extensively researched in relation to water deficit and heat stress in legumes (Guo and Oosterhuis, 1995; Matos et al., 2010). In our study, we found a significant increase of the total cyclic polyol levels in rainfed *vs*. irrigated plants, consistently every year and at all growth stages (Fig. 2.12). Among them, pinitol levels were approximately 30% higher in rainfed than in irrigated plants in 2011, but no differences between two water regimes were observed in 2012 (Fig. 2.13). Similarly, the total sugar content was higher in rainfed than in irrigated plants in both years and growth stages with only one exception, Stage R3 in 2012 (Fig. 2.15). Sucrose levels were lower in rainfed than in irrigated plants at Stage R1 in both years, but its levels were higher or similar in rainfed and irrigated plants at the other stages in 2011 and 2012 (Fig. 2.16).

Sugars and sugar alcohols are known for their osmoprotective and antioxidant functions in protecting the cells in response to abiotic stress (Bowne et al., 2012; Smirnoff and Cumbes, 1989; Stoop et al., 1996). In some cases, their accumulation is an indication of general plant stress rather than an adaptive response (Widodo et al., 2009). Based on this study, we cannot

conclude if the accumulation of sugars and cyclic polyols in rainfed plants in comparison with irrigated plants was an adaptive response or a general response to reduced tissue water content in rainfed plants. We did identify negative correlations between pod yield ($r = -0.20$, NS, in 2011 and $r = -0.44$, $p<0.01$, in 2012) and ELK ($r = -0.27$, $p<0.05$, in 2011) with total sugar content at Stage R3 in both years, and between pinitol and pod yield ($r = -0.40$, $p<0.01$) at Stage R3 in 2012. If they are adaptive traits, the accumulation of sugars and their alcohols and the reduction in the levels of organic acids are counterproductive adaptations with low value for the peanut crop production in our environment. To be productive in an agricultural setting, mechanisms that allow genotypes to maintain a balance of metabolites under stress similar with optimum conditions are desirable. Indeed Phillips, N05024J, and N05006 maintained higher citrate content more consistently than other genotypes under rainfed conditions across growth stages and years (Fig. 2.11). Phillips and N05024J also had lower pinitol content at Stages R1 and R3 than other genotypes in 2012 (Fig. 2.14). Phillips, N05024J, and N05006 had higher rainfed yields than the other genotypes in both years of this study. N04074FCT, CHAMPS, N05008, and at some stages Bailey, all had lower citrate and higher pinitol content in rainfed than irrigated plants in both years. With the exception of N05008, these genotypes yielded poorly in rainfed plots.

Fatty acids. Fatty acid composition of the membranes is critical during abiotic stresses (Dwivedi et al., 1996; Hashim et al., 1993; Upchurch, 2008; Zhang et al., 2005). Low content of saturated fatty acids and high content of unsaturated fatty acids in the membranes were often related to better tolerance to abiotic stresses (Upchurch, 2008; Zhang et al., 2005). In our study, saturated fatty acids negatively and unsaturated fatty acids positively ($p < 0.05$) correlated with pod yield regardless water regime, as shown by regression models (Table 2.8), indicating that low levels of saturated and high levels of unsaturated fatty acids may be related to increased

peanut yield under field conditions in the VC region. Although differences among genotypes in this study were relatively small, N05006, N05024J, and SPT 06-07 appeared to have higher content of unsaturated fatty acids in comparison with the other genotypes at several stages in 2011 and 2012 and in particular under rainfed conditions (Supplemental Table 2.14).

CONCLUSIONS

The present study investigated the agronomic, physiological and metabolic responses of eight virginia-type peanut cultivars and breeding lines grown under rainfed and irrigated field conditions in sub-humid environment. Growth stage and water regime had strong effects on all physiological characteristics and metabolite levels. Although years were different in terms of precipitation, temperature, radiation, and relative humidity, comparable results were obtained each year suggesting that water deficit of the rainfed plants is a realistic challenge for peanut production and physiology even in "rainy" years in the VC region. Genotypic differences existed for the majority but not for all physiological characteristics and metabolites. This could reflect the relatively low genetic diversity of the germplasm used in this study. However, we identified Phillips, SPT 06-07, and N05006 as genotypes showing potential tolerance and N04047FCT, CHAMPS, and Bailey susceptibility to water deficit in our environment. These data are in an agreement with the recent findings and current research performed at the North Carolina State University (Rosas-Anderson, 2012, personal communication). Among the physiological measurements, CTD and F_v/F_m ratio appeared to have the best potential for assessing peanut genotypes for water deficit stress in the mid-Atlantic region. Canopy temperature depression is an easy and affordable measurement that can accommodate a large number of plots in a relatively short period of time. However, usefulness of CTD as a screening

method requires validation when more genotypes and environments are included. The F_v/F_m ratio showed similar patterns each year. In both years, the F_v/F_m ratio was approximately 20% lower for the rainfed than for irrigated plants at early stages, which denotes more stress in rainfed than in irrigated plants. At Stage R5 in both years, the F_v/F_m ratio was higher for rainfed than irrigated plants, suggesting either a strong growth stage effect or adaptation mechanisms for rainfed plants in response to early season stress. In this study we could not clearly identify what metabolites associated with these effects, but these aspects of stress responses clearly deserve attention. We showed that steady-state levels of organic acids decreased, while sugar and cyclic polyols increased in rainfed relative to irrigated plants. In addition, citrate and pinitol could potentially be used as metabolic markers for water susceptibility in peanut. These changes, however, suggest general responses to different stress levels rather than adaptive mechanisms to water deficit, and they appeared to be counterproductive for peanut when considering overall yield.

Finally, we could not identify a single physiological and metabolic mechanism that was responsible for the differences in yield among the genotypes but rather a combination of several factors. In rainfed conditions, yield was repeatedly associated with increased levels of organic acids and cyclic polyols, reduced pinitol and sugar content, and cooler canopies. Regardless of water regime, higher yields in this study were associated with low saturated and high unsaturated fatty acid content. To our knowledge, there are no other similar studies to show the relationship between peanut yield and fatty acid composition in virginia-type peanut.

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Table 2.1. Pod yield and yield reduction of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) in 2011 and 2012. Negative sign means a reduction and positive means an increase in pod yield in rainfed *vs.* irrigated plants.

Genotype	2011			2012		
	Rainfed	**Irrigated**	**Reduction**	**Rainfed**	**Irrigated**	**Reduction**
	kg ha^{-1}		%	kg ha^{-1}		%
Bailey	6185 ab[1]	6713 a	-7.9	4283 a	4231 ab	1.2
CHAMPS	5563 ab	6007 ab	-7.4	4273 ab	3969 abc	7.7
N04074FCT	5072 b	5436 ab	-6.7	3440 bc	3203 c	7.4
N05006	5734 ab	6069 ab	-5.5	4685 a	4365 a	7.3
N05008	6360 ab	6888 a	-8.1	4642 a	4391 a	5.7
N05024J	6029 ab	5930 ab	1.7	4696 a	4696 a	0.0
Phillips	6193 ab	6442 ab	-3.9	4765 a	4020 abc	18.5
SPT 06-07	5125 b	5539 ab	-7.5	4091 ab	4607 a	-11.2
Mean	**5783 B[2]**	**6128 A**	**-5.6**	**4359 A**	**4185 B**	**4.2**

[1] Means followed by different small letters between water regimes and within a year are significantly different ($P < 0.05$ Tukey-HSD)

[2] Means followed by different capital letters between water regimes and within a year are significantly different ($P < 0.05$ student's t-test).

Table 2.2. Percentage of extra-large kernels (ELK) and sound-mature kernels (SMK) of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) in 2011 and 2012. Negative sign means a reduction and positive means an increase in pod yield in rainfed *vs.* irrigated plants.

	ELK (%)				SMK (%)			
	2011		2012		2011		2012	
Genotype	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**
Bailey	41.0 bc	45.5 ab	45.5 b-e	41.8 def	63.3 cde	65.9 a-d	68.1 abc	64.6 cd
CHAMPS	42.3 abc	46.8 ab	43.3 b-e	40.3 d-g	64.1 b-e	66.2 abc	67.0 abc	63.9 cde
N04074FCT	32.8 cde	39.5 bcd	42.0 cde	40.3 d-g	61.5 e	64.2 b-e	68.4 abc	68.9 abc
N05006	31.3 de	38.5 bcd	43.8 b-e	39.0 efg	61.4 e	65.1 b-e	65.4 bcd	64.9 cd
N05008	40.0 bcd	45.3 ab	41.0 def	40.5 def	64.7 b-e	69.3 a	67.1 abc	66.0 abc
N05024J	47.8 ab	51.5 a	48.3 a-d	50.8 abc	62.3 cde	61.8 de	58.8 e	61.0 de
Phillips	47.5 ab	51.8 a	55.3 a	51.3 ab	64.2 b-e	67.5 ab	70.3 ab	68.0 abc
SPT 06-07	25.8 e	27.8 e	31.5 g	33.0 fg	62.1 cde	62.9 cde	71.1 a	71.1 a
Mean	**38.5 B**	**43.3 A**	**43.8 A**	**42.1 A**	**63.0 B**	**65.4 A**	**67.0 A**	**66.0 A**

[1] Means followed by different small letters between water regimes and within a year are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a year are significantly different ($P < 0.05$ student's t-test).

Table 2.3. Leaf membrane injury of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R5, beginning seed) in 2011 and at three growth stages (R1, beginning flower; R3, beginningpod, and R5, beginning seed) in 2012 under field conditions.

Genotype	2011				2012					
	R1		R5		R1		R3		R5	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
	%									
Bailey	26.9 a[1]	21.2 a	57.0 c	78.5 abc	15.4 a	16.2 a	13.0 a	13.8 a	9.1 a	9.3 b
CHAMPS	41.9 a	19.6 a	69.7 abc	87.9 a	14.4 a	15.8 a	13.2 a	13.5 a	11.0 a	9.9 ab
N04074FCT	45.6 a	20.2 a	64.4 bc	70.2 abc	13.2 a	13.4 a	12.4 a	13.0 a	10.3 a	10.0 ab
N05006	33.2 a	31.2 a	82.9 ab	90.0 a	14.0 a	15.3 a	14.3 a	13.2 a	12.5 a	12.2 a
N05008	39.1 a	27.2 a	74.8 abc	89.1 a	13.9 a	15.0 a	13.6 a	12.7 a	10.3 a	10.9 ab
N05024J	53.5 a	33.0 a	74.1 abc	77.1 abc	13.1 a	14.0 a	12.6 a	12.3 a	11.5 a	11.1 ab
Phillips	45.9 a	39.6 a	71.9 abc	83.1 ab	13.3 a	13.5 a	12.5 a	14.0 a	12.2 a	10.2 ab
SPT 06-07	38.6 a	30.8 a	80.4 ab	87.6 a	14.2 a	14.7 a	13.2 a	13.5 a	10.7 a	10.5 ab
Average	**40.6 A[2]**	**27.9 B**	**71.9 B**	**82.9 A**	**13.9 B**	**14.7 A**	**13.1 A**	**13.2 A**	**10.9 A**	**10.5 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)

[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Table 2.4. Canopy temperature depression (CTD) of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R3, beginning pod; and R5, beginning seed) in 2011 and at three growth stages (R1, beginning flower and R5, beginning seed) in 2012 under field conditions.

Genotype	2011				2012			
	R3		R5		R1		R5	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
					°C			
Bailey	2.32 ab[1]	2.22 ab	2.40 a-d	3.44 a-d	3.39 abc[1]	2.11 abc	3.71 abc	2.23 f
CHAMPS	2.29 ab	2.01 b	1.73 d	4.08 a	3.78 abc	1.87 bc	3.63 a-e	2.46 def
N04074FCT	2.39 ab	2.59 ab	2.51 a-d	4.01 a	4.61 ab	2.05 bc	3.70 abc	2.74 a-f
N05006	2.09 ab	2.36 ab	1.75 d	3.94 ab	4.64 ab	2.37 abc	3.68 a-d	2.59 b-f
N05008	2.57 ab	2.33 ab	1.70 d	3.71 abc	4.16 abc	1.40 c	3.48 a-e	2.13 f
N05024J	2.23 ab	2.63 ab	1.74 d	3.98 a	4.88 a	2.09 abc	3.81 ab	2.57 c-f
Phillips	2.59 ab	2.70 ab	2.14 bcd	3.88 ab	4.20 abc	1.87 bc	3.89 a	2.59 b-f
SPT 06-07	2.78 a	2.57 ab	1.95 cd	3.80 ab	3.14 abc	1.60 c	3.57 a-e	2.43 ef
Average	**2.41A[2]**	**2.42 A**	**1.99 B**	**3.85 A**	**4.10 A[2]**	**1.92 B**	**3.68 A**	**2.47 B**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)

[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Table 2.5. Specific leaf area (SLA) of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at three growth stages (R1, beginning flower; and R5, beginning seed) in year 2011and 2012 under field conditions.

Genotype	2011						2012					
	R1		R3		R5		R1		R3		R5	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
	$cm^2\ g^{-1}$											
Bailey	146.0 a[1]	159.0 a	115.9 bc	113.2 c	128.9 ab	118.1 b	161.4 ab	157.2 ab	109.6 de	106.4 e	126.4 b	129.7 b
CHAMPS	146.0 a	143.0 a	112.0 c	113.7 c	129.9 ab	122.8 ab	132.0 ab	163.2 ab	111.2 de	111.9 cde	132.4 ab	135.0 ab
N04074FCT	149.0 a	161.7 a	133.3 a	125.5 abc	134.0 ab	123.5 ab	153.7 ab	180.4 a	118.3 bcde	119.4 bcde	136.3 ab	137.8 ab
N05006	145.4 a	139.4 a	125.0 abc	115.6 bc	133.9 ab	128.1 ab	116.1 b	162.4 ab	128.3 ab	124.0 abcd	139.2 ab	146.1 ab
N05008	142.7 a	147.5 a	123.1 abc	114.8 c	135.9 ab	129.4 ab	143.9 ab	156.5 ab	123.3 bcd	123.2 bcd	125.9 b	144.9 ab
N05024J	149.1 a	154.3 a	132.6 ab	122.8 abc	135.3 ab	129.0 ab	139.0 ab	166.9 a	131.6 ab	129.7 ab	140.2 ab	156.5 ab
Phillips	155.7 a	158.8 a	121.3 abc	116.6 abc	140.5 a	126.1 ab	143.7 ab	158.1 ab	123.0 bcd	127.4 abc	137.4 ab	156.9 ab
SPT 06-07	148.1 a	135.5 a	115.2 c	115.8 bc	142.1 a	135.6 ab	147.6 ab	169.4 a	121.3 bcde	139.3 a	145.5 ab	163.3 a
Mean (n=32)	**147.7 A[2]**	**149.9 A**	**122.3 A**	**117.3 B**	**135.1 A**	**126.6 B**	**142.2 B**	**164.3 A**	**120.8 A**	**122.7 A**	**135.4 B**	**146.3 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Table 2.6. SPAD chlorophyll reading of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at three growth stages (R1, beginning flower; and R5, beginning seed) in years 2011 and 2012 under field conditions.

Genotype	2011						2012					
	R1		R3		R5		R1		R3		R5	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
	Relative units											
Bailey	44.0 bc[1]	42.6 bc	49.5 a-d	44.1 fg	47.2 c-f	48.9 a-d	34.5 bc	38.5 abc	42.9 bcd	43.0 bcd	46.5 b	49.5 ab
CHAMPS	45.0 bc	43.8 bc	50.1 a-d	44.4 efg	46.33 c-g	47.3 b-f	36.6 bc	39.4 abc	43.1 bcd	43.3 bcd	46.6 b	48.9 ab
N04074FCT	42.7 bc	41.3 c	47.9 b-f	42.5 g	44.58 e-h	42.6 h	33.9 bc	32.8 c	40.7 d	41.8 cd	46.3 b	47.8 b
N05006	46.2 b	44.1 bc	50.3 abc	46.0 c-g	49.8 abc	48.0 a-e	40.9 ab	36.9 bc	46.2 ab	45.2 abc	51.1 ab	51.4 ab
N05008	45.5 b	43.4 bc	50.7 ab	45.7 c-g	47.9 b-e	49.5 abc	36.6 bc	38.7 abc	44.8 a-d	44.8 a-d	47.3 b	49.9 ab
N05024J	43.7 bc	44.5 bc	49.4 a-d	45.5 d-g	43.6 gh	44.0 fgh	37.0 bc	36.5 bc	43.1 bcd	42.0 bcd	46.1 b	48.7 b
Phillips	44.5 bc	43.7 bc	48.2 b-f	41.8 g	45.5 d-h	44.2 fgh	36.4 bc	39.3 abc	42.3 bcd	43.3 bcd	47.0 b	49.1 ab
SPT 06-07	50.8 a	50.2 a	53.1 a	49.0 a-e	50.8 ab	51.4 a	40.6 ab	44.8 a	45.2 abc	48.1 a	48.6 b	54.1 a
Mean (n=32)	**45.3 A[2]**	**44.2 B**	**49.9 A**	**44.9 B**	**47.0 A**	**46.9 A**	**37.1 A**	**38.4 A**	**43.5 A**	**43.9 A**	**47.5 B**	**49.9 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Table 2.7. List of the major metabolites found during the GCMS based metabolite profiling and fatty acid analysis of virignia-type peanut genotypes.

Organic acid	**Cyclic polyols**	**Sugars**	**Amino acids**	**Fattty acid**
Citrate	Myo-inositol	Glucose	Alanine	Palmitic acid (16:0)
Glycerate	Pinitol	Fructose	Glycine	Palmitoleic acid (16:1)
Malate		Sucrose		Stearic acid (18:0)
Maleate				Oleic acid (18:1)
Quinate				Linoleic acid (18:2)
Shikimate				Linolenic acid (18:3)
Succinate				Arachidic acid (20:0)
Tartrate				

Table 2.8. Linear regression and coefficient of determination (r^2) of physiological and metabolic traits observed on pod yield of eight virginia-type peanut cultivars and breeding lines, at two water regimes (rainfed and irrigated), three growth stages (R1, beginning flower; R3, beginning pod; R5 beginning seed) in 2011 and 2012.

Treatment	Intercept	SLA†	SCC‡	MI§	Organic acids	Sugar alcohols	Sugars	Amino acid	Unsat-FA¶	Sat-FA$	r^2
2011-R1-Rainfed	8731.1	-	-	-	-	-	-	-1167.3**	-	653.6*	0.46
2011-R1-Irrigated	8481.4	16.6*	-	-	-14.3*	-	5.7*	-	228.5*	-1629.0**	0.59
2011-R3-Rainfed	9740.8	-	-	-	-	69.8**	-12.9*	-	-	-1880.3*	0.56
2011-R3-Irrigated	10221.8	-	-134.2*	-	-	63.6*	-	-	510.1**	-4562.7**	0.48
2012-R1-Rainfed	3690.7	-14.2**	-	-	17.4*	102.5**	-7.5**	-	-	-	0.59
2012-R1-Irrigated	4939.3	-	-	-	-21.8*	-	-	-	429.3**	-870.7*	0.33
2012-R3-Rainfed	5352.2	-	-	-	16.9*	-	-4.9*	-	165.1*	-1288.1*	0.44
2012-R3-Irrigated	4810.0	-	-	-	12.6	-	-5.8*	-	-	-	0.26
2012-R5-Rainfed	2505.1	-	-	8952.0*	-	57.3	-	-	343.7**	-2152.9**	0.54
2012-R5-Irrigated	6032.1	10.4	-	-	-15.7*	-	-	-	-	-	0.24

**, * Significant at the 0.01 and 0.05 probability levels, respectively

† Specific Leaf Area

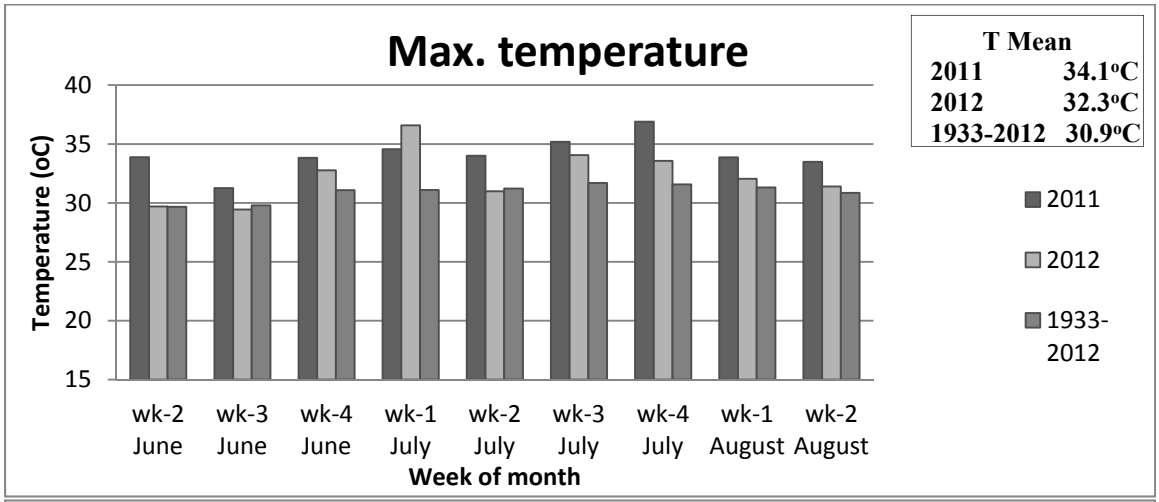
‡ SPAD Chlorophyll reading

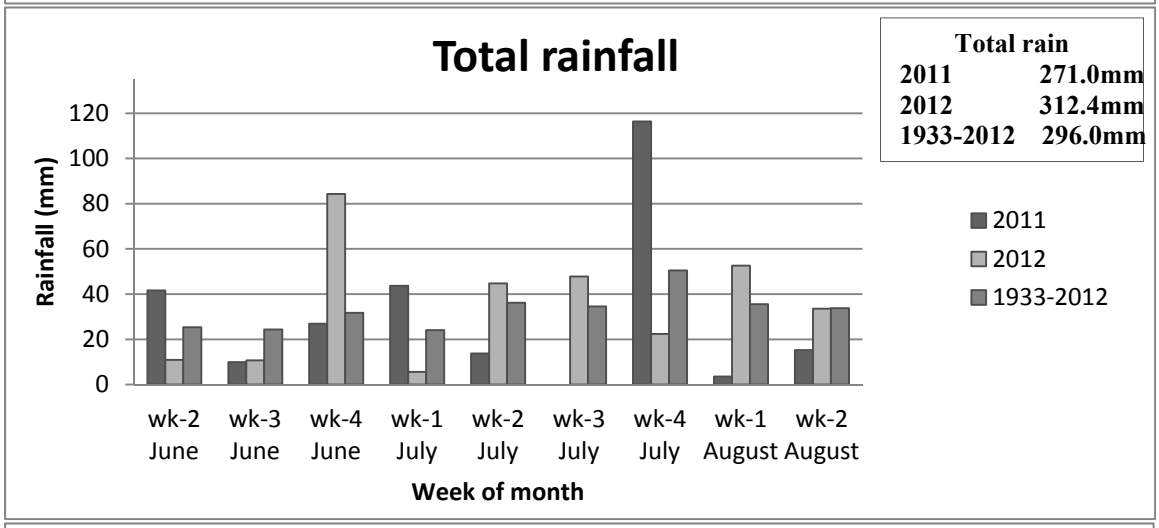
§ Membrane Injury

¶ Unsaturated Fatty Acid

$ Saturated Fatty Acid

Figure 2.1. Mean maximum temperature (T), total rainfall and mean relative humidity during June-August in Suffolk, VA in 2011 and 2012. For comparisons, long-term (1933-2012) maximum temperature and rainfall are also presented.





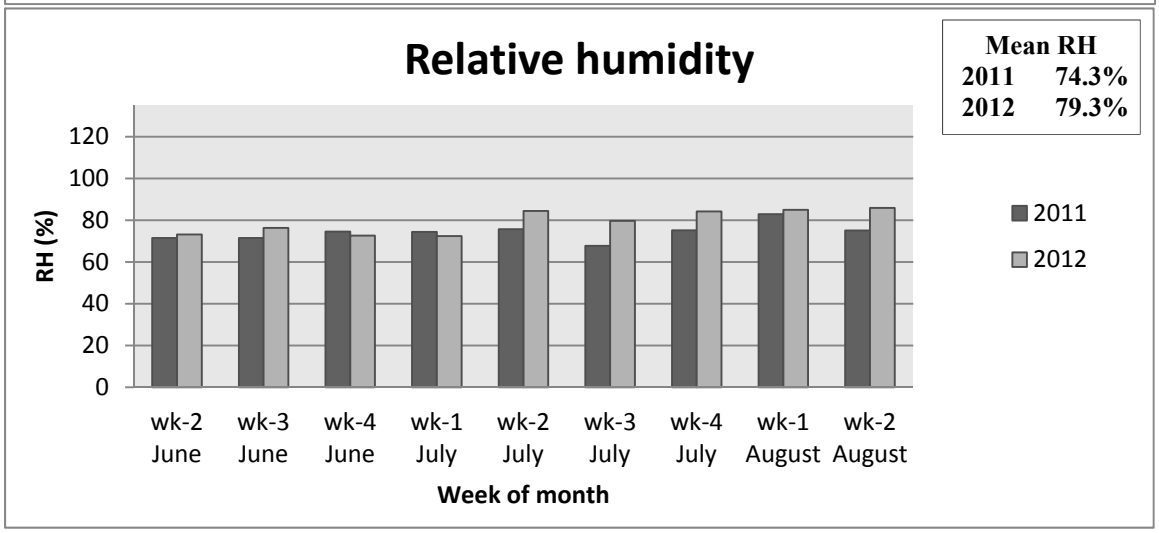
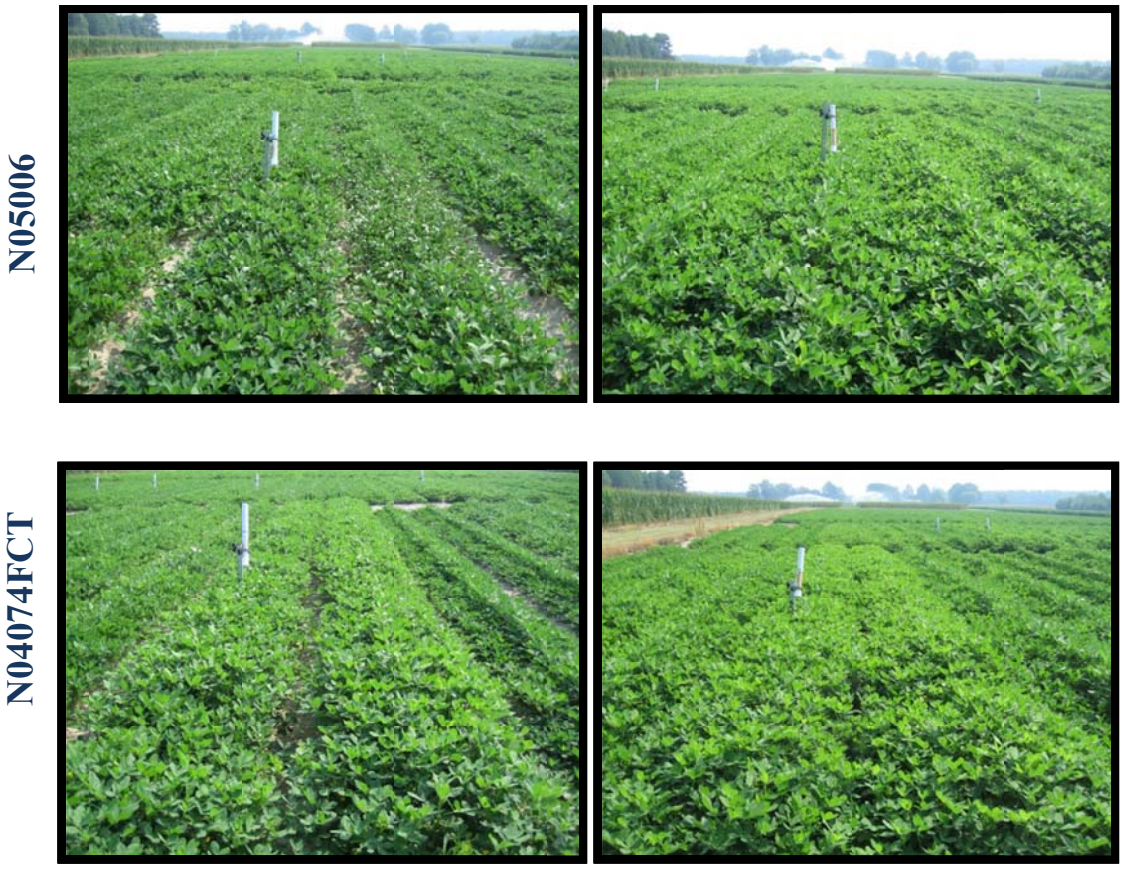


Figure 2.2. Visible phenotypes of two virginia-type peanut genotypes (N05006 and N04074FCT) under rainfed and irrigated conditions in 2011.



(Picture credit: Dr. Maria Balota)

Figure 2.3. Change in F_v/F_m (%) of virginia-typepeanut genotypes under two water regimes (rainfed and irrigated) at three growth stages (R1, beginning flower; R3, beginning pod; and R5, beginning seed) in year 2011 and 2012 under field conditions.

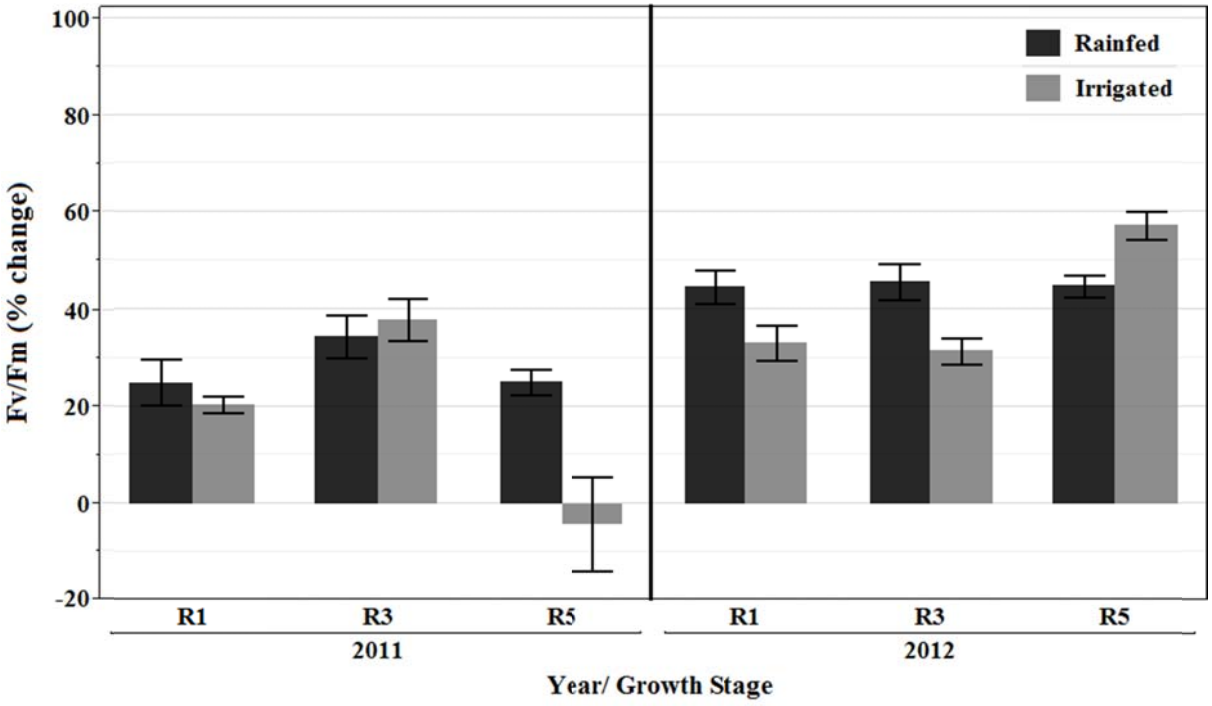


Figure 2.4. Change in F_v/F_m (%) of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at three growth stages (R1, beginning flower; R3, beginning pod; and R5, beginning seed) in year 2011 and 2012 under field conditions.

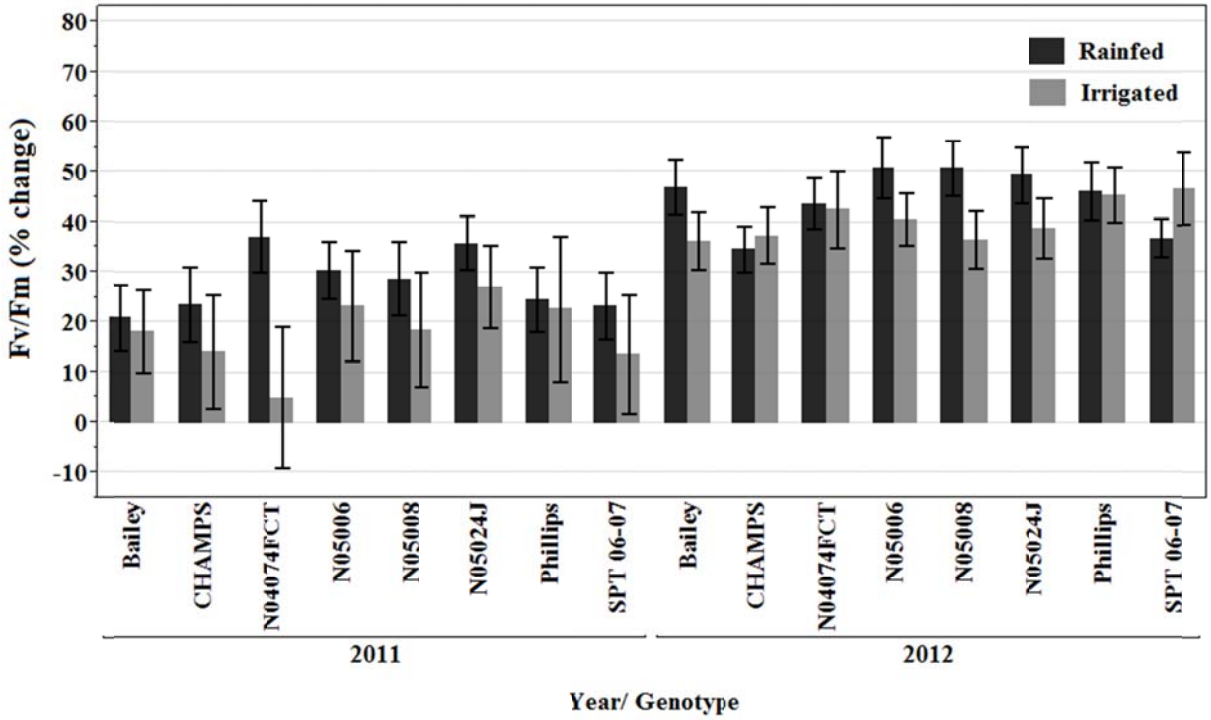


Figure 2.5. Plot of second vs. first principal component scores from principal component analysis (PCA) of leaf metabolite and fatty acid profiles of eight peanut genotypes at two water regimes (rainfed and irrigated) and two growth stages (R1 = beginning flowering and R3 = beginning pod), in 2011.

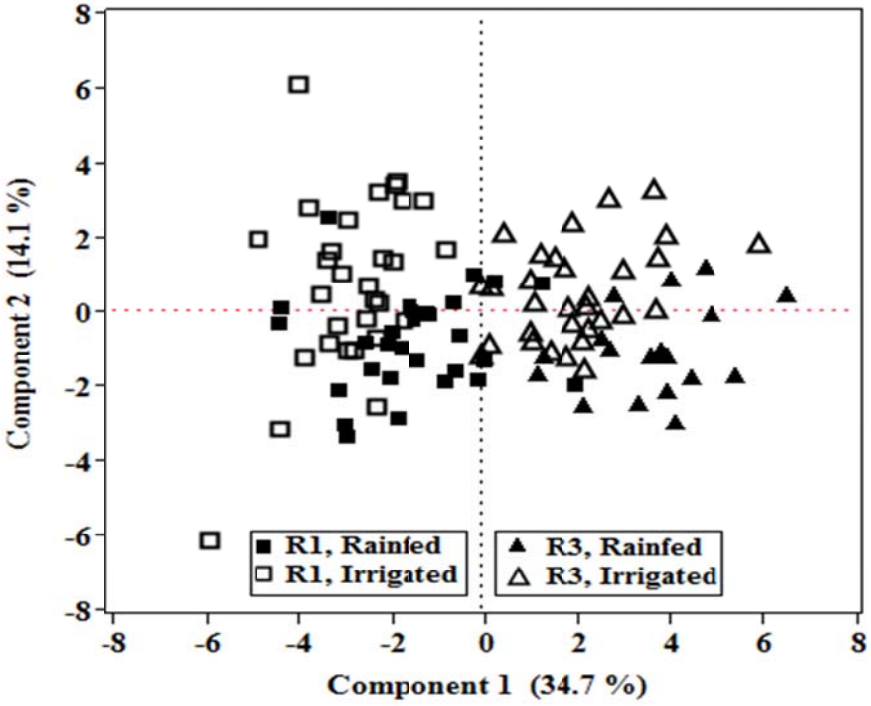


Figure 2.6. Plot of second vs. first principal component scores from principal component analysis (PCA) of leaf metabolite and fatty acid profiles of eight peanut genotypes at two water regimes (rainfed and irrigated) and three growth stages (R1 = beginning flowering, R3 = beginning pod and R5 = beginning seed), in 2012.

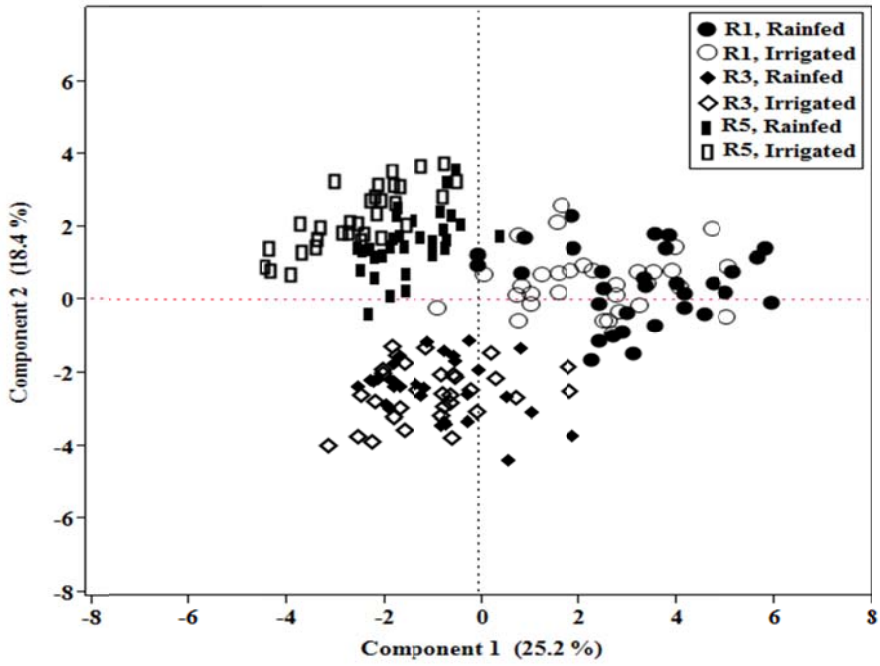


Figure 2.7. Loading plot from principal component analysis (PCA) of leaf metabolite and fatty acid profiles of eight peanut genotypes at two water regimes (rainfed and irrigated) and two growth stages (R1 = beginning flowering and R3 = beginning pod), in 2011.

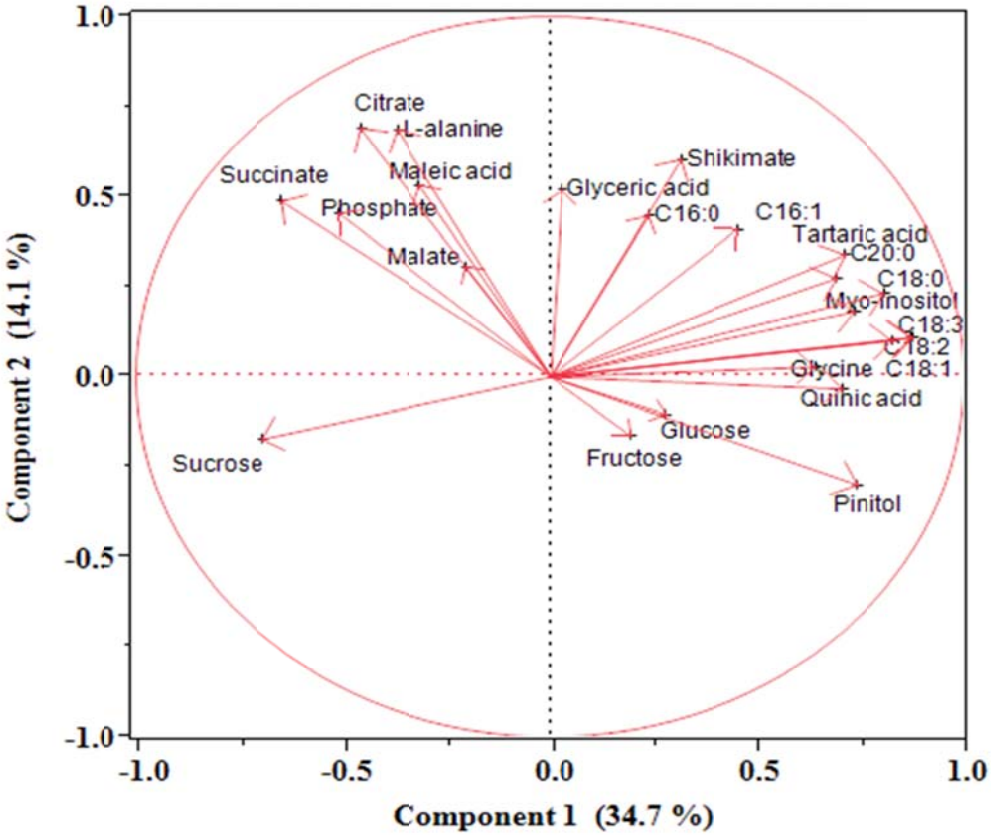


Figure 2.8. Loading plot from principal component analysis (PCA) of leaf metabolite and fatty acid profiles of eight peanut genotypes at two water regimes (rainfed and irrigated) and three growth stages (R1 = beginning flowering, R3 = beginning pod, and R5 = beginning seed), in 2012.

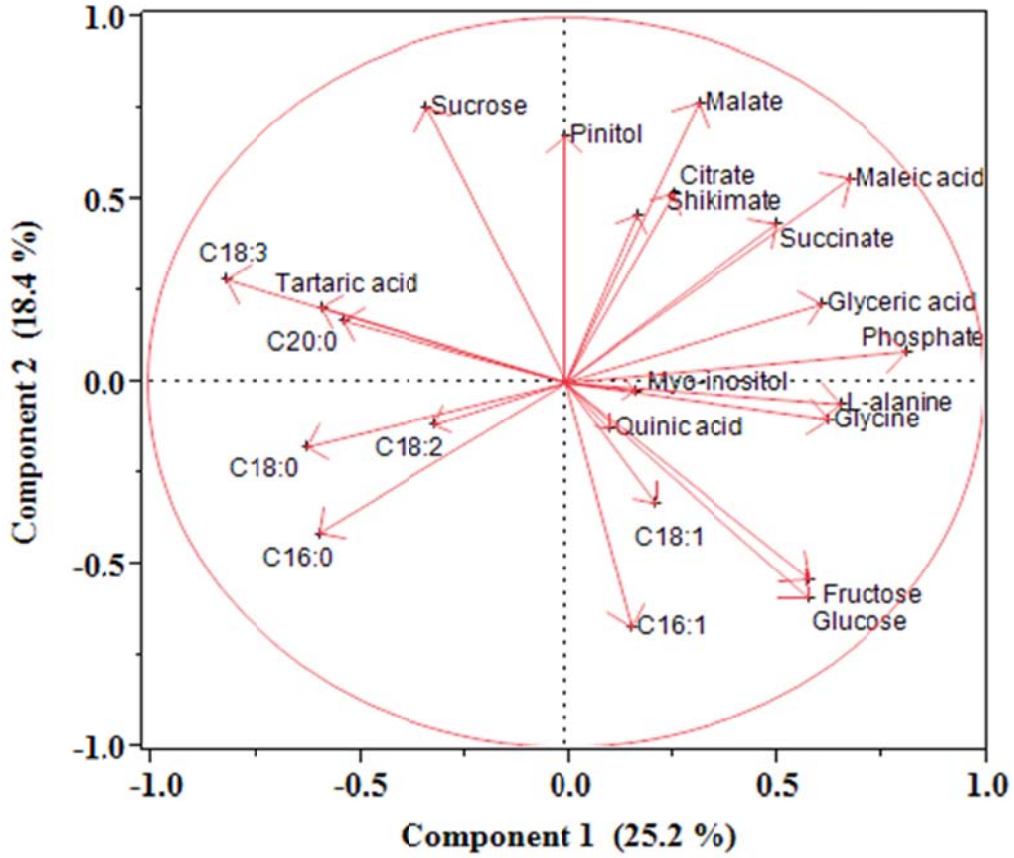


Figure 2.9. Relative organic acid levels (mg^{-1} of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) measured at two peanut growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and three growth stages (R1, R3, and R5, beginning seed) in 2012. Each error bar represent the standard error from mean (n=32).

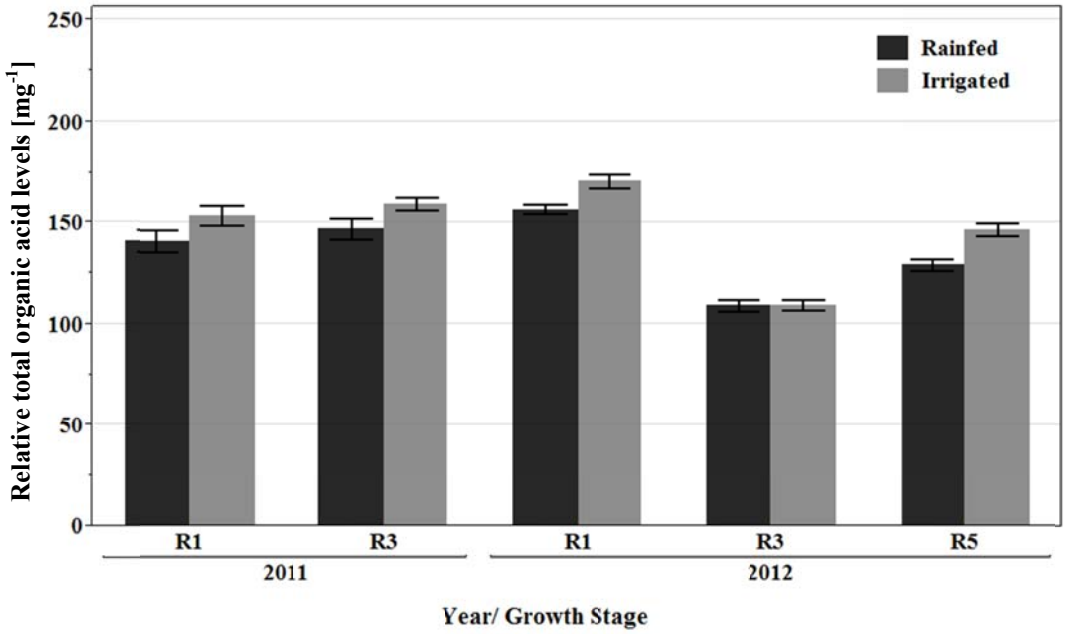


Figure 2.10. Relative citrate levels (mg^{-1} of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) measured at two peanut growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and three growth stages (R1, R3, and R5, beginning seed) in 2012. Each error bar represent the standard error from mean (n=32).

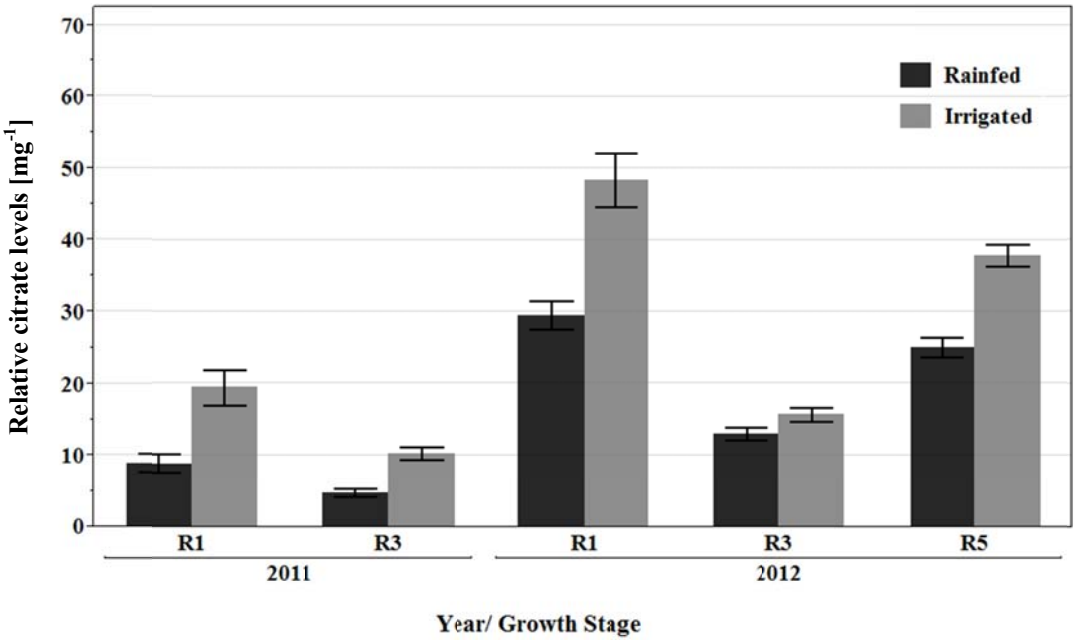


Figure 2.11. Relative citrate levels (mg-1 of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and three growth stages (R1, R3, and R5, beginning seed) in 2012. Each error bars represent the standard error from mean (n=32).

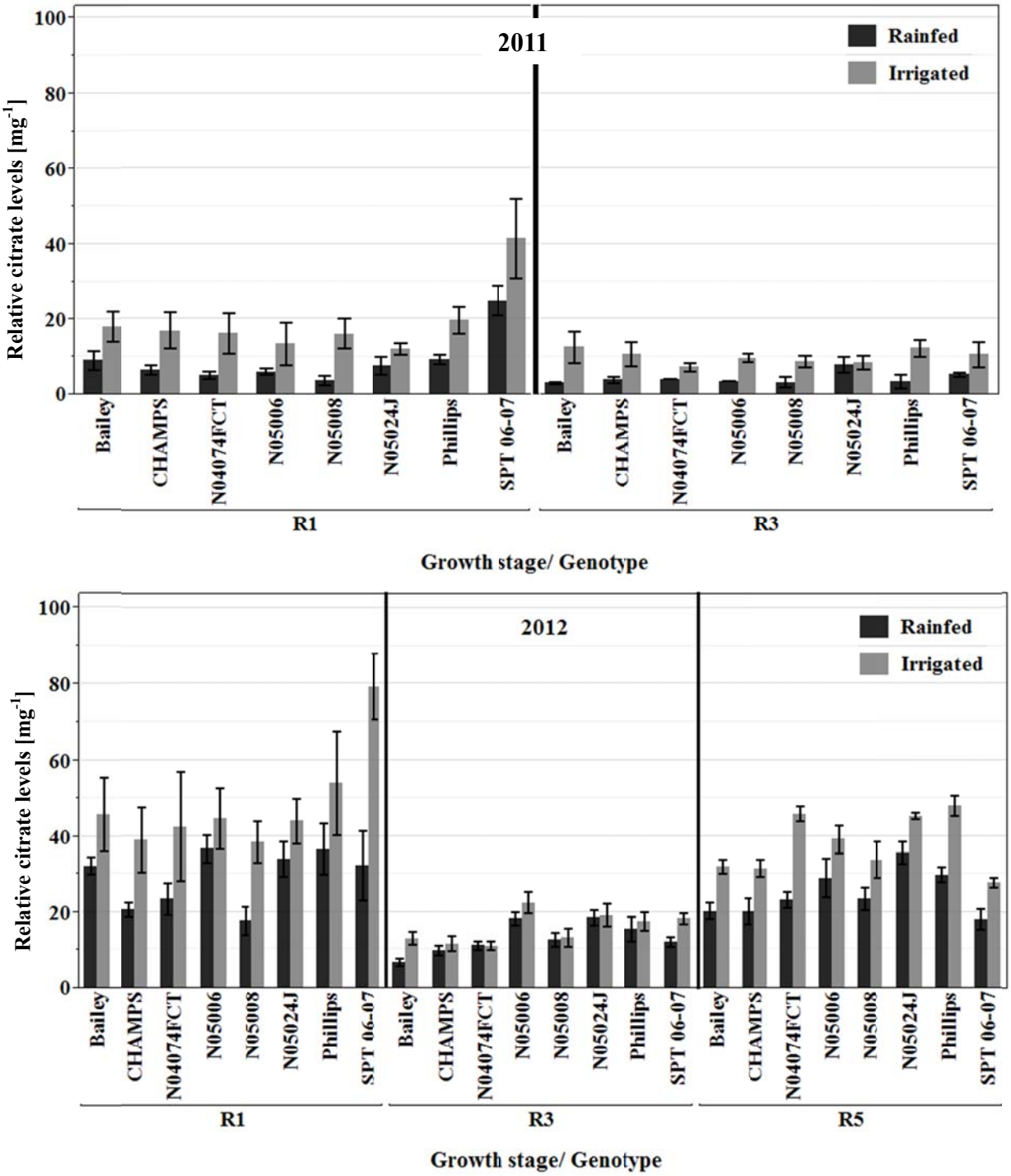


Figure 2.12. Relative sugar alcohol levels (mg^{-1} of leaf dry weight) in the eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) measured at two peanut growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and three growth stages (R1, R3, and R5, beginning seed) in 2012. Each error bars represent the standard error from mean (n=32).

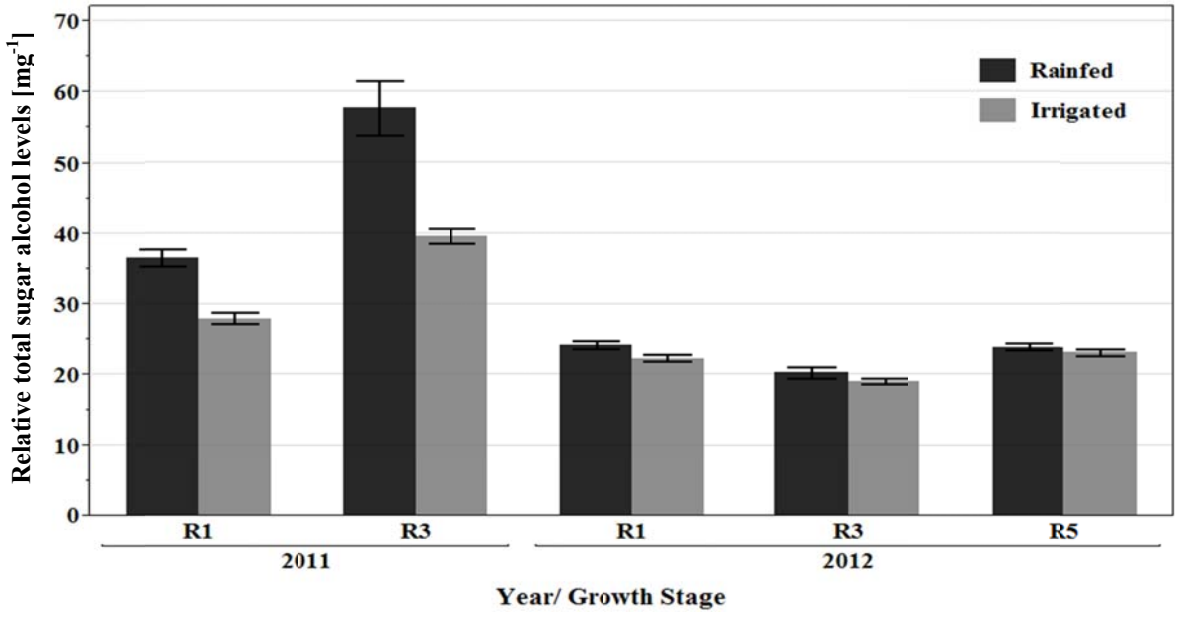


Figure 2.13. Relative pinitol levels (mg-1 of leaf dry weight**)** in the eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) measured at two peanut growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and three growth stages (R1, R3, and R5, beginning seed) in 2012. Each error bars represent the standard error from mean (n=32).

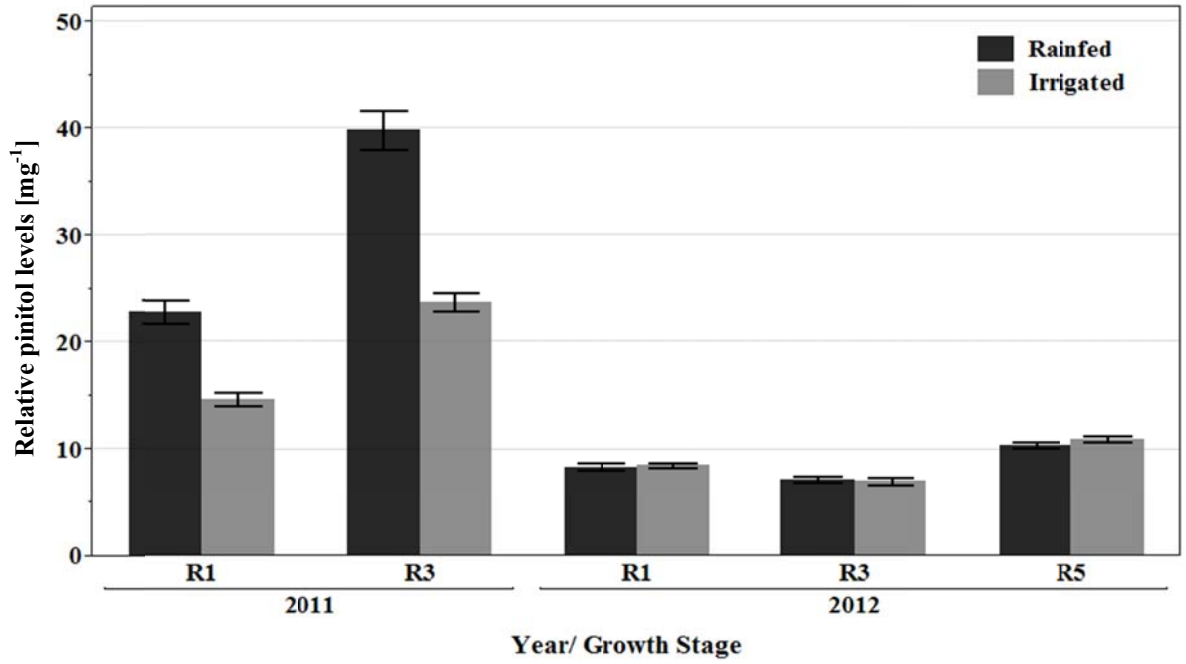


Figure 2.14. Relative pinitol levels (mg^{-1} of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and three growth stages (R1, R3, and R5, beginning seed) in 2012. Each error bars represent the standard error from mean (n=32).

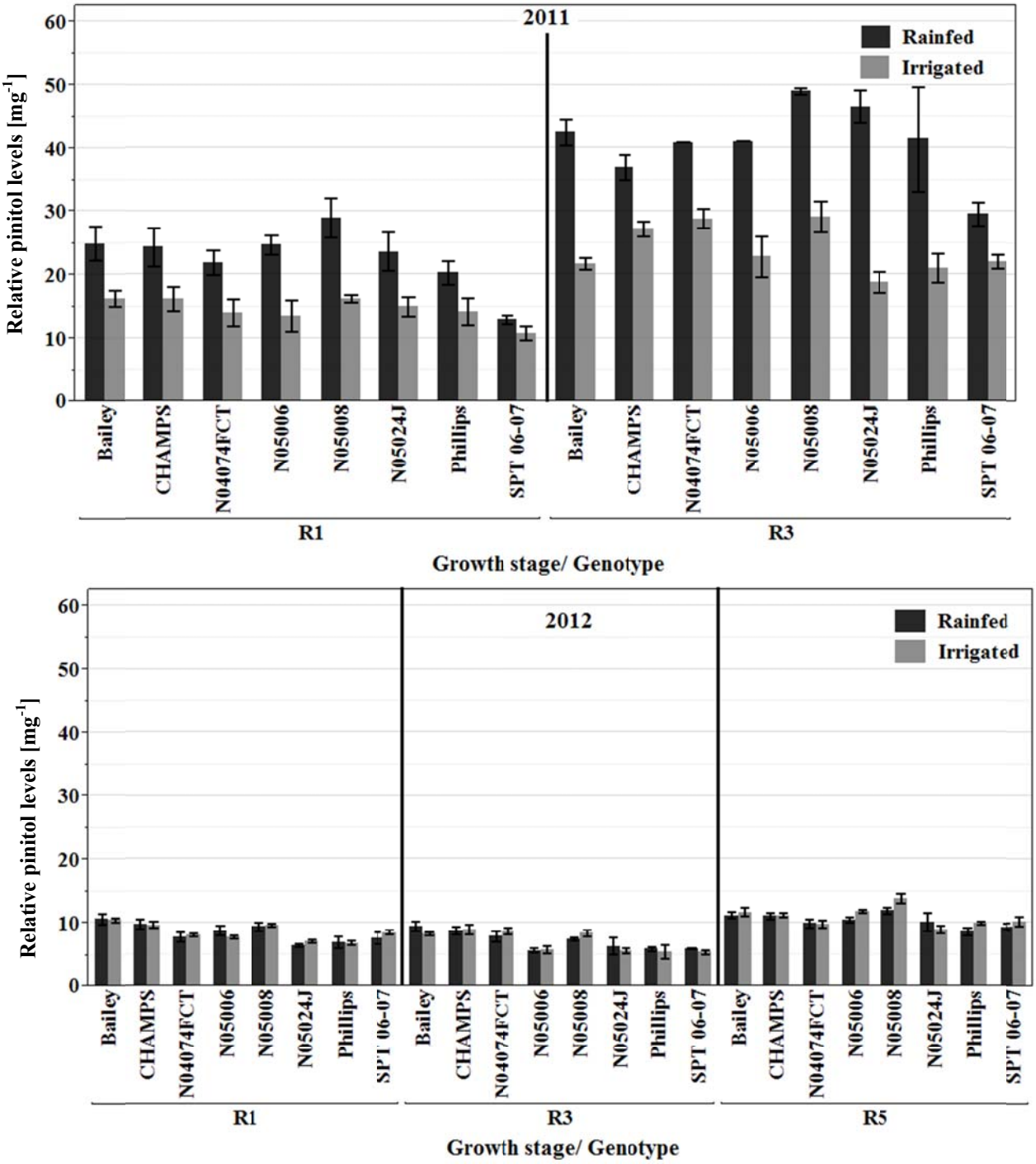


Figure 2.15. Relative total sugar levels (mg^{-1} of leaf dry weight) in the eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) measured at two peanut growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and three growth stages (R1, R3, and R5, beginning seed) in 2012. Each error bars represent the standard error from mean (n=32).

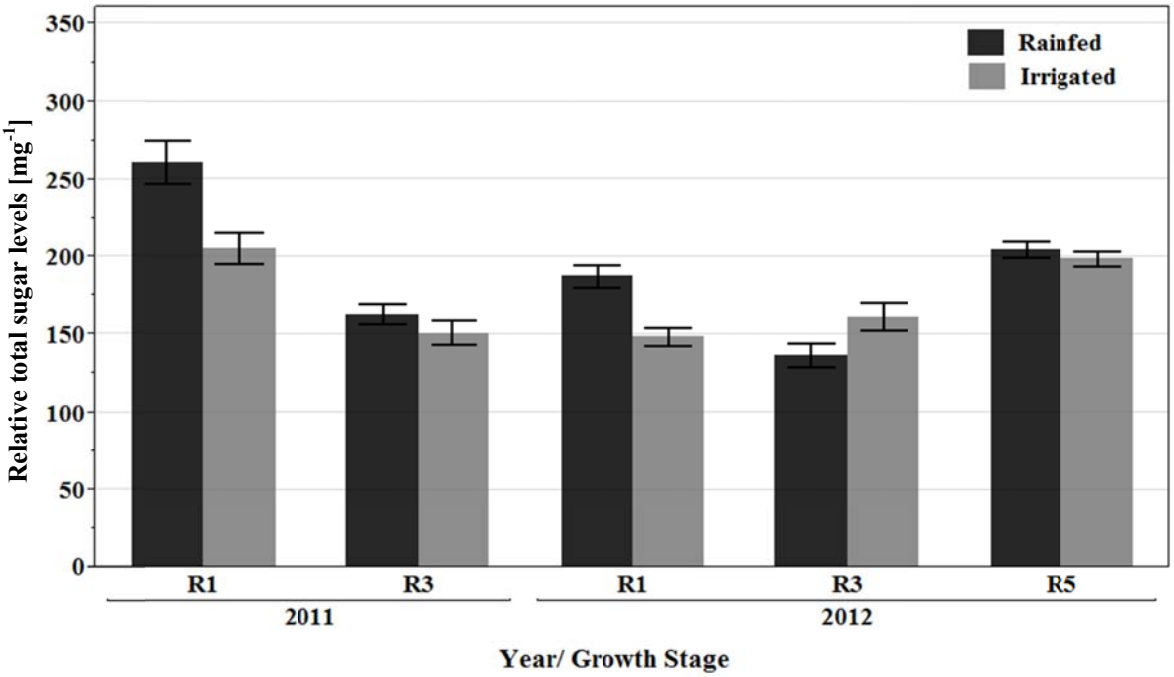


Figure 2.16. Relative sucrose levels (mg^{-1} of leaf dry weight) in the eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) measured at two peanut growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and three growth stages (R1, R3, and R5, beginning seed) in 2012. Each error bars represent the standard error from mean (n=32).

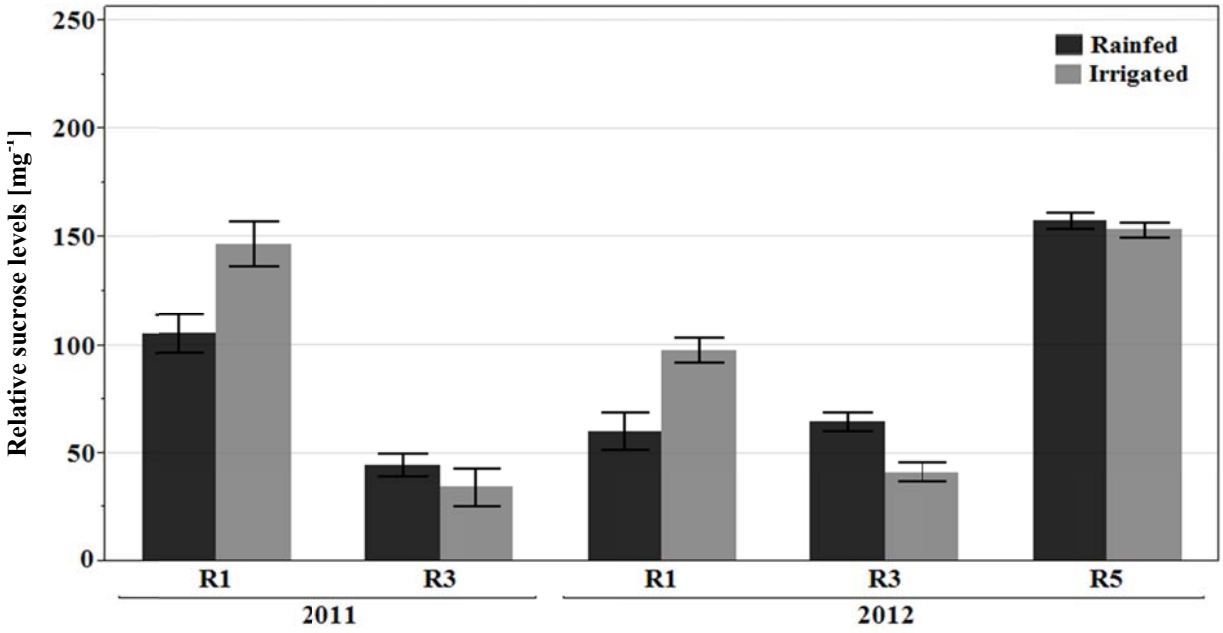


Figure 2.17. Relative total amino acid levels (mg^{-1} of leaf dry weight) in the eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) measured at two peanut growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and three growth stages (R1, R3, and R5, beginning seed) in 2012. Each error bars represent the standard error from mean (n=32).

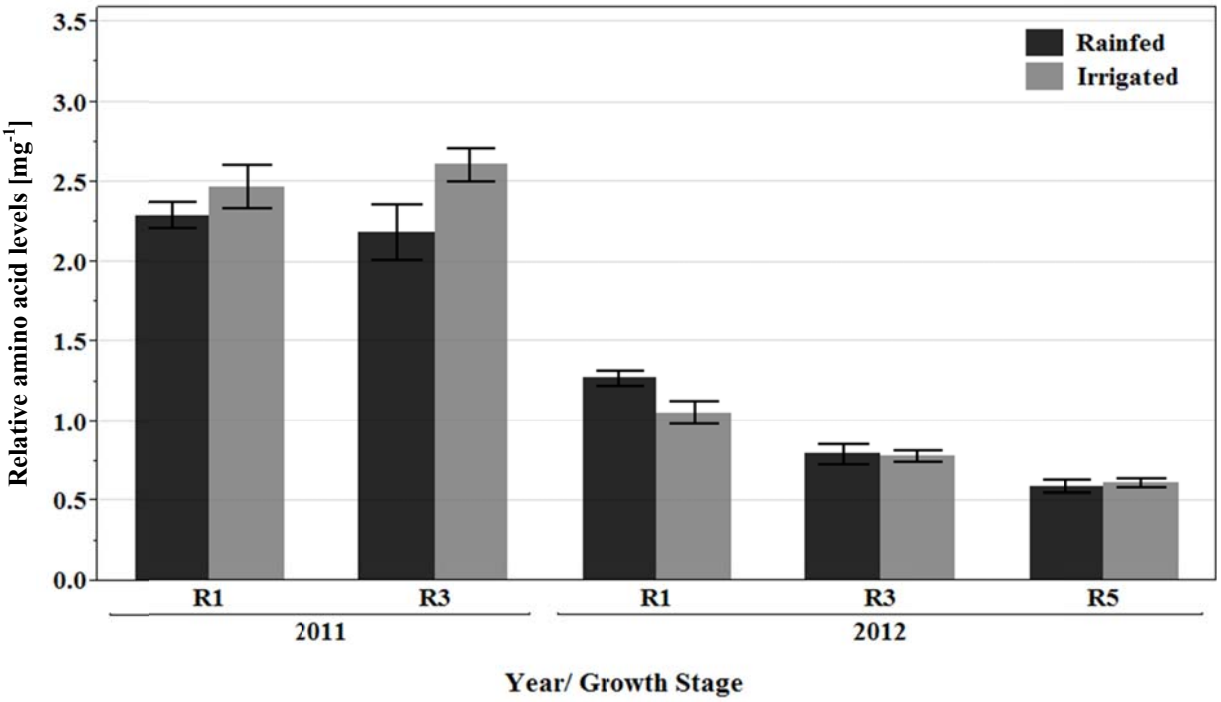


Figure 2.18. Unsaturated fatty acid levels (µg mg^{-1} leaf dry weight) in the eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) measured at two peanut growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and three growth stages (R1, R3, and R5, beginning seed) in 2012. Each error bars represent the standard error from mean (n=32).

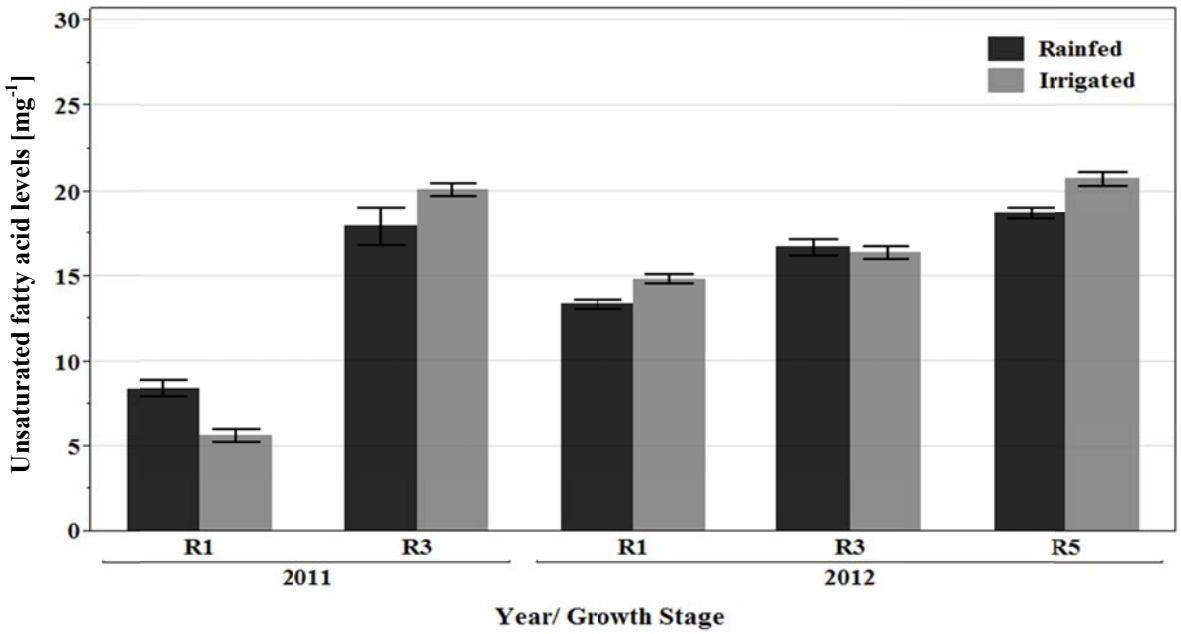
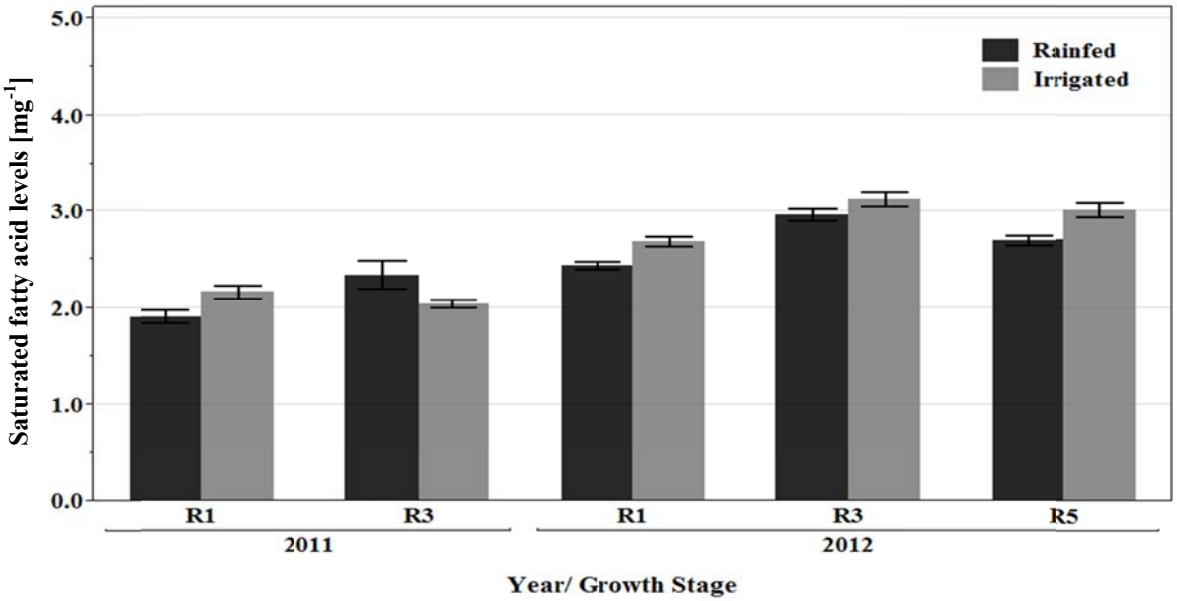


Figure 2.19. Saturated fatty acid levels (**µg mg^{-1} leaf dry weight**) in the eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) measured at two peanut growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and three growth stages (R1, R3, and R5, beginning seed) in 2012. Each error bars represent the standard error from mean (n=32).



Chapter 3 - Physiological and Metabolic Responses to Heat Stress of Virginia-type Peanut (*Arachis hypogaea* L.) Cultivars and Breeding Lines under Controlled Environment

ABSTRACT

Plants respond to stresses through a combination of biochemical, molecular, and physiological protective processes at cellular and whole plant levels. The objective of this study was to identify physiological and metabolic characteristics associated with the response to heat stress of eight Virginia-type peanut cultivars and breeding lines. Thirteen-day-old peanut seedlings were exposed to two temperature regimes 40/35°C (for heat treatment) and 30/25°C (optimum temperature) in controlled conditions. Physiological characteristics and metabolite levels were evaluated in peanut genotypes at four time-points (day 1, 2, 4, and 7) after the stress treatment initiation. Physiological characteristics included leaf membrane injury measured by the electrolyte leakage method and chlorophyll fluorescence measured as ratio of variable (F_v) vs. maximum (F_m) chlorophyll fluorescence (F_v/F_m). Membrane injury increased and chlorophyll fluorescence decreased under heat stress. Differential metabolite accumulation under heat and optimum temperature conditions was also observed. Metabolite profiling analysis revealed several amino acids, organic acids, sugars, and cyclic polyols as the most significant metabolites accumulating in the peanut leaves under heat stress. A general decrease in organic acid levels and increase in sugar alcohol levels was observed under heat stress. Genotype SPT 06-07 exhibited low membrane injury and high unsaturated fatty acid levels under heat stress. Uniqueness of SPT 06-07 in comparison with the other genotypes was evident from the hierarchical clustering on selected physiological characteristics, polar metabolites, and fatty acids. For the other genotypes, the results were inconsistent to ascertain their adaptability or

sensitivity to heat stress. Even though we were able to highlight some metabolites, *e.g.*, hydroxyproline, pinitol, and fatty acids, that could explain specific differential physiological (F_v/F_m ratio and membrane injury) responses in peanut seedlings, overall our data suggested general stress responses of peanut seedlings rather than adaptive mechanisms under heat stress.

INTRODUCTION

The Virginia-Carolina (VC) region including Virginia, North Carolina, and South Carolina, is the most important peanut production region for the large seeded, virginia-type peanut in the United States. Although the region has adequate conditions for high yields in terms of moisture and temperature, occurrence of heat during most part of the growing season was observed in the past few years. For example, average temperatures during June, July and August were almost 4 and 2 ºC in 2011 and 2012, respectively, above the 70-year multiannual average (Fig. 2.1 in Chapter 2), with predictions for even more increase by the end of the century (Solomon et al., 2009). Although peanut is generally fairly tolerant to abiotic stresses, yields can be substantially reduced at temperatures above 33 °C (Prasad et al., 2003).

High temperatures can negatively influence the physiological fitness of peanut plants and subsequently pod and seed quality and yield. Cellular membrane integrity is critical for maintaining optimum plant growth and physiology during abiotic stress. It is well established that under elevated abiotic stress condition, reactive oxygen species (ROS) production increases (Foyer and Noctor, 2005; Mittler, 2002; Moran et al., 1994). Although, ROS normally act as key signaling molecules regulating growth, development, and defense pathways, elevated ROS levels during abiotic stress can cause oxidative damage to the plant membranes and other cellular components (Dat et al., 2000; Kovtun et al., 2000; Mittler et al., 2004; Pei et al., 2000). For example, elevated levels of ROS can increase the membrane lipid peroxidation thus resulting in higher membrane damage in stressed plants (McKersie et al., 1990; Xu et al., 2006). In order to counter the increased levels of ROS, plants develop antioxidant (ROS scavengers) molecules to minimize or avoid the negative impacts of ROS. These protective compounds include, among others, compatible solutes such as proline, glycine betaine, and mannitol and antioxidants

ascorbate, glutathione, and carotenoids (Alscher et al., 2002; Apel and Hirt, 2004; Asada, 2006; Noctor and Foyer, 1998). For example, heat stress lead to significantly increased levels of pinitol in soybean (Guo and Oosterhuis, 1995) and galactinol and raffinose in *Arabidopsis* (Kaplan et al., 2004). It has been shown that tolerant cultivars of barley (Widodo et al., 2009) and turf grass (Du et al., 2011) accumulated higher levels of certain sugars, sugar alcohols, and organic acids in response to salt and heat stresses.

In the light of future temperature increases likely in the VC region, development of heat tolerant peanut cultivars could be an approach to avoid negative effects of heat on yield. This goal can be achieved by means of targeting the adaptive metabolic and physiological mechanisms of heat stress of the virginia-type commercial cultivars and breeding lines currently available. These mechanisms can further be used as screening tools in the breeding programs. To our knowledge no research in relation to heat stress has been done before on the Virginia-type peanut grown in the VC region. Therefore, our specific research objectives were to assess eight virginia-type peanut cultivars and breeding lines for physiological traits related to high temperature stress under controlled conditions, identify metabolites associated with high temperature stress using metabolite profiling analysis, evaluate the relationship between physiological characteristics and the metabolite levels during high temperature (40/35°C) stress, and identify metabolites that are important for peanut adaptation to high temperature.

MATERIALS AND METHODS

Plant materials, growth conditions, and temperature treatments. Seeds of virginia-type peanut (Arachis hypogaea L.) cultivars (CHAMPS, Bailey, and Phillips) and advanced breeding lines (N04074FCT, N05006, N05008, N05024J, and SPT 06-07) were surface sterilized with 70% ethanol for 1 min and rinsed twice with sterile water. Seeds were wrapped in moist

germination paper and allowed to germinate in the dark for 6 d in a growth chamber at 30°C. The seedlings were then transferred to plastic boxes (29.2 cm X 18.7 cm X 15.2 cm) containing half strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Before imposing the heat treatment, plants were allowed to grow in a growth chamber for 7 d at 30/25°C (day/night) temperature and 16/8 h (day/night) photoperiod, and light intensity of 300-400 µmol m-2 s-1 at the plant level. Thirteen-day-old seedlings were then exposed to two temperature regimes 40/35°C (for heat treatment) and 30/25°C (optimum temperature regime), sequentially using the same growth chamber. Seedlings were watered well to avoid drought stress during the heat treatment and the nutrient solution was replaced every 4 d to allow proper root aeration and to minimize microbial contamination. Each genotype within a temperature regime was replicated four times and the experiment was repeated twice. Youngest, fully mature leaves were harvested at 1, 2, 4, and 7 d after beginning of the heat treatment for the physiological characteristics (membrane injury and the Fv/Fm ratio), and all remaining leaves on the plant were harvested at the same time-points for metabolite measurements. One plant per replication and time-point was used for all measurements.

Membrane injury. Membrane injury (MI) of two leaflets per replication from the third youngest fully expanded leaf was measured based on the method by Blum and Ebercon (1981) with modifications. Leaf discs were sampled with 11.1mm diameter disc sampler. After being rinsed twice with distilled water, leaf segments were placed in 20 ml plastic vials containing 15 ml distilled water. After shaking for 24h, initial conductivity (C_i) of the bathing solution was measured with a conductivity meter (Seven-multi conductivity module and InLab® 741 electrode, Mettler-Toledo Inc., Columbus, OH). Leaves were later killed in an autoclave at

120°C for 45 minutes, and placed on a shaker for 24 h before recording the final conductivity (C_f) of the bathing $_s$olution. The percent MI was calculated using the formula:

$$MI = \frac{Ci}{Cf} X 100$$

Chlorophyll fluorescence. Chlorophyll florescence was measured as the F_v/F_m ratio, ratio of variable (F_v) to maximum (F_m) chlorophyll fluorescence [modified from Burke (2007)]. Two leaflets per replication from the third youngest fully expanded leaf were used for the measurement of the F_v/F_m ratio with a modulate*d* chlorophyll fluorometer (OS1p, OptiSciences Inc., NH, USA). Leaf discs were removed using a 11.1 mm disc sampler and subsequently arranged on a moist tissue paper in a Pyrex® glass dish, covered with GLAD® cling wrap transparent film (GLAD Products Company), and carefully pressed flat to remove air bubbles, and to ensure good contact between the leaf discs and film. Initial F_v/F_m values were recorded prior to incubation. Subsequently, leaf samples were dark incubated at 45°C for 3 h. The F_v/F_m ratios were recorded at hourly intervals.

Metabolite sampling and extraction. Three individual leaves from four single seedlings from each replication, genotype, and temperature regime were harvested at four different time-points, snap-frozen in liquid nitrogen, and stored at -80°C until further analysis. The leaves were freeze dried under vacuum for 48 h on a benchtop FreeZone lyophilizer (LABCONCO, Kansas City, MO). The dried leaf tissue was then disrupted to a fine powder with steel beads on a commercial paint shaker. Internal standards (2 µl each of 7.4 mM C17:0 and 10 mM ribitol) were used to account for losses during extractions and metabolite derivatizations for quantification purposes. Dry leaf tissue powder (4.00 ± 0.05 mg) was weighed and extracted with chloroform and water (400 µl each). After the addition of chloroform, tubes were vortexed

and briefly centrifuged to bring liquid down from the lids. Subsequently, water was added and tubes vortexed to partition polar and non-polar metabolites. After the final centrifugation for 5 min, the polar and non-polar extraction phases were physically separated by a completely white solid interphase containing insoluble material such as cell walls, starch, and proteins. The white color indicated a complete breakage of the membranes and extraction of metabolites, as all chlorophyll from the thylakoid membranes was extracted by chloroform. The top (clear) aqueous phase contained polar metabolites (sugars, sugar-alcohols, cyclic polyols, organic acids, and amino and carboxylic acids, organic amines, and major phenolics), while the bottom (green) organic phase contained the non-polar metabolites (lipids, chlorophyll, carotenoids, and other non-polar compounds such as sterols). These phases were used for metabolite profiling (MP) and fatty acid (FA) analysis, respectively. For polar metabolite profiling, 50 µl of the aqueous phase was transferred to 250-µl glass inserts in auto-sampler vials. For fatty acid analysis, a volume of 200 µl of non-polar extracts was transferred into glass derivatization vials for fatty acid analysis. Both water and chloroform were evaporated under a stream of N_2 gas at 50^{o}C.

Polar metabolites. After complete drying, polar metabolites were derivatized with 25 µl of methoxyamine.HCl in pyridine. The vials were rigorously vortexed and incubated at 50^{o}C for 2 hours. Subsequently, 25 µl of N-methyl-N-(trimetylsilyl) trifluoroacetamide containing 1% (v/v) trimethylchlorosilane was added. After thorough vortexing, the samples were incubated at 50^{o}C for 30 minutes.

The injection temperature was set at 280^{o}C. Helium was used as a carrier gas at a constant flow rate of 1.2 ml min^{-1}. The temperature program was: 60^{o}C for 1 min, 10^{o}C min^{-1} to 325^{o}C, and final hold at 320^{o}C for 5 min. Electron impact was used for molecule ionization and the

analysis was carried out in a positive scan mode using an Agilent 5975C series single quadrupole mass spectrometer (MS) (Agilent Technologies).

Individual peaks were characterized using both spectral and retention time information of a given compound against the commercially available reference databases such as spectral NIST library (National Institute of Standards and Technology, Gaithersburg, MD), FiehnLib (Kind et al., 2009), and the custom-built spectral and retention time library generated in Čolláková laboratory. Signals from coeluting compounds were deconvoluted using Automated Mass Spectrometry Deconvolution and Identification System (AMDIS, NIST). Individual metabolite identity and integration quality was checked using ChemStation software (Agilent Technologies). Individual metabolite quantitation recovery was optimized using relative peak areas of internal standards. Relative metabolite levels were determined after normalizing with internal standard ribitol and dry tissue weight.

Fatty acids. After drying with a stream of N_2, 500 µl of 1N methanolic HCl was added to each vial. After vortexing, the samples were incubated at 75^{o}C for 2 hours to hydrolyze membrane lipids into glycerol and fatty acids and generate volatile fatty acid methyl esters (FAME). Methanolic HCl was evaporated under a stream of N_2 and FAME were dissolved in 100 µl of heptane. After vortexing, the heptane containing FAME was transferred to 250-µl glass inserts placed in GC-MS vials and 1 µl was injected in a pulsed splitless mode on GC coupled to an Agilent flame ionization detector. The injection temperature was set at 250^{o}C. Helium was used as a carrier gas at a flow rate of 1 ml min^{-1}. Air and hydrogen flow rate was 350 and 30 ml min^{-1}, respectively. The temperature program was: 100^{o}C for 0.5 min, 20^{o}C min^{-1} to 250^{o}C, and final hold at 250^{o}C for 6 min. The individual fatty acids were identified based on the retention times. Relative peak areas of the internal standards were used to correct for

recovery in quantitation of individual fatty acids. The absolute fatty acid levels were computed after dividing with the dry tissue weight.

Statistical analysis. The two experiments were combined for statistical analysis. Data sets containing a total of eight replications per genotype and treatment were analyzed using JMP 9.0 software program (SAS Institute, Cary, NC). Analysis of variance (ANOVA) for individual metabolites and physiological characteristics was carried out and the model was:

$$y_{jkl} = \mu + g_j + t_k + d_l + (gt)_{jk} + (gd)_{jl} + (td)_{kl} + (gtd)_{jkl} + e_{jkl}$$

where μ is the overall mean effect, g_j the main effect of the j^{th} genotype (j = 1 to 7), t_k the main effect of the k^{th} temperature treatment (k = 1, 2), d_l the day of the l^{th} sampling effect (l = 1 to 4), $(gt)_{jk}$ the interaction of the j^{th} genotype and k^{th} treatment, $(gd)_{jl}$ the interaction of the j^{th} genotype and l^{th} day of sampling, $(td)_{kl}$ the interaction of the k^{th} treatment and l^{th} day of sampling, $(gtd)_{jkl}$ the interaction of the j^{th} genotype, k^{th} temperature treatment, and l^{th} day of sampling, and e_{jkl} the random error associated with the experimental unit.

Correlations between the physiological characteristics and changes in metabolite levels were also calculated. Step-wise variable selection analysis was carried out to identify the most discriminating variables that explained the largest proportion of variation in the data. These response variables were then used to construct a dendrogram of genotype clusters based on Ward's distance matrix.

Principal component analysis (PCA) was performed on the metabolite data. Variance component estimation was based on the restricted maximum likelihood (REML) method. Loading and score plots were used to reveal the correlations and degree of variation present in the metabolite data. First two principal components explained the highest percentage of

variation between samples, hence further analysis were based on these two principal components.

RESULTS

Membrane injury. The ANOVA for membrane injury showed temperature-by-genotype-by-day of measurement effect ($p < 0.05$) indicating that genotypes performed differently at each time-point and temperature treatments (Table 3.1). With the only exception of Day 2, seedlings under heat stress had higher membrane injury than control seedlings at all time-points (Fig. 3.1). For the heat stressed seedlings, membrane injury at Day 1 was 33%, at Day 4 30% and at Day 7 37% higher than for control seedlings (Table 3.2). N05008 at Days 1 and 7, Phillips at Day 1, and N05024J at Day 7 showed higher membrane injury in heat stressed than control seedlings (Table 3.2). When averaged across the time-points, genotypes N05008 and Phillips showed the highest (20 and 18%) and SPT 06-07, Bailey, and N04074FCT the lowest (14.5% each) membrane injury under heat stress.

Chlorophyll fluorescence. The F_v/F_m ratio decreased gradually over the 3 h of dark incubation period at 45°C for both control and heat stressed seedlings approximately 15% on Day 1, 13% on Day 2, 24% on Day 4, and 31% on Day 7 (Fig. 2). On Day 1, the F_v/F_m ratio decreased more for the control than for heat stressed seedlings ($p < 0.05$), but this trend was reversed on Day 2. After 4 d of differential temperature regime, the F_v/F_m ratio decreased similarly in control and heat stressed seedlings with time at 45ºC. Percent change in the F_v/F_m ratio from 0 to 3 h of incubation was calculated for individual genotypes and temperature regimes (Fig. 3.3). Because the temperature-by-genotype and temperature-by-genotype-by-day of measurement interactions were not statistically significant for the F_v/F_m ratio (Table 3.1), the

days of measurement or time-points were combined for each genotype. Overall, Bailey and Phillips exhibited the smallest (14 and 17%, respectively) and N05024J the largest (28%) decline in the F_v/F_m ratio among the eight genotypes (Fig. 3.2).

Metabolite profiling. Out of the total peaks identified during the metabolite profiling analysis, the 26 most abundant metabolites were chosen for further analysis (Table 3.1). These metabolites belong to four major groups. There were eight amino acids, nine organic acids, five sugars, and four sugar alcohols (Table 3.1). Principal component analysis (PCA) was performed on the combined metabolite and fatty acid levels and the physiological characteristics. Significant within-treatment and between-time-point variations were observed with the first two principal components explaining 37% of the total variance in the metabolite, fatty acid, and physiological data (Fig.3.4). The data cluster from Day 1 was separated from Day 7 by PC2, which was associated with tartrate, shikimate, citrate, quinate, threonate, myo-inositol, fructose, and glucose relative contents. The first three PCs along with their associated variables and component scores were presented (Table 3.3).

A total of nine organic acids were identified: glycerate, fumarate, citrate, malate, α-ketoglutarate, ascorbate, quinate, shikimate, threonate and tartrate. For glycerate and fumarate, there were no or small changes in response to temperature regime of the number of days of heat exposure (Table 3.4). However, changes with the day of heat exposure were significant, for malate, and citrate. Significant changes due to temperature regime were recorded for α-ketoglutarate and ascorbate levels ($p < 0.05$). For shikimate, tartrate, quinate, and threonate, temperature regime and the number of days to heat significantly changed their relative levels in the peanut plants (Table 3.4). For example, significant declines with time under heat treatment were recorded for shikimate levels (4.1 fold) and quinate (6.3 fold). Even shikimate levels

significantly decreased in heat-stressed seedlings of all genotypes, except for N04074FCT (Table 3.5). Among genotypes, N05006 had the lowest and N05024J the highest relative levels of α-ketoglutarate in heat-stressed seedlings. Quinate levels significantly ($p < 0.05$) decreased in heat-stressed Bailey, N05006, and N05008, and remained unchanged for all other genotypes (Table 3.5).

Pinitol, inositol, and galactinol were the major cyclic polyols and sugar alcohols identified in peanut samples. However, galactinol levels were significantly higher (over 100 fold) in heat-stressed than in control seedlings at all time-points. No significant differences in galactinol levels were observed among genotypes for galactinol in control seedlings. At high temperatures, Bailey, CHAMPS, Phillips, and SPT 06-07 had approximately 66% more galactinol accumulation than did N05006, N05008, and N04074FCT (Table 3.7). With the exception of N04074FCT and SPT 06-07, all other genotypes showed larger accumulation of pinitol in heat-stressed than in control seedlings. N05008 and CHAMPS had almost 70% more pinitol than N04074FCT and N05006 under heat treatment.

Among the sugars, sucrose and ribose changed their relative contents only in response to temperature, whereas fructose, glucose and maltose levels changed in response to both, temperature and day of exposure to high temperatures (Table 3.6). Glucose, fructose, and maltose also showed significant ($p < 0.05$) genotype-by-day of measurement and temperature-by-genotype-by-day interactions of measurements (Table 3.1). Fructose levels decreased at Days 1, 2, and 4 but no change was observed at Day 7 of the heat treatment (Table 3.6). Mean fructose levels decreased significantly for Bailey, CHAMPS, N05006, and SPT 06-07 in heat-stressed compared to their respective controls. N05006 and SPT 06-07 had the lowest and Phillips the highest levels of fructose among the eight genotypes under heat stress (Table 3.7).

In general, the amino acids showed increased accumulation in heat-stressed compared to the control seedlings (Table 3.8). For example, alanine, serine, threonine, glutamine, hydroxyproline, and asparagine were significantly higher in heat-stressed plants after one day of exposure to 40/35°C temperature regime. With the exception of alanine, aspartate, and glutamine, the other amino acids remained unchanged after Day 1 of the heat stress treatment initiation. Aspartate levels decreased gradually after 2, 4, and 7 d of heat stress whereas glutamate levels remained unchanged throughout the heat treatment time-course (Table 3.8). Total amino acid levels remained unchanged during Days 1, 2, and 4 and decreased significantly on Day 7 in both control and heat stressed seedlings. The genotype-by-day of measurement and temperature-by-genotype-by-day of measurement interactions were absent for all amino acids (Table 3.1), hence the time-points were combined for each genotype and important amino acids were presented in Table 3.9. Among the genotypes, N04074FCT and Phillips had the highest and SPT 06-07 and N05008 the lowest levels of hydroxyproline in heat stressed seedlings (Table 3.9). Similarly, threonine levels were 65% higher for heat stressed seedlings of N04074FCT and Phillips than for SPT 06-07.

Fatty acids. Seedlings under heat stress showed 64% increase of the unsaturated fatty levels at Days 2 and 4 compared to control; the levels remained unchanged at Days 1 and 7, although the trend was for higher production under heat (Table 3.10). Overall, seedlings from the heat treatment exhibited a 26% increase in unsaturated fatty acid levels in response to heat stress. Saturated fatty acids showed a 25% increase at Day 2 and 72% decrease at Day 7 in heat-stressed relative to the control seedlings. SPT 06-07, CHAMPS, and N05024J showed a increases ($p < 0.05$) in unsaturated fatty acid levels in heat compared to control seedlings. On

average SPT 06-07 had the highest, and Bailey and Phillips the lowest levels of unsaturated fatty acids under heat stress (Table 3.11).

Relationship of the physiological and metabolic characteristics. Correlations among the physiological characteristics and polar and non-polar metabolites for heat stressed seedlings were calculated for each time-point separately (Supplemental Table 3.1). The F_v/F_m decline was negatively correlated with the total organic acids (r= - 0.24, $p < 0.05$) and saturated fatty acid (r= - 0.35, $p < 0.01$) levels at day 1 of the heat treatment, but no other relationships were identified afterwards. Membrane injury was not correlated with any individual metabolite under heat stress. There was a negative relationship between membrane injury and the F_v/F_m decline in control, but not in heat stressed seedlings.

A dendrogram was constructed based on the physiological characteristics and the most significant metabolites through step-wise variable selection (Fig. 3.5). Based on the metabolic and physiological similarities, the eight genotypes were classified into two distinct groups. Group I consisted of seven genotypes and was significantly different from Group II with SPT 06-07 as the sole member. Within Group I, N05008 and Phillips were closely related to each other, N05006 was related phenotypically to N05024J, and Bailey was closely associated with N04074FCT.

DISCUSSION

Membrane injury. During stress, lipid peroxidation can cause severe membrane injury and as such, can be measured to assess the degree of abiotic stress in crops including peanuts (Bajji et al., 2002; Blum and Ebercon, 1981; Srinivasan et al., 1996). Membrane injury and the F_v/F_m ratio have previously been used in screening peanut genotypes for salt, heat, and drought

stress tolerance (Lauriano et al., 2000; Qin et al., 2011; Srinivasan et al., 1996). In our experiment, seedlings exposed to heat showed 33% more membrane injury than control seedlings after Day 1, 30% after Day 4, and 37% after 7 d (Fig. 3.1). N04074FCT, SPT 06-07, and Bailey exhibited the lowest and N05008 the highest percentage of membrane injury under heat stress. The responses for other genotypes were inconsistent and varied throughout the heat stress treatment. Although high levels of several amino acids, organic acids, sugar alcohols and cyclic polyols were previously associated with improved membrane stability during stress (Gounaris, 1984; Liu, 2000; Dhindsa, 1981), in this study we could not identify a single metabolite associated with reduced membrane injury. However, when multiple regression analysis was used, a combination of several metabolites including sugars fructose, glucose, and maltose, sugar alcohols inositol, and pinitol and amino acid aspartate were positively associated with membrane injury. In contrast, ribose, hydroxyproline, and saturated fatty acids were all negatively correlated with membrane injury and their combinations explained from 41 to 61% of the variation in the membrane injury during the heat stress treatment.

Chlorophyll fluorescence. Temperature effect was not significant for individual genotypes but overall Bailey and Phillips had the least (14 and 17%), and N05024J the greatest (28%) decline in the F_v/F_m ratio among eight genotypes (Fig. 3.2). Higher F_v/F_m ratio under drought stress compared to well-watered plants (Fig. 3.1, Day 1) was earlier reported in several crops including peanut (Burke, 2007; Kottapalli et al., 2009). Burke (2007) explained this to be the result of stress-induced sucrose accumulation, which provided respiratory metabolic substrate at high temperature stress. In our experiment, the negative correlations between the F_v/F_m ratio decline and total organic ($r= -0.24$, $p < 0.05$) and saturated fatty acid ($r= -0.35$, $p < 0.01$) levels at Day 1 of the heat treatment were observed, suggesting that higher levels of organic and saturated

fatty acids may be associated with a slow decline in the F_v/F_m ratio in peanut seedlings.. Although seedlings exhibited symptoms of heat stress in terms of increased membrane injury and change in levels of the majority of metabolites, the F_v/F_m ratio decline due to heat stress exposure was small for Bailey and absent for the other genotypes (Fig. 3.2). The lack of genotypic differences may be due to the short duration (7 d) of the heat stress period which may have been insufficient to cause significant damage to the photosystem II complex, often reflected by decreases in F_v/F_m in stressed plants (Balota and Lichtenthaler, 1999; Lichtenthaler et al., 1998; Maxwell, 2000).

Polar metabolites. Principal component analysis showed low variance among time points, indicating a weak differentiation of time-points on the basis of physiological characteristics and metabolites. In general, organic acids decreased under heat stress (Table 3.3). For example, major organic acids including quinate, ascorbate, and shikimate showed significant decreases in their relative steady-state levels in heat-stressed seedlings, especially during the last two time-points (Days 4 and 7). Similar results were reported in drought-stressed wheat (Bowne et al., 2012) and in our field study (Chapter 2). Increased levels of organic acids were often positively related to cell growth and respiration (Glassop et al., 2007; Vasquez-Robinet et al., 2008). The relationship between organic acid levels and maintenance of high F_v/F_m ratios was positive at Day 1 during the heat treatment, suggesting that higher levels of organic acids during early heat stress could be an indicator of increased photochemical efficiency during the stress (Widodo et al., 2009; Liu and Huang, 2000). Among the sugar alcohols identified during this study, galactinol increased over 100 fold as a result of heat stress (Table 3.5). The osmoprotective role of galactinol has been documented (Nishizawa et al., 2008). Galactinol and myo-inositol play a key role in raffinose biosynthesis, and their accumulation during abiotic stresses is known to

confer cellular protection against the oxidative stress (Kaplan et al., 2004; Peters et al., 2007). Increased levels of pinitol are also known to confer tolerance to various abiotic stresses in other crop plants (Matos et al., 2010; McManus et al., 2000). Overall, levels of cyclic polyols, such as pinitol and inositol did not change significantly in response to heat stress. In the present study, an increase of galactinol levels in peanut seedlings is in an agreement with the literature and highlights its role as a possible osmoprotectant during heat stress (Kaplan et al., 2004). Indeed in our research, a combination of several sugars, sugar alcohols, amino acids, and fatty acids better explained the variation in membrane injury, 41 to 61% of the total.

A steady decline in sugar content was observed following prolonged heat stress with the lowest levels at Day 7 of the heat treatment (Table 3.6). Sugars that contributed to this trend were the hexoses glucose and fructose and the disaccharide maltose. Abiotic stresses affect the process of photosynthesis through various means, *i.e.*, ROS-mediated membrane damage and reduced chlorophyll production (Chu et al., 1974; Dhindsa et al., 1981; Nishizawa et al., 2008). Lower sugar levels can be a direct consequence of reduced photosynthesis (Shah and Paulsen, 2003) and altered carbon metabolism under stress (Mangelsen et al., 2011). Decline in sugar content was seen in wheat seedlings exposed to drought (Bowne et al., 2012). Heat-induced accumulation of soluble sugars such as glucose, fructose, and sucrose was also reported in *Arabidopsis* and crop species (Du et al., 2011; Kaplan et al., 2004; Rizhsky et al., 2004). In the present study, a significant decrease in sugar levels during heat stress (7 d) was in an agreement with previous research.

Several studies reported increased amino acid levels in response to various stresses in sensitive cultivars (Du et al., 2011; Vasquez-Robinet et al., 2008; Zuther et al., 2007). In our study, the levels of hydroxyproline and asparagine were higher at Day 1, glutamate at Day 7, and

glutamine throughout the time course in the heat-stressed than in control seedlings (Table 3.8). Their increase is sometimes attributed to stress-induced protein degradation due to tissue damage and senescence (Diaz et al., 2005; Widodo et al., 2009). For example, increased hydroxyproline and glutamine levels for N04074FCT (Table 3.9) might indicate that this genotype is more sensitive to heat stress than other genotypes. However, regression analysis selected hydroxyproline as a component negatively associated with membrane injury in the heat-stressed seedlings. Other correlations between physiological characteristics and individual amino acids were non-existent.

Fatty acids. A General increases of unsaturated fatty acid levels during heat stress have been reported previously (Upchurch, 2008; Zhang et al., 2005). The unsaturated fatty acid levels increased significantly ($p < 0.05$) between Days 2 and 4 but not at Days 1 and 7 during heat stress relative to the controls (Table 3.10). Accumulation of unsaturated fatty acids is thought to help plants maintain intact membranes during stress (McKersie et al., 1990). For example, increased levels of unsaturated fatty acid levels in SPT 06-07 coincided with lower membrane injury for this genotype in comparison with other genotypes. As part of the cellular membrane complex, fatty acids play an important role in keeping the cellular membrane integrity during periods of environmental stress (McKersie et al., 1990; Simon, 1974).

CONCLUSIONS

This study investigated the physiological and metabolic responses of eight virginia-type peanut cultivars and breeding lines to high temperature stress in a controlled environment. Significant physiological and metabolic changes were observed in response to heat. From the physiological perspective, membrane injury increased and chlorophyll fluorescence (measured as

F_v/F_m ratio) decreased in response to high temperatures. Comparative metabolomics revealed changes in the levels of specific metabolites in heat-treated relative to control peanut seedlings. Genotype SPT 06-07 exhibited lower membrane injury and higher unsaturated fatty acids accumulation under heat stress. This is not surprising since SPT 06-07 has exhibited improved drought tolerance in other studies (Chapter 2; S.P. Tallury, 2013, personal communication). The uniqueness of SPT 06-07 in comparison with the other genotypes is also shown based on the step-wise variable classification analysis, where SPT 06-07 was selected as the sole genotype of Group II, which was significantly different from Group I, that included the remaining genotypes.. For the other genotypes, results were inconsistent to ascertain their adaptability or sensitivity to heat stress. Even though we were able to highlight some metabolites, ***e.g.***, hydroxyproline, galactinol, pinitol, and fatty acids, that could explain specific differential physiological (F_v/F_m ratio and membrane injury) responses in peanut seedlings, overall our data suggested general stress responses by plants rather than adaptive mechanisms under heat stress. Lack of genotypic differences was not surprising given the narrow genetic base of the plant materials with the exception of SPT 06-07, a cross with the diploid wild species *Arachis cardenasii* (Krapov. & W.C. Gregory) (S.P. Tallury, 2013, personal communication). This study also validates the use of the F_v/F_m ratio as a potential tool for assessing heat stress in peanut. In addition, as plants experience simultaneous multiple stresses in the field conditions, future investigations should focus on exploring multiple stresses at the field and controlled environment levels.

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Table 3.1. F-ratios from the ANOVA for the physiological and metabolite characteristics of eight virginia-type peanut genotypes after 1, 2, 4, and 7 d of exposure to control (30/25°C) and high temperature (40/35°C) in controlled conditions

Characteristics	Source of variation						
	Temperature (T)	Genotype (G)	DAY[§] (D)	T × G	T × D	G × D	T × G × D
df[†]	1	7	3	7	3	21	21
F_v/F_m	0.00	2.94**	12.89**	0.45	12.98**	2.00**	1.50
Membrane injury	8.07**	3.47**	4.26**	0.48	9.17**	1.67*	1.69*
Organic acids	6.26*	1.58	0.07	2.15	2.33	0.85	1.21
Glycerate	0.00	0.94	3.42*	0.31	0.52	1.56	1.20
Fumarate	10.41**	0.78	2.41	0.38	0.93	0.95	0.60
Malate	0.10	1.83	8.32**	3.35**	1.43	0.70	1.11
α-ketoglutarate	2.18	0.63	19.60**	1.47	7.50**	0.66	0.64
Ascorbate	471.44**	1.24	36.57**	0.44	20.55**	0.82	0.54
Tartrate	14.64**	2.62*	25.11**	0.42	2.18	0.81	1.11
Shikimate	124.57**	3.21**	25.05**	3.78**	3.49*	0.89	1.15
Citrate	3.33	2.47*	11.72**	0.55	3.50*	1.72*	0.79
Quinate	63.17**	4.63**	26.96**	3.39**	6.57**	0.49	1.08
Sugar alcohols	143.40**	5.32**	20.95**	1.69	2.30	0.98	0.71
Threonate	1.57	1.14	18.57**	0.88	16.46**	0.48	0.55
Pinitol	46.09**	7.04**	21.54**	2.30*	5.97**	1.41	1.15
Inositol	20.89**	2.25*	2.29	0.99	9.35**	0.77	1.11
Galactinol	46.75**	0.82	1.22	0.80	1.19	0.40	0.40
Sugars	91.73**	7.11**	65.91**	2.94**	5.10**	2.09**	3.27**
Glucose	20.42**	4.36**	94.62**	1.36	8.86**	1.59*	2.72**
Fructose	50.41**	4.85**	91.19**	1.18	8.41**	1.74*	2.20*
Sucrose	98.28**	6.97**	1.70	4.55**	6.00**	1.54	2.04**
Ribose	58.36**	5.08**	3.62*	0.70	0.67	0.41	0.32
Maltose	22.08**	1.57	28.51**	1.17	2.20	2.49**	1.59*
Amino acids	3.54	7.64**	13.20**	1.49	0.53	1.41	0.58
Alanine	9.82**	2.21*	3.48*	0.34	0.58	0.75	0.95
Serine	3.43	0.70	3.26*	0.15	0.74	0.84	0.51
Threonine	7.88**	0.63	3.24*	0.13	0.83	0.73	0.48
Aspartate	19.96**	2.67*	10.67**	2.25*	0.18	1.11	0.68
Glutamine	17.23**	3.23**	8.88**	2.15*	0.19	1.05	0.95
Hydroxyproline	2.36	8.62**	22.83**	0.73	2.19	1.24	0.54
Glutamate	2.29	3.98**	5.34**	1.55	0.59	0.74	0.54
Asparagine	5.60*	8.94**	13.03**	1.24	1.80	1.40	0.75
Unsaturated Fatty acids	30.99**	0.53	1.58	0.76	4.83**	0.49	0.56
Saturated Fatty acids	7.96**	3.41**	14.04**	1.30	14.66**	1.63*	1.27

**, * Significant at the 0.01 and 0.05 probability levels, respectively
† Degrees of freedom
§ Days after treatment initiation.

Table 3.2. Percentage membrane injury levels in leaves of eight virginia-type peanut cultivars and breeding lines after 1, 2, 4, and 7 d of exposure to control (30/25°C) and high temperature (40/35°C) under controlled conditions.

MI	Day 1		Day 2		Day 4		Day 7	
	Control	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**
				%				
Bailey	15.95 abc[1]	17.89 abc	16.35 bcd	12.69 bcd	14.73 abc	14.31 bc	11.15 c	13.19 bc
CHAMPS	15.75 bc	16.21 c	14.33 bcd	13.58 bcd	15.20 abc	22.48 a	14.80 bc	14.33 bc
N04074FCT	13.35 c	18.59 abc	-	9.51 d	-	15.43 abc	10.60 c	-
N05006	16.50 abc	18.55 abc	13.50 bcd	13.86 bcd	12.10 bc	19.80 ab	10.88 c	13.68 bc
N05008	14.85 c	25.45 a	37.55 a	18.08 b	-	15.64 abc	10.93 c	19.96 a
N05024J	14.45 c	19.10 abc	17.13 bc	14.85 bcd	13.00 bc	11.96 c	11.98 bc	20.04 a
Phillips	14.75 c	24.74 ab	15.65 bcd	12.68 bcd	14.23 abc	20.99 ab	11.60 bc	15.74 b
SPT 06-07	11.73 c	16.94 bc	15.23 bcd	11.05 cd	10.33 c	17.10 abc	10.00 c	13.84 bc
Average	**14.67 B[2]**	**19.68 A**	**18.53 A**	**13.29 B**	**13.26 B**	**17.21 A**	**11.49 B**	**15.75 A**

[1] Means followed by different small letters across genotypes within a day are significantly different ($P < 0.05$ student's t-test).
[2] Means followed by different capital letters within averages of days of treatment are significantly different ($P < 0.05$ student's t-test).
Each mean value calculated from at least (n=4) observations.

Table 3.3. Principal component analysis of metabolites and fatty acids exhibiting variance in eight virginia-type peanut cultivars and breeding lines exposed to heat stress (40/35°C) under controlled conditions.

PC1	PC2	PC3
Metabolites (PC Scores)		
Serine (0.86)	Tartrate (-0.39)	Ascorbate (0.76)
Threonine (0.86)	Shikimate (0.67)	Sucrose (0.41)
Aspartate (0.79)	Citrate (-0.49)	Ribose (-0.53)
Glutamine (0.76)	Quinate (0.76)	Unsaturated FA (0.52)
Hydroxyproline (0.79)	Threonate (0.78)	Saturated FA (0.66)
Glutamate (0.78)	Myo-Inositol (0.73)	
Asparagine (0.61)	Fructose (0.81)	
Glycerate (0.72)	Glucose (0.79)	
Fumarate (0.78)		
Malate (0.60)		
alpha-ketoglutarate (0.43)		

Table 3.4. Mean relative organic acid levels in the leaves of eight virginia-type peanut cultivars and breeding lines after 1, 2, 4, and 7 d of control (30/25°C) and high temperature (40/35°C) under controlled conditions.

Organic acid	Control-1	Heat-1	Control-2	Heat-2	Control-4	Heat-4	Control-7	Heat-7
Glycerate	1.09 ab[1]	1.20 a	1.05 ab	0.85 abc	0.38 bc	0.71 abc	0.54 abc	0.28 c
Fumarate	0.86 ab	1.62 a	0.79 ab	1.43 a	0.41 b	1.63 b	0.39 b	0.57 b
Malate	162.60 ab	171.13 a	161.25 ab	180.28 a	178.12 a	177.71 a	144.14 bc	125.61 c
α-ketoglutarate	1.99 a	1.43 cd	1.92 ab	1.65 bc	1.53 cd	1.54 c	0.86 e	1.25 d
Ascorbate	8.30 a	0.87 e	8.96 a	2.94 c	4.56 b	1.72 d	4.84 b	1.08 de
Tartrate	34.75 e	36.61 e	41.60 de	45.47 cd	40.82 de	52.37 b	53.17 bc	67.68 a
Shikimate	17.93 ab	13.88 c	20.33 a	10.92 d	15.97 bc	7.02 e	13.24 cd	3.43 f
Citrate	215.90 b	237.50 b	223.23 b	248.89 b	214.39 b	343.24 a	385.40 a	346.59 a
Quinate	209.00 ab	200.47 b	244.89 a	142.35 c	215.25 ab	75.01 d	128.42 c	31.82 e
Threonate	3.65 bc	6.34 a	3.56 bc	3.86 bc	4.36 b	3.22 c	3.14 cd	2.37 d

[1]Means followed by different letters within a row are significantly different within and between the days of treatment ($p < 0.05$, student t-test). Each mean value calculated from at least (n=30) observations.

Table 3.5. Mean relative organic acid levels in the leaves of eight virginia-type peanut cultivars and breeding lines under control (30/25°C) and high temperature (40/35°C) under controlled conditions. Data from days 1, 2, 4, and 7 were combined.

	Tartrate		**Shikimate**		**α- Ketoglutarate**		**Quinate**	
	Control	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**
Bailey	35.47 de[1]	50.85 ab	20.55 ab	10.16 de	1.53 ab	1.63 a	246.0 ab	135.0 ef
CHAMPS	33.90 e	42.05 b-e	15.23 c	8.94 e	1.62 ab	1.55 ab	208.4 bcd	147.1def
N04074FCT	47.83 a-e	53.64 a	14.23 cd	13.14 cd	1.64 a	1.60 a	161.4 c-f	124.0 efg
N05006	46.23 a-e	49.97 abc	22.57 a	7.37 e	1.71 a	1.19 b	294.7 a	102.0 ef
N05008	44.71 a-e	54.08 a	16.79 bc	7.98 e	1.67 a	1.43 ab	239.9 abc	109.2 efg
N05024J	48.28 a-e	53.54 a	14.07 cd	7.54 e	1.35 ab	1.64 a	140.2 d-g	105.0 efg
Phillips	46.86 a-e	53.01 a	17.54 abc	8.72 e	1.74 a	1.38 ab	132.3 d-g	100.2 fg
SPT 06-07	37.44 cde	47.69 a-d	13.97 cd	6.52 e	1.38 ab	1.34 ab	172.2 b-e	74.0 g
Average	**42.59 B[2]**	**50.60 A**	**16.87 A**	**8.80 B**	**1.58 A**	**1.47 A**	**199.4 A**	**111.9 B**

[1] Means followed by different small letters across genotypes and temperature regime are significantly different ($P < 0.05$ student's t-test).
[2] Means followed by different capital letters within averages of days of treatment are significantly different ($P < 0.05$ student's t-test).
Each mean value calculated from at least (n=16) observations.

Table 3.6. Mean relative sugar and sugar alcohol levels in the leaves of eight virginia-type peanut cultivars and breeding lines after 1, 2, 4, and 7 days of control (30/25°C) and high temperature (40/35°C) under controlled conditions.

Sugar alcohols	**Control-1**	**Heat-1**	**Control-2**	**Heat-2**	**Control-4**	**Heat-4**	**Control-7**	**Heat-7**
Pinitol	378.9 ab[1]	788.1 a	395.8 ab	453.3 ab	345.5 ab	423.8 ab	183.5 b	376.4 ab
Inositol	25.5 a	23.7 a	29.4 a	27.3 a	15.6 a	32.9 a	11.6 a	32.4 a
Galactinol	0.2 d	31.2 c	0.2 d	66.3 a	0.2 d	41.3 bc	0.7 d	55.9 ab
Sugars								
Fructose	196.0 a[1]	170.1 b	168.7 b	119.5 c	135.3c	90.2 d	77.7 d	83.7 d
Glucose	404.9 a	346.8 b	349.1 b	274.4 c	271.5 c	212.9 d	165.9 e	202.7 d
Sucrose	124.8 a	9.6 d	89.9 ab	52.9 c	76.8 bc	26.3 d	121.7 a	24.8 d
Ribose	2.3 c	4.2 a	2.2 c	3.7 ab	2.0 cd	3.3 b	1.2 d	3.3 b
Maltose	4.5 a	2.7 b	4.4 a	2.5 b	2.0 bc	1.3 cd	1.0 cd	0.6 d

[1]Means followed by different letters within a row are significantly different within and between the days of treatment ($p < 0.05$, student t-test). Each mean value calculated from atleast (n=30) observations.

Table 3.7. Mean relative sugar and sugar alcohol levels in the leaves of eight virginia-type peanut cultivars and breeding lines under control (30/25°C) and high temperature (40/35°C) under controlled conditions. Data from days 1, 2, 4, and 7 were combined.

	Glucose		Fructose		Galactinol		Pinitol	
	Control	**Heat**	**Control**	**Heat**	**Control**	**Heat**	**Control**	**Heat**
Bailey	308.3 ab[1]	253.4 bc	152.77 ab	111.94 cd	1.02 d	66.74 a	313.06 d-g[1]	405.40 bc
CHAMPS	305.6 ab	255.4 bc	152.98 ab	111.97 cd	0.18 d	62.95 ab	361.05 b-f	510.02 a
N04074FCT	300.0 abc	288.1 abc	137.82 a-d	125.61 bcd	0.28 d	34.90 bcd	359.82 b-f	325.58 def
N05006	303.5 abc	238.7 cd	146.51 ab	105.35 de	0.20 d	23.47 cd	238.73 g	353.33 c-f
N05008	274.0 abc	263.6 bc	135.76 a-d	112.48 cd	0.20 d	25.59 cd	404.73 bcd	513.58 a
N05024J	337.5 a	271.9 bc	163.54 a	120.95 bcd	0.38 d	51.64 abc	285.42 fg	417.60 bc
Phillips	300.0 abc	302.7 ab	148.50 ab	135.20 abc	0.23 d	63.00 ab	307.98 efg	441.01 b
SPT 06-07	254.3 bcd	196.4 d	117.41 bcd	81.10 e	0.21 d	60.55 ab	336.45 c-f	389.54 b-e
Average	**297.9 A[2]**	**258.7 B**	**144.41 A**	**113.08 B**	**0.34 B**	**48.76 B**	**325.91 B**	**419.51 A**

[1] Means followed by different small letters across genotypes and temperature regime are significantly different ($P < 0.05$ student's t-test).
[2] Means followed by different capital letters within averages of days of treatment are significantly different ($P < 0.05$ student's t-test).
Each mean value calculated from at least (n=16) observations.

Table 3.8. Mean relative amino acid levels in the leaves of eight virginia-type peanut cultivars and breeding lines after 1, 2, 4, and 7 days of control (30/25°C) and high temperature (40/35°C) under controlled conditions.

Amino Acids	Control-1	Heat-1	Control-2	Heat-2	Control-4	Heat-4	Control-7	Heat-7
Alanine	2.67 cd[1]	5.62 ab	3.88 bcd	4.59 bc	5.27 abc	7.32 a	1.81 d	4.72 bc
Serine	2.37 bc	4.88 a	1.96 bc	3.25 ab	1.21 bc	2.31 bc	1.03 bc	0.88 c
Threonine	0.69 bc	1.81 a	0.62 bc	1.31 ab	0.38 c	0.96 bc	0.26 c	0.38 c
Aspartate	34.63 ab	27.15 bc	37.20 a	26.27 bc	33.85 ab	21.60 c	20.75 c	9.89 d
Glutamine	59.05 cde	75.68 ab	67.80 bcd	87.14 a	59.43 cd	70.64 bc	41.75 e	58.04 d
Hydroxyproline	3.52 bc	4.96 a	4.57 ab	5.11 a	4.05 abc	3.47 c	1.57 d	1.93 d
Glutamate	73.31 ab	71.77 ab	68.28 bc	72.28 ab	72.53 ab	81.78 a	55.07 c	63.47 bc
Asparagine	63.21 bc	117.84 a	92.53 ab	115.32 a	88.20 ab	83.49 b	22.07 c	36.78 c

[1]Means followed by different letters within a row are significantly different within and between the days of treatment ($p < 0.05$, student t-test). Each mean value calculated from at least (n=30) observations.

Table 3.9. Mean relative amino acid levels in the leaves of eight virginia-type peanut cultivars and breeding lines under control (30/25°C) and high temperature (40/35°C) under controlled conditions. Data from days 1, 2, 4, and 7 were combined.

	Hydroxyproline		Glutamine	
	Control	**Heat**	**Control**	**Heat**
Bailey	3.54 b-f[1]	4.59 abc	52.5 de	90.0 a
CHAMPS	3.67 abc	4.75 abc	52.5 de	62.5 cde
N04074FCT	4.66 abc	5.33 a	60.0 b-e	93.7 a
N05006	3.13 c-f	2.90 def	56.2 de	58.2 de
N05008	2.13 f	2.10 f	72.3 a-d	65.8 b-e
N05024J	2.39 ef	4.06 a-e	53.6 de	79.8 abc
Phillips	4.61 a-d	5.06 ab	63.0 b-e	82.1 ab
SPT 06-07	2.32 f	2.06 f	45.9 e	50.3 de
Average	**3.43 A[2]**	**3.87 A**	**57.0 B**	**72.9 A**

[1] Means followed by different small letters across genotypes and temperature regime are significantly different ($P < 0.05$ student's t-test).
[2] Means followed by different capital letters within averages of days of treatment are significantly different ($P < 0.05$ student's t-test).
Each mean value calculated from at least (n=16) observations.

Table 3.10. Fatty acid levels (µg mg^{-1} leaf dry weight) in the leaves of eight virginia-type peanut cultivars and breeding lines after 1, 2, 4, and 7 days of control (30/25°C) and high temperature (40/35°C) under controlled conditions.

Fatty acid	Control-1	Heat-1	Control-2	Heat-2	Control-4	Heat-4	Control-7	Heat-7
Saturated	1.41 b[1]	1.27 b	1.03 c	1.29 b	1.35 b	1.31 b	1.90 a	1.29 b
Unsaturated	0.30 c	0.32 bc	0.23 d	0.35 ab	0.24 d	0.36 a	0.32 abc	0.34 abc

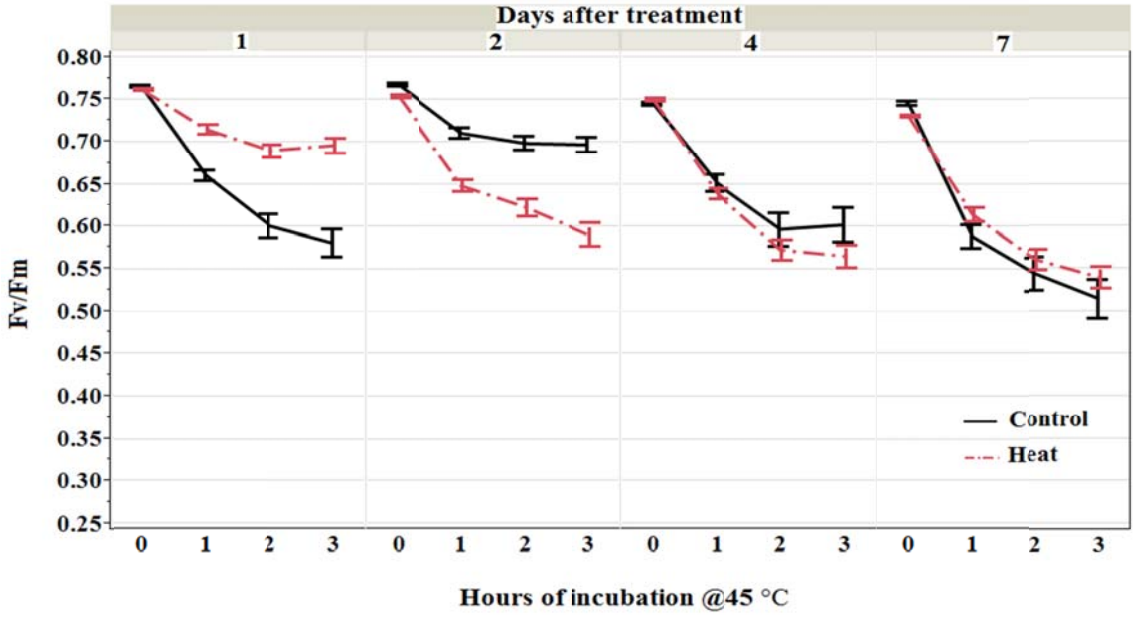
[1]Means followed by different letters within a row are significantly different within and between the days of treatment ($p < 0.05$, student t-test). Each mean value calculated from at least (n=30) observations.

Table 3.11. Fatty acid levels (μg mg^{-1} leaf dry weight) in the leaves of eight virginia-type peanut cultivars and breeding lines under control (30/25°C) and high temperature (40/35°C) under controlled conditions. Data from days 1, 2, 4, and 7 were combined.

	Saturated fatty acid		**Unsaturated fatty acid**	
	Control	**Heat**	**Control**	**Heat**
Bailey	1.60 a[1]	1.25 cde	0.28 c-f	0.32 a-e
CHAMPS	1.40 a-e	1.39 a-d	0.26 def	0.36 ab
N04074FCT	1.12 de	1.15 e	0.26 ef	0.33 a-e
N05006	1.53 abc	1.31 b-e	0.30 b-f	0.35 abc
N05008	1.38 a-e	1.24 cde	0.29 c-f	0.35 abc
N05024J	1.24 b-e	1.25 cde	0.23 f	0.33 a-d
Phillips	1.54 ab	1.20 de	0.29 c-f	0.32 a-e
SPT 06-07	1.56 ab	1.52 ab	0.25 f	0.37 a
Average	**1.42 A[2]**	**1.29 B**	**0.27 B**	**0.34 A**

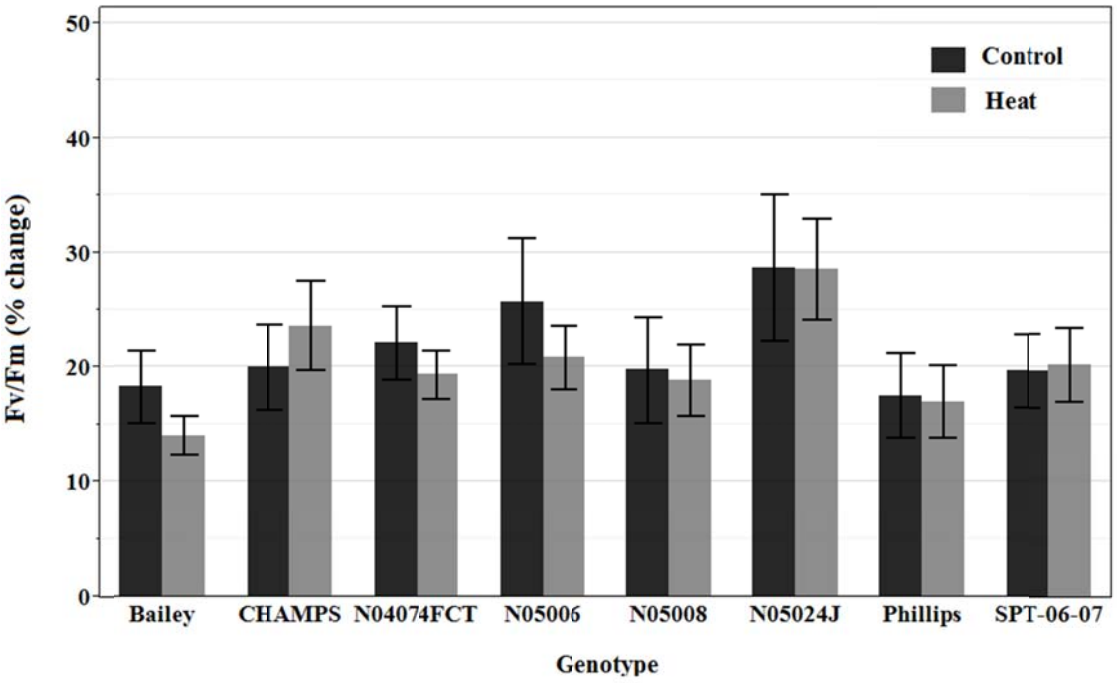
[1] Means followed by different small letters across genotypes and temperature regime are significantly different ($P < 0.05$ student's t-test).
[2] Means followed by different capital letters within averages of days of treatment are significantly different ($P < 0.05$ student's t-test).
Each mean value calculated from at least (n=16) observations.

Figure 3.1. F_v/F_m ratio of eight virginia-type peanut cultivars and breeding lines grown under two temperature treatments (heat, 40/35°C; control, 30/25°C), at four time-points after the temperature treatment initiation (day1, 2, 4, and 7), and after 3 hours of dark incubation at 45˚C.



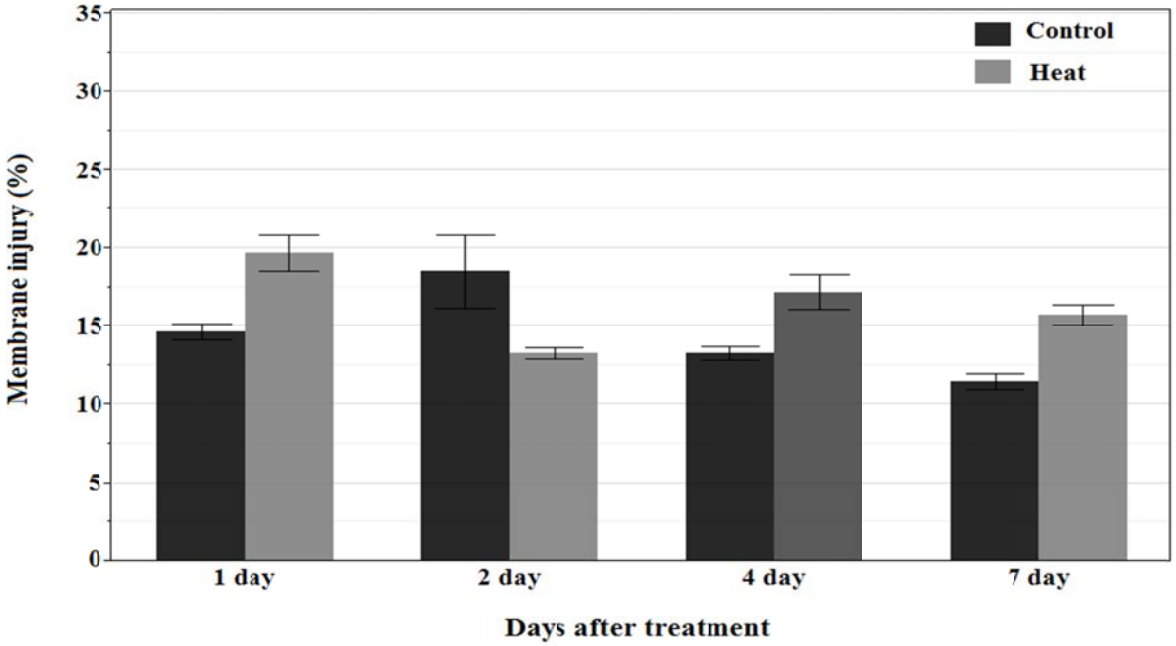
*Each error bar represent the standard error from mean (n=32)

Figure 3.2. Percentage change in F_v/F_m ratio of eight virginia-type peanut cultivars and breeding lines grown under two temperature regimes (heat, 40/35°C; control, 30/25°C) averaged at four time-points after the temperature treatment initiation (day1, 2, 4, and 7).



*Each error bar represent the standard error from mean (n=32)

Figure 3.3. Membrane injury (%) of eight virginia-type peanut cultivars and breeding lines grown under two temperature regimes (heat, 40/35°C; control, 30/25°C) measured at four time-points after the temperature treatment initiation (day1, 2, 4, and 7).



*Each error bar represent the standard error from mean (n=32)

Figure 3.4 . Principal component analysis of eight virginia-type peanut cultivars and breeding lines based on physiological characteristics and metabolite levels measured at day 1, 2, 4, and 7 of heat stress (40/35°C) under controlled conditions.

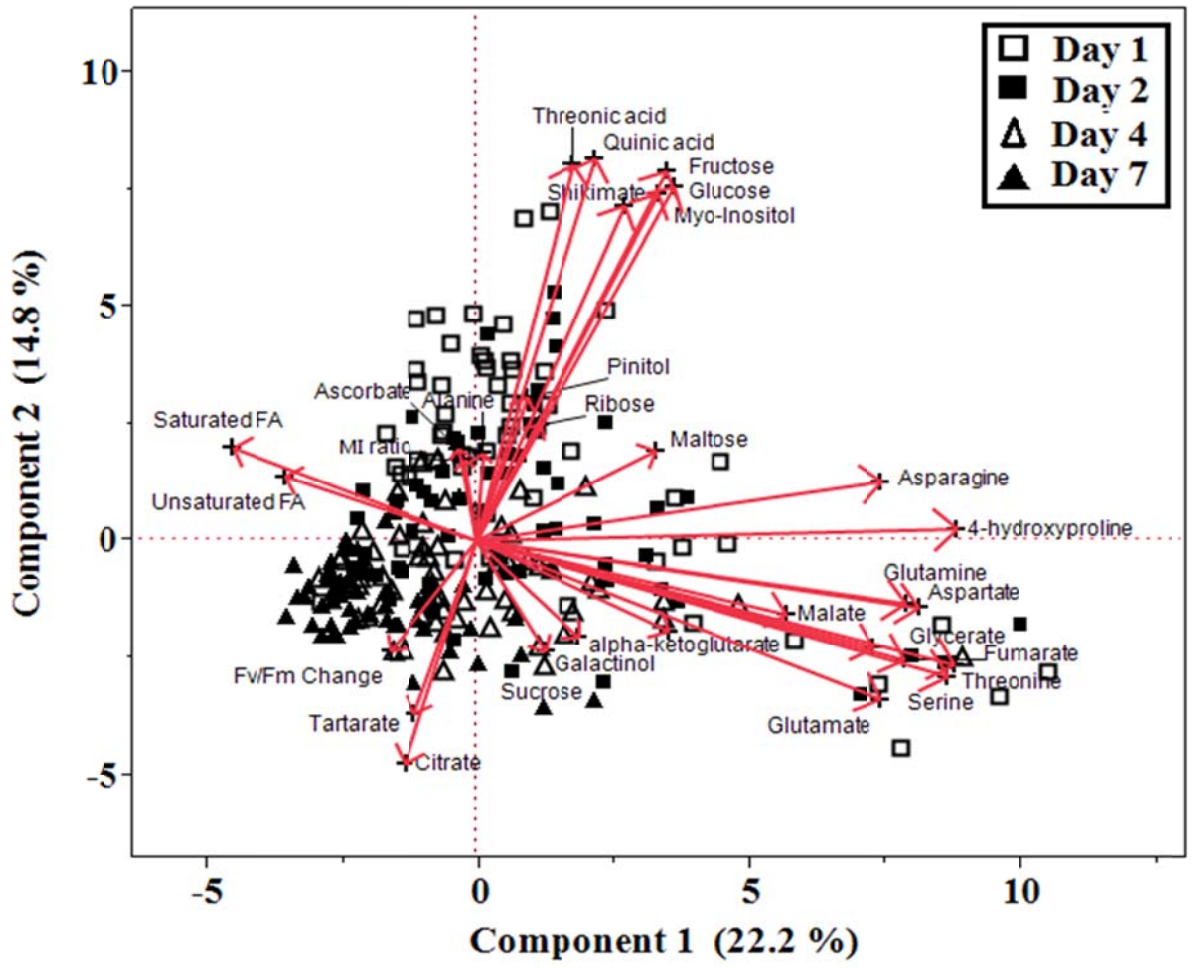
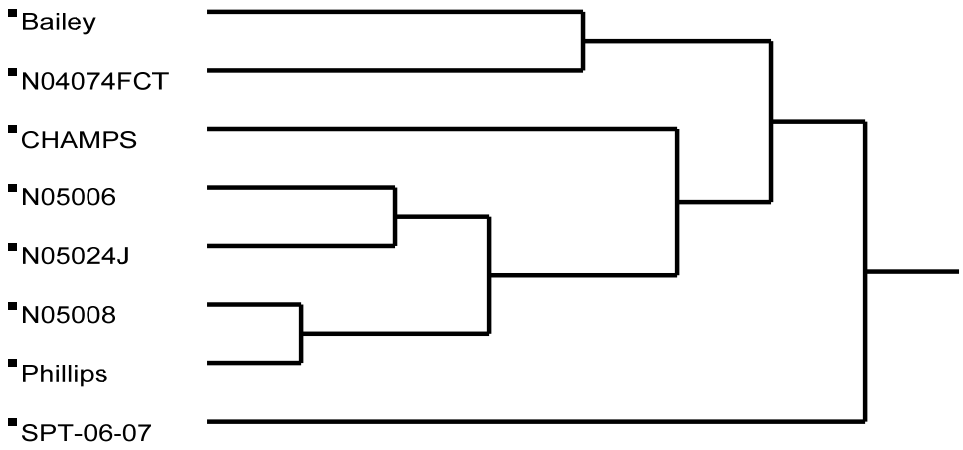


Figure 3.5. Dendrogram of the relationship among eight virginia-type peanut cultivars and breeding lines based on the selected physiological and metabolic variables from stepwise variable selection.



Appendix: Supplemental Tables

Supplemental Table 2.1. Analysis of variance for pod yield and grading factors (ELK, extra-large kernels and SMK, sound-mature kernels) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) in two years (2011 and 2012).

Source of Variation		F-ratio					
		Pod yield		ELK		SMK	
	df†	2011§	2012	2011	2012	2011	2012
Water regime (W)	1	5.24*	4.46*	26.21***	3.82	35.35***	3.83
Genotype (G)	7	5.44***	13.14***	35.47***	25.50***	9.61***	22.37***
G × W	7	0.23	2.36*	0.40	1.15	2.03	1.70

***, **, * Significant at the 0.001, 0.01 and 0.05 probability levels, respectively
† Degrees of freedom

Supplemental Table 2.2. Univariate repeated measures analysis of membrane injury (MI), canopy temperature depression (CTD), specific leaf area (SLA), SPAD chlorophyll reading, and of eight peanut genotypes under two water regimes in 2011 and 2012.

Source of Variation	2011			2012		
	df†	(SS_s/SS_e)‡	F-ratio§	df†	(SS_s/SS_e)‡	F-ratio§
Membrane Injury						
Genotype (G)	7	0.36	2.45*	7	0.24	1.66
Water regime (W)	1	0.00	0.13	1	0.01	0.46
G × W	7	0.12	0.79	7	0.02	0.11
Growth stage (GS)	1	4.78	229.38***	2	2.13	102.12***
GS × G	7	0.16	1.06	14	0.56	2.25*
GS × W	1	0.36	17.37***	2	0.06	2.80
GS × G × W	7	0.06	0.38	14	0.17	0.69
CTD						
Genotype (G)	7	0.18	1.20	7	0.25	1.69
Water regime (W)	1	2.37	113.93***	1	3.09	148.20***
G × W	7	0.16	1.10	7	0.07	0.46
Growth stage (GS)	1	0.48	23.03***	1	0.00	0.16
GS × G	7	0.08	0.55	7	0.07	0.51
GS × W	1	1.60	76.79***	1	0.19	9.16**
GS × G × W	7	0.10	0.68	7	0.07	0.47
SLA						
Genotype (G)	7	0.46	3.17**	7	0.52	3.54**
Water regime (W)	1	0.15	6.99*	1	0.58	27.67***
G × W	7	0.05	0.32	7	0.14	0.96
Growth stage (GS)	2	2.22	106.56***	2	1.91	91.47***
GS × G	14	0.43	1.71	14	0.65	2.61**
GS × W	2	0.08	3.64*	2	0.20	9.39***
GS × G × W	14	0.18	0.73	14	0.32	1.29
SPAD Chlorophyll reading						
Genotype (G)	7	5.80	39.76***	7	1.66	11.35***
Water regime (W)	1	1.40	67.22***	1	0.24	11.52**
G × W	7	0.16	1.13	7	0.31	2.15
Growth stage (GS)	2	1.21	57.98***	2	10.31	494.99***
GS × G	14	1.12	4.46***	14	0.39	1.54
GS × W	2	1.06	51.08***	2	0.09	4.36*
GS × G × W	14	0.28	1.01	14	0.23	0.92
F_v/F_m						
Genotype (G)	7	0.04	1.68	7	0.06	2.09*
Water regime (W)	1	0.06	18.01***	1	0.03	7.10**
G × W	7	0.05	1.94	7	0.07	2.69*
Growth stage (GS)	2	0.32	43.63***	2	0.16	28.50***
GS × G	14	0.94	1.71	14	0.90	1.12
GS × W	2	0.17	24.99***	2	0.15	27.15***
GS × G × W	14	0.94	1.39	14	0.90	1.32

***, **, * Significant at the 0.001, 0.01 and 0.05 probability levels, respectively

† Degrees of freedom

‡ Source sum of squares/error sum of squares
§ The adjusted univariate Greenhouse-Geisser (G-G) and Huynh-Feldt (H-F) F-ratio.

Supplemental Table 2.3. Univariate repeated measures analysis of organic acid levels in leaves of eight peanut genotypes under two water regimes (rainfed and irrigated) in 2011 and 2012.

Source of Variation	2011			2012		
	df[†]	(SS_s/SS_e)[‡]	F-ratio[§]	df[†]	(SS_s/SS_e)[‡]	F-ratio[§]
Total-Organic acid						
Genotype (G)	7	0.24	1.03	7	0.53	3.53**
Water regime (W)	1	0.19	5.63*	1	0.56	26.46***
G × W	7	0.16	0.69	7	0.21	1.44
Growth stage (GS)	1	0.00	0.05	2	5.42	254.89***
GS × G	7	0.25	1.05	14	0.55	3.70***
GS × W	1	0.01	2.0	2	0.16	7.64***
GS × G × W	7	0.18	0.76	14	0.15	1.03
Citrate						
Genotype (G)	7	0.72	3.07*	7	0.69	4.65***
Water regime (W)	1	0.60	18.00***	1	1.28	59.98***
G × W	7	0.09	0.40	7	0.16	1.09
Growth stage (GS)	1	0.74	22.13***	2	2.22	104.55***
GS × G	7	0.98	4.21**	14	0.47	3.14**
GS × W	1	0.10	2.93	2	0.24	11.49***
GS × G × W	7	0.07	0.32	14	0.18	1.24
Glycerate						
Genotype (G)	7	0.57	2.45*	7	0.20	1.31
Water regime (W)	1	0.15	4.40*	1	0.00	0.18
G × W	7	0.09	0.39	7	0.08	0.57
Growth stage (GS)	1	0.20	6.09*	2	2.45	115.12***
GS × G	7	0.16	0.68	14	0.14	0.91
GS × W	1	0.83	24.96****	2	0.03	1.55
GS × G × W	7	0.22	0.95	14	0.10	0.60
Malate						
Genotype (G)	7	0.87	3.75**	7	0.85	5.68
Water regime (W)	1	0.14	4.17*	1	0.29	13.50***
G × W	7	0.43	1.85	7	0.18	1.19
Growth stage (GS)	1	0.13	3.83	2	2.86	134.29***
GS × G	7	0.20	0.87	14	0.13	0.88
GS × W	1	0.08	2.47	2	0.29	13.43***
GS × G × W	7	0.14	0.61	14	0.09	0.61
Maleate						
Genotype (G)	7	1.04	4.45**	7	1.08	7.27***
Water regime (W)	1	0.00	0.00	1	0.18	8.39**
G × W	7	0.00	0.45	7	0.21	1.40
Growth stage (GS)	1	0.73	21.87***	2	9.42	442.56***
GS × G	7	0.10	0.43	14	1.40	3.07**
GS × W	1	0.04	1.31	2	0.03	1.36
GS × G × W	7	0.10	0.44	14	0.09	0.62
Quinate						
Genotype (G)	7	2.00	8.56***	7	0.67	4.53***
Water regime (W)	1	0.02	0.56	1	0.05	2.13
G × W	7	0.29	1.23	7	0.15	0.97

Growth stage (GS)	1	1.17	35.19***	2	0.09	4.36*
GS × G	7	0.11	0.48	14	0.35	2.38**
GS × W	1	0.05	1.65	2	0.03	1.43
GS × G × W	7	0.08	0.34	14	0.20	1.37
Shikimate						
Genotype (G)	7	0.59	2.53*	7	0.51	3.43**
Water regime (W)	1	0.67	20.00***	1	0.11	5.00*
G × W	7	0.05	0.20	7	0.11	0.73
Growth stage (GS)	1	0.25	7.53*	2	1.58	74.09***
GS × G	7	0.13	0.54	14	0.38	2.57**
GS × W	1	0.09	2.57	2	0.08	3.65*
GS × G × W	7	0.09	0.39	14	0.11	0.76
Succinate						
Genotype (G)	7	0.19	0.82	7	0.25	1.68
Water regime (W)	1	1.11	33.40***	1	0.06	3.21
G × W	7	0.10	0.41	7	0.17	1.13
Growth stage (GS)	1	2.57	77.12***	2	1.55	72.91***
GS × G	7	0.42	1.79	14	0.15	1.04
GS × W	1	0.01	0.16	2	0.07	3.15*
GS × G × W	7	0.18	0.77	14	0.03	0.21
Tartrate						
Genotype (G)	7	2.38	10.21***	7	2.87	19.24***
Water regime (W)	1	0.02	0.67	1	0.08	3.61
G × W	7	0.45	1.94	7	0.15	1.00
Growth stage (GS)	1	1.48	44.36***	2	1.97	92.63***
GS × G	7	0.41	1.75	14	0.41	2.78**
GS × W	1	0.01	0.36	2	0.17	7.86**
GS × G × W	7	0.18	0.76	14	0.20	1.34

***, **, * Significant at the 0.001, 0.01 and 0.05 probability levels, respectively

† Degrees of freedom

‡ Source sum of squares/ Error sum of squares

§ The adjusted univariate Greenhouse-Geisser (G-G) and Huynh-Feldt (H-F) F-ratio.

Supplemental Table 2.4. Relative total organic acid levels (mg^{-1} of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and at three growth stages (R1, beginning flower; R3, beginning pod, and R5, beginning seed) in 2012 under field conditions.

Genotype	2011				2012					
	R1		R3		R1		R3		R5	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
Bailey	159.2 a[1]	155.3 a	151.1 a	167.7 a	160.3 b	172.3 ab	102.5 ab	107.9 ab	137.0 a-d	152.3 abc
CHAMPS	142.7 a	150.5 a	136.9 a	157.3 a	150.3 b	159.4 b	111.4 ab	107.3 ab	136.8 a-d	150.7 a-d
N04074FCT	138.3 a	146.2 a	122.9 a	160.6 a	146.3 b	170.6 ab	93.2 b	96.5 b	118.6 cd	158.8 a
N05006	142.4 a	135.9 a	144.0 a	160.2 a	158.1 b	155.5 b	115.4 ab	121.7 ab	123.2 bcd	142.8 a-d
N05008	143.9 a	154.4 a	143.7 a	148.3 a	149.8 b	159.5 b	104.8 ab	109.7 ab	122.5 bcd	133.9 a-d
N05024J	137.6 a	149.4 a	177.5 a	151.6 a	158.2 b	168.1 ab	133.6 a	115.4 ab	148.8 a-d	152.0 abc
Phillips	148.6 a	154.2 a	135.8 a	171.6 a	165.4 ab	179.6 ab	108.9 ab	115.2 ab	130.7 a-d	156.5 ab
SPT 06-07	117.2 a	180.1 a	144.8 a	153.6 a	162.7 b	198.7 a	107.0 ab	104.1 ab	117.4 d	126.4 a-d
Average	**141.2 A[2]**	**153.3 A**	**144.6 B**	**158.9 A**	**156.4 B**	**170.5 A**	**109.6 A**	**109.7 A**	**129.4 B**	**146.7 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Supplemental Table 2.5. Relative citrate levels (mg^{-1} of leaf dry weight) of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and at three growth stages (R1, beginning flower; R3, beginning pod, and R5, beginning seed) in 2012 under field conditions.

Genotype	2011				2012					
	R1		R3		R1		R3		R5	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
Bailey	9.13 b[1]	18.17 b	3.29 a	12.78 a	32.12 b	45.78 ab	6.67 c	13.00 abc	20.35 ef	32.07 b-f
CHAMPS	6.62 b	17.20 b	4.08 a	10.81 a	20.75 b	39.06 b	9.83 bc	11.62 bc	20.26 ef	31.56 b-f
N04074FCT	5.32 b	16.40 b	4.23 a	7.51 a	23.53 b	42.54 ab	11.15 bc	11.05 bc	23.34 def	45.95 ab
N05006	6.28 b	13.59 b	3.72 a	9.92 a	36.69 b	44.70 ab	18.24 ab	22.58 a	29.02 c-f	39.22 abc
N05008	3.95 b	16.34 b	3.45 a	8.93 a	17.80 b	38.53 b	12.65 abc	13.11 abc	23.54 def	33.85 a-e
N05024J	7.76 b	12.32 b	8.14 a	8.59 a	34.10 b	44.03 ab	18.60 ab	19.22 ab	35.73 a-d	45.31 ab
Phillips	9.45 b	19.82 ab	3.50 a	12.53 a	36.62 b	53.90 ab	15.48 abc	17.55 ab	29.86 c-f	48.06 a
SPT06-07	25.01 ab	41.56 b	5.50 a	10.90 a	32.30 b	79.37 a	12.00 abc	18.25 ab	18.07 f	27.83 c-f
Average	**8.96 B[2]**	**19.51 A**	**4.84 B**	**10.29 A**	**29.23 B**	**48.49 A**	**13.08 B**	**15.80 A**	**25.02 B**	**37.98 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Supplemental Table 2.6. Univariate repeated measures analysis of cyclic polyol and sugar levels in leaves of eight peanut genotypes under two water regimes (rainfed and irrigated in 2011 and 2012.

Source of Variation	2011			2012		
	df[†]	(SS_s/SS_e)[‡]	F-ratio[§]	df[†]	(SS_s/SS_e)[‡]	F-ratio[§]
Total-Cyclic polyols						
Genotype (G)	7	3.26	13.97***	7	2.18	14.64***
Water regime (W)	1	9.52	285.49***	1	0.22	10.19**
G × W	7	0.91	3.89**	7	0.08	0.52
Growth stage (GS)	1	9.21	276.18***	2	0.97	45.42***
GS × G	7	0.12	0.52	14	0.14	0.97
GS × W	1	1.29	38.63***	2	0.02	0.92
GS × G × W	7	0.60	2.57*	14	0.08	0.53
Inositol						
Genotype (G)	7	0.93	3.96**	7	2.90	19.49***
Water regime (W)	1	0.95	28.49***	1	0.43	20.01***
G × W	7	0.22	0.96	7	0.11	0.72
Growth stage (GS)	1	2.49	74.92***	2	0.47	21.89***
GS × G	7	0.121	0.52	14	0.17	1.11
GS × W	1	0.67	20.01***	2	0.02	0.87
GS × G × W	7	0.15	0.64	14	0.14	0.91
Pinitol						
Genotype (G)	7	3.09	13.23***	7	3.56	23.91***
Water regime (W)	1	9.06	271.80***	1	0.03	1.27
G × W	7	1.11	4.76**	7	0.11	0.77
Growth stage (GS)	1	6.34	190.24***	2	2.48	116.51***
GS × G	7	0.19	0.81	14	0.41	2.78**
GS × W	1	0.65	19.54***	2	0.03	1.61
GS × G × W	7	0.64	2.73*	14	0.12	0.78
Total-Sugars						
Genotype (G)	7	0.43	1.84	7	0.70	4.69***
Water regime (W)	1	0.38	11.45**	1	0.03	1.33
G × W	7	0.21	0.91	7	0.06	0.39
Growth stage (GS)	1	1.29	38.57***	2	1.10	51.51***
GS × G	7	0.31	1.32	14	0.29	1.95*
GS × W	1	0.23	6.87*	2	0.40	18.73***
GS × G × W	7	0.22	0.94	14	0.37	2.47**
Fructose						
Genotype (G)	7	0.78	3.35**	7	0.51	3.44**
Water regime (W)	1	0.32	9.71**	1	0.027	1.27
G × W	7	0.38	1.63	7	0.06	0.43
Growth stage (GS)	1	0.00	0.00	2	1.64	76.95***
GS × G	7	0.32	1.37	14	0.31	2.08*
GS × W	1	0.05	1.39	2	0.03	1.51
GS × G × W	7	0.62	2.64	14	0.18	1.21
Glucose						
Genotype (G)	7	0.71	3.05*	7	0.45	2.99*
Water regime (W)	1	0.32	9.73**	1	0.02	0.88
G × W	7	0.39	1.68	7	0.06	0.41

Growth stage (GS)	1	0.05	1.50	2	1.88	88.51***
GS × G	7	0.31	1.34	14	0.22	1.47
GS × W	1	0.00	0.05	2	0.03	1.20
GS × G × W	7	0.65	2.79*	14	0.12	0.83
Sucrose						
Genotype (G)	7	0.34	1.46	7	0.28	1.90
Water regime (W)	1	0.11	3.21	1	0.01	0.32
G × W	7	0.21	0.88	7	0.04	0.26
Growth stage (GS)	1	2.02	60.60***	2	4.77	224.31***
GS × G	7	0.33	1.41	14	0.08	0.50
GS × W	1	0.26	7.71**	2	0.41	19.39***
GS × G × W	7	0.10	0.42	14	0.15	1.03

***, **, * Significant at the 0.001, 0.01 and 0.05 probability levels, respectively
† Degrees of freedom
‡ Source sum of squares/ Error sum of squares
§ The adjusted univariate Greenhouse-Geisser (G-G) and Huynh-Feldt (H-F) F-ratio.

Supplemental Table 2.7. Relative total cyclic polyol levels (mg^{-1} of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and at three growth stages (R1, beginning flower; R3, beginning pod, and R5, beginning seed) in 2012 under field conditions.

Genotype	2011				2012					
	R1		R3		R1		R3		R5	
	Rainfed	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**
Bailey	36.1 a-d[1]	28.6 bcd	61.7 ab	37.1 ef	25.4 abc	23.1 bc	17.8 a	18.7 a	22.9 cd	21.6 cd
CHAMPS	37.8 abc	29.5 bcd	58.1 abc	42.5 def	24.1 abc	20.4 c	18.9 a	19.5 a	23.5 cd	22.0 cd
N04074FCT	34.7 a-d	27.5 bcd	62.0 a-d	44.7 c-f	23.1 bc	21.3 c	20.7 a	19.0 a	22.6 cd	21.9 cd
N05006	39.8 ab	26.2 cd	64.0 abc	39.3 ef	24.1 abc	22.6 bc	19.3 a	18.6 a	23.8 bcd	23.9 bcd
N05008	45.5 a	30.7 bcd	70.2 a	48.4 b-f	29.5 a	27.1 ab	24.9 a	22.1 a	28.6 ab	29.2 a
N05024J	37.4 abc	28.9 bcd	70.2 a	35.1 f	25.0 abc	23.3 bc	24.9 a	20.1 a	25.9 abc	23.3 cd
Phillips	35.1 a-d	27.9 bcd	64.7 a	36.4 ef	22.2 bc	20.8 c	18.4 a	18.5 a	23.1 cd	23.7 cd
SPT 06-07	25.6 cd	24.2 d	48.3 b-e	35.9 ef	21.0 c	20.4 c	18.0 a	16.5 a	21.2 cd	20.2 d
Average	**36.5 A[2]**	**27.9 B**	**62.4 A**	**39.9 B**	**24.3 A**	**22.4 B**	**20.4 A**	**19.1 A**	**24.0 A**	**23.2 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Supplemental Table 2.8. Relative pinitol levels (mg^{-1} of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and at three growth stages (R1, beginning flower; R3, beginning pod, and R5, beginning seed) in 2012 under field conditions.

Genotype	2011				2012					
	R1		R3		R1		R3		R5	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
Bailey	25.0 ab[1]	16.3 bcd	42.5 ab	21.7 e	10.6 a	10.5 ab	9.5 a	8.4 a-e	11.3 ab	11.8 ab
CHAMPS	24.4 ab	16.3 bcd	37.0 a-d	27.2 de	9.8 abc	9.7 abc	8.8 abc	9.0 ab	11.1 ab	11.3 ab
N04074FCT	21.9 a-d	14.1 bcd	40.9 a-d	28.9 cde	7.8 a-d	8.2 a-d	7.9 a-e	8.7 a-d	9.9 b	9.8 b
N05006	24.7 ab	13.5 cd	41.1 a-d	22.8 e	8.8 a-d	7.8 a-d	5.7 b-e	5.8 b-e	10.5 b	11.9 ab
N05008	29.0 a	16.3 bcd	49.2 a	29.2 b-e	9.5 abc	9.7 abc	7.5 a-e	8.4 a-e	12.0 ab	13.9 a
N05024J	23.7 abc	15.1 bcd	46.7 a	18.9 e	6.4 d	7.1 cd	6.4 a-e	5.6 cde	10.2 b	9.0 b
Phillips	20.3 a-d	14.3 bcd	41.6 abc	21.1 e	7.0 cd	6.9 cd	5.9 b-e	5.4 de	8.7 b	10.0 b
SPT 06-07	13.0 cd	10.9 d	29.7 b-e	22.0 e	7.6 bcd	8.6 a-d	5.9 b-e	5.4 e	9.4 b	10.1 b
Average	**22.9 A[2]**	**14.6 B**	**39.9 A**	**23.8 B**	**8.5 A**	**8.5 A**	**7.2 A**	**7.1 A**	**10.4 A**	**11.0 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Supplemental Table 2.9. Relative total sugar levels (mg^{-1} of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and at three growth stages (R1, beginning flower; R3, beginning pod, and R5, beginning seed) in 2012 under field conditions.

Genotype	2011				2012					
	R1		R3		R1		R3		R5	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
Bailey	333 a[1]	252 a	177 a	132 a	192 abc	165 abc	192 ab	174 ab	206 a	213 a
CHAMPS	278 a	221 a	194 a	128 a	186 abc	150 bc	170 ab	187 ab	203 a	200 a
N04074FCT	274 a	161 a	143 a	194 a	242 a	153 bc	153 ab	217 a	203 a	199 a
N05006	224 a	167 a	159 a	131 a	190 abc	115 c	102 b	146 ab	190 a	183 a
N05008	235 a	205 a	159 a	194 a	198 ab	139 bc	115 b	185 ab	221 a	209 a
N05024J	248 a	221 a	149 a	122 a	159 bc	128 bc	128 ab	116 b	212 a	188 a
Phillips	229 a	191 a	134 a	155 a	165 abc	137 bc	105 b	136 ab	178 a	184 a
SPT 06-07	267 a	212 a	167 a	165 a	170 abc	202 ab	126 ab	133 ab	228 a	212 a
Average	**261 A[2]**	**204 B**	**160 A**	**153 A**	**188 A**	**149 B**	**136 B**	**162 A**	**205 A**	**199 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Supplemental Table 2.10. Relative sucrose levels (mg^{-1} of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and at three growth stages (R1, beginning flower; R3, beginning pod, and R5, beginning seed) in 2012 under field conditions.

Genotype	2011				2012					
	R1		R3		R1		R3		R5	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
Bailey	190 a[1]	128 a	9 a	37 a	103 a	77 a	63 a	75 a	160 a	175 a
CHAMPS	167 a	129 a	79 a	54 a	91 a	70 a	58 a	74 a	159 a	163 a
N04074FCT	126 a	63 a	39 a	33 a	140 a	60 a	48 a	87 a	153 a	158 a
N05006	129 a	83 a	33 a	42 a	104 a	42 a	27 a	66 a	141 a	143 a
N05008	90 a	104 a	9 a	79 a	95 a	49 a	31 a	71 a	157 a	159 a
N05024J	144 a	104 a	23 a	38 a	86 a	48 a	40 a	49 a	158 a	155 a
Phillips	133 a	104 a	23 a	39 a	90 a	54 a	29 a	47 a	132 a	146 a
SPT 06-07	187 a	118 a	38 a	39 a	67 a	79 a	30 a	48 a	164 a	161 a
Average	**146 A[2]**	**104 B**	**34 A**	**44 A**	**97 A**	**60 B**	**41 B**	**64 A**	**153 A**	**157 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Supplemental Table 2.11. Univariate repeated measures analysis of relative amino acid levels in leaves of eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) in 2011 and 2012.

Source of Variation	2011			2012		
	df†	(SS_s/SS_e)‡	F-ratio§	df†	(SS_s/SS_e)‡	F-ratio§
Total-Amino acids						
Genotype (G)	7	0.65	2.80*	7	0.08	0.55
Water regime (W)	1	0.14	4.15	1	0.05	2.19
G × W	7	0.21	0.90	7	0.08	0.52
Growth stage (GS)	1	0.00	0.11	2	1.69	79.48***
GS × G	7	0.30	1.30	14	0.22	1.43
GS × W	1	0.01	0.15	2	0.08	3.61*
GS × G × W	7	0.16	0.70	14	0.09	0.59
Alanine						
Genotype (G)	7	0.59	2.54*	7	0.12	0.80
Water regime (W)	1	2.01	60.26***	1	0.88	41.33***
G × W	7	0.24	1.03	7	0.09	0.57
Growth stage (GS)	1	0.54	16.10***	2	1.84	86.40***
GS × G	7	0.18	0.79	14	0.23	1.57
GS × W	1	0.02	0.49	2	0.52	24.65***
GS × G × W	7	0.12	0.50	14	0.09	0.63
Glycine						
Genotype (G)	7	0.34	1.45	7	0.08	0.51
Water regime (W)	1	1.52	45.58***	1	0.02	1.03
G × W	7	0.13	0.55	7	0.07	0.49
Growth stage (GS)	1	1.04	31.18***	2	0.71	33.49***
GS × G	7	0.46	1.97	14	0.20	1.31
GS × W	1	0.00	0.02	2	0.00	0.14
GS × G × W	7	0.33	1.43	14	0.11	0.74

***, **, * Significant at the 0.001, 0.01 and 0.05 probability levels, respectively

† Degrees of freedom

‡ Source sum of squares/ Error sum of squares

§ The adjusted univariate Greenhouse-Geisser (G-G) and Huynh-Feldt (H-F) F-ratio.

Supplemental Table 2.12. Relative total amino acid levels (mg^{-1} of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and at three growth stages (R1, beginning flower; R3, beginning pod, and R5, beginning seed) in 2012 under field conditions.

Genotype	2011				2012					
	R1		R3		R1		R3		R5	
	Rainfed	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**
Bailey	1.9 b[1]	1.8 b	2.9 a	2.3 a	1.2 a	1.1 a	1.2 a	0.9 a	0.5 a	0.73 a
CHAMPS	2.3 ab	2.5 ab	2.1 a	2.8 a	1.4 a	1.1 a	0.7 a	0.8 a	0.4 a	0.70 a
N04074FCT	2.8 ab	2.5 ab	2.1 a	2.7 a	1.3 a	1.1 a	0.6 a	0.8 a	0.6 a	0.52 a
N05006	2.4 ab	2.1 b	1.8 a	3.0 a	1.4 a	1.0 a	0.8 a	0.8 a	0.7 a	0.56 a
N05008	2.1 b	2.6 ab	2.5 a	2.9 a	1.1 a	0.9 a	0.7 a	0.7 a	0.6 a	0.61 a
N05024J	2.0 b	2.4 ab	2.2 a	2.1 a	1.1 a	0.9 a	1.1 a	0.8 a	0.7 a	0.55 a
Phillips	2.2 b	2.2 ab	2.4 a	2.6 a	1.1 a	1.0 a	0.7 a	0.8 a	0.7 a	0.64 a
SPT 06-07	2.9 ab	3.6 a	2.3 a	2.6 a	1.5 a	1.4 a	0.7 a	0.8 a	0.6 a	0.61 a
Average	**2.3 A[2]**	**2.5 A**	**2.3 A**	**2.6 A**	**1.3 A**	**1.1 B**	**0.8 A**	**0.8 A**	**0.6 A**	**0.59 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Supplemental Table 2.13. Univariate repeated measures analysis of fatty acid levels in leaves of eight peanut genotypes under two water regimes (rainfed and irrigated) in 2011 and 2012.

Source of Variation	2011			2012		
	df[†]	(SS_s/SS_e)[‡]	F-ratio[§]	df[†]	(SS_s/SS_e)[‡]	F-ratio[§]
Total- unsaturated fatty acids						
Genotype (G)	7	0.27	1.16	7	1.24	8.48***
Water regime (W)	1	0.06	1.92	1	0.27	13.09***
G × W	7	0.62	2.66*	7	0.19	1.28
Growth stage (GS)	1	16.39	491.80***	2	6.24	293.06***
GS × G	7	0.12	0.49	14	0.22	1.51
GS × W	1	0.37	11.08**	2	0.24	11.42***
GS × G × W	7	0.07	0.30	14	0.18	1.21
Total-saturated fatty acids						
Genotype (G)	7	0.46	1.96	7	1.96	13.43***
Water regime (W)	1	0.03	0.90	1	0.65	31.06***
G × W	7	0.23	0.99	7	1.52	1.04
Growth stage (GS)	1	0.26	7.81**	2	1.33	62.30***
GS × G	7	0.19	0.80	14	0.18	1.18
GS × W	1	0.82	24.72***	2	0.03	1.59
GS × G × W	7	0.22	0.93	14	0.16	1.06
Palmitic acid (16:0)						
Genotype (G)	7	0.50	2.13	7	1.89	12.95***
Water regime (W)	1	0.00	0.05	1	0.61	29.15***
G × W	7	0.23	0.99	7	0.13	0.92
Growth stage (GS)	1	0.03	0.82	2	1.36	63.93***
GS × G	7	0.14	0.58	14	0.19	1.26
GS × W	1	1.54	46.20***	2	0.04	0.31
GS × G × W	7	0.20	0.85	14	0.14	0.92
Palmitoleic acid (16:1)						
Genotype (G)	7	0.62	2.66*	7	1.32	9.04***
Water regime (W)	1	0.05	1.34	1	0.27	12.89***
G × W	7	0.30	1.28	7	0.11	0.74
Growth stage (GS)	1	0.40	11.96**	2	1.10	51.84***
GS × G	7	0.21	0.89	14	0.08	0.56
GS × W	1	1.17	35.10***	2	0.12	5.45
GS × G × W	7	0.13	0.54	14	0.06	0.43
Stearic acid (18:0)						
Genotype (G)	7	0.33	1.40	7	0.66	4.54
Water regime (W)	1	0.27	8.15**	1	0.26	12.50***
G × W	7	0.14	0.59	7	0.11	0.76
Growth stage (GS)	1	1.58	47.24***	2	0.54	25.37***
GS × G	7	0.40	1.73	14	0.08	0.53
GS × W	1	0.00	0.12	2	0.18	8.36***
GS × G × W	7	0.30	1.31	14	0.19	1.26
Oleic acid (18:1)						

Genotype (G)	7	0.09	0.41	7	0.32	2.22*
Water regime (W)	1	0.20	5.96*	1	0.03	1.46
G × W	7	0.38	1.61	7	0.04	0.28
Growth stage (GS)	1	2.85	85.63***	2	0.15	4.20*
GS × G	7	0.22	0.94	14	0.10	0.67
GS × W	1	0.62	18.46***	2	0.13	5.96**
GS × G × W	7	0.04	0.16	14	0.05	0.31
Linoleic acid (18:2)						
Genotype (G)	7	0.13	0.56	7	1.19	8.18***
Water regime (W)	1	0.02	0.47	1	0.33	15.62***
G × W	7	0.35	1.49	7	0.15	1.04
Growth stage (GS)	1	5.62	168.61***	2	0.04	1.63
GS × G	7	0.25	1.06	14	0.20	1.34
GS × W	1	0.35	10.61**	2	0.04	1.86
GS × G × W	7	0.02	0.10	14	0.15	1.01
Linolenic acid (18:3)						
Genotype (G)	7	0.40	1.71	7	1.11	7.58***
Water regime (W)	1	0.06	1.89	1	0.22	10.66**
G × W	7	0.59	2.55	7	0.17	1.14
Growth stage (GS)	1	22.33	670.04***	2	6.89	323.63***
GS × G	7	0.07	0.32	14	0.22	1.48
GS × W	1	0.34	10.16	2	0.31	14.60***
GS × G × W	7	0.13	0.54	14	0.19	1.26
Arachidic acid (20:0)						
Genotype (G)	7	0.24	1.05	7	0.82	5.12***
Water regime (W)	1	0.01	0.20	1	0.04	1.58
G × W	7	0.20	0.85	7	0.08	0.47
Growth stage (GS)	1	1.02	30.50***	2	0.58	27.16***
GS × G	7	0.21	0.91	14	0.20	1.34
GS × W	1	0.04	1.12	2	0.18	8.50***
GS × G × W	7	0.24	1.01	14	0.12	0.79

***, **, * Significant at the 0.001, 0.01 and 0.05 probability levels, respectively

† Degrees of freedom

‡ Source sum of squares/ Error sum of squares

§ The adjusted univariate Greenhouse-Geisser (G-G) and Huynh-Feldt (H-F) F-ratio.

Supplemental Table 2.14. The unsaturated fatty acid levels (μg mg^{-1} of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and at three growth stages (R1, beginning flower; R3, beginning pod, and R5, beginning seed) in 2012 under field conditions.

Genotype	2011				2012					
	R1		R3		R1		R3		R5	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
Bailey	9.2 a[1]	5.8 a	17.2 a	20.8 a	12.5 b	13.9 b	13.6 a	14.9 a	17.4 de	19.6 b-e
CHAMPS	6.7 a	6.0 a	17.2 a	21.3 a	12.7 b	13.3 b	14.5 a	15.1 a	16.6 e	18.0 cde
N04074FCT	7.3 a	5.8 a	19.4 a	20.1 a	13.2 b	13.7 b	16.4 a	14.7 a	18.8 b-e	21.1 abc
N05006	9.3 a	4.5 a	17.5 a	20.3 a	14.2 ab	14.7 ab	18.5 a	17.6 a	20.3 bcd	19.8 b-e
N05008	9.0 a	6.3 a	12.4 a	19.8 a	13.9 b	15.2 ab	17.9 a	15.8 a	18.6 cde	21.3 abc
N05024J	8.6 a	3.7 a	20.2 a	19.1 a	14.3 ab	14.9 ab	18.1 a	17.5 a	18.6 cde	22.1 ab
Phillips	8.4 a	7.3 a	19.8 a	19.4 a	13.6 b	15.8 ab	16.8 a	16.9 a	19.2 b-e	20.3 a-d
SPT 06-07	8.9 a	6.2 a	20.2 a	20.1 a	12.9 b	17.4 a	17.9 a	18.8 a	20.7 a-d	23.8 a
Average	**8.4 A[2]**	**5.7 B**	**18.0 A**	**20.1 A**	**13.4 B**	**14.9 A**	**16.7 A**	**16.4 A**	**18.8 B**	**20.8 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Supplemental Table 2.15. The saturated fatty acid levels (μg mg^{-1} of leaf dry weight) in eight virginia-type peanut genotypes under two water regimes (rainfed and irrigated) at two growth stages (R1, beginning flower; and R3, beginning pod) in 2011 and at three growth stages (R1, beginning flower; R3, beginning pod, and R5, beginning seed) in 2012 under field conditions.

Genotype	2011				2012					
	R1		R3		R1		R3		R5	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
Bailey	1.6 a[1]	2.1 a	2.3 a	2.1 a	2.3 cd	2.40 bcd	2.53 c	2.78 abc	2.38 d	2.70 bcd
CHAMPS	1.9 a	2.0 a	2.3 a	2.2 a	2.2 d	2.32 bcd	2.55 bc	2.81 abc	2.40 d	2.44 d
N04074FCT	1.9 a	2.6 a	2.8 a	2.2 a	2.6 a-d	2.85 ab	3.13 abc	3.05 abc	2.93 a-d	3.17 abc
N05006	2.0 a	1.9 a	2.4 a	2.0 a	2.7 a-d	2.79 ab	3.11 abc	3.34 abc	2.80 bcd	2.77 bcd
N05008	2.1 a	2.2 a	1.7 a	2.0 a	2.5 a-d	2.73 abc	3.16 abc	3.01 abc	2.65 cd	3.17 abc
N05024J	2.0 a	2.2 a	2.8 a	2.0 a	2.5 a-d	2.72 abc	3.10 abc	3.38 ab	2.70 bcd	3.31 ab
Phillips	2.0 a	2.5 a	2.6 a	1.9 a	2.5 a-d	2.77 abc	2.95 abc	3.16 abc	2.79 bcd	3.08 abc
SPT 06-07	1.9 a	2.2 a	2.4 a	2.0 a	2.3 bcd	2.99 a	3.20 abc	3.50 a	2.99 a-d	3.51 a
Average	**1.9 B[2]**	**2.2 A**	**2.4 A**	**2.1 B**	**2.4 B**	**2.70 A**	**2.97 A**	**3.13 A**	**2.71 B**	**3.02 A**

[1] Means followed by different small letters between water regimes and within a growth stage are significantly different ($P < 0.05$ Tukey-HSD)
[2] Means followed by different capital letters between water regimes and within a growth stage are significantly different ($P < 0.05$ student's t-test).

Supplemental Table 3.1. The correlation coefficients of selected physiological, metabolic, and agronomic traits of eight virginia-type peanut genotypes at a) R1, beginning flower, b) R3, beginning pod, and c) R5, beginning seed growth stage during field conditions in 2011. Here $r \geq 0.25$, 0.32 is significant at $p \leq 0.05$ and 0.01 respectively.

a) R1-2011

	Pod Yield (kg/ha)	ELK	SMK	SLA	SPAD	MI, %	Fv/Fm % change	L-alanine	Total- Amino Acid	Citrate	Total-Org acid	Pinitol	Total- Sugar Alcohols	Sucrose	Total- Sugars	Unsaturated FA	Saturated FA	CTD (-1)
Pod Yield (kg/ha)	1.00	0.54	0.62	0.08	-0.28	-0.11	-0.00	-0.09	-0.35	-0.08	0.09	-0.08	-0.13	-0.00	-0.03	-0.12	-0.20	0.00
ELK	0.54	1.00	0.53	0.26	-0.58	-0.07	-0.04	-0.30	-0.46	-0.41	0.01	0.10	0.06	0.02	0.04	-0.18	-0.01	0.00
SMK	0.62	0.53	1.00	0.05	-0.27	-0.26	-0.04	0.04	-0.15	-0.04	0.14	-0.15	-0.18	0.10	0.04	-0.18	0.02	0.00
SLA	0.08	0.26	0.05	1.00	-0.20	-0.00	-0.28	0.00	0.07	-0.10	-0.01	-0.03	-0.00	0.02	0.05	-0.11	0.02	0.00
SPAD	-0.28	-0.58	-0.27	-0.20	1.00	0.13	0.12	0.37	0.38	0.40	0.00	-0.19	-0.18	0.22	0.09	0.15	-0.01	0.00
MI, %	-0.11	-0.07	-0.26	-0.00	0.13	1.00	0.07	-0.06	0.02	-0.25	-0.34	0.27	0.27	0.15	0.10	-0.04	-0.14	0.00
Fv/Fm % change	-0.00	-0.04	-0.04	-0.28	0.12	0.07	1.00	0.10	0.01	-0.10	0.04	0.10	0.05	0.03	-0.01	0.07	-0.04	0.00
L-alanine	-0.09	-0.30	0.04	0.00	0.37	-0.06	0.10	1.00	0.82	0.63	0.53	-0.46	-0.31	-0.17	-0.23	-0.16	0.43	0.00
Total- Amino Acid	-0.35	-0.46	-0.15	0.07	0.38	0.02	0.01	0.82	1.00	0.49	0.54	-0.26	-0.10	0.03	-0.00	0.04	0.36	0.00
Citrate	-0.08	-0.41	-0.04	-0.10	0.40	-0.25	-0.10	0.63	0.49	1.00	0.72	-0.60	-0.48	-0.17	-0.27	-0.04	0.34	0.00
Total-Org acid	0.09	0.01	0.14	-0.01	0.00	-0.34	0.04	0.53	0.54	0.72	1.00	-0.14	0.03	-0.04	0.05	0.18	0.40	0.00
Pinitol	-0.08	0.10	-0.15	-0.03	-0.19	0.27	0.10	-0.46	-0.26	-0.60	-0.14	1.00	0.91	0.19	0.42	0.25	-0.18	0.00
Total- Sugar Alcohols	-0.13	0.06	-0.18	-0.00	-0.18	0.27	0.05	-0.31	-0.10	-0.48	0.03	0.91	1.00	0.03	0.22	0.37	0.08	0.00
Sucrose	-0.00	0.02	0.10	0.02	0.22	0.15	0.03	-0.17	0.03	-0.17	-0.04	0.19	0.03	1.00	0.86	0.07	-0.41	0.00
Total- Sugars	-0.03	0.04	0.04	0.05	0.09	0.10	-0.01	-0.23	-0.00	-0.27	0.05	0.42	0.22	0.86	1.00	0.08	-0.39	0.00
Unsaturated FA	-0.12	-0.18	-0.18	-0.11	0.15	-0.04	0.07	-0.16	0.04	-0.04	0.18	0.25	0.37	0.07	0.08	1.00	0.30	0.00
Saturated FA	-0.20	-0.01	0.02	0.02	-0.01	-0.14	-0.04	0.43	0.36	0.34	0.40	-0.18	0.08	-0.41	-0.39	0.30	1.00	0.00
CTD (-1)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

b) R3-2011

	Pod Yield (kg/ha)	ELK	SMK	SLA	SPAD	MI, %	Fv/Fm % change	L-alanine	Total- Amino Acid	Citrate	Total-Org acid	Pinitol	Total- Sugar Alcohols	Sucrose	Total- Sugars	Unsaturated FA	Saturated FA	CTD (-1)
Pod Yield (kg/ha)	1.00	0.54	0.62	-0.10	-0.35	0.00	-0.15	0.41	0.38	0.50	0.45	-0.08	0.02	-0.07	-0.20	0.03	-0.23	-0.22
ELK	0.54	1.00	0.53	0.02	-0.51	0.00	-0.09	0.13	0.15	0.15	0.20	-0.03	0.08	-0.01	-0.27	0.09	0.00	-0.11
SMK	0.62	0.53	1.00	-0.28	-0.45	0.00	-0.18	0.51	0.44	0.21	0.08	-0.15	-0.06	0.13	-0.05	0.22	-0.11	-0.13
SLA	-0.10	0.02	-0.28	1.00	0.12	0.00	-0.10	-0.03	-0.10	-0.26	-0.06	0.35	0.13	-0.34	-0.14	0.01	0.19	-0.08
SPAD	-0.35	-0.51	-0.45	0.12	1.00	0.00	-0.20	-0.37	-0.05	-0.42	-0.33	0.56	0.50	-0.01	0.21	-0.21	0.13	0.03
MI, %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fv/Fm % change	-0.15	-0.09	-0.18	-0.10	-0.20	0.00	1.00	-0.39	-0.49	-0.07	-0.09	-0.08	-0.12	-0.03	0.03	-0.11	0.10	0.05
L-alanine	0.41	0.13	0.51	-0.03	-0.37	0.00	-0.39	1.00	0.72	0.42	0.27	-0.45	-0.49	0.15	-0.01	0.52	-0.41	-0.10
Total- Amino Acid	0.38	0.15	0.44	-0.10	-0.05	0.00	-0.49	0.72	1.00	0.12	0.32	0.03	0.24	-0.06	0.06	0.42	-0.10	-0.06
Citrate	0.50	0.15	0.21	-0.26	-0.42	0.00	-0.07	0.42	0.12	1.00	0.55	-0.47	-0.45	0.19	-0.07	0.23	-0.35	0.04
Total-Org acid	0.45	0.20	0.08	-0.06	-0.33	0.00	-0.09	0.27	0.32	0.55	1.00	-0.15	-0.13	-0.18	-0.07	0.28	-0.03	-0.04
Pinitol	-0.08	-0.03	-0.15	0.35	0.56	0.00	-0.08	-0.45	0.03	-0.47	-0.15	1.00	0.98	-0.12	0.24	-0.16	0.67	-0.05
Total- Sugar Alcohols	0.02	0.08	-0.06	0.13	0.50	0.00	-0.12	-0.49	0.24	-0.45	-0.13	0.98	1.00	-0.12	0.20	-0.13	0.70	0.02
Sucrose	-0.07	-0.01	0.13	-0.34	-0.01	0.00	-0.03	0.15	-0.06	0.19	-0.18	-0.12	-0.12	1.00	0.62	-0.21	-0.40	-0.14
Total- Sugars	-0.20	-0.27	-0.05	-0.14	0.21	0.00	0.03	-0.01	0.06	-0.07	-0.07	0.24	0.20	0.62	1.00	-0.38	-0.17	0.11
Unsaturated FA	0.03	0.09	0.22	0.01	-0.21	0.00	-0.11	0.52	0.42	0.23	0.28	-0.16	-0.13	-0.21	-0.38	1.00	0.71	-0.04
Saturated FA	-0.23	0.00	-0.11	0.19	0.13	0.00	0.10	-0.41	-0.10	-0.35	-0.03	0.67	0.70	-0.40	-0.17	0.71	1.00	0.03
CTD (-1)	-0.22	-0.11	-0.13	-0.08	0.03	0.00	0.05	-0.10	-0.06	0.04	-0.04	-0.05	0.02	-0.14	0.11	-0.04	0.03	1.00

c) R5-2011

	Pod Yield (kg/ha)	ELK	SMK	SLA	SPAD	MI, %	Fv/Fm % change	L-alanine	Total- Amino Acid	Citrate	Total-Org acid	Pinitol	Total- Sugar Alcohols	Sucrose	Total- Sugars	Unsaturated FA	Saturated FA	CTD (-1)
Pod Yield (kg/ha)	1.00	0.54	0.62	-0.27	0.00	0.15	-0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33
ELK	0.54	1.00	0.53	-0.26	-0.50	-0.06	-0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23
SMK	0.62	0.53	1.00	-0.30	0.05	0.22	-0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.41
SLA	-0.27	-0.26	-0.30	1.00	0.18	-0.01	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.44
SPAD	0.00	-0.50	0.05	0.18	1.00	0.40	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.09
MI, %	0.15	-0.06	0.22	-0.01	0.40	1.00	-0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25
Fv/Fm % change	-0.26	-0.09	-0.33	0.34	0.05	-0.07	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.26
L-alanine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total- Amino Acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Citrate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total-Org acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pinitol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total- Sugar Alcohols	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sucrose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total- Sugars	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Unsaturated FA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Saturated FA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CTD (-1)	0.33	0.23	0.41	-0.44	-0.09	0.25	-0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Supplemental Table 3.2. The correlation coefficients of selected physiological, metabolic, and agronomic traits of eight virginia-type peanut genotypes at a) R1, beginning flower, b) R3, beginning pod, and c) R5, beginning seed growth stage during field conditions in 2012. Here $r \geq 0.25$, 0.32 is significant at $p \leq 0.05$ and 0.01, respectively.

a) R1-2012

	Pod Yield (kg/ha)	ELK	SMK	SLA	SPAD	MI, %	Fv/Fm % change	L-alanine	Total- Amino Acid	Citrate	Total-Org acid	Pinitol	Total- Sugar Alcohols	Sucrose	Total- Sugars	Unsaturated FA	Saturated FA	CTD (-1)
Pod Yield (kg/ha)	1.00	0.16	-0.25	-0.24	0.32	-0.00	0.07	0.16	-0.06	0.12	0.11	-0.13	0.20	-0.11	-0.21	0.27	0.07	0.12
ELK	0.16	1.00	-0.16	-0.19	-0.28	-0.22	0.06	0.04	-0.23	-0.13	-0.05	-0.21	0.15	0.09	-0.09	-0.03	-0.01	0.23
SMK	-0.25	-0.16	1.00	0.06	0.03	-0.04	0.12	0.21	0.19	0.02	0.19	0.12	-0.11	0.18	0.38	-0.03	0.09	-0.14
SLA	-0.24	-0.19	0.06	1.00	-0.30	0.25	-0.24	-0.31	-0.04	0.36	0.34	-0.06	-0.22	-0.35	-0.22	0.23	0.22	-0.38
SPAD	0.32	-0.28	0.03	-0.30	1.00	0.23	0.26	0.12	0.35	0.59	0.39	0.08	-0.15	-0.16	-0.06	0.44	0.00	0.05
MI, %	-0.00	-0.22	-0.04	0.25	0.23	1.00	0.14	-0.03	0.13	0.20	0.13	0.37	-0.00	-0.08	-0.01	-0.03	-0.23	-0.21
Fv/Fm % change	0.07	0.06	0.12	-0.24	0.26	0.14	1.00	0.28	0.38	0.17	0.03	0.12	0.26	0.14	0.27	-0.05	-0.14	0.39
L-alanine	0.16	0.04	0.21	-0.31	0.12	-0.03	0.28	1.00	0.59	-0.07	-0.01	0.14	0.14	0.08	0.25	-0.37	-0.37	0.49
Total- Amino Acid	-0.06	-0.23	0.19	-0.04	0.35	0.13	0.38	0.59	1.00	0.22	0.23	0.26	0.16	-0.27	0.18	-0.21	-0.38	0.41
Citrate	0.12	-0.13	0.02	0.36	0.59	0.20	0.17	-0.07	0.22	1.00	0.76	-0.05	-0.27	-0.39	-0.27	0.72	0.28	-0.05
Total-Org acid	0.11	-0.05	0.19	0.34	0.39	0.13	0.03	-0.01	0.23	0.76	1.00	0.06	-0.21	-0.23	0.01	0.55	0.33	-0.22
Pinitol	-0.13	-0.21	0.12	-0.06	0.08	0.37	0.12	0.14	0.26	-0.05	0.06	1.00	0.41	0.13	0.30	-0.28	-0.37	-0.01
Total- Sugar Alcohols	0.20	0.15	-0.11	-0.22	-0.15	-0.00	0.26	0.14	0.16	-0.27	-0.21	0.41	1.00	-0.06	0.09	-0.13	-0.15	0.31
Sucrose	-0.11	0.09	0.18	-0.35	-0.16	-0.08	0.14	0.08	-0.27	-0.39	-0.23	0.13	-0.06	1.00	0.80	-0.20	-0.02	0.19
Total- Sugars	-0.21	-0.09	0.38	-0.22	-0.06	-0.01	0.27	0.25	0.18	-0.27	0.01	0.30	0.09	0.80	1.00	-0.22	-0.11	0.26
Unsaturated FA	0.27	-0.03	-0.03	0.23	0.44	-0.03	-0.05	-0.37	-0.21	0.72	0.55	-0.28	-0.13	-0.20	-0.22	1.00	0.67	-0.18
Saturated FA	0.07	-0.01	0.09	0.22	0.00	-0.23	-0.14	-0.37	-0.38	0.28	0.33	-0.37	-0.15	-0.02	-0.11	0.67	1.00	-0.41
CTD (-1)	0.12	0.23	-0.14	-0.38	0.05	-0.21	0.39	0.49	0.41	-0.05	-0.22	-0.01	0.31	0.19	0.26	-0.18	-0.41	1.00

b) R3-2012

	Pod Yield (kg/ha)	ELK	SMK	SLA	SPAD	MI, %	Fv/Fm % change	L-alanine	Total- Amino Acid	Citrate	Total-Org acid	Pinitol	Total- Sugar Alcohols	Sucrose	Total- Sugars	Unsaturated FA	Saturated FA	CTD (-1)
Pod Yield (kg/ha)	1.00	0.16	-0.25	0.24	0.42	0.16	-0.01	0.20	0.06	0.38	0.36	-0.40	0.05	-0.34	-0.44	0.25	0.09	0.00
ELK	0.16	1.00	-0.16	0.07	-0.32	-0.21	0.13	0.09	0.13	0.14	0.25	-0.10	0.17	-0.18	-0.19	-0.00	-0.03	0.00
SMK	-0.25	-0.16	1.00	0.02	0.10	-0.03	0.01	-0.10	-0.10	-0.24	-0.30	0.04	-0.19	-0.11	0.06	-0.03	0.06	0.00
SLA	0.24	0.07	0.02	1.00	0.43	-0.12	0.19	0.21	-0.02	0.53	0.16	-0.60	0.10	-0.28	-0.45	0.63	0.59	0.00
SPAD	0.42	-0.32	0.10	0.43	1.00	0.06	-0.01	0.11	0.05	0.36	0.11	-0.35	-0.09	-0.14	-0.22	0.47	0.33	0.00
MI, %	0.16	-0.21	-0.03	-0.12	0.06	1.00	-0.06	-0.03	-0.10	-0.15	-0.16	-0.04	-0.24	0.16	0.04	-0.16	-0.25	0.00
Fv/Fm % change	-0.01	0.13	0.01	0.19	-0.01	-0.06	1.00	0.40	0.26	0.03	0.13	-0.04	0.16	-0.11	-0.12	0.19	-0.08	0.00
L-alanine	0.20	0.09	-0.10	0.21	0.11	-0.03	0.40	1.00	0.43	0.04	0.28	0.00	0.49	-0.22	-0.18	0.26	0.01	0.00
Total- Amino Acid	0.06	0.13	-0.10	-0.02	0.05	-0.10	0.26	0.43	1.00	0.01	0.23	0.16	0.23	-0.03	0.04	-0.02	-0.11	0.00
Citrate	0.38	0.14	-0.24	0.53	0.36	-0.15	0.03	0.04	0.01	1.00	0.52	-0.61	0.06	-0.17	-0.43	0.69	0.61	0.00
Total-Org acid	0.36	0.25	-0.30	0.16	0.11	-0.16	0.13	0.28	0.23	0.52	1.00	0.06	0.47	-0.08	0.01	0.25	0.22	0.00
Pinitol	-0.40	-0.10	0.04	-0.60	-0.35	-0.04	-0.04	0.00	0.16	-0.61	0.06	1.00	0.35	0.40	0.68	-0.59	-0.46	0.00
Total- Sugar Alcohols	0.05	0.17	-0.19	0.10	-0.09	-0.24	0.16	0.49	0.23	0.06	0.47	0.35	1.00	-0.22	-0.10	0.24	0.30	0.00
Sucrose	-0.34	-0.18	-0.11	-0.28	-0.14	0.16	-0.11	-0.22	-0.03	-0.17	-0.08	0.40	-0.22	1.00	0.81	-0.37	-0.44	0.00
Total- Sugars	-0.44	-0.19	0.06	-0.45	-0.22	0.04	-0.12	-0.18	0.04	-0.43	0.01	0.68	-0.10	0.81	1.00	-0.58	-0.48	0.00
Unsaturated FA	0.25	-0.00	-0.03	0.63	0.47	-0.16	0.19	0.26	-0.02	0.69	0.25	-0.59	0.24	-0.37	-0.58	1.00	0.81	0.00
Saturated FA	0.09	-0.03	0.06	0.59	0.33	-0.25	-0.08	0.01	-0.11	0.61	0.22	-0.46	0.30	-0.44	-0.48	0.81	1.00	0.00
CTD (-1)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

c) R5-2012

	Pod Yield (kg/ha)	ELK	SMK	SLA	SPAD	MI, %	Fv/Fm % change	L-alanine	Total- Amino Acid	Citrate	Total-Org acid	Pinitol	Total- Sugar Acohols	Sucrose	Total- Sugars	Unsaturated FA	Saturated FA	CTD (-1)
Pod Yield (kg/ha)	1.00	0.16	-0.25	0.06	0.17	0.33	0.07	0.07	0.00	-0.07	-0.13	0.09	0.30	-0.10	0.04	0.04	0.02	0.05
ELK	0.16	1.00	-0.16	0.01	-0.22	0.09	0.11	0.24	0.11	0.32	0.27	-0.18	0.16	-0.26	-0.34	-0.18	-0.14	0.21
SMK	-0.25	-0.16	1.00	0.05	0.10	-0.19	0.05	-0.01	-0.17	-0.31	-0.32	-0.15	-0.28	-0.03	0.06	0.13	0.18	0.14
SLA	0.06	0.01	0.05	1.00	0.48	-0.01	0.27	-0.11	0.05	0.35	0.13	-0.19	-0.14	-0.13	-0.18	0.60	0.60	-0.34
SPAD	0.17	-0.22	0.10	0.48	1.00	-0.10	0.29	-0.13	0.31	0.25	0.10	0.12	-0.20	0.06	-0.02	0.51	0.27	-0.38
MI, %	0.33	0.09	-0.19	-0.01	-0.10	1.00	-0.03	-0.02	-0.21	0.02	-0.20	0.01	0.21	-0.27	-0.16	-0.03	0.04	0.03
Fv/Fm % change	0.07	0.11	0.05	0.27	0.29	-0.03	1.00	-0.16	-0.05	0.37	0.32	0.02	-0.05	0.05	0.02	0.27	0.30	-0.20
L-alanine	0.07	0.24	-0.01	-0.11	-0.13	-0.02	-0.16	1.00	0.36	-0.04	-0.15	-0.07	0.23	-0.18	-0.11	0.06	-0.09	0.33
Total- Amino Acid	0.00	0.11	-0.17	0.05	0.31	-0.21	-0.05	0.36	1.00	0.13	0.07	0.03	-0.00	-0.08	-0.15	0.12	-0.13	0.06
Citrate	-0.07	0.32	-0.31	0.35	0.25	0.02	0.37	-0.04	0.13	1.00	0.69	-0.14	0.00	-0.10	-0.30	0.36	0.33	-0.49
Total-Org acid	-0.13	0.27	-0.32	0.13	0.10	-0.20	0.32	-0.15	0.07	0.69	1.00	0.13	0.04	0.04	-0.17	0.00	-0.02	-0.33
Pinitol	0.09	-0.18	-0.15	-0.19	0.12	0.01	0.02	-0.07	0.03	-0.14	0.13	1.00	0.63	0.21	0.19	-0.20	-0.22	-0.24
Total- Sugar Alcohols	0.30	0.16	-0.28	-0.14	-0.20	0.21	-0.05	0.23	-0.00	0.00	0.04	0.63	1.00	-0.20	-0.08	-0.11	-0.03	-0.00
Sucrose	-0.10	-0.26	-0.03	-0.13	0.06	-0.27	0.05	-0.18	-0.08	-0.10	0.04	0.21	-0.20	1.00	0.90	-0.19	-0.14	-0.03
Total- Sugars	0.04	-0.34	0.06	-0.18	-0.02	-0.16	0.02	-0.11	-0.15	-0.30	-0.17	0.19	-0.08	0.90	1.00	-0.18	-0.07	0.14
Unsaturated FA	0.04	-0.18	0.13	0.60	0.51	-0.03	0.27	0.06	0.12	0.36	0.00	-0.20	-0.11	-0.19	-0.18	1.00	0.87	-0.39
Saturated FA	0.02	-0.14	0.18	0.60	0.27	0.04	0.30	-0.09	-0.13	0.33	-0.02	-0.22	-0.03	-0.14	-0.07	0.87	1.00	-0.37
CTD (-1)	0.05	0.21	0.14	-0.34	-0.38	0.03	-0.20	0.33	0.06	-0.49	-0.33	-0.24	-0.00	-0.03	0.14	-0.39	-0.37	1.00

Supplemental Table 3.3. Correlations among physiological characteristics and metabolite and fatty acid levels in eight virginia-type peanut cultivars and breeding lines after 7 days of heat stress.

	F_v/F_m Change	MI	Total-OA	Total-SA	Total-Sug	Total-AA	Unsat FA	Sat FA
Time-point (DAT)=1 day								
F_v/F_m change	1							
MI	-0.02	1						
Total- OA	**-0.24***	-0.05	1					
Total- SA	-0.08	0.20	0.05	1				
Total-Sug	-0.15	0.11	0.06	**0.47****	1			
Total- AA	0.06	-0.08	-0.07	-0.05	0.15	1		
Unsat FA	-0.01	0.05	0.22	**0.33****	-0.08	**-0.40****	1	
Sat FA	**-0.35****	-0.04	**0.27***	0.03	0.14	**-0.52****	**0.41****	1
Time-point (DAT)=2 day								
F_v/F_m change	1							
MI	0.10	1						
Total- OA	0.02	-0.03	1					
Total- SA	0.05	0.01	-0.14	1				
Total-Sug	0.19	-0.11	-0.02	**0.48****	1			
Total- AA	-0.08	-0.10	0.03	**0.39****	0.2	1		
Unsat FA	0.00	-0.01	**0.28***	0.05	-0.03	**-0.37****	1	
Sat FA	0.18	-0.02	**0.44****	-0.04	**0.24***	**-0.42****	**0.59****	1
Time-point (DAT)=4 day								
F_v/F_m change	1							
MI	-0.12	1						
Total- OA	-0.09	-0.02	1					
Total- SA	0.21	-0.06	0.05	1				
Total-Sug	0.08	-0.16	**0.29***	0.04	1			
Total- AA	0.00	-0.06	**0.26***	-0.04	0.15	1		
Unsat FA	0.02	0.01	-0.11	0.13	-0.13	-0.07	1	
Sat FA	-0.02	0.11	**-0.45****	0.02	**-0.38****	**-0.40****	**0.64****	1
Time-point (DAT)=7 day								
Fv/Fm Change	1							
MI	0.19	1						
Total- OA	-0.01	-0.17	1					
Total- SA	0.03	0.03	0.06	1				
Total-Sug	0.08	0.04	**0.34****	-0.16	1			
Total- AA	-0.20	0.13	**0.27***	0.13	0.02	1		
Unsat FA	0.11	0.05	-0.16	-0.06	-0.12	-0.01	1	
Sat FA	0.19	0.04	-0.22	-0.14	-0.08	-0.17	**0.87****	1

Supplemental Table 3.4 . Correlations among physiological characteristics and metabolite and fatty acid levels in eight virginia-type peanut cultivars and breeding lines after 7 days of heat stress with days of treatment combined.

	Fv/Fm Change	MI	Total-OA	Total-SA	Total-Sug	Total-AA	Unsat FA	Sat FA
Heat								
Fv/Fm change	1							
MI	**-0.19***	1						
Total- OA	0.12	0.07	1					
Total- SA	**-0.24****	**0.30****	**-0.43****	1				
Total-Sug	-0.14	**0.19***	**-0.27****	**0.33***	1			
Total- AA	**-0.18***	0.04	**-0.18***	**0.39****	0.03	1		
Unsat FA	**0.27****	-0.07	0.16	**-0.29****	-0.07	0.02	1	
Sat FA	**0.30****	-0.14	**0.23****	**-0.50****	**-0.26****	**-0.24****	**0.87****	1
Control								
Fv/Fm change	1							
MI	-0.08	1						
Total- OA	-0.09	-0.02	1					
Total- SA	0.02	0.03	0.01	1				
Total-Sug	-0.13	0.05	0.11	**0.37****	1			
Total- AA	-0.11	-0.09	0.12	0.17	**0.23***	1		
Unsat FA	0.07	-0.01	0.06	0.11	-0.10	**-0.21***	1	
Sat FA	0.06	0.01	0.08	-0.03	0.05	**-0.37****	**0.64****	1