ABSTRACT

ROSAS-ANDERSON, PABLO JOSE. Peanut Genetic Variation for Recovery from Severe Soil Drying and Low Epidermal Conductance as a Drought Tolerance Trait. (Under the direction of Dr. Thomas R. Sinclair).

Peanut (*Arachis hypogaea* L.) is an important food crop that is often grown in areas prone to intermittent drought. After drought is relieved, plant recovery from soil drying is an important factor for continued productivity. This thesis sought to identify genotypic variation for transpiration recovery and leaf maintenance in peanut and identify traits responsible for superior plant recovery from soil drying. In the first study, two greenhouse experiments were conducted to evaluate the transpiration recovery of 18 peanut genotypes subjected to a well-defined level of water-deficit stress before being re-watered. In addition, a visual rating scale was used to evaluate the maintenance of leaves during severe water-deficit stress one day after re-watering; where a rating of 1.0 indicated that plants had greater than 80% dead leaves and 5.0 meant that there was minimal leaf damage. A subsequent field experiment was conducted to verify the results of plant recovery observed in the greenhouse. Considerable genotypic variation for transpiration recovery from soil drying and leaf maintenance was found. Four genotypes with both superior transpiration recovery and leaf maintenance were identified: TMV 2, ICGV 86388, PI 497579, and N05006. These genotypes had transpiration ratios of at least 0.5 by the second day after re-watering and a visual rating of at least 4 or higher in almost all cases.

In the second study, two greenhouse experiments and a field experiment were conducted to identify genetic variation for epidermal conductance (EC) in peanut. In addition, the effect of water-deficit stress on peanut EC and the association of two leaf

anatomical traits with EC were investigated in the field. Genetic variation for EC in peanut was found among genotypes evaluated in the greenhouse and the field. Results indicated that water-deficit stress did not affect the EC of peanut. Neither stomatal density nor specific leaf weight were associated with peanut EC. TMV 2 and PI 298639 had the least EC in the first greenhouse experiment with 1.72 and 1.71 mmol m^{-2} s^{-1}, respectively. In the second greenhouse experiment VA 98R had the least EC of 2.16 mmol m^{-2} s^{-1}. In the Field, SPT 06-07 had the least or second least EC and Bailey consistently had low EC throughout all measurements. When results from the first and second study are considered together, low EC was in part responsible for higher leaf retention and consequently better recovery in TMV 2, SPT 06-07, PI 298639 and to some extent, Bailey.

Peanut Genetic Variation for Recovery from Severe Soil Drying and Low Epidermal Conductance as a Drought Tolerance Trait

by
Pablo Jose Rosas-Anderson

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Crop Science

Raleigh, North Carolina

2013

APPROVED BY:

Dr. Thomas R. Sinclair Committee Chair	Dr. Thomas Rufty
Dr. Thomas G. Isleib	Dr. Shyamalrau P. Tallury
Dr. Maria Balota	

BIOGRAPHY

Pablo Jose Rosas-Anderson was born in Lima, Peru and lived there until he was about six years old. His family emigrated to the U.S.A., and he along with his two brothers and one sister was raised by his two parents in Orlando, FL. After graduating from high school, he enrolled at Valencia Community College and received an A.A. degree in Business Administration with the wavering intention of pursuing a B.S. degree in Business. Instead, he enrolled in the education program at the University of Central Florida. After one year, he decided to take a semester off from school and travel to the Middle East and Europe. After much thought and discussion about the direction in life he wanted to take, he enrolled at the University of Florida and received a B.S. degree in Horticultural Sciences. After completing the Epcot Science Professional Internship Program at the Walt Disney World company in Orlando, FL, he attended North Carolina State University to complete his M.S. degree in Crop Science.

ACKNOWLEDGMENTS

This thesis was made possible because of the advice and support of many people. The author is grateful for the advice and direction of his Committee Chair, Dr. Thomas R. Sinclair, who shared his experiences and offered his guidance throughout the last two and a half years and without whose advice this study would not have been successful. The author is exceedingly grateful for his committee members, Dr. Thomas Rufty, for his knowledge and encouragement and providing the financial means to complete this research study, Dr. Thomas Isleib, for providing greenhouse space, a wealth of information and guidance, Dr. Shyam Tallury, for offering his laboratory facilities and knowledge, and Dr. Maria Balota, for providing field space and laboratory facilities and guidance throughout this research study. The author wishes to thank the staff at Tidewater Research Station, Suffolk, VA for their invaluable assistance and hospitality. Thanks to all of the author's laboratory friends and especially Jyostna Devi and Avat Shekoofa, who offered their encouragement, knowledge, and assistance throughout this research study. The author wishes to express his love and gratitude to his family and friends for their love and support through the duration of his studies and especially to his parents for their support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 2

CHAPTER 3

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

CHAPTER 1

INTRODUCTION

Background

Peanut (*Arachis hypogaea* L.) is one of the most important and widely grown legumes and oilseed crops throughout the world. Its great multitude of uses and value-added processed goods has made this crop one of the most important commodities around the world. About 50% of worldwide peanut production is used directly for oil extraction (Sanders, 2001). Utilization of peanut, whether crushed for oil or used directly as food, varies with each country. Peanut is popularly used for snacking, confectionaries, and peanut butter.

Ongoing studies of certain functional compounds found in peanut are proving this food source to be healthier than previously perceived (Maria Leonora and Resurreccion, 2008). Maria Leonora and Resurreccion (2008) examined five large epidemiological studies on the effect of frequent nut consumption on coronary heart disease and found that peanuts and other various nuts are associated with a reduced risk of coronary heart disease. Indeed, peanut is a rich source of protein as well as vitamins and minerals, and contains many beneficial compounds such as flavonoids and plant sterols and has antioxidant activity (Yang et al., 2009, Awad et al., 2000, Hwang et al., 2001). When compared with the ten most commonly consumed nuts in the US, peanut is among the greatest in total flavonoid content and potentially reduces the risk of cardiovascular disease (Yang et al., 2009; Vita, 2005). Many countries depend on peanut as an important food source.

Origin and Distribution

While known to be in tropical South America, the exact location of peanut's origin is unknown. There is speculation that the State of Mato Grosso in southwest Brazil is a possibility as it appears to contain the greatest diversity of wild *Arachis* (Ferguson et al., 2005). Genetic studies indicate that two wild species, *A. duranensis* and *A. ipaensis* hybridized in the wild and gave rise to the allotetraploid *A. monticola* which was then domesticated as *A. hypogaea* (Kochert et al., 1996; Seijo et al., 2004; Seijo et al., 2007). Domestication may have happened somewhere between northern Argentina and southern Bolivia (Kochert et al., 1996). Carbon dated fragments of peanut hulls found near ancient garden sites on the western slopes of the Andes in northern Peru indicated that the cultivation and domestication of peanut occurred over 7,500 years ago (Dillehay et al., 2007).

Peanut Production

The production of peanut extends to most countries of the world. In 2010 the world produced over 37.5 million MT of peanut, with China by far being the biggest contributor (14.7 million MT) (FAO, 2011). Average yields worldwide have increased from 1,169 to 1,563 kg ha^{-1} in the last 20 years (FAO, 2011). Peanut production around the world continues to grow as it is such an important food crop.

However, peanut is mainly grown on sandy soils with low water holding capacity and often without irrigation. Many farmers are dependent on rainfall to irrigate much of their lands. In many arid and semi-arid regions the rain is often sparse and erratic throughout the growing season. Even in the U.S.A. about a quarter of peanut acreage is grown without irrigation (USDA, 2007). The average percentage of peanut crop losses due to drought and heat from 1962 to 2009 was about 43% (USDA, 2010). Agriculture in Sub-Saharan Africa and where peanut is grown is almost completely dependent on rainfall (Rockström and Falkenmark, 2000). Peanut is often the preferred crop to grow in semi-arid areas, and as a consequence considerable yield reductions are realized. If drought is severe and soil water is exhausted, farmers also risk total crop failure unless the plant can survive and then recover after a rain event.

Plant Response to Soil Drying

Three stages in plant response to soil drying have been proposed by Sinclair and Ludlow (1985): Stage I occurs when ample water is present in the soil, stomatal conductance is at maximum and water uptake is unhindered. Stomatal behavior follows a regular daily pattern of opening in the early morning, reaching peak transpiration in the afternoon followed by stomatal closure into the night. Stage I can continue even as soil dries until a threshold fraction of transpirable soil water (FTSW) is reached. Once the threshold is reached, the next stage of stress begins. Plant processes can be affected during Stage I as the soil begins to dry though not yet reaching the threshold FTSW. For example, in soybean genotypes (*Glycine max* L. Merr.), nitrogen fixation rates are reduced as the soil dries yet while plants are still under Stage I (Sinclair, 1986; Sall and Sinclair, 1991). Some peanut genotypes also have greater nitrogen fixation sensitivity to soil drying and reduced fixation rates while still under Stage I (Devi et al., 2009; Devi et al., 2010).

Stage II occurs as the soil dries to a fraction of transpirable soil water threshold where plants respond by closing their stomata for longer periods during the day to maintain the water balance between water uptake and loss. The onset of Stage II is a measurable response of the plant to a decreasing soil water supply. Generally, a plant's ability to produce acceptable yields during this stage can be considered to have some level of drought tolerance (Passioura, 1996). The FTSW at which Stage II occurs is generally between 0.35 and 0.45 though differences among species and within species have been identified (Ray and Sinclair, 1997; Devi et al., 2009). Ray and Sinclair (1997) suggested that maize hybrids which close their stomata when the fraction of transpirable soil water is high, *i.e.*, close them earlier in the drying cycle and conserve soil water for future use, are better positioned to deal with long term drought than those which close stomata later in soil drying. However this comes at the expense of reduced CO_2 uptake and a reduction in growth. Such a response would probably be beneficial when drought is long term or locations with low average precipitation. Under short and frequent drought events it seems more beneficial for the plant to close stomata later in the drying cycle and continue normal growth. As a result, a plant considered to be drought tolerant may indeed tolerate and survive in environments with long drought events, however yield poorly in environments where droughts occur frequently and are short.

A number of peanut drought studies that impose a water deficit stress treatment by withholding water for a number of days consequently impose a stress comparable to Stage II stress (Bennett et al., 1984; Nautiyal et al., 1995). Other studies grow peanut plants with available soil water reduced to 1/3 or 2/3 (Pimratch et al., 2008; Arunyanark et al., 2008; Songsri et al., 2008). However, peanut genotypic differences have been identified for the fraction of transpirable soil water at which plants respond by reducing their transpiration, *i.e.*, when they enter Stage II and were found to be between 0.22 and 0.71 (Devi et al., 2009). Therefore, studies which grow peanuts under reduced soil water will most likely be imposing Stage II stress with a chance that plants will have actually remained in Stage I depending on the genotypes tested.

Stage III begins when the soil dries further, exhausting transpirable soil water. At this point, stomata remain at minimum aperture until soil water is replenished. Experimentally in the greenhouse, plants are considered to have exhausted all transpirable soil water when their relative transpiration ratios are measured to be about 0.1 of well-watered control plants. Plants are unable to control water loss through any further stomatal closure and regulation of water loss is by the vapor resistance of the leaf epidermis and the difference in vapor pressure between the leaves and atmosphere (Muchow and Sinclair, 1989). There is very limited information on the physiological mechanisms that are involved at Stage III that may lead to superior recovery in peanut.

During Stage III, many crops will sustain such severe stress that yields may not be high enough to achieve profits. In this case it may be more economical to let the crop fail than further invest inputs. Crops that are able to survive prolonged durations under Stage III must have adequate recovery that is agronomically relevant in order to achieve greater productivity potential (Lawn and Likoswe, 2008). For example, crops that survive severe droughts by leaf-firing or experience leaf dehydration will not be agronomically favorable since regrowth will most likely be slow and additional inputs will be needed. However, if adequate leaf retention is achieved under severe stress and leaf rehydration and transpiration recovery is accomplished after soil water is replenished, whole plant recovery will most likely be adequate and timely, and the productivity potential will be higher. Some plants such as pigeonpea (*Cajanus cajan* (L.) Millsp.) and cowpea (*Vigna unguiculata* (L.) Walp.) can survive for extended periods under Stage III while others such as soybean have limited survival at this stage (Sinclair and Ludlow, 1986).

The physiological mechanisms involved in plant survival and recovery from Stage III are still being investigated. The rapid recovery of plant function and growth is certainly a beneficial trait once soil water has been replenished. A plant's ability to survive for any period of time during Stage III and its subsequent recovery after re-hydration may depend on its dehydration tolerance and ability to delay leaves from reaching a lethal dehydration state (Flower and Ludlow, 1986) by limiting water loss from the leaf epidermis (Sinclair and Ludlow, 1986; James et al., 2008).

Physiological Mechanism Under Stage III

Dehydration tolerance is understood as the ability of a plant to survive and continue functioning at low water contents within tissues. Tolerance is associated with low lethal relative water content (RWC) of leaves (Ludlow and Muchow, 1988). Plants considered to be more dehydration tolerant usually have lower values of RWC when leaves are nearly senescing (Flower and Ludlow, 1986; Sinclair and Ludlow, 1986). Leaf RWC is the percentage of water content of a leaf relative to the total water present in the leaf at full turgor (Hsiao, 1973). RWC has been used to indicate plant water status though it is more appropriately used when severe water deficits are imposed because RWC is insensitive to moderate water-deficits (Hsiao, 1973). Leaf necrosis begins when the RWC falls below a lethal value that is dependent on the species. It has been reported that the lethal RWC in some grasses can be as low as 25% (Chai et al., 2010). Lethal RWC between 40 and 60% has been reported in soybean (James et al., 2008) and 32-36% in pigeonpea (Flower and Ludlow, 1986; Likoswe and Lawn, 2008).

When plants reach Stage III, the RWC in leaves act as a water reservoir that is slowly depleted to the air and because soil water has been exhausted, water uptake by roots is minimal and the reservoir may not readily be replenished. It is possible that the RWC of different species may differ when Stage III is reached. Thus some may have more water available to be lost until the lethal RWC is reached (Sinclair and Ludlow, 1986). Plants with mechanisms that delay reaching the lethal RWC are most likely to survive longer under severe water stress than plants that lack such mechanisms.

Epidermal conductance refers to the water vapor conductance of the leaf epidermis after stomata have closed or reached minimum aperture in response to exhausted soil water supply. Plants with lower epidermal conductance (EC) experience less water loss after stomata are closed and as a result may be considered more tolerant of severe drought (Sinclair and Ludlow, 1986; Muchow and Sinclair, 1989; Saito and Futakuchi, 2010). Stomatal density in sorghum (*Sorghum bicolor* (L.) Moench) has been correlated with EC indicating that the majority of water loss is from either the pore due to incompletely closed stomata or directly from the guard cells (Muchow and Sinclair, 1989). However, Araus et al. (1991) measured EC and stomatal density in wheat (*Triticum durum* L.) and could not find a strong association between them. They attributed differences in EC to an interrelation of leaf traits. Jordan et al. (1984) found that epidermal conductance in sorghum is influenced by the amount of epicuticular wax when the amount is below a certain threshold. Other researchers have also reported the importance of epicuticular wax for limiting water loss (Hull et al., 1978; Bengtson et al., 1978; Paje et al., 1988). Among other species, genetic variation for epidermal conductance has been reported in soybean (Paje et al., 1988; James et al., 2008;), rice (*Oryza* spp.) (Saito and Futakuchi, 2010), and oat (*Avena sativa* L.) (Bengtson et al., 1978). Surprisingly, genetic variation for EC in peanut has not been reported in the literature.

Low epidermal conductance would limit water loss during Stage III and allow plants to remain hydrated prolonging leaf maintenance and giving plants a better chance to recover when soil water is replenished. Indeed, soybean recovery from severe water deficit has been associated with leaf area maintenance attributed to low EC (Lawn and Likoswe, 2008).

Studies on the sensitivity of EC of different species to environmental factors are somewhat limited. Paje et al. (1988) reported that EC in soybean is higher under cool and moist conditions and is reduced by water stress. O'Toole et al. (1979) measured the cuticular resistances of rice leaves grown under water stressed conditions and found they were higher than their well-watered counterparts.

While the interrelation of survival traits such as low lethal RWC and low EC has been studied in soybean (James et al., 2008), researchers could not determine which of these was most important. They did note however that the rate of decline in relative water content (RWC) per day was more important than the RWC at which leaf death occurred. This may be evidence that low EC is more beneficial than a low lethal RWC. Indeed, Sinclair (2000) used a model to determine crop survival based on lethal RWC and epidermal conductance and found that to obtain substantial survival lengths under Stage III it was first necessary to have EC below 10.35 mmol m^{-2} s^{-1}. The potential to breed for lower EC below this amount with the goal of extending survival might be limited in some crops. For example, the EC of durum wheat has been reported to be in the range of 11 to 30.5 mmol m^{-2} s^{-1} (Araus et al., 1991), and in some rice species 11.6-17.2 mmol m^{-2} s^{-1} (Saito and Futakuchi, 2010). However, as more genotypes of each species are studied, sources of lower EC may be found. By comparison, severe drought adapted plants such as sabal palm (*Sabal palmetto*) can have EC as low as 0.32 mmol m^{-2} s^{-1} (Holbrook and Sinclair, 1992). Information on EC for peanut could not be found in the literature. Because peanut is grown in areas that are prone to droughts, low EC might be a beneficial trait that extends survival and consequently improves the recovery of peanut under Stage III.

Plant Recovery

The recovery of leaf gas exchange from severe water stress will undoubtedly be one of the most important factors to whole plant recovery and productivity of a crop. The recovery of stomatal conductance and photosynthesis varies among species and severity of water-deficit stress (Ludlow et al., 1980; Souza et al., 2004; Miyashita et al., 2005). In cowpea, non-stomatal inhibition of photosynthetic rates may happen for the first few days after recovery. Kidney beans (*Phaseolus vulgaris* L.) have faster recovery of photosynthetic rate than stomatal conductance as the stress becomes more severe (Miyashita et al., 2005). Some plants may not achieve the full recovery of transpiration rates after re-watering;

presumably as an adaptive mechanism for recurring droughts. The benefits or drawbacks of this response depend on the environmental conditions in which the crop is grown. However, if transpiration never fully recovers after a drought event, the productivity potential will be greatly reduced. Prolonged duration under Stage III stress is likely to limit transpiration recovery due to leaf dehydration and possibly damage to guard cells or chemical signals.

It has been previously reported that peanut is able to rapidly recover from severe water deficit stress and that this rapid recovery is an adaptive mechanism (Black et al., 1985). However, the transpiration recovery of peanut under prolonged Stage III stress has not been thoroughly investigated. Severe water deficit stress studies on peanut indicate differential transpiration recovery from Stage III stress and therefore imply that there is genetic variation for the ability to recover from Stage III stress (Lauriano et al., 2004).

Leaf maintenance due to dehydration tolerance or avoidance traits, *i.e.,* low epidermal conductance coupled with high transpiration recovery from Stage III would greatly improve the whole plant recovery potential of peanut. Identification of genotypes possessing either of these traits or in combination is needed in order to develop genotypes with improved plant performance under drought prone environments.

The objectives of this thesis were to identify genetic variation in peanut for plant recovery from soil drying and to identify sources of low epidermal conductance as a physiological mechanism that could be used to develop commercial peanut genotypes with improved tolerance to severe water deficit stress.

The objective of the first study presented in this thesis was to identify genotypic differences for leaf maintenance and transpiration recovery among nineteen peanut genotypes subjected to a soil drying cycle. In two greenhouse experiments, individual peanut plants were taken through the three stages proposed by Sinclair and Ludlow (1985) and re-watered after a number of days under Stage III stress. Their transpiration rates were measured for a number of days following re-watering in order to determine their recovery. To determine leaf maintenance, plants were also visual rated based on the amount of leaf damage one day after re-watering. A field experiment was conducted to verify results found in the greenhouse experiments.

The second study presented in this thesis aimed to identify low epidermal conductance as a trait for delaying leaf dehydration leading to improved recovery of peanut from severe water-deficit stress, *i.e.,* Stage III. The first objective was to identify genetic variation for epidermal conductance in the peanut germplasm. A second objective was to investigate the effects of water-deficit stress on the stability of epidermal conductance in peanut. And a third objective sought to determine the association between epidermal conductance and two anatomical leaf traits: stomatal density and specific leaf weight. To fulfill the first objective and determine the association between epidermal conductance and stomatal density, two greenhouse experiments were conducted which evaluated the epidermal conductance of 22 peanut genotypes chosen for their drought tolerance, origin and species. A field experiment was conducted to confirm genetic variation found in the greenhouse experiments and to fulfill the second and third objectives.

REFERENCES

Araus, J., Febrero, A., and Vendrell, P. (1991). Epidermal conductance in different parts of durum wheat grown under mediterranean conditions: The role of epicuticular waxes and stomata. *Plant, Cell and Environment, 14*(6), 545-558.

Arunyanark, A., Jogloy, S., Akkasaeng, C., Vorasoot, N., Kesmala, T., Nageswara Rao. R.C., Wright, G.C., Patanothai, A. (2008). Chlorophyll stability is an indicator of drought tolerance in peanut. *Journal of Agronomy and Crop Science, 194*(2), 113-125.

Awad, A. B., Chan, K. C., Downie, A. C., and Fink, C. S. (2000). Peanuts as a source of β-sitosterol, a sterol with anticancer properties. *Nutrition and Cancer, 36*(2), 238-241.

Bengtson, C., Larsson, S., and Liljenberg, C. (1978). Effects of water stress on cuticular transpiration rate and amount and composition of epicuticular wax in seedlings of six oat varieties. *Physiologia Plantarum, 44*(4), 319-324.

Bennett, J., Boote, K., and Hammond, L. (1984). Relationships among water potential components, relative water content, and stomatal resistance of field-grown peanut leaves 1. *Peanut Science, 11*(1), 31-35.

Black, C., Tang, D., Ong, C., Solon, A., and Simmonds, L. (1985). Effects of soil moisture stress on the water relations and water use of groundnut stands. *New Phytologist, 100*(3), 313-328.

Chai, Q., Jin, F., Merewitz, E., and Huang, B. (2010). Growth and physiological traits associated with drought survival and post-drought recovery in perennial turfgrass species. *Journal of the American Society for Horticultural Science, 135*(2), 125-133.

Devi, M. J., Sinclair, T., Vadez, V., and Krishnamurthy, L. (2009). Peanut genotypic variation in transpiration efficiency and decreased transpiration during progressive soil drying. *Field Crops Research, 114*(2), 280-285.

Devi, M. J., Sinclair, T. R., and Vadez, V. (2010). Genotypic variability among peanut (*Arachis hypogaea* L.) in sensitivity of nitrogen fixation to soil drying. *Plant and Soil, 330*(1), 139-148.

Dillehay, T. D., Rossen, J., Andres, T. C., and Williams, D. E. (2007). Preceramic adoption of peanut, squash, and cotton in northern Peru. *Science, 316*(5833), 1890-1893. doi:10.1126/science.1141395

Ferguson, M. E., Jarvis, A., Stalker, H. T., Williams, D. E., Guarino, L., Valls, J. F. M., Pittman, R. N., Simpson, C. E., Bramel, P. J. (2005). Biogeography of wild *Arachis* (leguminosae): Distribution and environmental characterisation. *Biodiversity and Conservation, 14*(7), 1777-1798.

Flower, D., and Ludlow, M. M. (1986). Contribution of osmotic adjustment to the dehydration tolerance of water-stressed pigeon pea (*Cajanus cajan* (L.) Millsp.) leaves. *Plant, Cell and Environment, 9*(1), 33-40.

Food and Agriculture Organization of the United Nations, Statistics Division. 2011. Retrieved February/14, 2013, from http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Holbrook, N., and Sinclair, T. (1992). Water balance in the arborescent palm, sabal palmetto. II. transpiration and stem water storage. *Plant, Cell and Environment, 15*(4), 401-409.

Hsiao, T. C. (1973). Plant responses to water stress. *Annual Review of Plant Physiology, 24*(1), 519-570.

Hull, H. M., Wright, L. N., and Bleckmann, C. A. (1978). Epicuticular wax ultrastructure among lines of *Eragrostis lehmanniana* Nees developed for seedling drought tolerance. *Crop Science, 18*(5), 699-704.

Hwang, J., Shue, Y., and Chang, H. (2001). Antioxidative activity of roasted and defatted peanut kernels. *Food Research International, 34*(7), 639-647.

James, A., Lawn, R. J., and Cooper, M. (2008). Genotypic variation for drought stress response traits in soybean. I. Variation in soybean and wild *Glycine* spp. for epidermal conductance, osmotic potential, and relative water content. *Crop and Pasture Science, 59*(7), 656-669.

Jordan, W., Shouse, P., Blum, A., Miller, F., and Monk, R. (1984). Environmental physiology of sorghum. II. Epicuticular wax load and cuticular transpiration. *Crop Science, 24*(6), 1168-1173.

Kochert, G., Stalker, H. T., Gimenes, M., Galgaro, L., Lopes, C. R., and Moore, K. (1996). RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (Leguminosae). *American Journal of Botany*, 1282-1291.

Lauriano, J., Ramalho, J., Lidon, F., and do Céu Matos, M. (2004). Peanut photosynthesis under drought and re-watering. *Photosynthetica, 42*(1), 37-41.

Lawn, R. J., and Likoswe, A. A. (2008). Genotypic differences in leaf area maintenance contribute to differences in recovery from water stress in soybean. *Crop and Pasture Science, 59*(12), 1075-1085.

Likoswe, A. A., and Lawn, R. J. (2008). Response to terminal water deficit stress of cowpea, pigeonpea, and soybean in pure stand and in competition. *Crop and Pasture Science, 59*(1), 27-37.

Ludlow, M. M., and Muchow, R. C. (1988). Critical evaluation of the possibilities for modifying crops for high production per unit precipitation, p. 178-204. In F. R. Bidinger and C. Johansen (Eds.), *Drought research priorities for the dryland tropics*). ICRISAT, Patancheru, India.

Ludlow, M. M., Ng, T., and Ford, C. (1980). Recovery after water stress of leaf gas exchange in *Panicum maximum* var. trichoglume. *Functional Plant Biology, 7*(3), 299-313.

Maria Leonora, D., and Resurreccion, A. (2008). Functional components in peanuts. *Critical Reviews in Food Science and Nutrition, 48*(8), 715-746.

Miyashita, K., Tanakamaru, S., Maitani, T., and Kimura, K. (2005). Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environmental and Experimental Botany, 53*(2), 205-214.

Muchow, R. C., and Sinclair, T. R. (1989). Epidermal conductance, stomatal density and stomatal size among genotypes of *sorghum bicolor* (L.) Moench. *Plant, Cell and Environment, 12*(4), 425-431.

Nautiyal, P., Ravindra, V., and Joshi, Y. (1995). Gas exchange and leaf water relations in two peanut cultivars of different drought tolerance. *Biologia Plantarum, 37*(3), 371-374.

O'toole, J., Cruz, R., and Seiber, J. (1979). Epicuticular wax and cuticular resistance in rice. *Physiologia Plantarum, 47*(4), 239-244.

Paje, M.C.M., Ludlow, M. M., and Lawn, R. J. (1988). Variation among soybean (*Glycine max* (L.) Merr.) accessions in epidermal conductance of leaves. *Crop and Pasture Science, 39*(3), 363-373.

Passioura, J. (1996). Drought and drought tolerance. *Plant Growth Regulation, 20*(2), 79-83.

Pimratch, S., Jogloy S., Vorasoot N., Toomsan B., Patanothai A., and Holbrook C.C. (2008). Relationship between biomass production and nitrogen fixation under drought-stress conditions in peanut genotypes with different levels of drought resistance. *Journal of Agronomy and Crop Science*, 194(1), 15-25.

Ray, J. D., and Sinclair, T. R. (1997). Stomatal closure of maize hybrids in response to drying soil. *Crop Science, 37*(3), 803-807.

Rockström, J., and Falkenmark, M. (2000). Semi-arid crop production from a hydrological perspective: Gap between potential and actual yields. *Critical Reviews in Plant Sciences, 19*(4), 319-346.

Saito, K., and Futakuchi, K. (2010). Genotypic variation in epidermal conductance and its associated traits among *Oryza sativa* and *O. glaberrima* Cultivars and their interspecific progenies. *Crop Science, 50*(1), 227-234.

Sall, K., and Sinclair, T. R. (1991). Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration. *Plant and Soil, 133*(1), 31-37.

Sanders, T. (2001). Individual oils: Peanut oil. Paper presented at the *Proceedings of the World Conference on Oilseed Processing and Utilization,* pp. 141-144.

Seijo, G., Lavia, G. I., Fernández, A., Krapovickas, A., Ducasse, D. A., Bertioli, D. J., et al. (2007). Genomic relationships between the cultivated peanut (*Arachis hypogaea*, lLeguminosae) and its close relatives revealed by double GISH. *American Journal of Botany, 94*(12), 1963-1971.

Seijo, J. G., Lavia, G. I., Fernández, A., Krapovickas, A., Ducasse, D., and Moscone, E. A. (2004). Physical mapping of the 5S and 18S–25S rRNA genes by FISH as evidence that *Arachis duranensis* and *A. ipaensis* are the wild diploid progenitors of *A. hypogaea* (Leguminosae). *American Journal of Botany, 91*(9), 1294-1303.

Sinclair, T. R. (2000). Model analysis of plant traits leading to prolonged crop survival during severe drought. *Field Crops Research, 68*(3), 211-217.

Sinclair, T. R., and Ludlow, M. M. (1985). Who taught plants thermodynamics? The unfulfilled potential of plant water potential., *Functional Plant Biology, 12*(3), 213-217.

Sinclair, T. R. (1986). Water and nitrogen limitations in soybean grain production I. Model development. *Field Crops Research, 15*(2), 125-141.

Sinclair, T. R., and Ludlow, M. M. (1986). Influence of soil water supply on the plant water balance of four tropical grain legumes. *Functional Plant Biology, 13*(3), 329-341.

Songsri, P., Jogloy, S., Vorasoot, N., Akkasaeng, C., Patanothai, A., and Holbrook, C. (2008). Root distribution of drought-resistant peanut genotypes in response to drought. *Journal of Agronomy and Crop Science, 194*(2), 92-103.

Souza, R., Machado, E., Silva, J., Lagôa, A., and Silveira, J. (2004). Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea V*igna unguiculata* during water stress and recovery. *Environmental and Experimental Botany, 51*(1), 45-56.

U.S. Department of Agriculture. (2007). 2007 Census of Agriculture. *Farm and Ranch Irrigation Survey, volume 3.* Washington, DC: U.S. Department of Agriculture, 2009. Retrieved 02/14, 2013, from http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Farm_and_Ranch_Irrigation_Survey/fris08.pdf

U.S. Department of Agriculture. (2010). National Agricultural Statistics Service. *Agricultural Statistics, 2010. U.S.* Government Printing Office. Washington, DC. Retrieved 02/15, 2013, from http://www.nass.usda.gov/Publications/Ag_Statistics/2010/2010.pdf

Vita, J. A. (2005). Polyphenols and cardiovascular disease: Effects on endothelial and platelet function. *The American Journal of Clinical Nutrition, 81*(1), 292S-297S.

Yang, J., Liu, R. H., and Halim, L. (2009). Antioxidant and antiproliferative activities of common edible nut seeds. *LWT-Food Science and Technology, 42*(1), 1-8.

CHAPTER 2

Genetic Variation in Peanut Leaf Maintenance and Transpiration Recovery From Severe Soil Drying

Introduction

Peanut is usually grown on sandy soils with low water holding capacity and often grown without irrigation. In non-irrigated fields, long dry periods between rainfall events may cause plants to experience severe water-deficit stress. This can often lead to substantial marketable yield losses or even complete crop failure. The ability to recover from a severe dry spell upon re-watering and to maintain productivity resulting in acceptable yields would be beneficial to farmers.

Three stages in plant response to soil drying have been proposed by Sinclair and Ludlow (1985): Stage I occurs when plants are unstressed, stomatal conductance is at maximum and water uptake is unhindered. Stage II occurs as the soil dries and plants begin to close stomata for longer periods during the day, maintaining the water balance between water uptake and water loss, and as a result, a reduction in overall transpiration concurrent with decreasing soil water. Stage III begins when the soil further dries exhausting transpirable soil water. At this point, stomata remain at minimum aperture until soil water is replenished. Plants are unable to control water loss through any further stomatal adjustments and regulation of water loss is by the vapor resistance of the leaf epidermis and the difference in vapor pressure between the leaves and atmosphere (Muchow and Sinclair, 1989). During severe water-deficit, a plant's ability to survive for any period of time and subsequently recover after re-watering may depend on its ability to delay leaves from reaching a lethal dehydration state by limiting water loss from the leaf epidermis (Flower and Ludlow, 1986; Sinclair and Ludlow, 1986; James et al., 2008a).

Genetic variation for plant recovery from severe water-deficit has been shown in soybean (James et al., 2008b), among species of bentgrass (*Agrostis capillaries* L.; *A. stolonifera* L., *A. canina* L.) (DaCosta and Huang, 2007) and among various legumes (Sinclair and Ludlow, 1986; Likoswe and Lawn, 2008). Variation for recovery of physiological mechanisms such as nitrogen fixation, gas exchange, and photosynthetic capacity from various levels of water stress has been previously reported in peanut (Lauriano et al., 2004; Puangbut et al., 2010). However, only limited studies have looked at the recovery of gas exchange and leaf maintenance from a well-defined water-deficit stress level such as Stage III. Often, a water-deficit treatment is imposed on all plants by withholding water for a certain number of days once all plants have grown for a period of time. For example, Lauriano et al. (2004) measured the response of gas exchange in peanut after a severe water-deficit treatment was imposed; however, the treatment included withholding water for a certain number of days for all plants without making a distinction between plants that are possibly experiencing differential stress. Consequently, the genotypes they compared for recovery from the stress treatment may have experienced differential reduction in gas exchange immediately before re-watering. Other studies imposed treatments that reduce soil water content to moderate or even low amounts (Awal and Ikeda, 2002; Puangbut et al., 2010). However, genotypic differences in peanut have been reported for transpiration decline in response to a drying soil (Devi et al., 2010). Some peanut genotypes will begin to reduce their transpiration rates when soil water is as high as 70% of available water and others when available soil water is as low as 25%. It is clear that in terms of reduced gas exchange as a measure of stress, a treatment of withholding water until 2/3 or even 1/3 of available soil water is left will not significantly stress some peanut genotypes. If plant recovery from water-deficit stress is measured on the basis of transpiration recovery, it is plausible to take each plant to a well-defined level of stress based on transpiration in response to decreasing soil water before re-watering.

Therefore, the objective of this study was to identify genetic variation in recovery based on a visual rating scale and recovery of transpiration among nineteen peanut genotypes experiencing Stage III stress during a soil drying cycle. An initial greenhouse experiment screened eighteen peanut lines for variation in recovery from a soil drying cycle while a second greenhouse experiment involved five selected lines from the first Greenhouse Experiment plus an additional peanut wild species to confirm that variation exists within the peanut germplasm. In addition, a third experiment was conducted in the field to evaluate the recovery of gas exchange of six selected peanut genotypes from a water-deficit treatment.

Materials and Methods

Two greenhouse experiments were conducted at North Carolina State University, Raleigh, NC and a field experiment at Tidewater Agricultural Research and Extension Center (TAREC) in Suffolk, VA to evaluate the recovery of a total of nineteen peanut genotypes from Stage III stress. The genotypes were chosen based on drought tolerance, origin, commercial status, and seed availability. Detailed information for all genotypes is presented in Table 1.

Plant Culture and Treatments

Greenhouse Experiment 1 (GH 1) was designed to evaluate the recovery of eighteen peanut genotypes after re-watering from Stage III stress based on visual ratings and transpiration recovery. Three seeds from each genotype were sown in 2.8-L, 16.5-cm tall plastic pots in April 2011. Pots were prepared by placing a screen mesh in the inside bottom to prevent soil from leaching through drainage holes. Then, pots were filled with a sandy loam soil to about 2.5 cm from the top. In addition, 20 g of pulverized $CaCO_3$ was added to each pot to neutralize soil acidity. Two weeks after sowing, pots were thinned to one plant to make all pots as uniform as possible. One week after thinning, 1.02 g of 5-13-20 fertilizer diluted in 88 ml of water was added to each pot. Each genotype was represented by a total of twelve replicate pots evenly assigned to three treatments. The greenhouse was sprayed with a pesticide once every two weeks to control thrips. Later in the season flowers were removed daily to keep plants in the vegetative stage.

Greenhouse Experiment 2 (GH 2) was designed to evaluate the extent of variation in recovery under more severe stress and to validate results obtained in GH 1. Five genotypes with contrasting response to re-watering after Stage III stress from GH 1 were selected and an additional genotype, PI 298639 (*A. batizocoi*) was added to widen the genetic diversity of the material evaluated in GH 2. Seeds were sown in June 2011 and maintained as those in GH 1. The only difference was that the soil did not need to be fertilized or limed because the soil was recycled from GH 1.

Drying cycle

In both GH 1 and GH 2, plants were grown under well-watered conditions for about four weeks. On the day prior to starting the drying cycle, pots were over-watered and allowed to drain overnight. The following morning all pots were enclosed in white plastic bags with the opening of the bag bunched around the base of the plant and firmly held together by twist ties. A small plastic tube was also inserted in the opening for irrigating the pot. The bags ensured that no evaporative water loss occurred and any water loss from the pots was via transpiration. All pots were weighed after placing them in the bags to determine initial weight. All pots were individually weighed on a daily basis usually between 1300 and 1600 EST and watered between 1600 and 1700 EST.

To prevent plants from experiencing stress too soon from rapid soil dehydration, soil moisture was controlled by restoring any transpirational water loss that exceeded 70 g over any single day. Well-watered control plants for each genotype were re-watered to maintain a daily weight 100 g below the initial weight. The 100 g deficit was to prevent saturated conditions in the well-watered pots.

Transpiration ratios were calculated daily for each stress treatment plant by dividing its daily weight loss by the mean weight loss of well-watered plants. In addition, a normalized transpiration ratio (NTR) was calculated for each plant by dividing the daily transpiration rate by the average transpiration rate of the same plant from the first two to four days when the plant was still under well-watered conditions. This was done to account for any plant to plant variation for transpirational differences. Plants were sorted and assigned as either well-watered or stressed treatments based on their initial transpiration rates. This ensured that replications among well-watered plants and stressed plants were distributed equally. Pots assigned to the stressed treatment were later divided into two stressed treatments (below).

Stressed plants eventually reached an NTR below 0.11 concurrent with soil water exhaustion and was defined as the endpoint of the drying cycle and beginning of Stage III dehydration (Sinclair and Ludlow, 1986). Plants reached this point on different days depending on their size and how much water they lost. In GH 1, a treatment was imposed on individual plants by withholding water for one day after Stage III had been reached. A second treatment was imposed on a second set of replicate plants by withholding water for three days after Stage III had been reached. In GH 2, treatments were imposed for four and six days. Re-watering was done at the end of each treatment. The entire drying cycle which started when pots were bagged and ended upon re-watering lasted approximately 13-16 days. NTR was determined for four to five additional days following re-watering in GH 1 and three days in GH 2. However, because plants reached Stage III on different days, treatments were not imposed on all plants at the same time and NTR was not calculated for some genotypes in the latter days after re-watering.

Transpirable soil water was calculated as the initial weight of a pot minus the weight on the day the endpoint was reached. Fraction of transpirable soil water in the pot on each day was calculated as the weight on that day minus the weight when the endpoint was reached divided by transpirable soil water.

Field

A field experiment was conducted at TAREC to evaluate the recovery of stomatal conductance of six selected genotypes after re-watering from a water-deficit stress treatment. These genotypes were selected from GH 1 and GH 2 based on their contrasting response to re-watering after a drying cycle and seed availability. Seeds were sown in 3.5 m x 2 m subplots on 14 May 2012 on a sandy soil at a depth of 3.8 cm in four rows spaced at 0.9 m. Due to poor machine sowing, most of the plots were re-sown by hand three weeks later. The field was divided into three plots: two plots were designated as a water-deficit stress treatment and one as a well-watered treatment. The stress treatment was split into two plots in order to accommodate the size of the rain-shelters used to impose the water-deficit stress. Two border rows on each side of the plots were also sown. Each plot was divided into three replicate blocks and each block contained six randomized subplots of all six peanut genotypes. Therefore, the stress treatment split between the two rain-shelter plots was represented by a total of six replicate blocks.

When the plant canopies of all genotypes reached at least 80% closure the two rain-shelters measuring 21.5 m in length and 6-m wide were moved over each stress plot while the plot designated as well-watered was not covered. Each shelter took at least a day to move and as a result the second stress plot received an additional 3.6 cm of rain. The structural frame of the rain-shelters was aluminum and shaped like a gable and covered with 0.15 mm thick polyethylene (Atlas Manufacturing, Atlanta, GA). The rain-shelters remained over the plots for the duration of the experiment. The experiment began on 7 August 2012. Stress plots were re-irrigated on the 39th day of the experiment when midday stomatal conductance measurements indicated that conductance was only 0.11 or less of the well-watered plot in at least one replication. The well-watered plot was kept well-watered by continuous rains experienced throughout the duration of the experiment.

Stomatal conductance (Gs) was measured on Day 7 and 8 (one measurement), 18, 21, 31 and 32 (one measurement), 35, 38, 41, and 44 of the experiment on two leaflets per subplot using a LI-6400 (LICOR, Inc., Lincoln, Nebraska). This occurred around mid-day, usually between 1000 and 1500 EST. A Gs ratio was calculated for each genotype replicate subplot by dividing the Gs of each subplot by the mean Gs of the corresponding well-watered replicate subplots. A normalized Gs ratio (NGR) was calculated by dividing the Gs ratio of each stress subplot by the Gs ratio of that same subplot at the first Gs measurement (Day 7 and 8). This was done to adjust Gs to be 1.0 at the start of the Experiment while plants were still under well-watered conditions. Two data loggers collecting temperature and humidity information every five minutes were equally spaced within each plot. The Experiment ended on Day 44 when the last Gs measurements were taken five days after sheltered plots were re-watered.

Comparisons of transpiration and Gs recovery from water deficit stress between the greenhouse and the field were done for the second day after re-watering because Gs measurements were taken two days after plots were irrigated.

Visual Rating

A visual rating scale was developed for both greenhouse experiments to rate the recovery of the plant canopy after re-watering from stress. Each plant in the greenhouse experiments was visually evaluated one day after re-watering and given a rating of one to five. A rating of one represented plants which were either dead or had very poor recovery based on the amount of leaf death (greater than 80% death). Plants with a rating of two were considered to have somewhat poor recovery or were at the bottom end of moderate recovery and specifically had leaf death between 50-80%, discoloration, and greatly reduced size overall. Plants that received a rating of three generally had around 25-50% of leaves in the plant dead and/or heavily discolored. Plants which received a rating of four had some leaf death (less than 25%) or discoloration and had moderate reduction in size compared with well-watered plants. A rating of five meant that plants had minimal leaf death or discoloration and were fully recovered. The mean of each genotype replicate represented the final rating value.

Statistical Analysis

Analysis of variance was performed on overall genotypic NTR for each day after re-watering and visual rating after re-watering. All genotypic comparisons in GH 2 were performed using Tukey's HSD test with significance at $p < 0.05$. NTR and visual rating from selected genotypes in GH 1 that were repeated in GH 2 were also tested using Tukey's HSD test. A two-piece linear regression was performed between FTSW and NTR and a breakpoint calculated from the FTSW at which the two lines intersect. The breakpoint represents the start of Stage II stress. All statistical analyses were conducted on the statistical software JMP 10.01 (SAS Institute, Cary, NC) and Prism 2.0 (GraphPad Software, Inc., La Jolla, CA).

Results

Greenhouse Experiments

Plant Response to Soil Drying

Plant response to soil drying followed the typical two-segmented response curve reported in other studies (Ray and Sinclair, 1997; Devi et al., 2009) (Figure 1). Severe wilting occurred as plants reached Stage III in both greenhouse experiments. Genotype PI 298639 exhibited a different response to the drying cycle than expected. Typically, once plants have reached Stage III they will remain in this stage until re-watering. However, when PI 298639 reached Stage III, it reverted back to Stage II after one day and then back to Stage III cycling back and forth until re-watering. In addition, the wilting seen in this genotype during Stage III was light compared with the other genotypes.

Visual Rating and Recovery of Transpiration

The NTR recovery could not be calculated for genotypes N05006 and PI 497579 in the one-day treatment of GH 1 and for genotypes PI 576636, PI 404020, ICGV 86388, and Georgia Green in the three-day treatment of GH 1 due to an error in the timing of re-watering. In GH 2, ICGV 86388 failed to germinate properly and provided limited replicate pots. As a result, the NTR recovery of ICGV 86388 could not be calculated for either the four or six-day treatment because one replicate pot in the four-day treatment failed to reach the endpoint before the experiment was terminated and a replicate pot in the six-day treatment was re-watered the day before the experiment was terminated. However, a visual rating for the six-day treatment for ICGV 86388 was still possible because the one replicate pot that was delayed was rated the day after re-watering but before the experiment was terminated. In addition, the NTR recovery of PI 497579 and N05008 could not be calculated for the six-day treatment of GH 2 because the experiment was terminated one day after they were re-watered. However, as in ICGV 86388, a visual rating for all genotypes was still possible for the six-day treatment.

Normalized Transpiration Ratio

In GH 1, significant differences in the recovery of NTR for the one-day treatment in Stage III stress were detected among genotypes two days after re-watering (Table 2a). The greatest NTR recovery from the one-day treatment under Stage III stress two days after re-watering was achieved by ICGV 86015, with a value of 0.92. Among the same genotypes that were repeated in the field experiment (except for N05006), in the one-day treatment under Stage III stress, Bailey had the greatest NTR recovery of 0.68 two days after re-watering while HTS 02-05 had the least NTR of 0.24. By the fourth day after re-watering, significant differences among genotypes could not be detected for the one-day treatment under Stage III stress.

Significant differences in NTR recovery for the three-day treatment under Stage III stress in GH 1 were also detected among genotypes (Table 2b) on Days 1 and 2 after re-watering. PI 497579 had the greatest NTR of 1.17 two days after re-watering while NC-V11 had the least NTR of 0.09 which remained unchanged for the three days that NTR was calculated. Among the same genotypes that were repeated in the field experiment (except for

Georgia Green), N05006 had the greatest NTR of 0.57 while Bailey had the least NTR of 0.05 two days after re-watering from the three-day treatment under Stage III stress.

Among the five genotypes that had NTR recovery calculated in GH 2, plants in the four and six-day treatment under Stage III stress had significant differences in NTR on the first day after re-watering (Table 3). Genotype PI 298639 had NTR above 1 by the first day of recovery and maintained a high NTR through the third day and was significantly different from HTS 02-05. Because of the extended six-day treatment and the different days in which plants reached Stage III, only NTR recovery data from PI 298639, TMV 2, and HTS 02-05 could be obtained for the six-day treatment. In this case, differences among these genotypes occurred for the first two days. TMV 2 was not significantly different from HTS 02-05 in the first day after re-watering, however was significantly greater than from HTS 02-05 on the second day. Only NTR data for TMV 2 could be obtained on the third day of recovery, however TMV 2 maintained a moderate NTR for all days in the six-day treatment under Stage III stress.

Visual Rating

Visual ratings were meant to evaluate the leaf maintenance of plants based on the approximate percentage of damaged leaves after re-watering from Stage III. In GH 1, significant differences in visual rating were only found in the three-day treatment (Table 4a). Genotype PI 497579 had the greatest rating of 5.0, *i.e.*, minimal leaf damage, in the three-day treatment while Bailey received the least rating of 1.0, *i.e.*, greater than 80% dead leaves.

The relationship between visual ratings and NTR recovery for the one and three-day treatments in GH 1 on Day 1 after re-watering was significantly correlated (Figure 1).

In GH 2, significant differences in visual rating were only detected for the six-day treatment under Stage III (Table 4b). ICGV 86388 and PI 298639 had visual ratings of 5.0 and were significantly different from HTS 02-05.

Field

Rainwater infiltrated one end of both shelters and replicate subplots under both rain-shelters experienced differential stress levels based on NGR throughout the experiment. As a result, only one or no subplots for each genotype reached the desired NGR of 0.11, *i.e.*, Stage III stress. An NGR of 0.11 was chosen because it would give the best estimation of plants in the field reaching Stage III based on the endpoint NTR of 0.11 in the greenhouse experiments. To estimate the recovery NGR from Stage III, a regression was done to extrapolate results to a stress of NGR = 0.11. Therefore, a linear regression between NGR one day before re-watering (NGR1) and NGR two days after re-watering (NGR2) using all six replications from the two water-deficit treatment plots was done for each genotype. The regressions allowed the influence of Stage III stress to estimate the NGR recovery on a common basis for all genotypes.

Recovery of Normalized Stomatal Conductance Ratio

The linear regression between NGR-before (NGR1) versus NGR-after (NGR2) re-watering yielded significant correlations of 0.74 or higher for five of the six genotypes tested

(Figure 3). When one data point is removed as an outlier from genotype N05008, a highly significant correlation of 0.97 ($p < 0.003$) was also obtained for this genotype. In genotype N05006, a correlation of 0.53 is significant at $p = 0.099$.

When 0.11 is substituted for x in each regression equation, a predicted NGR two days after re-watering is obtained (Table 5). N05006 had the greatest predicted NGR recovery of 0.61 while HTS 02-05 had the least with -0.1. The negative NGR in HTS 02-05 was indicative of a prediction of non-recovery from Stage III stress.

Discussion

Previous studies on peanut recovery from drought stress have either focused on low to moderate stress recovery or imposed a severe water-deficit stress treatment on peanut plants that is not well defined in terms of plant transpiration response to decreasing soil water (Awal and Ikeda, 2002; Lauriano et al., 2004; Puangbut et al., 2010). In this study, individual plant response to soil drying was characterized and used to determine when re-watering would occur so that recovery was from the same level of stress, *i.e.*, days subjected to Stage III stress.

Transpiration and Visual Rating

The objective of this study was to identify differences among peanut genotypes in their ability to recover from Stage III stress determined from visual ratings and recovery of transpiration. Plant recovery from Stage III remained fairly consistent among genotypes evaluated in both greenhouse experiments (Tables 2, 3, 4). NTR ranking among genotypes evaluated throughout both greenhouse experiments were fairly consistent. Genotypes TMV 2 and PI 497579 always had relatively high recovery NTR and high visual rating and almost always, HTS 02-05 had poor NTR and visual rating after re-watering. PI 404020 also had high NTR recovery and high visual rating. Our findings for PI 404020 (73-30) agree with a study by Lauriano et al. (2004) who also found that this genotype had high recovery of stomatal conductance after a water-deficit treatment. The consistency in visual ratings and the transpiration response of genotypes between both greenhouse experiments indicates that the mechanisms involved in recovery are stable throughout prolonged water-deficits.

Bailey had the greatest decrease in NTR to prolonged water-deficit in GH 1. In the one-day treatment under Stage III stress, Bailey had relatively high NTR recovery (0.68, Day 2); however after the three-day treatment it was not able to recover (NTR 0.05, Day 2). In addition the visual rating of Bailey dropped from 5 in the one-day treatment, to 1 in the three-day treatment under Stage III stress. It seems that while Bailey can have substantial recovery from only a brief exposure to Stage III, in the event of a prolonged water-deficit stress Bailey may not be able to recover. In contrast, TMV 2 maintained its ability to recover NTR between treatments and experiments. In addition, the visual rating of TMV 2 remained relatively high between experiments indicating the maintenance of leaves was high even as soil water was exhausted.

The visual rating was associated with the NTR recovery (Figure 2) when plotted against each other in GH 1. However, at least in the first few days, the NTR of genotypes does not recover as much as the visual ratings might indicate, especially for those with a rating between 4 and 5. This implies that the rehydration of leaves takes priority before full transpiration recovery is obtained (Lauriano et al., 2004) or that stomata have not completely responded to re-watering because of damaged guard cells or perhaps a chemical signal triggering this response.

The maintenance of leaves would be beneficial for recovery upon re-watering after a water-deficit stress however the plant is also exposed to greater amounts of radiation which may intensify the stress experienced. Lawn and Likoswe (2008) reported that higher recovery from 90% plant available water depletion was attributed to higher leaf area maintenance in soybean. Using a visual rating scale after re-watering provided a simple and practical way of evaluating leaf maintenance under Stage III stress. Compared with other legumes, peanut seems to better maintain its leaves when soil water is depleted (Likoswe and Lawn, 2008). Among the genotypes evaluated, TMV 2, SPT 06-07, ICGV 86388, and PI 298639 have the greatest maintenance of leaves under severe water-deficit which presumably may result in greater initial recovery of physiological function after re-watering. The maintenance of leaves during Stage III could be attributed to a decrease in chlorophyll content resulting in lower photo-oxidation (Havaux and Tardy, 1999), higher dehydration tolerance of leaves, *i.e.*, lower lethal relative water content (Flower and Ludlow, 1987), osmotic adjustment (Lilley and Ludlow, 1996; Ali-Ahmad and Basha, 1998), or dehydration avoidance by limiting water loss from the leaf epidermis, *i.e.*, low epidermal conductance (Sinclair and Ludlow, 1986). Further study to determine the mechanisms of leaf maintenance on genotypes which displayed a high visual rating after re-watering is warranted.

SPT 06-07 had an interesting recovery response. While SPT 06-07 had a high visual rating in GH 1 under both treatments, the NTR recovery was only moderate, and never was higher than 0.53 even in the latter days. Compared with other genotypes that achieved a similar visual rating, the NTR recovery was the least. SPT 06-07 seems to have a mechanism that maintains leaves during stress, quickly rehydrates after re-watering and retains a low NTR. This implies that leaves would be able to withstand the next drought event and this genotype may be best adapted to conditions of frequent severe drought events. This response however would restrict C uptake if transpiration is restricted, and ultimately limit growth and yield. In fact, this genotype has been reported to have low yields under drought while still retaining a visually healthy canopy (M. Balota, personal communication, 2012). The best response would be to have both a high visual rating (high leaf maintenance) and a high NTR recovery which would presumably give the greatest potential for recovery of metabolic function and photosynthesis upon re-watering. The genotypes with the greatest rating and NTR throughout both greenhouse experiments were TMV 2, ICGV 86388, PI 497579, and N05006.

In the field experiment, the range of stress experienced by replicate blocks under the shelters allowed for the use of a regression to predict the recovery of NGR of genotypes after re-watering from Stage III stress (Figure 4, Table 5). The regression model tended to overestimate the recovery in genotypes when compared to NTR recovery in GH 1 except in HTS 02-05. This was probably because stress in the GH became more severe the longer the plant remained under Stage III stress, while plants in the field were not necessarily stressed to that level of severity. However, overall, the predicted field results were fairly consistent with the greenhouse results. Bailey and HTS 02-05 were predicted to have relatively poor NGR recovery while N05006 was predicted to have good recovery with similar results for NTR seen in GH 1. An exception was Bailey during the one-day treatment in GH 1, which as previously mentioned seems to have substantial recovery if re-watering is done early into Stage III. Therefore, the results in the field give further support for genetic variation for recovery from Stage III and for the stability of mechanisms which impart drought tolerance and recovery under different environmental conditions.

We have presented evidence for consistent differential recovery from Stage III stress in both greenhouse studies among peanut genotypes. In addition, the field experiment complemented the results found in the greenhouse experiments based on the predicted stomatal conductance recovery for selected genotypes. We report genetic variation for recovery from Stage III in the peanut germplasm. The stability of the differential response of genotypes after re-watering throughout varying experimental conditions is encouraging for further study of possible mechanisms leading to improved recovery from Stage III stress.

REFERENCES

Ali-Ahmad, M., and Basha, S. (1998). Effect of water stress on composition of peanut leaves. *Peanut Science, 25*(1), 31-34.

Awal, M. A., and Ikeda, T. (2002). Recovery strategy following the imposition of episodic soil moisture deficit in stands of peanut (*Arachis hypogaea* L.). *Journal of Agronomy and Crop Science, 188*(3), 185-192.

DaCosta, M., and Huang, B. (2007). Drought survival and recuperative ability of bentgrass species associated with changes in abscisic acid and cytokinin production. *Journal of the American Society for Horticultural Science, 132*(1), 60-66.

Devi, M. J., Sinclair, T. R., and Vadez, V. (2010). Genotypic variability among peanut (*Arachis hypogaea* L.) in sensitivity of nitrogen fixation to soil drying. *Plant and Soil, 330*(1), 139-148.

Devi, M. J., Sinclair, T., Vadez, V., and Krishnamurthy, L. (2009). Peanut genotypic variation in transpiration efficiency and decreased transpiration during progressive soil drying. *Field Crops Research, 114*(2), 280-285.

Flower, D., and Ludlow, M. M. (1986). Contribution of osmotic adjustment to the dehydration tolerance of water-stressed pigeon pea (*Cajanus cajan* (L.) Millsp.) leaves. *Plant, Cell and Environment, 9*(1), 33-40.

Havaux, M., and Tardy, F. (1999). Loss of chlorophyll with limited reduction of photosynthesis as an adaptive response of syrian barley landraces to high light and heat stress. *Functional Plant Biology, 26*(6), 569-578.

James, A., Lawn, R. J., and Cooper, M. (2008a). Genotypic variation for drought stress response traits in soybean. I. Variation in soybean and wild *Glycine* spp. for epidermal conductance, osmotic potential, and relative water content. *Australian Journal of Agricultural Research*, 59, 656-669.

James, A., Lawn, R. J., and Cooper, M. (2008b). Genotypic variation for drought stress response traits in soybean. II. Inter-relations between epidermal conductance, osmotic potential, relative water content, and plant survival. *Australian Journal of Agricultural Research*, 59, 670-678.

Lauriano, J. A., Ramalho, J., Lidon, F., and do Céu Matos, M. (2004). Peanut photosynthesis under drought and re-watering. *Photosynthetica, 42*(1), 37-41.

Lawn, R. J., and Likoswe, A. A. (2008). Genotypic differences in leaf area maintenance contribute to differences in recovery from water stress in soybean. *Crop and Pasture Science, 59*(12), 1075-1085.

Likoswe, A. A., and Lawn, R. J. (2008). Response to terminal water deficit stress of cowpea, pigeonpea, and soybean in pure stand and in competition. *Crop and Pasture Science, 59*(1), 27-37.

Lilley, J., and Ludlow, M. M. (1996). Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. *Field Crops Research, 48*(2), 185-197.

Muchow, R. C., and Sinclair, T. R. (1989). Epidermal conductance, stomatal density and stomatal size among genotypes of *Sorghum bicolor* (L.) Moench. *Plant, Cell and Environment, 12*(4), 425-431.

Puangbut, D., Jogloy, S., Toomsan, B., Vorasoot, N., Akkasaeng, C., Kesmala, T., Rachaputi R.C.N., Wright, G.C. Patanothai, A. (2010). Drought Stress: Physiological basis for genotypic variation in tolerance to and recovery from pre-flowering drought in peanut. *Journal of Agronomy and Crop Science, 196*(5), 358-367.

Ray, J. D., and Sinclair, T. R. (1997). Stomatal closure of maize hybrids in response to drying soil. *Crop Science, 37*(3), 803-807.

Sinclair, T. R., and Ludlow, M. M. (1985). Who taught plants thermodynamics? the unfulfilled potential of plant water potential., *Functional Plant Biology, 12*(3), 213-217.

Sinclair, T. R., and Ludlow, M. M. (1986). Influence of soil water supply on the plant water balance of four tropical grain legumes. *Functional Plant Biology, 13*(3), 329-341.

Table 1. For all peanut genotypes evaluated in this study, information on the origin, type or species, use, whether they have been released, and in which experiment they were used is provided in this table.

Genotype	Use	Type/species	Origin	Release	Greenhouse/Field
N05006	Breeding line	Virginia	NCSU	Unreleased	Field, GH 1
SPT 06-07	Breeding line	Species-derived*	NCSU	Unreleased	Field, GH 1
Bailey	Commercial	Virginia	NCSU	Released	Field, GH 1
Georgia Green	Commercial	Runner	Univ. of GA	Released	Field, GH 1
HTS 02-05	Breeding line	Species-derived*	NCSU	Unreleased	Field, GH 1, 2
N05008	Breeding line	Virginia	NCSU	Unreleased	Field, GH 1, 2
N04074FCT	Breeding line	Virginia	NCSU	Unreleased	GH 1
ICGV 86015	Commercial	Spanish	ICRISAT	Released	GH 1
NC-V11	Commercial	Virginia	NCSU	Released	GH 1
HTS 02-01	Breeding line	Species-derived*	NCSU	Unreleased	GH 1
Phillips	Commercial	Virginia	NCSU	Released	GH 1
PI 433525	Germplasm	Peruviana	Peru	-	GH 1
CHAMPS	Commercial	Virginia	VPIandSU	Released	GH 1
PI 576636	Germplasm	Hirsuta	Mexico	-	GH 1
PI 404020	Germplasm	Spanish	Senegal	-	GH 1
PI 497579	Wild species	*A. stenosperma*	Brazil	-	GH 1, 2
ICGV 86388	Commercial	Spanish	ICRISAT	Released	GH 1, 2
TMV 2	Commercial	Spanish	Tamil Nadu, India	Released	GH 1, 2
PI 298639	Wild species	*A. batizocoi*	Bolivia	-	GH 2

*Can be traced back to a wild species within a few generations

Table 2. Daily normalized transpiration ratio (NTR) for all genotypes evaluated in Greenhouse Experiment 1 after re-watering from Stage III. Plants were subjected to a) one day and b) three days under Stage III before re-watering. Significant differences over all genotypes were detected by analysis of variance test in Day 2 (n=3 or 4). Tukey's HSD test was performed on genotypes repeated in the Greenhouse Experiment 2. Genotypes followed by the same letter are not statistically different (n=3 or 4) for NTR.

a) One-Day Stage III Stress

Genotype	Day 1	Day 2	Day 3	Day 4	Day 5
ICGV 86015	0.64 ± 0.09	0.92 ± 0.34	0.89 ± 0.13	-	-
PI 404020	0.53 ± 0.04	0.88 ± 0.07	0.83 ± 0.04	0.91 ± 0.07	0.92 ± 0.01
TMV 2	0.64 ± 0.02 ab	0.73 ± 0.10 a	0.71 ± 0.07 a	0.87 ± 0.10 a	-
Bailey	0.56 ± 0.11	0.68 ± 0.09	0.71 ± 0.10	0.77 ± 0.10	0.79 ± 0.11
ICGV 86388	0.75 ± 0.17 a	0.59 ± 0.05 a	0.67 ± 0.04 a	0.84 ± 0.06 a	0.87 ± 0.06
PI 576636	0.48 ± 0.19	0.51 ± 0.16	0.46 ± 0.16	0.58 ± 0.17	0.72 ± 0.11
PI 433525	0.55 ± 0.09	0.51 ± 0.04	0.56 ± 0.02	0.63 ± 0.02	0.75 ± 0.07
Georgia Green	0.67 ± 0.33	0.50 ± 0.22	0.58 ± 0.30	0.60 ± 0.27	-
SPT 06-07	0.34 ± 0.03	0.41 ± 0.03	0.43 ± 0.02	0.53 ± 0.03	-
Phillips	0.30 ± 0.05	0.39 ± 0.16	0.43 ± 0.18	0.46 ± 0.20	0.60 ± 0.21
N05008	0.32 ± 0.10 ab	0.34 ± 0.17 a	0.37 ± 0.17 a	0.44 ± 0.20 a	0.51 ± 0.21
N04074FCT	0.29 ± 0.05	0.34 ± 0.09	0.34 ± 0.09	0.41 ± 0.11	-
CHAMPS	0.26 ± 0.09	0.33 ± 0.17	0.37 ± 0.21	0.44 ± 0.24	-
NC-V11	0.25 ± 0.13	0.26 ± 0.11	0.37 ± 0.15	0.49 ± 0.14	0.45 ± 0.19
HTS 02-05	0.17 ± 0.14 b	0.24 ± 0.16 a	0.22 ± 0.19 a	0.30 ± 0.24 a	-
HTS 02-01	0.64 ± 0.25	0.24 ± 0.12	0.31 ± 0.18	0.38 ± 0.17	0.32 ± 0.17
ANOVA	-	*	-	-	-

*Significant at $p < 0.05$

Table 2 continued

b) Three-Day Stage III Stress

Genotype	Day 1	Day 2	Day 3
PI 497579	0.81 ± 0.25 a	1.17 ± 0.30 a	-
N05006	0.38 ± 0.11	0.57 ± 0.11	0.52 ± 0.22
ICGV 86015	0.30 ± 0.03	0.53 ± 0.18	-
TMV 2	0.28 ± 0.09 a	0.53 ± 0.06 ab	-
PI 433525	0.31 ± 0.10	0.41 ± 0.15	-
Phillips	0.35 ± 0.07	0.38 ± 0.21	0.43 ± 0.38
SPT 06-07	0.48 ± 0.09	0.35 ± 0.09	-
CHAMPS	0.26 ± 0.10	0.34 ± 0.16	0.34 ± 0.40
N05008	0.31 ± 0.12 a	0.29 ± 0.21 b	-
HTS 02-05	0.34 ± 0.08 a	0.28 ± 0.12 b	-
HTS 02-01	0.19 ± 0.07	0.21 ± 0.09	0.10 ± 0.06
NC-V11	0.09 ± 0.02	0.09 ± 0.03	0.09 ± 0.05
Bailey	0.18 ± 0.04	0.05 ± 0.03	-
N04074FCT	0.11 ± 0.01	0.05 ± 0.02	0.08 ± 0.03
ANOVA	**	***	-

**Significant at $p < 0.01$. ***Significant at $p < 0.001$

Table 3. Normalized transpiration ratios for five peanut genotypes in Greenhouse 2 for the days following re-watering from Stage III. Water was withheld for four days in the first treatment and for six days in the second treatment after plants reached Stage III. Genotypes with NTR followed by the same letter are not significantly different (n=3 or 4).

Genotype	**4-d under Stage III Stress**			**6-d under Stage III Stress**		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
PI 298639	1.1 a	1.05 a	1.04 a	1.09 a	-	-
PI 497579	0.84 ab	0.95 a	1.17 a	-	-	-
TMV 2	0.51 ab	0.68 a	0.81 a	0.46 b	0.51 a	0.63
N05008	0.5 ab	0.58 a	0.62 a	-	-	-
HTS 02-05	0.37 b	0.61 a	0.82 a	0.08 b	0.05 b	-

Table 4. Visual rating of genotypes one day after re-watering for both treatments in a) Greenhouse 1 and b) Greenhouse 2. A rating of 1 meant that more than 80% of leaves were dead after re-watering while a rating of 5 meant that minimal leaf damage from stress was observed after re-watering (see text for details). Significant differences over all genotypes were detected in the second treatment by analysis of variance.

a) Greenhouse 1

	Days under Stage III Stress	
Genotype	1 Day	3 Days
PI 497579	-	5
SPT 06-07	5	4.67
PI 433525	5	4.5
TMV 2	4.5	4.25
N05006	-	4.25
Phillips	3	3.67
ICGV 86015	5	2.75
CHAMPS	3	2.75
NC-V11	3	2
HTS 02-05	2.33	1.75
HTS 02-01	3.33	1.75
N04074FCT	3.67	1.67
N05008	2.75	1.5
Bailey	5	1
PI 576636	3.67	-
PI 404020	4.75	-
ICGV 86388	4.75	-
Georgia Green	3.67	-
ANOVA	-	***

***Significant at $p < 0.001$

b) Greenhouse 2

	Days under Stage III Stress	
Genotype	4 Days	6 Days
ICGV 86388	-	5.00 a
PI 298639	4.33 a	5.00 a
TMV 2	5.0 a	4.33 ab
PI 497579	4.33 a	3.67 ab
N05008	3.67 a	3.00 ab
HTS 02-05	4.5 a	1.00 b

Genotypic ratings followed by the same letter are not significantly different ($p < 0.05$).

Table 5. Regression equation of the NGR before and after re-watering for each genotype and its predicted NGR recovery from theoretical Stage III (endpoint, x = 0.11).

Genotype	**Regression Equation**	**Predicted NGR Recovery from Stage III**
Bailey	y = 0.027 + 1.64x	0.21
Georgia Green	y = 0.39 + 0.67x	0.46
HTS 02-05	y = -0.25 + 1.37x	-0.1
N05006	y = 0.58 + 0.25x	0.61
N05008	y = 0.34 + 0.84x	0.43
SPT 06-07	y = 0.55 + 0.44x	0.60

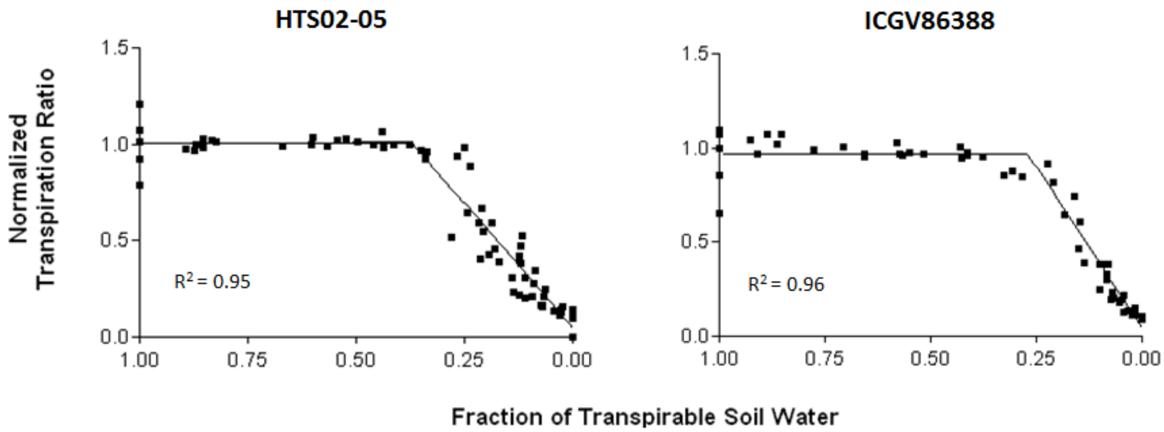

Figure 1. Representative fraction of transpirable soil water (FTSW) curve of two genotypes with contrasting responses after re-watering from Stage III: HTS 02-05 and ICGV 86388. Where the two linear portions intersect is the initiation of Stage II which begins at 0.37 FTSW for HTS 02-05 and 0.27 for ICGV 86388. Stage III begins as the NTR reaches 0.11, at which point FTSW is 0.0.

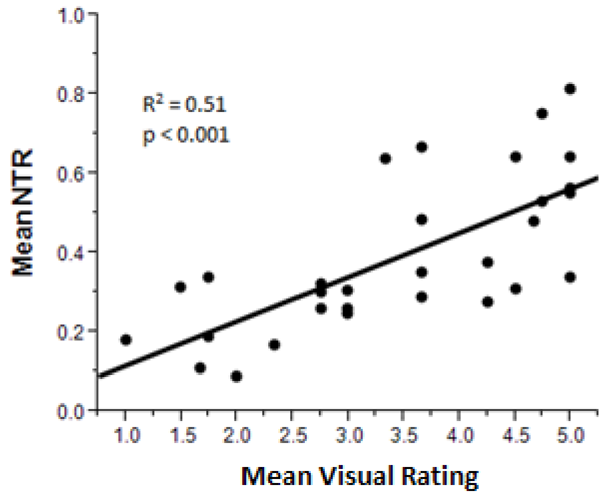

Figure 2. Relationship between all genotypic mean visual ratings and NTR of both treatments in GH 1 on Day 1 after re-watering.

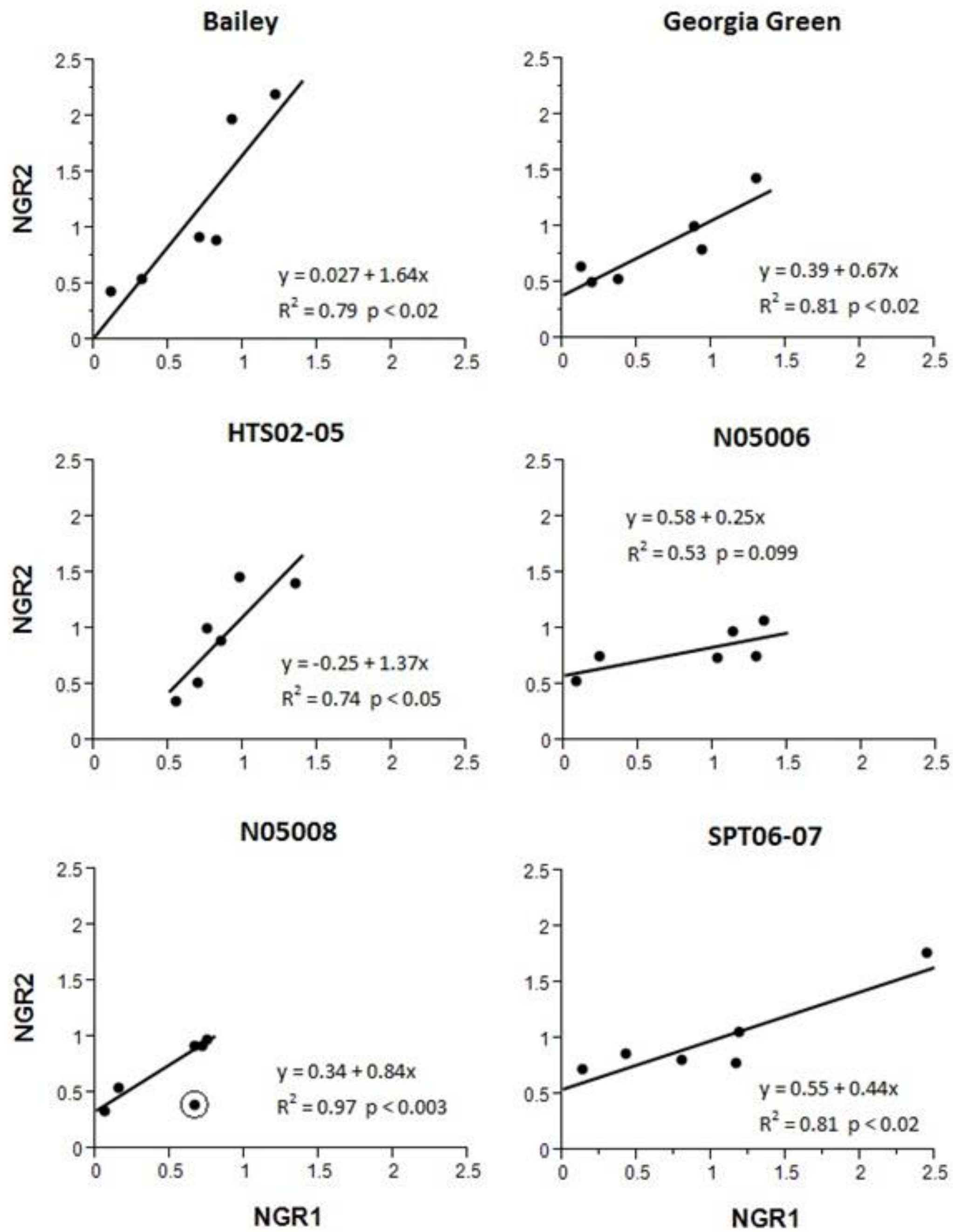

Figure 3. Linear regression of normalized stomatal conductance ratio the day before re-watering (NGR1) versus normalized stomatal conductance ratio two days after re-watering (NGR2) for six peanut genotypes in the field. Circled data point in N05008 was excluded from the regression.

CHAPTER 3

Genetic Variation for Epidermal Conductance in Peanut

Introduction

Some crops are continuously grown in areas were severe droughts may occur. Survival and subsequent recovery from such conditions becomes an increasingly important factor for crop productivity. This may be the case with peanut (*Arachis hypogaea* L.), where it is grown in drought-prone areas and on sandy soils. Research on physiological mechanisms in peanut for survival and the ability to recover from severe drought are therefore warranted to improve the chances of avoiding crop loss and improving recovery during times of intermittent drought and rainfall.

When soil water is exhausted during a severe drought event and plants can no longer adjust stomata to limit transpirational water loss, limitation on leaf water loss is dictated by the vapor resistance of the leaf epidermis and the difference in vapor pressure between the leaves and atmosphere (Muchow and Sinclair, 1989). At this point, the extraction of water by plants from the soil will be minimal and insufficient to keep leaves hydrated. During a severe drought event, a plant's ability to survive for any period of time may depend on its ability to delay leaves from reaching a lethal dehydration state by limiting water loss from the leaf epidermis (Flower and Ludlow, 1986; Sinclair and Ludlow, 1986; James et al., 2008).

Epidermal conductance (EC) refers to the water vapor conductance of the leaf epidermis after stomata have reached minimum aperture in response to an exhausted soil water supply. Plants with low EC experience less water loss after stomata are closed and as a result may be considered more tolerant of severe drought (Sinclair and Ludlow, 1986; Muchow and Sinclair, 1989; Saito and Futakuchi, 2010).

The EC of several crop plants has been extensively studied. Genetic variability has been reported in soybean (*Glycine max* (L.) Merr.) (James et al., 2008), cotton (*Gossypium hirsutum* L.) (Quisenberry et al., 1982), sorghum (*Sorghum bicolor* L.) (Muchow and Sinclair, 1989), wheat (*Triticum turgidum* var durum L.) (Araus et al., 1991), and rice (*Oryza* spp.) (Saito and Futakuchi, 2010). Other studies have also looked at EC in sabal palm (*Sabal palmetto* (Walt.) Lodd) (Holbrook and Sinclair, 1992), two species of grasses (Smith et al., 2006), and in three other tropical legumes (Sinclair and Ludlow, 1986) for drought tolerance. Table 1 shows the range of EC among the crops mentioned.

The potential of low EC to extend plant survival has long been implicated. Sinclair and Ludlow (1986) evaluated the length of survival in four tropical legumes under a severe water-deficit treatment. They found that cowpea survived the longest, followed by pigeonpea, black gram, and soybean. Coincidently, cowpea and pigeonpea had very low EC while black gram had moderate EC values and soybean had the greatest EC. The low EC values of 2.73-3.85 mmol m^{-2} s^{-1} in desert grasses (Smith et al., 2006) and EC of 0.32-0.56 mmol m^{-2} s^{-1} in sabal palm (Holbrook and Sinclair, 1992) indicate low EC is an adaptation to extreme drought or normally dry areas. Sinclair (2000) looked at two traits for modeling plant survival: low EC and lethal relative water content. He found that it is first necessary to obtain low EC and then decrease lethal relative water content in order to significantly extend plant survival. Surprisingly, there have been little to no reports on the EC of peanut and its implication on breeding for severe drought tolerance.

Epidermal conductance is sensitive to environmental conditions. Quisenberry et al. (1982) found that EC decreased in dryland cotton vs. irrigated cotton and that the range in EC was greater under lower water availability. Soybean has also been found to have reduced EC under water-deficit stress (Paje et al., 1988). The differential sensitivity of EC to changes in different environmental parameters (Holmgren et al., 1965; James et al., 2008) lend support that EC is influenced by multiple morphological (leaf width and leaf area), physiological (increased epicuticular wax load in response to water stress) and/or anatomical parameters (specific leaf weight, stomatal density) (Araus et al., 1991). Because these parameters in the leaf are interrelated in response to the environment and especially to drought stress, it is necessary to understand the sensitivity of EC to environmental changes and how they may be associated with the aforementioned leaf parameters.

The objectives of this study were i) to determine the genetic variability for EC in 22 peanut genotypes, ii) to investigate the effect of water-deficit on the stability of EC, and iii) to investigate two anatomical characteristics of peanut: stomatal density and specific leaf weight and their association with EC. To fulfill these objectives two greenhouse experiments were conducted to evaluate the EC of well-watered plants and one field experiment was conducted to verify greenhouse results and further evaluate EC of peanut plants experiencing a water-deficit treatment.

Materials and Methods

Two greenhouse experiments were conducted at North Carolina State University, Raleigh, NC and a field experiment at Tidewater Agricultural Research and Extension Center in Suffolk, VA to screen for genetic variation in epidermal conductance of peanut. The genotypes were chosen based on drought tolerance, origin, commercial status, and seed availability. Detailed information for all genotypes is presented in Table 2. Six genotypes were evaluated in Greenhouse 1 Experiment (GH 1), fourteen in Greenhouse 2 Experiment (GH 2) and six in the Field Experiment.

Plant Material and Culture

Greenhouse Experiment 1

GH 1 was designed to evaluate the EC of five genotypes picked from a preliminary study that were found to have contrasting responses after re-watering from severe water-deficit stress and one additional genotype not previously evaluated. Genotype PI 298639 was added because it represented a different species of peanut (*A. batizocoi* Krapov. & W.C. Gregory) and widened the genetic diversity of the material studied. Three seeds from each genotype were sown in 2.8 L, 16.5 cm tall plastic pots in June 2011. Pots were prepared by placing a screen mesh in the inside bottom to prevent soil from leaching through drainage holes and then filled with a sandy loam soil to about 2.5 cm from the top. Each genotype was represented by four replicate pots. Two weeks after sowing, pots were thinned to 1 plant per pot keeping all pots as uniform as possible. The greenhouse was sprayed with a pesticide once every two weeks to control thrips. Later in the season, flowers were removed daily to keep plants in the vegetative stage.

Greenhouse Experiment 2

GH 2 was designed to evaluate the extent of variation in EC and stomatal density of fourteen peanut genotypes. Two genotypes with contrasting EC values were selected from GH 1 plus twelve genotypes representing mostly commercial types and breeding lines (Table 2). Thirteen genotypes were Virginia types and one was a Spanish type. Plants were sown in March 2012 and maintained as those in GH 1. The soil used in GH 1 was recycled and used in this experiment. All genotypes were represented by four replicate pots arranged in a randomized block design. Plants were kept well-watered for the duration of the experiment.

Field Experiment

A field experiment was conducted to compare with results from the greenhouse studies. Six genotypes were selected from a preliminary study that were found to have contrasting response to severe soil water-deficit, and also based on seed availability (Table 2). The preliminary study is described in detail in Chapter 2 and initially screened 18 peanut genotypes for recovery from soil drying. Seeds were sown in 3.5-m x 2-m subplots on 14 May 2012 on a sandy soil at a depth of 3.8 cm in four rows spaced at 0.9 m. Due to poor machine sowing, most of the plots were re-sown by hand 3 weeks later. The field was divided into three plots: two plots were designated as water-deficit stress treatment and one as a well-watered treatment. The stress treatment was split into two plots in order to accommodate the size of the rain-shelters used to impose a water-deficit stress. Two border rows on each side of the plots were also sown. Each plot was divided into three replicate blocks which contained six randomized subplots to accommodate six peanut genotypes. The water-deficit stress treatment contained in two plots was represented by a total of six replicate blocks. There was a gentle downward slope from west to east in the field. Wooden boards were positioned on the outside of the uphill blocks to prevent water run-off from infiltrating the rain-shelters. The downhill end of the rain-shelters remained unprotected and water became trapped between the downhill end of the rain-shelters and an adjacent hill that separated the field and a nearby road.

When the plant canopies of all genotypes reached at least 80% closure (6 August 2012), two rain-shelters measuring 21.5 m in length and 6 m wide were moved over each stress plot while the plot designated as well-watered was not covered. Each shelter took at least a day to move and as a result the second stress plot received an additional 3.5 cm of rain. The structural frame of the rain-shelters was aluminum and shaped like a gable and covered with 0.15 mm thick polyethylene (Atlas Manufacturing, Atlanta, GA). The rain-shelters remained over the plots for the duration of the experiment. Stress plots were irrigated on the 39th day of the experiment when midday stomatal conductance measurements indicated that conductance was only 0.11 or less of the well-watered plot in at least one replicate block. The well-watered plot was kept well-watered by continuous rains experienced throughout the duration of the experiment.

Stomatal conductance (Gs) was measured on the following days after the rain-shelters were moved into position: Day 7 and 8 (one measurement day during a 2-d period due to weather conditions), 18, 21, 31 and 32 (one measurement day during a 2-d period due to weather conditions), 35, and 38. Stomatal conductance was measured on two leaflets of each subplot using a LI-6400 (LICOR, Inc., Lincoln, NE). This occurred around mid-day, usually between 1000 and 1500 EST. A Gs ratio was calculated for each genotype replicate subplot by dividing the Gs of each subplot by the mean Gs of the three corresponding well-watered replicate subplots. Two data loggers collecting temperature information every five minutes were equally spaced within each plot. The experiment ended on Day 41 when the last EC measurements were taken.

Leaf Sampling

In both greenhouse experiments, three youngest fully expanded leaves from the main stem of each well-watered plant were collected between 1400 and 1600 EST. Leaves were collected in plastic bags with high humidity to prevent water loss while being transported to the laboratory and were immediately refrigerated upon reaching the laboratory. Epidermal conductance (EC) measurements were performed within 24 hours in GH 1 and within 48 hours in GH 2. Once EC was measured, leaves were kept in the refrigerator for later determining stomatal density.

In the field experiment, leaflets were sampled on five occasions for the well-watered plot and three on the water-deficit stress plots. Two representative leaflets were sampled from the middle two rows of each subplot and immediately placed in bags with high humidity. Sampling usually was performed by replicate blocks across plots to minimize temporal variation. Generally, sampling was done between 0930 and 1200 EST. Leaflets were immediately refrigerated upon reaching the laboratory and EC was always measured within 12 hours of sampling. For stomatal density determinations, the same leaflets were also used once EC had been measured. Following EC measurements on leaflets, they were put in paper bags and oven-dried at 60 C for 24 h for specific leaf weight determinations.

Epidermal Conductance Measurements

The procedure described by Sinclair and Ludlow (1986) was used to determine epidermal conductance (EC). Leaf area was first measured using a LI-3000 (LICOR Inc., Lincoln, Nebraska). Moist leaves were then dried with paper towels and placed in semi-transparent 32.2-L chambers filled with a layer of drierite at the bottom. The drierite had been previously dried in an oven at about 65 C for 24 h. Leaves were laid on a metal grate inside the chamber exposing both surfaces to the air but without making direct contact to the drierite (W.A. Hammond Drierite Company Ltd., Xenia, OH). About 35 leaves per chamber could be measured at one time. In the field experiment, leaflets were used instead and each chamber held about 55 leaflets during the measurements. Each leaf or leaflet was weighed on a digital balance every 20 min for the greenhouse experiments and 30 min for the field experiment over about 150 min while keeping the chamber closed as much as possible. Temperature in the room remained at or approximately 25C.

Epidermal conductance was determined from the plot of a leaf/leaflet weight over time. Sinclair and Ludlow (1986) interpreted the multiphasic result, with the first phase showing an initial decrease in weight as the time in which stomata close in response to decreasing water content. The second phase of the graph, which was linear, was interpreted to be the slow linear decline in weight loss due to epidermal conductance once stomata had reached minimum aperture. A regression was performed on the second phase of the graph and the slope of the regression equation was used to calculate EC. In the greenhouse experiments, the mean slope of replicate plants was used to calculate genotypic EC values. In the field, the mean slope of replicate subplots was used to calculate genotypic EC values. The following formula was used to calculate epidermal conductance:

$$EC = \frac{\Delta W}{T} * \frac{1}{A} * \frac{1}{\Delta E} * 2.31x10^6$$

Where EC is epidermal conductance (mmol m^{-2} s^{-1}), ΔW is the weight change (g) of a leaf over time 'T' (s); A is leaf area (m^2), ΔE is the difference in absolute humidity between the air inside of the chamber and the leaf. The absolute humidity of water vapor in the leaf at 25 C is 1280 mmol m^{-3} and was used as the value for ΔE because the absolute humidity of the air in the chamber is less than 1 mmol m^{-3}. Finally, 2.31 x 10^6 is a coefficient with the units mmol water x mmol air x g^{-1} water x m^{-3} air (Sinclair and Ludlow, 1986). After EC was determined in the field, leaves were oven dried at 60C for 24 h and subsequently weighed. Specific leaf weight (SLW) was calculated by dividing the dry weight (mg) of each leaflet by the leaflet area (cm^2).

Stomatal Density Determination

For both greenhouse experiments, leaves which had been collected for EC measurements were used to determine stomatal density (SD). A section of the epidermal layer of both adaxial and abaxial surfaces was removed with a scalpel and placed under the microscope. Because there were no significant differences between abaxial and adaxial surfaces for any genotypes, only abaxial epidermis was counted in GH 2.

Direct counts were made on photographs taken of the area under the microscope of 0.332 mm2. Any stoma on the edge of the microscope's viewing area that looked like more than 50% of its area was visible was counted as one.

For the field experiment, SD was determined on two sampling occasions for well-watered subplots (Day 31 and Day 36) on selected genotypes. Imprints of the epidermal layer were done by gently applying nail polish to the abaxial surface of leaflets and allowing them to dry for approximately 3 min (Balota et al., 2012). A piece of clear tape was attached to the area where nail polish had been applied and subsequently removed gently. The tape removed the nail polish along with an imprint of the epidermal layer of the leaflet and was then taped to a microscope slide to be viewed under the microscope. Stomatal counts were made directly from photographs taken of 0.338 mm^2 of leaflet area in the same way as the leaves from the greenhouse experiments were counted.

Statistical Analysis

Tukey's HSD test was used to compare means among genotypic EC and SD. Data for SD was not statistically different between the two sampling dates in the field and in order to compare all genotypes, replicate data was pooled for each genotype to determine mean differences.

A linear regression was used for plotting genotypic mean EC values vs. SD and EC vs. SLW for the well-watered treatment and positions under the rain-shelters. For SLW, temporal data was combined when performing linear regression. All statistical analyses were conducted on the statistical software JMP 10.01 (SAS Institute, Cary, NC).

Results

Epidermal Conductance

Greenhouse Experiments

Leaf water loss showed the expected multiphasic curve shown in previous studies (Muchow and Sinclair, 1989; James et al., 2006) (Figure 1). Significant differences for EC were apparent among the six genotypes evaluated in GH 1 (Table 3). *Arachis stenosperma* genotype PI 497579 had the greatest EC of 3.17 mmol m^{-2} s^{-1} and TMV 2 and PI 298639 had the least EC values of 1.72 and 1.71 mmol m^{-2} s^{-1}, respectively.

The range in EC between GH 1 and 2 was similar. In GH 2, genotype CHAMPS had the greatest EC of 3.67 mmol m^{-2} s^{-1} while VA 98R had the least with 2.16 mmol m^{-2} s^{-1}. While a moderate range was evident in GH 2, significant differences among genotypes were very limited (Table 3). Only two genotypes with the smallest EC were different from two genotypes with the largest EC in GH 2.

Field Experiment

The field was at a gentle slope and consistent rains led to water infiltrating the downhill edge of each shelter. As a result, plants in the downhill replicate block in each shelter and plants in the middle replicate block in each shelter developed little to no water-deficit stress as evidenced by Gs measurements (Figure 2). To evaluate any water-deficit stress effects on peanut, the water-deficit stress treatment plots were divided into three positions: Uphill, middle and downhill replicate blocks. Plants in the middle and downhill blocks retained a Gs ratio of about 1.0 or higher throughout the experiment while plants in the uphill blocks showed signs of water-deficit stress by Day 21. Plants in the uphill blocks reached the least Gs ratio of 0.11 by Day 35. As a result, EC comparisons among genotypes experiencing a water-deficit stress could not be accomplished. However, genotype subplots in each position of the water-deficit stress treatment plots were taken as replicates and compared among positions in order to determine the effect of water-deficit stress on EC in peanut as a whole.

Significant differences in EC occurred in the well-watered treatment plot on Day 1 between HTS 02-05 and all genotypes except N05008; however no significant differences could be detected thereafter (Table 4). While no significant increase in EC was detected between Days 1 and 18 in the well-watered plot, a significant increase in EC occurred in all genotypes except N05006 from Day 18 to Day 41. However, genotype N05006 did experience a significant increase in EC between Days 18 and 36.

The comparison among positions in the water-deficit stress treatment for peanut yielded significant differences on Day 1 and Day 41 (Table 5). In addition, all positions had significant decreases in EC between Day 1 and Day 41 compared with the well-watered treatment plot.

Daily high air temperatures throughout the experiment were similar both inside and outside the rain-shelters (Figure 3).

Stomatal Density

Significant differences among genotypes were observed for stomatal density (SD) in both greenhouse experiments and in the field experiment for the well-watered treatment (Table 6). In GH 1, genotype PI 497579 had the greatest SD with 301 mm^{-2} which was almost twice the SD of the other five genotypes. In GH 2, the range in SD was 137 to 186 mm^{-2} with genotype N04074FCT having the greatest and Gregory the least SD. In the Field, SPT 06-07 had the greatest SD and was significantly different from the last two genotypes. The correlation between EC and SD is shown on Figure 4 and the correlation between EC and SLW is shown on Figure 5.

Discussion

Genetic Variation for Epidermal Conductance

The first objective of this study was to identify genetic variation in peanut EC. Significant differences in EC among genotypes were detected in both greenhouse experiments and to a less extent in the field (Tables 3, 4). The range in EC for GH 1 was 1.71 to 3.17 mmol m^{-2} s^{-1} while in GH 2 it was 2.16 to 3.67 mmol m^{-2} s^{-1}. The range for EC in the field for the well-watered plot at the start of the experiment was 6.30 to 9.09 mmol m^{-2} s^{-1}. A moderate yet significant range in EC among the greenhouse experiments and the Field Experiment give strong evidence for genetic variation for EC in peanut. However, it appears the variation is somewhat limited and more erratic in the field. The variation among blocks in the well-watered plot made it difficult to statistically determine differences after Day 1 despite the EC in HTS 02-05 being 18.02 mmol m^{-2} s^{-1} and SPT 06-07 being 12.24 mmol m^{-2}

s^{-1} on Day 41. A study involving more genotypes and more replicate plots in the field may produce greater significant differences among peanut genotypes in the field.

The relative ranking of HTS 02-05 in the field remained fairly consistent among days. HTS 02-05 had the greatest EC in Day 1 and Day 41 and was ranked among the top three of genotypes having the greatest EC in all other days. Therefore, based on this trait, HTS 02-05 would be less suited for environments where severe droughts occur. We reported in Chapter 2 that in almost all cases, most leaves in HTS 02-05 became necrotic during severe water-deficit stress. It seems the high EC reported for HTS 02-05 in this study is able to at least in part explain the inability of HTS 02-05 to maintain leaves during severe water-deficit stress. Though SPT 06-07 was not evaluated in the greenhouse, it consistently had the least or second least EC in almost all cases in the field. Bailey was also moderately consistent in having low EC in most cases. Therefore, based on the low EC of these genotypes in the field, SPT 06-07 and Bailey may be well suited for environments where severe droughts may occur. Indeed, it was reported in Chapter 2 that SPT 06-07 was able to greatly maintain leaves during severe water-deficit stress and rehydrate after re-watering even after prolonged water-deficit stress. Low EC would mean delayed leaf dehydration in this genotype improving the ability to maintain leaves during severe water-deficit stress. In the study in Chapter 2, Bailey was also able to maintain leaves during severe water-deficit stress, however when the stress was prolonged most leaves in Bailey became necrotic. SPT 06-07 may have additional mechanisms for leaf retention such as higher relative water content when severe stress begins or has greater dehydration tolerance, *i.e.,* lower lethal relative water content which gives SPT 06-07 greater survivability and recovery from prolonged droughts than Bailey. In addition, results from Chapter 2 indicate that EC may have greatly improved the recovery potential of TMV 2 and PI 298639 by delaying leaf dehydration. These two genotype showed superior recovery from severe soil drying while having low EC values of 1.72 and 1.71 mmol m^{-2} s^{-1} in GH 1. Lawn and Likoswe (2008) also reported a similar result in a soybean cultivar and concluded that leaf retention on the plant due to low EC substantially improved crop survival and subsequent recovery from severe water-deficit.

Interestingly, genotype PI 497579 also had good recovery (Chapter 2) yet the EC measured in this study was among the greatest of genotypes in both greenhouse experiments. It appears this genotype may be utilizing alternate mechanism(s) for leaf maintenance and recovery from soil drying. In GH 2, VA98R and Phillips had the least EC of 2.16 and 2.23 mmol m^{-2} s^{-1}(Table 3), respectively, and represent a source of low EC among commercial lines. Phillips was also evaluated in the previous study (Chapter 2) and consistently had moderate leaf maintenance.

Compared to other crops, peanut was found in these experiments to have low EC but a smaller range among genotypes. At first this may indicate that the breeding potential is limited. However, modeling by Sinclair (2000) suggests otherwise. He showed that when EC was below 10.35 mmol m^{-2} s^{-1} the benefit of decreasing EC even by small amounts greatly increases the length of survival of crops. Peanut falls within this value, therefore decreasing the EC through breeding may substantially delay leaf dehydration, extend survival and improve the recovery of peanut from severe water deficits.

The extremely low EC of PI 298639, which has 1.71 mmol m^{-2} s^{-1} indicates the potential benefit of including wild species into the peanut breeding population for the goal of decreasing EC in advanced lines of *A. hypogaea*. Furthermore, the least and greatest EC in GH 1 was obtained from two wild species, PI 298639 and PI 497579, respectively (Tables 2 and 3). In addition, the greatest and least EC obtained in the field were from two species-derived genotypes: SPT 06-07 and HTS 02-05, respectively. The range in EC in GH 1 produced by the only two wild species and the range in EC in the field by the only two species-derived lines are similar to the range produced by the fourteen accessions of *A. hypogaea* in GH 2 and indicate that greater genetic variation for EC may be found in wild species of peanut. A study involving a greater number of peanut wild species may reveal even greater variability in EC and a source of genetic material for this trait.

Water-Deficit Stress

The second objective of this study was to investigate the effects of water-deficit stress on the stability of EC in peanut. Consistent rains leading to water infiltration in to the water-deficit stress plots made it impossible to make comparisons among genotypes for EC sensitivity to water-deficit stress. Instead, comparisons among field positions in the water-deficit stress plots made it possible to determine the effects of water-deficit stress on peanut as a whole.

Differences in Gs ratio among the three positions were statistically significant on Day 7 (*i.e.*,, when the first Gs measurements were taken), however these differences were relatively small (Figure 2). In general, the Gs ratio of plants in the downhill and middle positions in the water-deficit treatment plots remained close to or higher than 1.0 throughout the experiment and was indicative of the well-watered conditions of the subplots in these two positions. Between Day 1 and Day 18, EC significantly decreased in the uphill and downhill positions, however Gs ratios on Day 18 were near well-watered conditions for all positions (Table 5). It is evident from the Gs ratio that peanut is experiencing a decrease in EC that is not caused by water-deficit stress between Day 1 and Day 18 in the uphill and downhill positions. Between Day 18 and Day 41, EC significantly decreased in the middle and uphill positions while the Gs ratio of the middle position remained close to well-watered conditions and the Gs ratio of the uphill position decreased to 0.11 on Day 38. It is evident from the Gs ratio of plants in the middle position that a decrease in EC is not a result of water-deficit stress. Taken together, these results indicate that the decrease in EC seen in the water-deficit treatment plots was not a result of water-deficit stress. This is in contrast to previous studies on soybean (*Glycine max* L. Merr.) (Paje et al., 1988) and sorghum (Premachandra et al., 1992) but consistent with other studies on wheat (Clarke et al., 1991).

Paje et al. (1988) reported sensitivity of EC to temperature in soybean. Plants growing in the well-watered plot experienced an increase in EC while plants under the rain-shelters experienced a decrease in EC by the end of the experiment (Table 5), though daily high temperatures between the inside and outside of the shelters followed a similar trend of increasing and decreasing temperatures throughout the experiment (Figure 3). This indicates that differences in temperature did not cause the decrease in EC seen in peanut under the shelters. It remains unclear which condition(s) under the rain-shelters caused the decrease in EC in the water-deficit treatment plots.

Leaf Anatomical Characteristics and Epidermal Conductance

The third objective of this study was to investigate the association between two leaf anatomical characteristics of peanut and epidermal conductance: stomatal density (SD) and specific leaf weight (SLW). Significant differences in SD among peanut genotypes were detected (Table 6). Pallas (1982) reported similar peanut abaxial stomatal densities between 99 and 220 mm^{-2}. While studies on EC in relation to various leaf traits are somewhat limited, mixed results among crops are evident. Improperly closed stomata as a result of the architecture of the guard cell or water loss directly from guard cell teichodes could be a source of water loss when stomata reach minimum aperture (Muchow and Sinclair, 1989). Muchow and Sinclair (1989) measured the EC of 10 sorghum lines and found a high correlation (0.82) with stomatal density. They concluded that the source of water loss was from peristomatal transpiration, *i.e.*,, water loss from guard cells. Saito and Futakuchi (2010) came to a similar conclusion when they found a high correlation (0.75) between stomatal area index and EC in certain cultivars of rice. They concluded that water loss was mainly from the stomatal pore.

When the EC means of the peanut genotypes in this study are plotted against the mean SD in GH 1, an R^2 value of 0.46 is produced (p= 0.14, NS). The R^2 is 0.04 in GH 2 (p= 0.51, NS, Figure 4). In the field the R^2 was greater but still not significant (R^2= 0.49, p= 0.12, NS). The results presented in this study indicate that water loss from the stomatal pore or guard cells do not account for the variation in EC in peanut. Araus et al. (1991) also reported no relationship between stomatal density and EC in durum. Similarly, Paje et al. (1988) reported very low correlations of 0.18 under well-watered conditions and 0.29 under stressed conditions in soybean. Similar results have also been reported for two species of desert grasses (*Digitaria californica* and *Eragrostis lehmanniana*, Smith et al., 2006). While significant differences in SD between peanut genotypes in this study were detected, any attempt to manipulate stomatal traits directly or indirectly based on these results will not significantly influence epidermal water loss during severe water-deficit in peanut.

The correlation between mean EC and mean SLW for the well-watered treatment plot was 0.09 and not significant (Figure 5). However, when regressions between EC and SLW are performed for comparisons among positions under the rain-shelters, highly significant correlations of 0.42, 0.33, and 0.52 were detected for the uphill, middle, and downhill positions, respectively ($p < 0.001$). However, the correlations under the shelters are most likely an associated response to the conditions under the rain-shelters as no significant correlations were found for the well-watered treatment plot.

The influence of leaf anatomical characteristics on EC in peanut remains unclear. In tobacco (*Nicotiana tabacum* L.) and oat seedlings, higher epicuticular wax load can be seen in response to water stress resulting in lower epidermal conductance (Bengtson et al., 1978; Cameron et al., 2006). In contrast, sorghum and wheat epicuticular wax has little to no influence on epidermal conductance (Jenks et al., 1994; Jordan et al., 1984; Araus et al., 1991) unless the amount of wax in sorghum is below 0.067 g m^{-2}. Genetic variation has been reported in peanut for epicuticular wax load in response to drought stress (Samdur et al., 2003) however there is no evidence that differential wax load in peanut influences EC. The least amount of wax detected in that study was 0.145 g m^{-2} which is above the threshold mentioned for sorghum, thus, based on the results for sorghum, it is unlikely that epicuticular wax explains variation in EC of peanut.

REFERENCES

Araus, J., Febrero, A., and Vendrell, P. (1991). Epidermal conductance in different parts of durum wheat grown under mediterranean conditions: The role of epicuticular waxes and stomata. *Plant, Cell and Environment, 14*(6), 545-558.

Balota, M., Isleib, T. G., Tallury, S. (2012). Variability for drought related traits of Virginia-type peanut cultivars and advanced breeding lines. *Crop Science*, 52(6), 2702-2713.

Bengtson, C., Larsson Stig, and Liljenberg, C. (1978). Effects of water stress on cuticular transpiration rate and amount and composition of epicuticular wax in seedlings of six oat varieties. *Physiologia Plantarum, 44*(4), 319-324.

Cameron, K. D., Teece, M. A., and Smart, L. B. (2006). Increased accumulation of cuticular wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco. *Plant Physiology, 140*(1), 176-183.

Clarke, J., Richards, R., and Condon, A. (1991). Effect of drought stress on residual transpiration and its relationship with water use of wheat. *Canadian Journal of Plant Science, 71*(3), 695-702.

Flower, D. J., and Ludlow, M. M. (1986). Contribution of osmotic adjustment to the dehydration tolerance of water-stressed pigeon pea (cajanus cajan (L.) millsp.) leaves. *Plant, Cell and Environment, 9*(1), 33-40.

Holbrook, N., and Sinclair, T. R. (1992). Water balance in the arborescent palm, sabal palmetto. II. transpiration and stem water storage. *Plant, Cell and Environment, 15*(4), 401-409.

Holmgren, P., Jarvis, P. G., and Jarvis, M. S. (1965). Resistances to carbon dioxide and water vapour transfer in leaves of different plant species. *Physiologia Plantarum, 18*(3), 557-573.

James, A. T., Lawn, R., and Cooper, M. (2008). Genotypic variation for drought stress response traits in soybean. I. variation in soybean and wild glycine spp. for epidermal conductance, osmotic potential, and relative water content. *Crop and Pasture Science, 59*(7), 656-669.

Jenks, M. A., Joly, R. J., Peters, P. J., Rich, P. J., Axtell, J. D., and Ashworth, E. N. (1994). Chemically induced cuticle mutation affecting epidermal conductance to water vapor and disease susceptibility in sorghum bicolor (L.) moench. *Plant Physiology, 105*(4), 1239-1245.

Jordan, W., Shouse, P., Blum, A., Miller, F., and Monk, R. (1984). Environmental physiology of sorghum. II. epicuticular wax load and cuticular transpiration. *Crop Science*, *24*(6), 1168-1173.

Lawn, R. J., and Likoswe, A. A. (2008). Genotypic differences in leaf area maintenance contribute to differences in recovery from water stress in soybean. *Crop and Pasture Science*, *59*(12), 1075-1085.

Muchow, R. C., and Sinclair, T. R. (1989). Epidermal conductance, stomatal density and stomatal size among genotypes of sorghum bicolor (L.) moench. *Plant, Cell and Environment*, *12*(4), 425-431.

O'toole, J., Cruz, R., and Seiber, J. (1979). Epicuticular wax and cuticular resistance in rice. *Physiologia Plantarum*, *47*(4), 239-244.

Paje, M. C. M., Ludlow, M. M., and Lawn, R. J (1988). Variation among soybean (*Glycine max* (L.) merr.) accessions in epidermal conductance of leaves. *Crop and Pasture Science*, *39*(3), 363-373.

Pallas Jr, J. E. (1982). Photosynthetic traits of selected peanut genotypes 1, 2. *Peanut Science*, *9*(1), 14-17.

Premachandra, G. S., Saneoka, H., Fujita, K., and Ogata, S. (1992). Leaf water relations, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing water deficits in sorghum. *Journal of Experimental Botany*, *43*(12), 1569-1576.

Quisenberry, J., Roark, B., and McMichael, B. (1982). Use of transpiration decline curves to identify drought-tolerant cotton germplasm. *Crop Science*, *22*(5), 918-922.

Saito, K., and Futakuchi, K. (2010). Genotypic variation in epidermal conductance and its associated traits among oryza sativa and *O. glaberrima* Cultivars and their interspecific progenies. *Crop Science*, *50*(1), 227-234.

Samdur, M., Manivel, P., Jain, V., Chikani, B., Gor, H., Desai, S., et al. (2003). Genotypic differences and water-deficit induced enhancement in epicuticular wax load in peanut. *Crop Science*, *43*(4), 1294-1299.

Sinclair, T. R. (2000). Model analysis of plant traits leading to prolonged crop survival during severe drought. *Field Crops Research*, *68*(3), 211-217.

Sinclair, T. R., and Ludlow, M. M. (1986). Influence of soil water supply on the plant water balance of four tropical grain legumes. *Functional Plant Biology*, *13*(3), 329-341.

Smith, S., Fendenheim, D., and Halbrook, K. (2006). Epidermal conductance as a component of dehydration avoidance in digitaria californica and eragrostis lehmanniana, two perennial desert grasses. *Journal of Arid Environments*, *64*(2), 238-250.

Table 1. Epidermal conductance among selected crops.

Crop	**EC range (mmol m^{-2} s^{-1})**	**Growth Environment**	**Source**
Cotton (*Gossypium hirsutum* L.)	6.2 to 19.3	Greenhouse	Fish and Earl 2009
Rice (*Oryza* spp.)	11.6 to 17.2	Pots outside	Saito and Futakuchi 2010
Wheat (3 leaf types) (*Triticum turgidum var* durum L.)	11 to 30.5	Pots outside	Araus et al., 1991
Soybean (*Glycine max* L. Merr.)	8.6 to 18.5	Greenhouse	Hufstetler et al., 2007
Sorghum (*Sorghum bicolor* L.)	6.13 to 17.4	Field	Muchow and Sinclair 1989
Sabal Palm (*Sabal palmetto*)	0.32 to 0.56	Greenhouse	Holbrook and Sinclair 1992

Table 2. For all peanut genotypes evaluated in this study, information on the origin, type or species, use, whether they have been released, and in which experiment it was used is provided in this table.

Genotype	Use	Type/species	Origin	Release	Greenhouse/ Field
Bailey	Commercial	Virginia	NCSU	Released	Field
Georgia Green	Commercial	Runner	Univ. of Georgia	Released	Field
SPT 06-07[A]	Breeding line	Species-derived	NCSU	Unreleased	Field
HTS 02-05[B]	Breeding line	Species-derived	NCSU	Unreleased	Field, GH 1
N05008	Breeding line	Virginia	NCSU	Unreleased	Field, GH 1, 2
N05006	Breeding line	Virginia	NCSU	Unreleased	Field, GH 2
PI 298639	Wild species	*A. batizocoi*	Bolivia	-	GH 1
PI 497579	Wild species	*A. stenosperma*	Brazil	-	GH 1
ICGV 86388	Commercial	Spanish	ICRISAT	Released	GH 1
TMV 2	Commercial	Spanish	Tamil Nadu, India	Released	GH 1, 2
CHAMPS	Commercial	Virginia	VPIandSU	Released	GH 2
Florida Fancy	Commercial	Virginia	Univ. of Florida	Released	GH 2
Gregory	Commercial	Virginia	NCSU	Released	GH 2
N04074FCT	Breeding line	Virginia	NCSU	Unreleased	GH 2
NC-V11	Commercial	Virginia	NCSU	Released	GH 2
Perry	Commercial	Virginia	NCSU	Released	GH 2
Phillips	Commercial	Virginia	NCSU	Released	GH 2
Sugg	Commercial	Virginia	NCSU	Released	GH 2
VA 98R	Commercial	Virginia	VPIandSU	Released	GH 2
VT004152	Breeding line	Virginia	VPIandSU	Unreleased	GH 2
VT024051	Breeding line	Virginia	VPIandSU	Unreleased	GH 2

[A] and [B], SPT 06-07 and HTS 02-05 can be traced back within a few generations to wild species (contain *A. cardenasii* germplasm)

Table 3. Epidermal conductance (mmol m^{-2} s^{-1}) for Greenhouse 1 and 2 for all peanut genotypes. For each experiment, means followed by the same letter are not statistically different among genotypes ($p < 0.05$). All measurements were conducted on well-watered plants.

Genotype	**Greenhouse 1**	**Greenhouse 2**
CHAMPS	-	3.67 a
Sugg	-	3.56 a
VT024051	-	3.20 ab
Florida Fancy	-	3.15 ab
N05008	2.51 b	2.97 ab
Perry	-	2.96 ab
N05006	-	2.89 ab
Gregory	-	2.78 ab
NC-V11	-	2.77 ab
VT004152	-	2.71 ab
N04074FCT	-	2.70 ab
TMV 2	1.72 c	2.68 ab
Phillips	-	2.23 b
VA 98R	-	2.16 b
PI 497579	3.17 a	-
PI 298639	1.71 c	-
ICGV 86388	2.50 b	-
HTS 02-05	2.43 b	-

Table 4. Field epidermal conductance (mmol m^{-2} s^{-1}) means of genotypes in the well-watered plot for measurement days taken throughout the experiment. Means followed by the same letter are not statistically different among days ($p < 0.05$, n=3. HTS 02-05 was different from all other genotype except N05008 on Day 1. All other genotypes were not statistically different. No statistical differences were detected among genotypes after Day 1.

Day	HTS 02-05	N05008	N05006	Georgia Green	SPT 06-07	Bailey
1	9.09 b	7.88 ab	6.84 ab	6.65 b	6.43 b	6.30 b
18	6.42 b	6.68 b	5.68 b	6.23 b	5.19 b	5.89 b
31	10.25 b	13.51 ab	13.00 ab	7.86 ab	9.18 ab	9.96 ab
36	11.68 b	11.54 ab	14.51 a	10.63 ab	8.84 ab	13.17 a
41	18.02 a	14.50 a	13.29 ab	16.11 a	12.42 a	13.17 a

Table 5. Field peanut epidermal conductance (EC, mmol m^{-2} s^{-1}) means among the well-watered plot and positions and under the water-deficit stress treatment and among days.

Position	**Day 1**	**Day 18**	**Day 41**
Well-watered	7.20 bc B	6.02 a B	14.59 a A
Downhill	9.89 a A	6.50 a B	5.46 b B
Middle	5.95 c A	6.26 a A	4.62 b B
Uphill	7.44 b A	5.79 a B	4.36 b C

a, b, c Means within a column followed by the same lowercase letters are not significantly different among positions.
A, B, C Means within a row followed by the same uppercase letters are not significantly different among days ($p < 0.05$).

Table 6. Abaxial stomatal density (mm^{-2}) means of all peanut genotypes under well-watered conditions in Greenhouse Experiments 1 and 2 (GH 1, GH 2), and in the Field Experiment. For GH 1 and the Field, means followed by the same letter are not statistically different among genotypes ($p < 0.05$).

Genotype	GH 1	GH 2	Field
N04074FCT	-	186	-
TMV 2	137 c	170	-
Sugg	-	169	-
VA98R	-	165	-
CHAMPS	-	164	-
N05024J	-	164	-
Phillips	-	164	-
Florida Fancy	-	159	-
N05008	146 bc	153	187 b
VT024051	-	152	-
N05006	-	144	192 ab
Perry	-	141	-
NC-V11	-	140	-
Gregory	-	137	-
SPT 06-07	-	-	226 a
Georgia Green	-	-	206 ab
Bailey	-	-	188 ab
PI 497579	301 a	-	-
HTS 02-05	143 bc	-	180 b
ICGV 86388	162 bc	-	-
PI 298639	177 b	-	-
ANOVA	***	*	*

*Significant differences at $p < 0.05$, ***Significant differences at $p < 0.001$

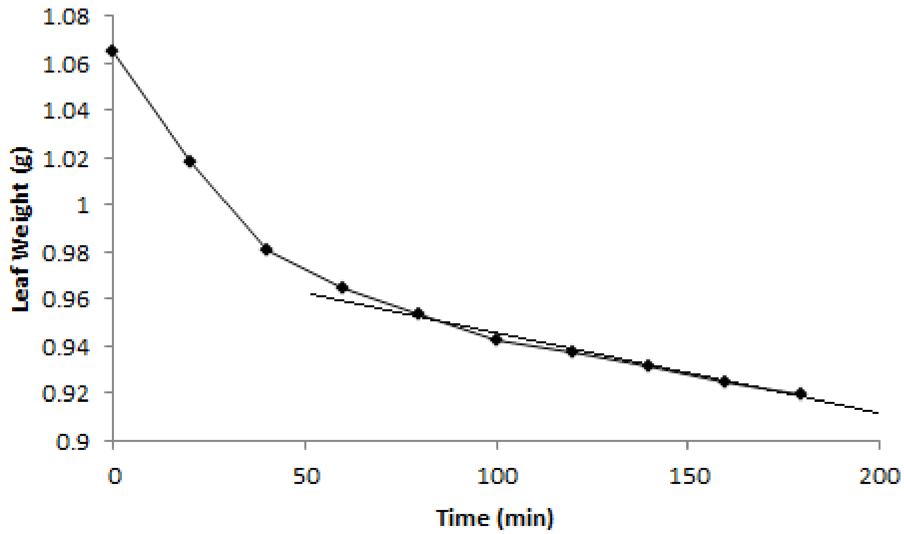

Figure 1. Multiphasic curve of a detached leaf of genotype ICGV 86388. The first linear portion represents active stomatal closure while the second linear portion represents water loss after stomata have reached minimum aperture. The slope of the second linear portion determined from a regression between leaf weight and time between 80 and 180 min is used to calculate epidermal conductance.

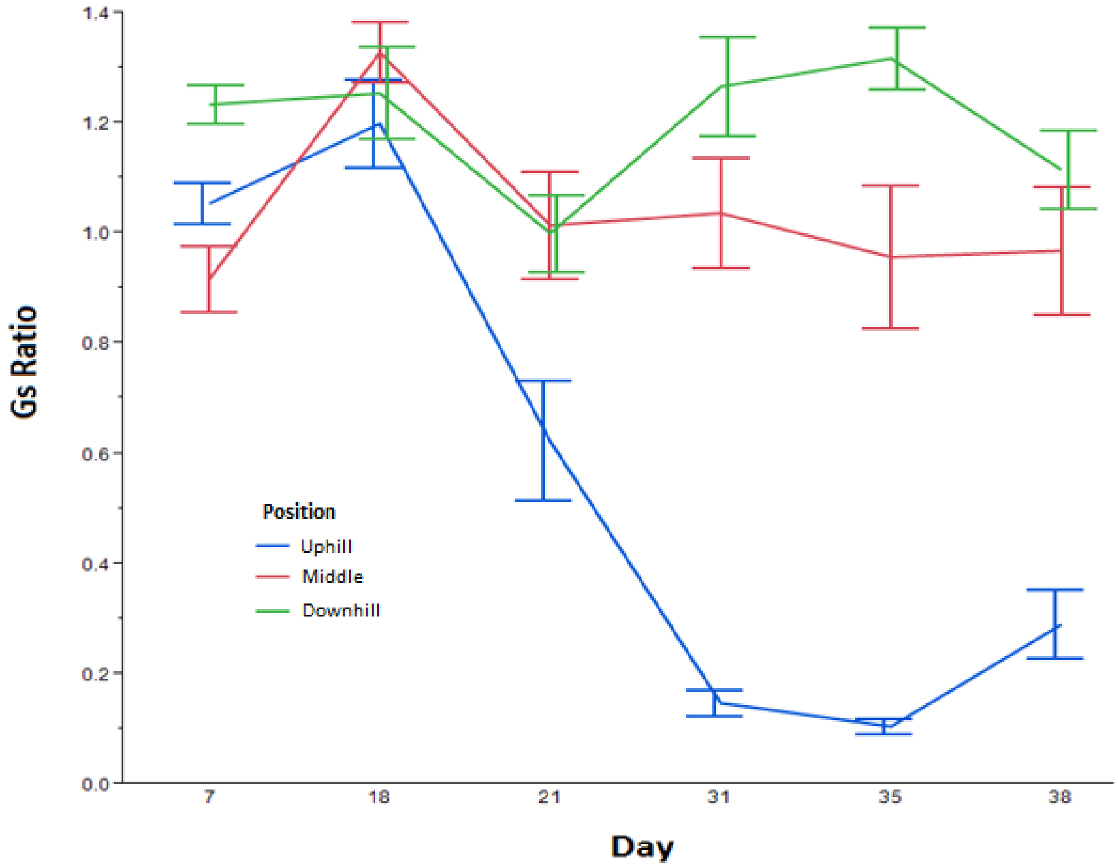

Figure 2. Mean stomatal conductance ratio (Gs ratio) of peanuts in three positions in the water-deficit treatment throughout measurement days. Error bars represent standard error of the mean (n = 11 or 12).

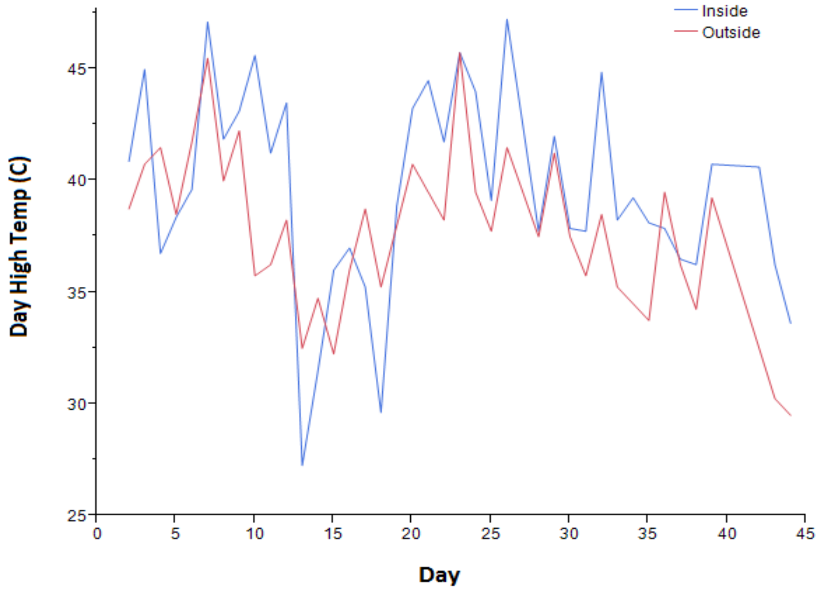

Figure 3. Greatest daily air temperatures (Celsius) inside and outside rain-shelters.

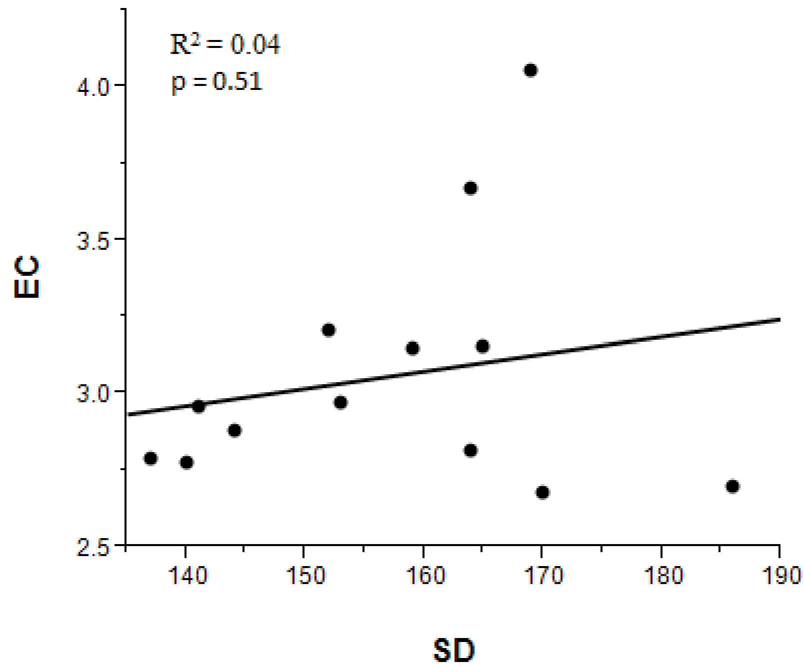

Figure 4. The relationship between genotypic epidermal conductance (EC, mmol m^{-2} s^{-1}) and genotypic stomatal density (SD, mm^{-2}) in Greenhouse 2.

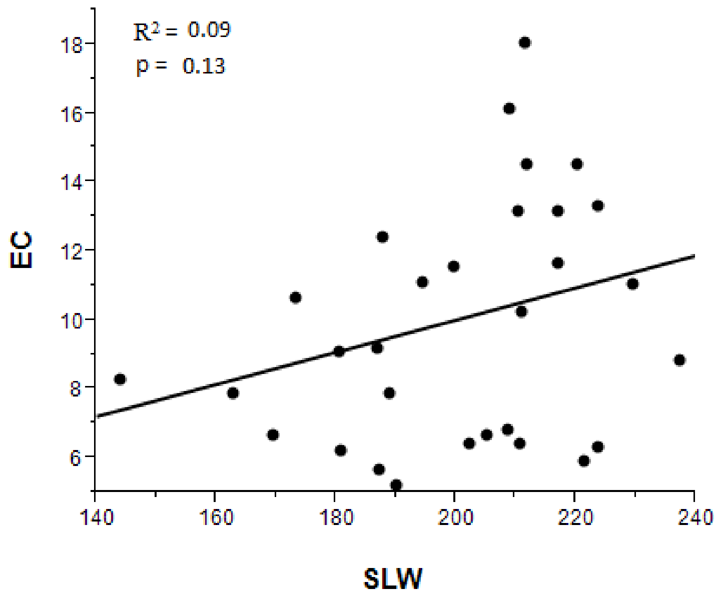

Figure 5. The relationship between genotypic epidermal conductance (EC, mmol m^{-2} s^{-1}) and specific leaf weight (SLW, mm^{-2}) for all measurement days in the well-watered treatment plot.

CHAPTER 4

CONCLUSION

Recovery from severe drought is an important characteristic of peanut for achieving adequate yields. Identifying traits which lead to improved recovery from soil drying in the peanut germplasm is important for crop improvement. The objectives of this thesis were to identify genotypic differences for plant recovery after re-watering from a soil drying cycle and to identify traits that may be used to improve the recovery of peanut from severe water deficit stress. These objectives were fulfilled in two separate studies in Chapters 2 and 3.

The objective of the study in Chapter 2 was to identify genetic variation in peanut (*Arachis* spp.) for leaf maintenance and transpiration recovery from a soil drying cycle. A total of nineteen peanut genotypes were evaluated in two greenhouse experiments and a field experiment was conducted to verify results from the greenhouse experiments. In the greenhouse experiments, plants were subjected to a well-defined level of stress based on the proposed stages of plant response to soil drying by Sinclair and Ludlow (1985). Plants experiencing soil drying respond in three successive stages: Stage I occurs when there is enough soil water to support normal transpiration rates. Plants enter Stage II when the soil dries to a threshold fraction of transpirable soil water and plants reduce their transpiration rates by adjusting their stomata to close for longer periods during the day. Plants enter Stage III when they close their stomata in response to an exhausted soil water supply and stomata remain at minimum aperture until soil water is replenished.

While other studies have investigated the recovery of physiological mechanism such as photosynthesis and gas exchange from a water deficit stress treatment, a majority of these studies have not imposed a well-defined level of water deficit stress on individual plants before re-watering. As a result, differential recovery response to re-watering from different levels of stress may occur making it more difficult to make accurate comparisons of the physiological response among genotypes. In addition, there are limited studies evaluating the transpiration recovery response involving a wide selection of peanut genotypes.

In the greenhouse experiments of the study in Chapter 2, transpirable soil water was calculated and individual plants were taken through the three stages and allowed to remain under Stage III stress for a determined number of days before re-watering. The advantage of this methodology is that each plant experienced relatively the same level of stress before being re-watered. Therefore, transpiration recovery comparisons among genotypes after re-watering were presumed to be more accurate since all genotypes recovered from relatively the same level of stress. In the field experiment, a water deficit stress treatment was imposed by moving rain-shelters over treatment plots. Complications from unusually high rainfall and field conditions discussed in Chapter 2 limited the amount of stress experienced by genotypes. However, differential stress experienced by genotypes allowed for the use of a regression analysis to estimate the recovery of stomatal conductance from Stage III by extrapolating stress levels to 0.11 using the regression equation produced for each genotype. The estimated stomatal conductance recovery verified to some degree the transpiration recovery results from the greenhouse experiments.

Leaf maintenance during water-deficit stress can improve the recovery of plants upon re-watering (Lawn and Likoswe, 2008). This would be an important trait for increasing the potential of a crop to remain productive under intermittent droughts. In order to evaluate leaf maintenance during Stage III stress in peanut, a visual rating scale was implemented. The visual rating scale was a practical method of rating leaf damage on each plant one day after re-watering.

In Chapter 2, we reported genetic variation for transpiration recovery among the nineteen peanut genotypes evaluated in the greenhouse. High transpiration recovery is beneficial for continued CO_2 uptake and growth, nutrient uptake, and presumably the recovery of nitrogen fixation leading to greater whole plant recovery. In addition, differences among genotypes for leaf maintenance during Stage III stress were identified based on the visual rating scale. Superior genotypes were identified for their capacity to retain a high percentage of leaves during Stage III stress (high visual rating) and their ability to recover transpiration rates after re-watering. These genotypes were TMV 2, ICGV 86388, PI 497579, and N05006. These genotypes are likely to have greater productivity potential due to greater whole plant recovery from severe water deficit stress. These genotypes are recommended for future studies on the mechanisms involved in recovery from water-deficit stress and as a source of material for crop improvement for areas that are prone to drought.

The maintenance of leaves under water deficit stress may be due to different physiological mechanisms. Dehydration avoidance by limiting water loss from the leaf epidermis, *i.e.,* low epidermal conductance, has been identified as one such mechanism which may extend the hydration of leaves and the survival of certain legumes experiencing severe water deficit stress (Sinclair and Ludlow, 1986; Likoswe and Lawn, 2008).

The study in Chapter 3 aimed to identify low epidermal conductance as a trait for delaying the dehydration of leaves and as a result improving the recovery of peanut genotypes from severe water-deficit. The first objective was to identify genetic variation among 22 peanut genotypes for epidermal conductance. A second objective was to investigate the effects of water-deficit stress on the stability of epidermal conductance. A third objective sought to determine the association between epidermal conductance and two anatomical leaf traits, stomatal density and specific leaf weight. Two greenhouse experiments and a field experiment were conducted to fulfill these objectives.

A comparison among genotypes in each experiment revealed genotypic differences for epidermal conductance in peanut. In addition, peanut has relatively low epidermal conductance compared with other crops such as cotton or wheat. Modeling by Sinclair (2000) indicates that the potential for increasing the survival of leaves during severe stress is improved in genotypes that have epidermal conductance lower than 10.35 mmol m^{-2} s^{-1}. When field epidermal conductance values at the start of the experiment are considered, all genotypes evaluated had lower than 10.35 mmol m^{-2} s^{-1}. Genotypes which had the least epidermal conductance in the first greenhouse experiment were: TMV 2 and PI 298639, in the second Greenhouse Experiment: VA 98R and Phillips, and in the Field: SPT 06-07 and Bailey were consistently ranked as having the least or second least epidermal conductance. In the previous study (Chapter 2), SPT 06-07, TMV 2, and PI 298639 had superior leaf maintenance in all cases where they were evaluated; Bailey had superior leaf maintenance for a shorter duration under Stage III; and Phillips had consistently moderate leaf maintenance; VA 98R was not evaluated. When the results of both studies are taken together, it is evident that low epidermal conductance is a beneficial trait which in part is responsible for improved leaf maintenance during severe water deficit stress in the peanut genotypes mentioned. It is recommended that these genotypes be included in a future study in the field to confirm these results and considered as a source of low epidermal conductance in a breeding program that aims to improve the recovery of peanut from severe water deficit stress.

Further studies for identifying additional mechanisms which improve the recovery of peanut from Stage III are also warranted. An example of a genotype that may be utilizing an alternate mechanism for recovery is *A. stenosperma* accession PI 497579. This genotype had excellent leaf maintenance and transpiration recovery in almost all cases in the study on plant recovery. However, PI 497579 also had one of the greatest epidermal conductance of all genotypes evaluated in both greenhouse experiments. This genotype may have greater dehydration tolerance, *i.e.,* lower lethal relative water content than other genotypes or is maintaining a higher relative water content before reaching Stage III stress and consequently able to maintain more leaves. ICGV 86388 also had superior recovery yet its epidermal conductance was only mid-range indicate a separate mechanism for leaf maintenance.

The epidermal conductance of peanut as a whole was not affected by water deficit stress indicating a limited ability of peanut to acclimate by further limiting leaf water loss during Stage III stress. However a considerable decrease in epidermal conductance was shown in the plots covered by the rain-shelters compared to the uncovered plot by the end of the field experiment. Values of epidermal conductance in the greenhouse experiments were also substantially lower than those in the uncovered plot in the field indicating that some similar conditions between the greenhouse and rain-shelters may have increased the vapor resistance of the leaf epidermis. An experiment where plants are grown in different light intensities may reveal an effect on the vapor resistance of the leaf epidermis.

There was a lack of association between epidermal conductance and stomatal density. It was concluded that water loss from the stomatal pore or directly from guard cells does not explain the variation in epidermal conductance among genotypes. Based on these results, using stomatal density as a selection criterion, as has been previously suggested with sorghum (Muchow and Sinclair, 1989) will be ineffective in selection for low epidermal conductance. While it is speculated from previous studies on other species that differences in epicuticular wax may not explain the variation in epidermal conductance of peanut, it is recommended that this be investigated experimentally.

The results of the study in Chapters 2 and 3 are encouraging for improving the recovery of peanut from droughts. However, further study is needed to determine how the recovery of transpiration and leaf maintenance affects the long term productivity of peanut genotypes. In addition, the ability of peanut genotypes to recover nitrogen fixation after such severe stress is experienced may be just as important as the recovery of transpiration in terms of yield. Differences in the sensitivity of nitrogen fixation among peanut genotypes to soil drying has been reported (Devi et al., 2010), however no reports on the recovery of nitrogen fixation from Stage III stress in peanut have been made.

Some recommendations for future studies on the physiological mechanisms for whole plant recovery can be made from this thesis. While low epidermal conductance was identified as a positive trait for dehydration avoidance in some peanut genotypes, other genotypes with alternate mechanisms for leaf maintenance were identified. Low lethal relative water content, leaf osmotic adjustment, nitrogen fixation recovery and root osmotic adjustment are physiological mechanisms that deserve attention for future studies on peanut recovery from Stage III stress. Low lethal relative water content in combination with low epidermal conductance and low leaf osmotic adjustment may prolong the maintenance of leaves during such severe stress leading to rehydration and leaf recovery upon re-watering. The recovery of nitrogen fixation is of great importance especially for droughts early in the season and may be a greater determinant of yields. Root osmotic adjustment may reduce the water potential of roots and nodules during severe water-deficit stress and delay nodule dehydration which would aid in the maintenance and recovery of nodule activity.

The collective implementation of leaf maintenance and recovery traits in a peanut breeding program may result in increased yields for drought prone environments. Attention to the traits mentioned here should be given when considering whole plant recovery leading to increased recovery and yields.

REFERENCES

Devi, J. M., Sinclair, T. R., and Vadez, V. (2010). Genotypic variability among peanut (*Arachis hypogaea* L.) in sensitivity of nitrogen fixation to soil drying. *Plant and Soil, 330*(1), 139-148.

Lawn, R. J., and Likoswe, A. A. (2008). Genotypic differences in leaf area maintenance contribute to differences in recovery from water stress in soybean. *Crop and Pasture Science, 59*(12), 1075-1085.

Likoswe, A. A., and Lawn, R. J. (2008). Response to terminal water deficit stress of cowpea, pigeonpea, and soybean in pure stand and in competition. *Crop and Pasture Science, 59*(1), 27-37.

Muchow, R., and Sinclair, T. R. (1989). Epidermal conductance, stomatal density and stomatal size among genotypes of *Sorghum bicolor* (L.) Moench. *Plant, Cell and Environment, 12*(4), 425-431.

Sinclair, T. R. (2000). Model analysis of plant traits leading to prolonged crop survival during severe drought. *Field Crops Research, 68*(3), 211-217.

Sinclair, T. R., and Ludlow, M. M. (1985). Who taught plants thermodynamics? the unfulfilled potential of plant water potential., *Functional Plant Biology, 12*(3), 213-217.

Sinclair, T. R, and Ludlow, M. M. (1986). Influence of soil water supply on the plant water balance of four tropical grain legumes. *Functional Plant Biology, 13*(3), 329-341.