IDENTIFYING ADDITIONAL VARIATION IN SOYBEAN WATER-USE PHENOTYPES-SCREENING OF EXOTIC GERMPLASM

by

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(Under the Direction of Hugh J. Earl)

ABSTRACT

Twenty-three soybean genotypes were screened in greenhouse studies for vegetative-phase variation in three potential drought resistance traits: Water use efficiency (WUE), response of whole plant transpiration defined as normalized transpiration ratio (NTR) to soil water deficits, and epidermal conductance (g_e). A computer-automated lysimeter was used to impose distinct levels of water stress on potted soybean plants in the first experiment; significant variation was found between genotypes for both WUE and response of NTR to soil water content. In a second experiment, significant variation was found between soybean genotypes under well-watered conditions for g_e measured by leaf gas exchange, and a strong negative correlation was found between WUE (measured in the first experiment) and g_e. Results from the two experiments demonstrated that genetic variability exists in the twenty-three genotypes for all three traits examined.

INDEX WORDS: epidermal conductance, drought stress, *Glycine max*, lysimeter, leaf gas exchange, normalized transpiration ratio, stomatal closure, water stress, water use efficiency

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DEDICATION

I would like to dedicate this thesis in its entirety to my father, John W. Lutz, Sr., who encouraged me to pursue my education in agriculture.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Soybean

The soybean [*Glycine max* L. Merr.], a legume, most likely emerged as a domesticated plant during the Zhou dynasty in the eastern half of north China. Since domestication involves much trial and error, an exact date is difficult to establish, but the process probably occurred between 1500-1100 B.C. The soybean was brought to North America by Samuel Bowen in 1765. Specifically, Mr. Bowen who was a former seaman of the East India Company introduced soybean seeds into Savannah, Georgia, from China. Because Mr. Bowen did not yet have land of his own to sow seeds he asked the Surveyor-General of Georgia, Henry Yonge, to plant the first U.S. crop in 1765. By 1766, Bowen acquired a plantation called Greenwich located at Thunderbolt (today a cemetery) where he grew soybean to manufacture soy sauce and vermicelli (Hymowitz and Harlan, 1983; Hymowitz, 2004). Since then, the United States has become the world's leading producer of soybean with 34% of the global market followed closely by Brazil with 28% of the market (Soy Stats, 2004). In 2003, soybean plants were grown on 29.7 million hectares producing 65.81 million metric tons with a total crop value of $18.4 billion (Soy Stats, 2004). In Georgia, 77,000 hectares of soybeans were planted last year with a cash value of $43 million (Soy Stats, 2004). However, the high temperatures of the southern U.S. or water deficits of some duration or a combination of these factors limit crop growth and development on a yearly basis. The single greatest limitation to soybean productivity in the southeast is drought (Palmer, 1996).

Cultural practices that improve water use can help improve productivity; these include reduced tillage to limit evaporative losses, proper crop residue management to enhance water infiltration, and effective weed control (Unger, 1983). Additional measures can also benefit crops such as increasing irrigation water application efficiency, and reducing late season irrigation to ensure complete utilization of available soil water so that the soil water storage capacity for off-season rainfall is maximized (Bordovsky and Lyle, 1996). Even so, as water shortages continue in Georgia, future restrictions may be placed on water use for irrigation purposes, and heavy fees may be implemented for surface or ground water use . Under these conditions, the development of crop cultivars that can withstand the water stress and still produce acceptable seed yields should be a high priority. According to Palmer et al. (1996) "these drought-tolerant [soybean] varieties will not solve all of our problems when facing dry conditions, but they will give us a few more bushels per acre when there is a drought." One way to increase yields under water limited conditions is to improve water use efficiency (WUE).

WUE

WUE may be defined at the level of the whole plant or whole crop as the quantity of dry matter produced per unit of water used. Genetic variability for WUE has been found in several crop species including peanut (*Arachis hypogaea;* Hubick et al., 1988; Wright et al., 1994), cowpea (*Vigna unguiculata;* Ismail and Hall, 1993; Ashok et al., 1999), cotton (*Gossypium spp.;* Quisenberry and McMichael, 1991; Saranga et al., 1998), sorghum (*Sorghum bicolor;* Donatelli et al., 1992), barley (*Hordeum vulgare;* Hubick and Farquhar, 1989), wheat (*Triticum aestivum;* Ehdaie and Waines, 1993; Van Den Boogaard et al., 1997), and soybean (Mian et al., 1996,1998).

WUE may be expressed in terms of diffusion theory for instantaneous leaf gas exchange:

$$\mathrm{WUE} = \frac{A_N}{E} = \frac{g_c\,(c_a - c_i)}{g_w\,(w_i - w_a)}$$

Which may be rewritten as:

$$\frac{A_N}{E} = \frac{c_a\,(1 - c_i/c_a)}{1.6\,(w_i - w_a)} \qquad \text{(Turner, 1997)}$$

Where c_a = ambient CO_2 concentration

c_i = leaf intercellular CO_2 concentration

g_c = gas phase conductance to CO_2

g_w = gas phase conductance to water vapor

w_i = concentration of water vapor inside leaf

w_a = concentration of water vapor surrounding leaf

1.6 = the factor that accounts for the difference in the binary diffusivity of water vapor and air and CO_2 and air. The binary diffusivity of water vapor and air is 1.6-fold greater than that of CO_2 and air (Farquhar et al., 1989). Since g_w can be approximated as 1.6 g_c, the equation was rewritten above to replace g_w with a constant.

Plants have little control over w_i, which is determined by leaf temperature, or over the ambient water vapor and CO_2 concentrations. However, plants do have the ability to modify c_i and therefore the ratio of c_i/c_a, which is one of the determinants of WUE. According to theory, leaves that maintain lower c_i will display higher WUE (Jones, 1992). Reduced c_i may arise from reduced stomatal conductance (g_s) which reduces both net carbon assimilation (A_N) and water transpired (E) or from increased A_N at a given stomatal conductance. Clearly, WUE is controlled by stomatal and non-stomatal factors, but only increased intrinsic photosynthetic capacity leads to an increase in A_N without an increase in E. Thus, only increased leaf photosynthetic response can increase potential productivity (Saranga et al., 2001). Therefore, to achieve the best results in breeding, maximum leaf intrinsic photosynthetic capacity should be combined with an optimum stomatal response to the environment.

At the whole plant level, WUE may also be quantified as the total biomass produced including both the root and shoot on a dry weight basis per unit water lost to transpiration by the plant (Quisenberry and McMichael, 1991).

$$\text{Dry matter-based (DM-based) WUE} = \frac{\text{g DM}}{\text{kg } H_2O}$$

In the present work, WUE will be determined using the DM-based definition.

The Compromise Between Productivity and WUE

Higher water use efficiency is a desirable trait in agriculture because more carbon can be assimilated for growth with the use of less water. Increased WUE is a water conservation mechanism (Nilsen and Orcutt, 1996). High WUE cultivars could be of value in non-irrigated soybean hectarage in the Southeast where rainfall is often limiting.

However, the relationship between WUE and yield is determined by many factors and will vary for different species in different defined habitats. Hence, breeding simply for high WUE in certain crops may not necessarily lead to increased yields in water restricted sites (Nilsen and Orcutt, 1996). Often, maximum WUE is synonymous with low productivity. Furthermore, breeding for efficiency alone can be inadequate because greater efficiency is frequently linked with a slow rate of water use. Under this scenario, high WUE plants and their faster-growing counterparts might compete for potentially available water with the faster-growing plants winning the water war. Even so, this is only occasionally a problem in a typical agricultural monoculture, but efficiency mechanisms can have other related drawbacks. For example, it is useless to merely have high efficiency of water use if the availability of water is non-limiting. If other factors are equal, productivity in water limited environments will likely be greater for a plant that maximizes assimilation in relation to the availability of water than for a plant that only has a high ratio of assimilation to water lost. Maximum production (yield) from the best use of available water is more favorable than maximum WUE - i.e., it is of no gain to maximize WUE if some water remains unused. Optimal stomatal aperture might be achieved in such situations if the aperture is between fully open and that providing maximum WUE (Jones, 1992). Therefore, in order for WUE to be a beneficial agronomic trait that can be selected for in breeding programs, the compromise between water conservation and productivity must reach an optimum balance.

Stomatal Closure Under Drought Stress and its Relationship to WUE

The Effect of Stomatal Closure on Instantaneous WUE: Simple and Complex Leaf Models

Often, when a plant is affected by a negative change in soil water potential, stomata will close and transpiration and carbon accumulation will decrease. Upon stomatal closure during the early stages of drought stress, transpiration will be inhibited more than photosynthesis, c_i will decrease, and instantaneous WUE will increase in accordance with simple leaf models (Nilsen and Orcutt, 1996). Water loss through the cuticle is often ignored in simple leaf models perhaps because stomatal conductance of normal healthy leaves may be two or more orders of magnitude greater than the cuticular component (Lendzian and Kersteins, 1991; van Gardingen and Grace, 1991). However, these leaves must be in an environment which does not promote stomatal closure to safely ignore cuticular water loss. Leaves that are water stressed or dark adapted experience a shift from stomatal to cuticular control Boyer et al. (1997) and the cuticular conductance may exceed the stomatal conductance (van Gardingen and Grace, 1992). Therefore, the magnitude of cuticular resistance (r_c) should not be ignored under water stress and is a crucial component to complex leaf models (Jones, 1992).

In complex leaf models, when cuticular resistance is infinite (cuticular water loss is negligible), instantaneous WUE increases as stomata close and net photosynthesis decreases. However, when cuticular resistance has a definite value, the relationship changes. As r_c decreases and cuticular conductance (g_c) increases, the advantage of stomatal closure is altered so that an optimum stomatal resistance becomes apparent. This effect arises because the long liquid-phase pathway from the epidermis to the chloroplast means that CO_2 uptake through the cuticle is negligible even where water loss may be extensive (Jones, 1992). Therefore, it is possible for instantaneous WUE to increase to an optimum as stomata close, then to decrease as stomata close further.

The Compromise Between Early and Late Stomatal Closure

In addition to the complication of having optimum levels of stomatal closure and hence the highest WUE, it is difficult to know whether early stomatal closure (at higher relative soil water content (RSWC)) or later stomatal closure (at lower RSWC) will benefit plants. Whether or not early or late stomatal closure will be of greater benefit to the plant will depend on the duration of the drought, which is of course difficult to know in advance. A plant that closes stomata early will be at a disadvantage under a short-term drought because of lost productivity. However, by closing its stomata early, a plant could increase its chances for survival in a long-term drought. On the other hand, delayed closure of stomata would be more favorable under a short-term drought. Delayed closure would yield a higher stomatal conductance though under a long-term drought and the plant would use the available water more quickly and thus would be at a disadvantage. Ray and Sinclair (1997) have discussed these scenarios in relation to maize (*Zea mays*) hybrids.

The Role of Epidermal Conductance

When experiments are done to measure the value of cuticular resistance, often it is preferred to consider its reciprocal, cuticular conductance, or a more appropriate term which includes incompletely closed stomata: epidermal conductance (g_e). Epidermal conductance values have been measured by Sinclair and Ludlow (1986) and modeled by Sinclair (2000). According to Sinclair (2000), g_e represents the restriction of water loss from the plant from two sources: epidermal cells through the cuticle and from the stomatal complexes. This may include water loss directly from the guard cells or from incompletely closed stomatal pores. Under severe drought, crop survival becomes an issue when stomatal conductance reaches its minimum and crop CO_2 assimilation has virtually stopped. This period of severe drought stress has been defined as Stage III by Sinclair and Ludlow (1986), and follows Stage I in which leaf conductance is not suppressed by soil water deficiency and Stage II in which stomatal conductance progressively declines as the soil water shortage intensifies. Stage III is assumed to begin when stomatal conductance reaches its lowest values; therefore, water loss from the plant is restricted by epidermal conductance (Sinclair, 2000). One plant trait that has been suggested for increasing plant tolerance and prolonging plant survival during Stage III stress is lowered g_e. Plants that have lower g_e (and higher epidermal resistance) have decreased water loss rate per unit leaf area, and this results in water conservation during severe drought. Sinclair (2000) predicted, by computer modeling, that decreasing g_e is advantageous in prolonging crop survival, and Paje et al. (1988) found a very large range in g_e values among soybean lines.

Drought Stress Simulation

Methods for simulating drought stress in a controlled environment or greenhouse should achieve the following: i) maintenance of uniform soil moisture around the roots, ii) precise control over the rate at which moisture stress develops in spite of differences in plant size and variation in environmental conditions, and iii) imposition of clearly definable levels of stress within a distinct range. Few methods have met all of these criteria. Simply withholding water is not appropriate because water deficits usually develop much more quickly in pots than they do in the field which can affect the types of physiological responses observed (Cornic et al., 1987; Saccardy et al., 1996; Farrant et al., 1999). Two methods have been successful at preventing water stress from developing quickly in pots. The first consists of increasing the total plant-available water, either by using large containers (Allen et al., 1994) or by using a rooting medium with an unusually high water holding capacity (Pennypacker, 1990; Nissanka et al., 1997). Yet, the actual rate of the development of water stress will depend on the environmental conditions as well. The second alternative involves the determination of water loss from each pot gravimetrically by frequent recording of pot weights, and replacing part of the transpired water manually to control the rate of soil dry-down (Sinclair and Ludlow, 1986; Ekanayake et al., 1993; Ray and Sinclair 1997, 1998). Unfortunately, this method is labor intensive and not widely used.

As an alternative, Earl (2003) described a completely automated method for determining responses of whole plant water use to soil water content in greenhouse experiments. In this method [an adaptation of the Hunter and Tonks (1979) technique], each pot rests on an electronic balance, and its weight is monitored continuously by a computer. Transpired water is replaced in individual pots as required by a computer-automated watering system, so that each pot can be maintained within a very narrow pre-programmed weight range. Realistic rates of soil drying can then be simulated by strictly controlling the maximum amount of soil water depletion on any single day of an experiment. Specific advantages of this approach are i) small pots can be used, thus ensuring that the root system explores the entire soil volume, and ii) very uniform soil water content can be maintained within the pots. Together, these two factors make pot weight a reliable indicator of the soil water content experienced by the roots. Also, the frequent, as-needed replacements of transpired water allow the soil water deficit to develop at the same rate in each experimental unit, even if the plants differ substantially in their transpiration rates, and at a pre-determined rate that is not dependent on environmental conditions (Earl, 2003).

Normalized Transpiration Ratio (NTR), Relative Soil Water Content (RSWC), and Fraction of Transpirable Soil Water (FTSW)

In the present work, the daily transpiration rates and relative soil water contents were measured by the automated lysimeter. Whole plant transpiration of stressed plants was normalized first to transpiration rates of control plants then to evaporative demand to yield normalized transpiration ratio (NTR) as described by Ray and Sinclair (1997). Previously, relative soil water content (RSWC) has been expressed as the fraction of transpirable soil water (FTSW) (Sinclair and Ludlow, 1986; Ray and Sinclair, 1997). RSWC when gravimetrically determined represents the weight of water in the soil as a fraction of the amount of water in the soil at pot capacity (Gardner, 1986). The FTSW is equivalent to the current soil water content (RSWC) minus the RSWC when the plant can no longer extract any additional water ($\mathrm{RSWC}_{\mathrm{end}}$) divided by the difference between the saturated water content and the $\mathrm{RSWC}_{\mathrm{end}}$. Because of the very low rate of plant water use at low soil water contents, in practice it is difficult to experimentally determine the soil water content at which plant available soil water is fully depleted (Earl, 2003). Instead, this point has been arbitrarily defined as the soil water content at which NTR drops below 0.1 (Sinclair and Ludlow, 1986; Ray and Sinclair, 1997).

Objectives

The objectives of the present work were to:

i) Screen a selection of 23 soybean plant introductions, breeding lines, and cultivars (genotypes) for vegetative phase dry-matter based water use efficiency,

ii) Identify genetic variation in this selection of soybean genotypes for regulation of whole plant water use in response to soil water deficits using the "null balance lysimetry" technique, and

iii) A) Quantify genetic variation for leaf epidermal conductance in the 23 soybean genotypes using gas exchange measurement techniques and

B) Determine whether a correlation between WUE and g_e was present in the 23 soybean genotypes.

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CHAPTER 2

IDENTIFYING ADDITIONAL VARIATION IN SOYBEAN WATER-USE PHENOTYPES-SCREENING OF EXOTIC GERMPLASM[1]

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ABSTRACT

Two physiological traits that may affect drought tolerance in plants are water use efficiency (WUE) and the response of whole plant water use to soil water deficits. Twenty-three soybean [*Glycine max* (L.) Merr.] genotypes were screened for variability in these two traits during vegetative growth in a greenhouse study. WUE was estimated as the ratio of total plant dry weight to total water used. Whole plant water use, normalized both to plant size and evaporative demand (the normalized transpiration ratio, NTR), was monitored during a 10-d cycle of gradually increasing drought stress by a computer-automated pot weighing and watering system. After 2 d under severe stress, pots were rewatered and NTR was monitored for two more days. Soil water content was expressed as fraction of transpirable soil water (FTSW), and the point where each plant began to reduce its water use ($FTSW_C$) was identified via plateau regression. Substantial variation was found among genotypes for both WUE (21%) and $FTSW_C$ (45%). Genotypes also differed significantly for the extent to which NTR recovered upon rewatering. These results indicate that there is considerable variability within the available soybean germplasm for traits related to drought tolerance.

INTRODUCTION

Drought is the leading cause of soybean yield loss in the southeastern USA (Palmer et al., 1996), and so increasing productivity under water deficit stress is an important goal of soybean breeding efforts in this region. One strategy involves developing soybean lines with better drought tolerance through higher water use efficiency (WUE, the quantity of crop dry matter accumulated per unit of soil water transpired). Genetic variability for WUE has been found in several crop species including peanut (*Arachis hypogaea;* Hubick et al., 1988; Wright et al., 1994), cowpea (*Vigna unguiculata;* Ismail and Hall, 1993; Ashok et al., 1999), cotton (*Gossypium spp.;* Quisenberry and McMichael, 1991; Saranga et al., 1998), sorghum (*Sorghum bicolor;* Donatelli et al., 1992), barley (*Hordeum vulgare;* Hubick and Farquhar, 1989), wheat (*Triticum aestivum;* Ehdaie and Waines, 1993; Van Den Boogaard et al., 1997), and soybean (Mian et al., 1996,1998). In the work of Mian et al. (1996), a high-WUE genotype, ‘Young,’ and a low-WUE genotype, PI416937, were crossed to form an F_4-derived population in which several quantitative trait loci associated with WUE were found. They also observed that Young used 16.5% less water to produce a given mass of dry matter than did PI416937.

Another physiological trait that may affect drought tolerance is whole plant water use during a soil water deficit event, which is regulated primarily by modulation of stomatal resistance, i.e., late vs. early stomatal closure. Earl (2003) found that NTR and stomatal conductance were highly correlated in soybean by linear regression $r^2 = 0.99$. Intraspecific differences in stomatal regulation during a water deficit have been studied much less extensively than differences in WUE, and in only a few species. Ray and Sinclair (1997) evaluated various maize, *Zea mays,* hybrids for stomatal response to a drying soil. They measured daily soil water content (expressed as the fraction of transpirable soil water, (FTSW)) and transpiration (by weighing each pot regularly as the soil dried) and used a plateau regression to examine the relationship between whole plant water use (transpiration) and FTSW. A specific FTSW value where whole plant water use began to decline was identified for each maize hybrid as the breakpoint in the regression and defined as the $FTSW_C$ (critical FTSW value). There was significant variability for $FTSW_C$ among the maize hybrids (Ray and Sinclair, 1997).

Plateau regression analysis was also used by Sinclair and Ludlow (1986) to identify interspecific variation for the $FTSW_C$ for each of four grain legumes: black gram (*Vigna mungo)*, soybean, cowpea, and pigeonpea (*Cajunus cajan)*. For both soybean and cowpea, the transpiration rate was unchanged until FTSW dropped to about 0.3. The logistic curve fit to the relationship between transpiration and FTSW indicated that black gram reduced whole plant transpiration sooner than did cowpea as the soil dried.

The effect of pot size on transpiration in maize and soybean was described by Ray and Sinclair (1998), and they found that the dominant factor determining regulation of whole plant water use in response to water deficit stress was soil water content. They also established that regardless of pot size, the relationship between transpiration and soil water content was maintained. In all of these experiments, pots were weighed on a daily basis to estimate transpiration.

Earl (2003) described another method for determining responses of whole plant water use to soil water content in greenhouse experiments. In this method, each pot rests on an electronic balance, and its weight is monitored continuously by a computer. Transpired water is replaced in individual pots as required by a computer-automated watering system, so that each pot can be maintained within a very narrow pre-programmed weight range. Realistic rates of soil drying can then be simulated by strictly controlling the maximum amount of soil water depletion on any single day of an experiment. Specific advantages of this approach include: i) small pots can be used, thus ensuring that the root system explores the entire soil volume, and ii) very uniform soil water content can be maintained within the pots. Together, these two factors make pot weight a reliable indicator of the soil water content experienced by the roots. Also, the frequent, as-needed replacements of transpired water allow the soil water deficit to develop at the same rate in each experimental unit, even if the plants differ substantially in their transpiration rates, and at a pre-determined rate that is not dependent on environmental conditions (Earl, 2003).

Given that genetic variation exists for WUE, the first objective of the present study was to identify additional genetic variability for WUE in soybean by screening additional lines. Twenty-three soybean cultivars, breeding lines, and plant introductions (genotypes) were compared for vegetative stage WUE. The second objective was to identify genetic variation in soybean for the regulation of whole plant water use in response to soil water deficits, using the technique of "null balance lysimetry." Finally, the genotypes were compared for their ability to re-establish high rates of whole plant water use upon relief of a severe soil water deficit by rewatering.

MATERIALS AND METHODS

Plant Culture

All experiments were conducted in a greenhouse between August 2001 and November 2002 at the Univ. of Georgia in Athens, GA (33.9°N, 88.3°W). Temperatures were maintained at 27±4°C during the day and 20±2°C during the night. Photoperiod was extended to 16 h with overhead 400-W metal halide lamps that produced a supplemental photosynthetic photon flux density (PPFD) of approximately 230 μmol m^{-2} s^{-1} at the tops of plants.

Soybean plants were grown using the greenhouse culture system described by Mian et al. (1996). Plant material consisted of 23 genotypes chosen in consultation with Dr. T.E. Carter (USDA-ARS, Raleigh, NC), based on the results of prior field screening and putative water use characteristics (Table 2.1). The growth medium was a Pacolet sandy loam soil (a member of the clayey, kaolinitic, thermic family of Typic Hapludults) amended with sand to a texture of 800 g kg^{-1} sand, 120 g kg^{-1} silt, and 80 g kg^{-1} clay, and 3.2 kg of this mixture was placed into each 2.5-L pot without drainage holes (actually white plastic food containers from Berry Plastics Corp., Evansville, IN). Seeds were sown four to a pot and fertilized with 50 ml of a 0.8% (w/v) solution of 20-20-20 (N-P-K) fertilizer plus micronutrients (Miller Greenhouse Special, Miller Chemical and Fertilizer Co. Corp., Hanover, PA). Cotyledons were expanded and horizontal at 10 to 12 d after sowing (DAS) and plants were at the VC to V1 stage (Ritchie et al., 1994); at this time, plants were thinned to one per pot, an additional 50 ml of fertilizer solution was added, and each pot was capped with a plastic lid to reduce evaporation of water from the soil surface. Each lid had two holes - one to accommodate the plant stem, and another to permit water additions.

After capping, all water added to the pots was recorded by weight, and pots were maintained between 55 and 85% relative soil water content (RSWC – see below) prior to placement on the lysimeter balances. In this culture system, 55% RSWC is equal to approximately 50% FTSW as defined by Ray and Sinclair (1998), and is above the soil water content where soybean plants first begin to reduce their water use (Ray and Sinclair, 1998; Earl, 2003).

Relative Soil Water Content and Soil Water Holding Capacity

Before planting, soil water holding capacity was determined. In addition to the pots that were prepared for the soybean plants, two extra pots with mesh-covered drainage holes were filled, then watered to excess, capped, and allowed to drain until reaching a constant weight. The constant weight was the wet weight of the soil + pot + lid (W_W). A third soil-filled sample pot was emptied into a pan and the soil placed in an 80º C forced air dryer until it had reached constant weight. The oven-dried soil weight + pot weight + lid weight (W_D) was subtracted from W_W to calculate the amount of water held by the soil at 100% pot capacity (maximum amount of water held after free drainage has stopped). This was calculated for each of the two pots, and the mean value was used as the soil water holding capacity estimate. Plants were watered by hand and maintained at above 55% RSWC for approximately 30 d, then placed on the lysimeter.

Lysimeter Design and Operation

The gravimetric lysimeter consisted of 16 electronic balances with 6-kg maximum capacity and 0.1-g readability (Model XL-6100, Denver Instruments, Arvada, CO), connected to a personal computer that also operated 16 mechanical relays. Each relay operated a normally closed two-way solenoid valve, each of which controlled water flow from a 20-L reservoir to one of the 16 pots on the lysimeter balances. When a solenoid valve was activated by the computer, water was conducted by gravity flow from the reservoir to the watering hole in the lid of the appropriate pot, via vinyl tubing. The computer ran custom control software that was designed to read the weights from each balance approximately every 2 s, and then activate the appropriate solenoid valve to replace transpired water if any pot weight had fallen below the predetermined target weight for that balance. The lysimeter added water based on a 30-g threshold so that each pot was maintained between 15 g below and 15 g above its current target weight. The target weight (W_T) for each balance was calculated by the computer software as:

$W_T = W_D + W_P + RSWC\ (W_W - W_D)$ where;

W_D is the dry weight of soil + pot + lid,

W_P is total plant fresh weight estimated from measuring the shoot and root weights of two extra pots for each run of the experiment,

RSWC is the desired relative soil water content expressed as a fraction between 0 and 1, and

W_W is the wet weight of soil + pot + lid.

New target RSWC values were entered manually on a daily basis as required by the experimental protocol. The control software included logic to reject anomalous data caused by occasional faulty communication, balance malfunction, tampering, etc., and posted error alerts whenever such events occurred. Pot weights were recorded every 10 min by the software, and each time water was added to a pot the amount added was also recorded in the data file (Earl, 2003).

Drought Stress Simulation

After pots were placed on the lysimeter at approximately 30 DAS, drought stress was imposed and water use of both drought pots and control pots was monitored. For the first 2 d (the first day was the setup day), all pots were maintained at 80% RSWC (±3%) by the lysimeter to determine initial water use under water replete conditions. Four pots of 'Boggs' were maintained at 80% RSWC (water-replete controls) throughout the experiment. For drought pots, the system was programmed to allow RSWC to decline by 15% per day until 50% RSWC was reached, then 10% per day until 40% was reached. Subsequently, the RSWC was allowed to decline by 5% per day until 10% was reached. After one complete day at 10% RSWC, the pots were returned to 80% RSWC, and the whole plant water use was recorded for an additional 2 d.

Calculation of NTR

The normalized transpiration ratio (NTR) was calculated for each drought pot for every day of the dry-down phase, and for the two recovery days following rewatering. To calculate NTR, two steps were required as described by Ray and Sinclair (1997). First, daily water use of each plant was divided by that same plant's water use on the initial day of the experiment, when all pots were at 80% RSWC. This value is the transpiration ratio (TR), and it adjusts for any initial differences in plant size. Second, the daily TR of each drought stressed plant was divided by the mean daily TR of the control plants. This value is the NTR, and it adjusts for day-to-day differences in environmental conditions affecting transpiration such as solar radiation, humidity, and temperature. Mean daily RSWC (adjusted for actual plant fresh weights determined at the end of the experiment) was also calculated from lysimeter data for each day of the experiment. The first day was the normalization day. The NTR by definition has a value of 1.0 under water-replete conditions on the normalization day. On subsequent days, RSWC of drought pots was allowed to decline as described above and as shown in Figure 2.1.

Dry Matter-Based WUE

When each run of the lysimeter protocol was finished, plants were harvested, and shoot and root fresh weights were determined in order to adjust RSWC based on actual plant weight for each pot. After the shoots were removed and weighed, roots were thoroughly washed with water to remove soil, blotted dry with paper towels, and weighed. Shoots and roots were dried at 80°C to constant weight. Water use efficiency was estimated as the ratio of total plant dry weight to total water used since capping, including water use recorded by the lysimeter.

Experimental Design

The availability of 16 lysimeter units required grouping the 23 soybean genotypes into an early and a late maturing group (Table 2.1). ‘Boggs’ was included in both groups. Ultimately, six replications of both groups of genotypes were completed. Each run of the experiment required about 14 d of lysimeter time (dry-down and recovery period) after 30 d of maintaining the plants at non-stress water levels via hand watering. During the hand watering portion of the experiment, pots were positioned on the greenhouse bench in a randomized complete block experimental design. Eighteen pots were planted for each run – sixteen for the lysimeter (12 genotypes to be exposed to drought stress, plus four pots of Boggs to serve as water-replete controls), and two additional pots of Boggs for fresh weight determination. Plants were placed on the 16-balance lysimeter in two groups of 12 different genotypes based on maturity – Maturity Group (MG) IV to VI in Group 1 and MG VI to IX in Group 2 as described in Table 2.1. Both groups contained five pots of Boggs (MG VI), four of which were used as controls and one of which was a drought entry. Each run of the experiment constituted a replication of either Group 1 or Group 2, and groups were randomly ordered with respect to time. Genotypes were randomly assigned to the balances within each group. Six replications were completed for Group 1; however, for Group 2 there were six replications of WUE data but only five replications of NTR data due to a technical failure. In addition, one replication of Group 1 is lacking the NTR data for day 13 (second recovery day after rewatering) due to an error in data recording.

Data Analysis

Plateau regression was applied to the response of NTR to RSWC in order to find the threshold for the decline in evapotranspiration. This technique assumes that NTR is unaffected by soil drying until the RSWC reaches some critical value (usually between 0.2 and 0.3). As soil water content declines below this threshold, NTR is assumed to decline in a linear fashion. The intersection of the unaffected plateau region where NTR = 1 and the linear phase of the curve, as fit by PROC NLIN in SAS (SAS Institute, Cary, NC), is defined as the critical soil water content (that soil water content where the plant begins to reduce its evapotranspiration rate).

A third phase of the NTR-RSWC curve was revealed as a non-linear tail once NTR declined below about 0.1. Consistent with previous practice (Sinclair and Ludlow, 1986; Ray and Sinclair, 1998), all data for which NTR < 0.1 were removed so that these non-linear data would not unduly affect the plateau regression. As expected, removal of the "tail" significantly increased the average x-intercept for the regression (data not shown). In order to allow for convenient comparisons between the current and previous work, soil water content was also expressed as a fraction of transpirable soil water (FTSW) for each pot (Sinclair and Ludlow, 1986; Ray and Sinclair, 1997,1998). From the regression of NTR response to RSWC for each pot, the RSWC at which NTR is predicted to be 0.1 ($RSWC_{10}$) was determined. Then, estimates of RSWCc were converted to FTSWc as FTSWc = ($RSWCc-RSWC_{10}$) / ($1-RSWC_{10}$).

A few plants were missing due to lack of emergence or accidental damage prior to the end of the experiment; therefore, LSMeans were compared for each line for $FTSW_C$, recovery days NTR (measured on the last two days of the experiment when plants were returned to 80% RSWC), and WUE using the GLM procedure in SAS (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

WUE

Among the Group 1 genotypes, several lines differed significantly ($P < 0.05$) for WUE (Table 2.2). The percent difference from the lowest WUE (PI416937) to the highest WUE (Dillon) (calculated as [(highest WUE-lowest WUE)/ lowest WUE] x 100) was 21.4%. A comparison of all of the genotypes in Group 1 (with n = 6) shows that Dillon had significantly (P = 0.05) higher WUE than Hutcheson and all lines with WUE < 3.20 g DM kg^{-1} H_2O. PI416937 differed significantly (P = 0.05) from all lines with WUE values equal to or higher than 'Holladay' (n = 6). The WUE for control pots (Boggs) was 3.21 g DM kg^{-1} H_2O (standard error = 0.0349).

Previous authors induced cyclic drought stress in soybean by allowing Young and PI416937 to drop to 20 to 25% pot capacity before rewatering. In the current work, these genotypes were well watered for 30 d and were then exposed to a dry down (to 10% RSWC) and recovery (to 80% RSWC) period. This resulted in a 14.6% difference in WUE between Young and PI416937, which is between the values found by Earl in 2002 (9.1%) and Mian et al. in 1996 (18.9%) for cyclic stress (Table 2.3). Technique disparities in dry down and recovery periods, lysimeter apparatus, and age of the plants at harvest might account for some of the difference in the WUE values found for this experiment and previous ones. In the other two experiments a lysimeter was not used, and the microclimate around the balances (higher temperature around metal balance tops) might account for some of the difference observed. The harvest age for the prior experiments was from 34 to 38 d, while the harvest age in this experiment was 42 to 46 d. Even so, the relationship between Young and PI416937 was maintained in this experiment with Young being more water use efficient than PI416937 despite the fact that no significant differences in total plant dry weight or total water use were found between the two (data not shown).

In Group 2, several genotypes also differed in WUE (Table 2.4). Boggs had the highest WUE value, 3.24 g DM kg^{-1} H_2O, while 'Tokyo' had the lowest WUE, 2.72 g DM kg^{-1} H_2O, which amounted to a variation of 19.1% among the 12 genotypes in Group 2. Tokyo had been screened for WUE previously by Mian et al. (1996), but the value reported was 28.6% higher than that of this experiment (Table 2.3) perhaps for reasons cited above. Control pots (Boggs) had a WUE of 3.14 g DM kg^{-1} H_2O (standard error = 0.0446).

A comparison of the genotypes in Group 2 shows that Boggs was significantly different from 'Haskell,' and from all lines with WUE values < 2.97 (with n = 6). Tokyo was significantly (P = 0.05) different from N96-6809, N95-7424, 'Benning', and Boggs. Interestingly, Boggs had very similar WUE values in both groups (Group 1, 3.23; Group 2, 3.24), had the highest WUE in Group 2, and was not significantly different (P > 0.05) from the most water use efficient line, Dillon, in Group 1. Since the WUE value was so similar in both groups, a comparison of genotypes across groups where n = 6 could be considered. Total variation across groups from lowest to highest WUE was 25.4%.

Combining all of the data afforded the opportunity to identify extreme genotypes in one graph (Fig. 2.2). In this figure, dry weight is graphed against water used, and the WUE of any genotype is the slope of the line through the origin to that data point on the graph. The average of all genotypes was 3.0 g DM kg^{-1} H_2O. Identifying outliers with either very high or very low average WUE can assist in deciding which genotypes are worthy of further study and future planting in the field. Several lines emerged with interesting properties from regression analysis in SAS. Boggs, PI 407859-2, Dillon, PI416937, PI471938, and Tokyo were most notable. Boggs is mentioned because it was in both groups, and it showed low water using and low dry matter producing properties and near average WUE. PI 407859-2 also had a WUE near the average, but in contrast to Boggs, it was a very high water using and very high dry matter producing line. Dillon used a moderate amount of water but produced considerable dry matter. It was identified by Cook's distance measure (Neter et al., 1996) in SAS as an influential point in the regression, which could skew the regression when it is allowed to remain in the data set. PI416937 also was a moderate water user but produced little dry matter. PI471938 displayed moderate water use but high dry matter production. It was a leveraged point (extreme observation) because it had a studentized deleted residual or externally studentized residual (R-student value in SAS) of $> |2.0|$ at 2.02 (Belsley et al., 1980). Tokyo was a very high water user but moderate dry matter producer. It was an influential point like Dillon.

When the genotypes were evaluated within their respective groups (Fig. 2.3 and 2.4), similar results were found, but there were some differences. In Group 1, PI416937 was identified as a leveraged point by its R-student value (-2.13). In Group 2, Boggs was an extreme observation with an R-student of 2.34. Tokyo remained an influential point as it was when all of the data were grouped together.

Variation for WUE among genotypes has previously been reported under well-watered conditions in the following species: wheat, 20.6% difference, (Van den Boogaard et al., 1997); sorghum, 34% difference, (Donatelli et al., 1992); cowpea, 25% difference, (Ismail et al., 1993); and soybean, 8.1% difference, (Earl, 2002). Additionally, variation has been found under intermittent drought stress in peanut, 62.2%, (Wright et al., 1994) and in soybean between Young and PI416937, 18.9%, (Mian et al., 1996) and 9.1%, (Earl, 2002). Original percentage differences in WUE from these manuscripts were recalculated using the same method described previously in this section, for the purpose of accurate comparison.

NTR

One potential disadvantage of expressing NTR in terms of FTSW instead of RSWC is that by calculating FTSW independently for every pot, any real differences that may exist between genotypes in their abilities to extract water from very dry soil are obscured. If such differences exist, FTSW is a biased measure of soil water content that is affected by the genotype under consideration, and FTSWc is not a suitable measure of sensitivity of whole plant water use to soil drying when comparing different genotypes. However, analysis of the x-intercepts of the regression of NTR on RSWC revealed few significant differences among genotypes, indicating that there was little variability for the ability to extract water from dry soil. The only significant ($P < 0.05$) difference found was in Group 2, between Tokyo (intercept = 0.057) and Haskell (intercept = 0.091) (data not shown).

Figure 2.5 shows the relationship between NTR and FTSW and two different $FTSW_C$ values for Young and PI416937 in Group 1, and Tables 2.2 and 2.4 show critical values from the plateau regression of NTR on FTSW for Groups 1 and 2, respectively. These critical values represent the soil water content or fraction of transpirable soil water at which NTR declined, probably because stomates began to close (Ray and Sinclair, 1997; Earl, 2003), and they were, for the most part, in the range of 0.3 to 0.2 FTSW as previously reported for the soybean genotype CPI 26671 by Sinclair and Ludlow (1986). However, a mean FTSW threshold as high as 0.35 was reported for 'Biloxi' (Ray and Sinclair, 1998). In Group 1, values ranged from 0.287 (PI416937) to 0.217 (Young), which represents a 32.3% variation between the highest and lowest genotypes, while Group 2 values ranged from 0.270 (G2120) to 0.186 (Haskell) representing a 45.2% variation. Several pairs of genotypes were significantly different from each other in Group 1, but in Group 2, only two pairs of genotypes were significantly different (Benning and Haskell and G2120 and Haskell).

Small but significant differences were found among these genotypes in the FTSW at which NTR began to decline (Tables 2.2 and 2.4). These results indicate that some genotypes might have differed in their stomatal response to soil water content. For example in Group 1, PI416937 had the highest $FTSW_C$ and reduced whole plant water use earlier than N98-7265, PI 407859-2, N94-7784, and Young. In Group 2, Benning and G2120 had the highest $FTSW_C$ and reduced whole plant water use sooner than Haskell. The relative advantage or disadvantage of early stomatal closure in the field would depend on the duration of water stress or drought. Under a long term drought, PI416937 would be at an advantage over N98-7265, PI 407859-2, N94-7784, and Young because it could conserve water and increase its chances for survival. However, under short term stress PI416937 could be at a disadvantage by prematurely closing stomata and could thereby sacrifice potential productivity.

No significant ($P > 0.05$) genotype effect was found for normalized transpiration ratio recovery data among Group 1 genotypes. For Group 2 genotypes, on Recovery Day 1, the range in NTR between the highest and lowest entries in Group 2 was 0.144 (Table 2.5). Boggs demonstrated the highest NTR value which was more than double that of the lowest entry N96-7031. On Day 2, the range between highest and lowest entries was 0.207. N96-7031 had the lowest NTR value, while N96-6809 had the highest with an NTR of 0.542. Therefore, N96-6809 showed a 61.8% higher NTR value than N96-7031. A genotype with faster recovery could potentially regain full productivity sooner than one with slower recovery. This physiologic behavior could be of interest to breeders since yield is of utmost importance to them and N96-6809 and Boggs both had high WUE values, and high NTR values on recovery days.

SUMMARY

Substantial variation was found between soybean genotypes for vegetative stage WUE, while small but significant variation was found for down regulation of whole plant water use during drought stress. Among Group 1 genotypes, the variation was 21.4% for WUE, 32.3% for $FTSW_C$, and insignificant for recovery from stress as measured by NTR. In Group 2, the variation between genotypes was 19.1% for WUE, 45.2% for $FTSW_C$, 114.3% on Recovery Day 1 for NTR and 61.8% on Recovery Day 2 for NTR. When Groups 1 and 2 were combined, the variation across genotypes for WUE was 25.4%. Interestingly, some genotypes had extreme WUE values for different reasons. For example, Dillon used a moderate amount of water to produce a significant amount of dry matter while PI416937 used a moderate amount of water to produce little dry matter. Boggs on the other hand, while displaying near average WUE, used little water and created little dry matter.

Perhaps differences in WUE offer the greatest potential for future work. Several QTLs have already been identified to explain the difference in WUE between Young and PI416937 (Mian et al., 1996). Marker-assisted selection of desirable new QTLs (if found) could be used to complement phenotypic selection of other genotypes from the current work. By extending the work of Mian et al. (1996;1998), we have shown that additional intraspecific variation for WUE exists among soybean genotypes. While increased WUE could be beneficial in any climate, a high value for this trait in water-limited areas like the southeastern USA should certainly be advantageous if the same degree of variation also exists in field plots (Quisenberry and McMichael, 1991).

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Table 2.1. Grouping, maturity, and putative drought response characteristics of the 23 soybean genotypes.

Group	Name	Maturity Group	Putative drought characteristics/comments†
I	Fendou 34	IV	Slow wilter from China
I	Holladay	V	Adapted to high yield environments
I	Hutcheson	V	Very stable over environments
I	PI471938	V	Slow wilter from Nepal, high yield under stress
I	PI 407859-2	V	Slow wilter from Korea
I	N98-7265	V	High yield; progeny of PI471938
I	N94-7784	V	Slow wilter from Egypt; all U.S. pedigree
I	PI416937	V	Slow wilter from Japan, parent of QTL population
I	Dillon	VI	Adapted to high yield
I	N94-7589	VI	Slow wilter; progeny of PI416937
I	Young	VI	Parent of QTL population
I & II	Boggs	VI	Stable over environments; common check
II	Tokyo	VII	Source of N_2 fixation; possible tolerance to drought
II	Benning	VII	Commercial Cultivar
II	Jackson	VII	N_2 fixation; tolerant of drought
II	Haskell	VII	Commercial Cultivar
II	N95-7424	VII	Fast grow off
II	N90-7199	VII	High yield; progeny of PI416937; released as N7001
II	N96-6809	VII	Very high yield; grandchild of PI416937
II	Cook	VIII	Very high yield
II	N97-9765	VIII	Very slow wilter; grandchild of PI416937
II	N96-7031	VIII	Very slow wilter; grandchild of PI416937
II	G2120	IX	Indonesian type with possible unusual leaf properties or heat tolerance

† Personal communication, T.E. Carter.

Table 2.2. Water use efficiency (WUE) and critical values for the fraction of transpirable soil water ($FTSW_C$) at which normalized transpiration ratio begins to decline as estimated by plateau regression for soybean genotypes in Group 1 (Maturity Groups IV-VI).

Genotype	Replications	WUE	$FTSW_C$
	No.	(g DM kg^{-1} H_2O)	
Dillon	6	3.41	0.271
PI471938	6	3.38	0.244
N98-7265	6	3.30	0.226
Boggs	6	3.23	0.256
Young	6	3.22	0.217
Hutcheson	6	3.19	0.259
Holladay	6	3.17	0.239
Fendou 34	5	3.10	0.252
N94-7589	5	3.02	0.251
N94-7784	6	2.94	0.221
PI 407859-2	6	2.94	0.223
PI416937	6	2.81	0.287
LSD $_{(0.05)}$ WUE	0.209†, 0.219‡, 0.229§		
LSD $_{(0.05)}$ $FTSW_C$	0 .049†, 0.052‡, 0.054§		

† Comparisons between genotypes with n = 6.

‡ Comparisons between genotypes with n = 5 and n = 6.

§ Comparisons between genotypes with n = 5.

Table 2.3. Comparison of WUE values for several soybean genotypes by different researchers in 1996, 1998, and 2002 at Athens, GA.

Comparison of WUE Values

(g DM kg^{-1} H_2O)

Genotype		Mian et al. (1996,1998) Cyclic stress	Earl (2002) Cyclic stress	Hufstetler and Earl (Current work) Control then Stress	Earl (2002) Control
Young		4.40	3.70	3.22	3.20
PI416937		3.70	3.39	2.81	2.96
	% difference	18.9	9.1	14.6	8.1
	Days until harvest	36	34-38	42-46	34-38
Tokyo		3.50		2.72	

Table 2.4. Water use efficiency (WUE) and critical values for the fraction of transpirable soil water ($FTSW_C$) at which normalized transpiration ratio begins to decline as estimated by plateau regression for soybean genotypes in Group 2 (Maturity Groups VI-IX).

Genotype	**WUE**	**$FTSW_C$**
	(g DM kg^{-1} H_2O)	
Boggs	3.24 (6)†	0.246 (5)
Benning	3.14 (5)	0.270 (4)
N95-7424	3.04 (6)	0.215 (5)
N96-6809	3.01 (6)	0.243 (5)
Cook	2.98 (6)	0.212 (5)
Haskell	2.96 (6)	0.186 (5)
Jackson	2.94 (6)	0.229 (5)
N90-7199	2.91 (6)	0.234 (5)
N97-9765	2.90 (6)	0.251 (5)
G2120	2.87 (6)	0.270 (5)
N96-7031	2.82 (6)	0.255 (5)
Tokyo	2.72 (6)	0.241 (5)
$LSD_{(0.05)}$ WUE	0.268‡, 0.281§	
$LSD_{(0.05)}$ $FTSW_C$	0 .079¶, 0.084#	

† ( ) = sample size (n)

‡ Comparisons between genotypes with n = 6.

§ Comparisons between genotypes with n = 6 and n = 5.

¶ Comparisons between genotypes with n = 5.

Comparisons between genotypes with n = 5 and n = 4.

Table 2.5. Late maturing (Group 2) soybean recovery day data for normalized transpiration ratio (NTR) after returning pots to 80% relative soil water content.

Genotype	Replications	NTR, first recovery day	NTR, second recovery day
	No.		
Boggs	5	0.270	0.515
N96-6809	5	0.229	0.542
N90-7199	5	0.216	0.512
Cook	5	0.190	0.448
Jackson	5	0.184	0.398
Tokyo	5	0.175	0.366
Benning	4	0.165	0.388
N97-9765	5	0.163	0.452
G2120	5	0.161	0.402
N95-7424	5	0.156	0.338
Haskell	5	0.136	0.374
N96-7031	5	0.126	0.335

$LSD_{(0.05)}$ Recovery Day 1 0.073†, 0.077‡
$LSD_{(0.05)}$ Recovery Day 2 0.125†, 0.132‡

† Comparisons between genotypes with n = 5.

‡ Comparisons between genotypes with n = 5 and Benning n = 4.

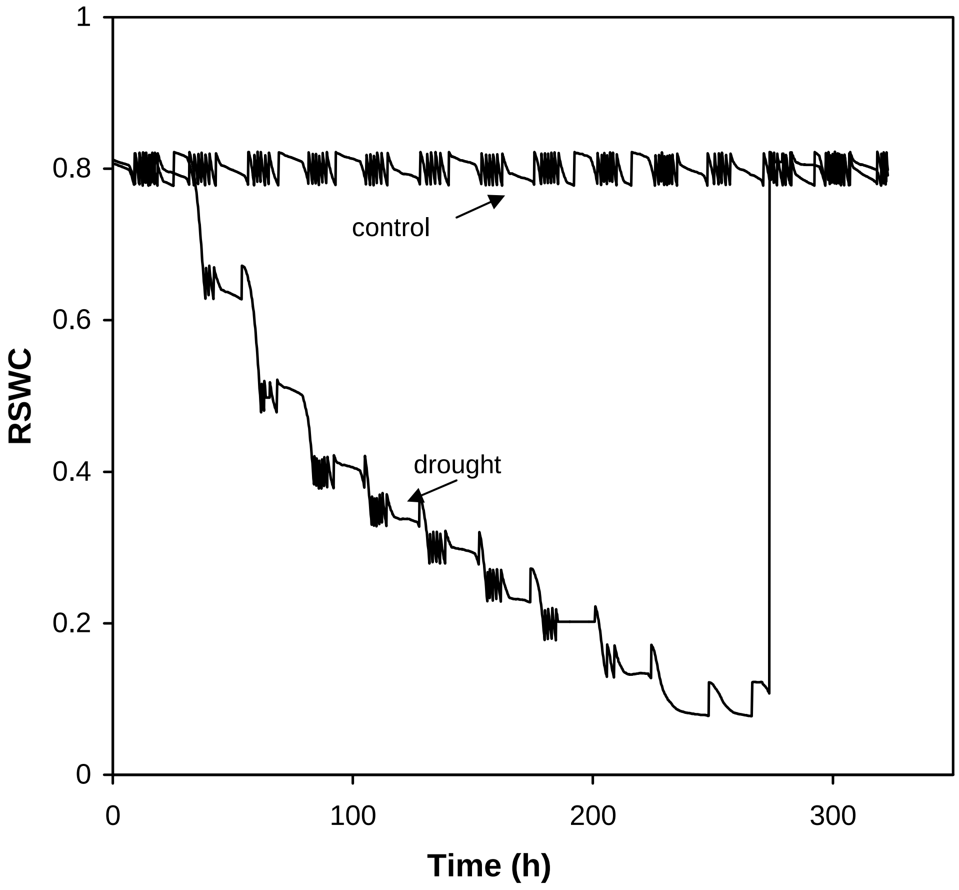

Figure 2.1. Relative soil water content (RSWC) vs. Time for a drought entry Fendou 34 and a control entry Boggs. The normalization day spans the first 24 h.

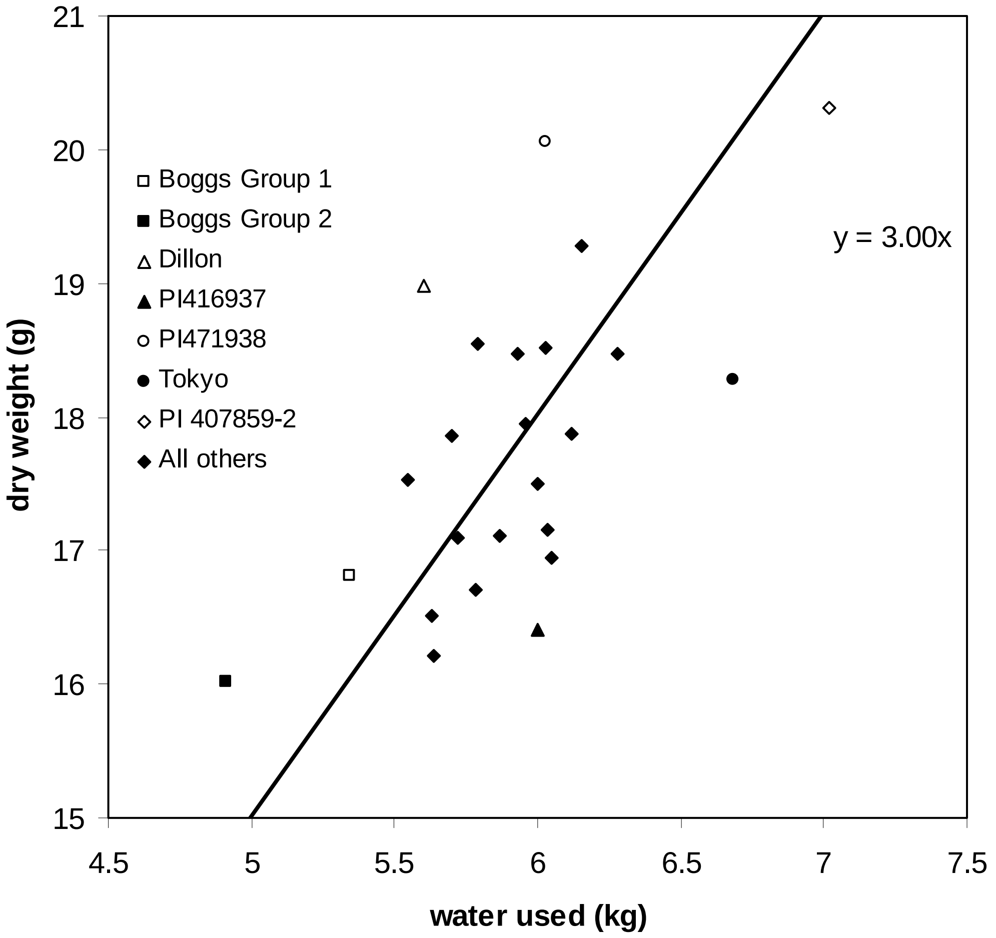

Figure 2.2. Average soybean dry matter production vs. average water used for 23 soybean genotypes. The slope of the regression line through the origin closely estimates mean water use efficiency (WUE). The WUE of any genotype can be estimated from the slope of its data point on the graph to the origin.

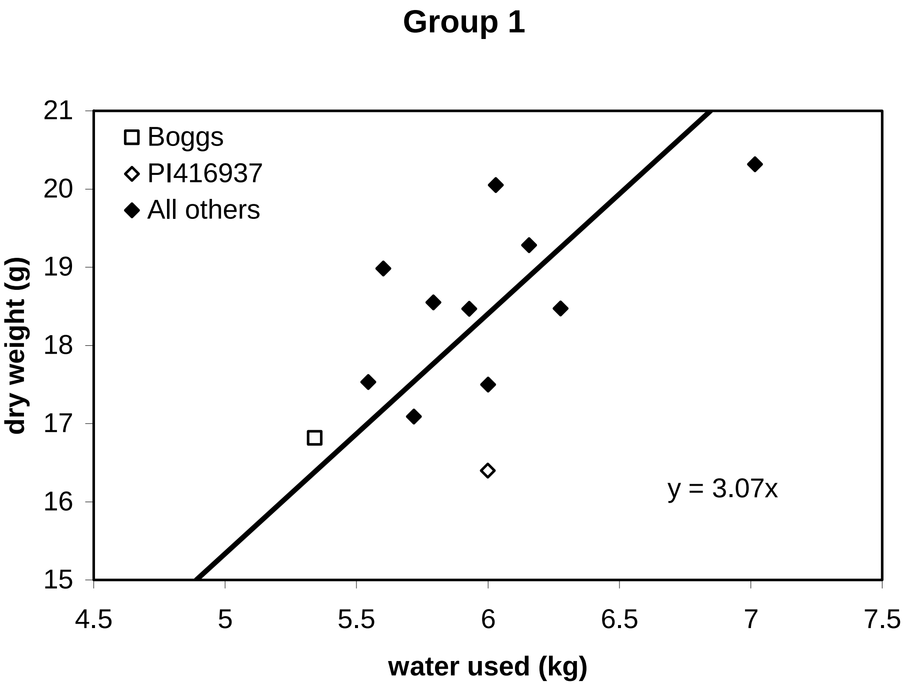

Figure 2.3. Average soybean dry matter production vs. average water used after six replications for early maturing genotypes (Group 1). The slope of the regression line through the origin closely estimates mean water use efficiency (WUE). The WUE of any genotype can be estimated from the slope of its data point on the graph to the origin.

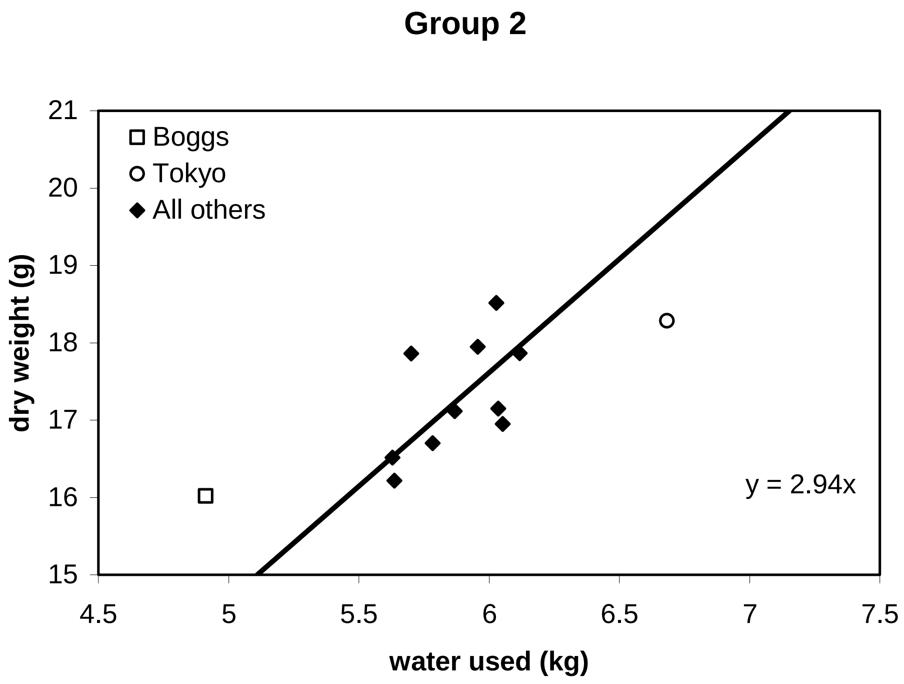

Figure 2.4. Average soybean dry matter production vs. average water used after six replications for late maturing genotypes (Group 2). The slope of the regression line through the origin closely estimates mean water use efficiency (WUE). The WUE of any genotype can be estimated from the slope of its data point on the graph to the origin.

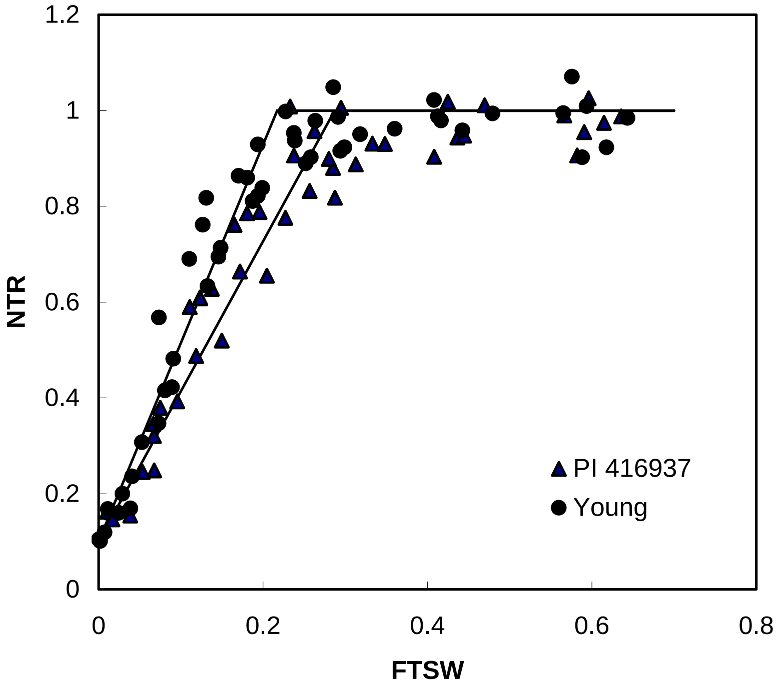

Figure 2.5. Normalized transpiration ratio (NTR) vs. fraction of transpirable soil water (FTSW) for two soybean genotypes in Group 1 with different critical values of fraction of transpirable soil water ($FTSW_C$) based on plateau regression. $FTSW_C$ values are 0.287 and 0.217 for PI416937 and Young, respectively. Data with NTR values < 0.1 and recovery data (days 12 and 13) have been excluded.

CHAPTER 3

USING GAS EXCHANGE TECHNIQUES TO MEASURE AND IDENTIFY VARIATION IN MINIMUM LEAF EPIDERMAL CONDUCTANCE OF 23 SOYBEAN GENOTYPES[1]

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[1] Hufstetler, E.V. and H.J. Earl. To be submitted to *Crop Science*.

ABSTRACT

Leaf conductance to water vapor is very low under conditions when stomata are closed. Since lower minimum epidermal conductance values (g_e) have been implicated in imparting drought resistance, 23 soybean (*Glycine max* (L.) Merr) genotypes, (cultivars, breeding lines, and plant introductions) with putative differences in drought survival traits were screened for variation in vegetative phase $g_{e.}$ Plants were grown under water-replete conditions in a greenhouse, and 30 d after sowing were dark-adapted for 36 h. Leaf gas exchange techniques were used to measure total leaf conductance to water vapor in the dark, which was taken as g_e. Significant variability for the trait was found among these genotypes, with g_e values ranging from 8.60 to 18.5 mmol m^{-2} s^{-1}. A concurrent experiment was being conducted to screen these same lines for variation in whole plant water use efficiency (WUE). The range of values for WUE was 2.72 to 3.41 g DM kg^{-1} H_2O. A strong negative correlation $r = -0.736$ was found between these two traits. Such a relationship was not expected and is not predicted by current theory. This result raises new questions about the physiological basis of soybean genotype differences in WUE.

INTRODUCTION

Severe soil water deficits can threaten both the yield and survival of soybean (*Glycine max* L. Merr.) crops in the southeastern USA. The uncertainty of crop survival begins when stomatal conductance has reached its physiological minimum and CO_2 assimilation has virtually stopped (Sinclair and Ludlow, 1986). One physiological trait that has been suggested for increasing drought tolerance and prolonging crop survival during severe water stress is reduced epidermal conductance (g_e), which reflects leaf water loss both via epidermal cells through the cuticle and via incompletely closed stomata (Sinclair and Ludlow, 1986; Ludlow and Muchow, 1990). When stomata close, a leaf's water loss is controlled by g_e and the leaf-to-air vapor pressure differential. Leaf survival depends on the rate of water loss and the difference between the relative water content (RWC) at which stomata close and the RWC at which leaves die. Low g_e promotes leaf survival, and hence plant survival, by improving dehydration avoidance (Ludlow and Muchow, 1990).

Total leaf surface conductance to water vapor is composed of the stomatal and cuticular pathways acting in parallel (van Gardingen and Grace, 1992). Cuticular conductance to CO_2 and H_2O is often negligible unless stomata are at minimum apertures (Lambers et al., 1998). The stomatal conductance of unstressed leaves may be two or more orders of magnitude greater than the cuticular constituent (Lendzian and Kersteins, 1991; van Gardingen and Grace, 1991). However, the cuticular conductance of water-stressed leaves or dark-adapted leaves may exceed the stomatal conductance (van Gardingen and Grace, 1992), and Boyer et al. (1997) observed that these water stressed or dark-adapted leaves will experience a shift from stomatal to cuticular control when stomata close. In water stressed wheat (*Triticum aestivum* L. and *Triticum turgidum* L. var. *durum*) plants, non-stomatally controlled water loss through the leaf epidermis accounted for up to 50% of total daytime transpiration and 100% of night transpiration (Rawson and Clarke, 1988). Therefore, after stomata close, a plant's ability to withstand severe water deficits is largely determined by its capacity to reduce water loss through the leaf epidermis (Araus et al., 1991).

Genetic variability for epidermal transpiration has been identified in several species including oats (*Avena sativa*, Bengston et al., 1978); maize (*Zea mays*, Dube et al., 1975); sorghum (*Sorghum bicolor*, Jordan et al., 1984); and rice (*Orzya sativa*, O'Toole et al., 1979). Genetic variation in minimum epidermal conductance has been found in soybean (Paje et al., 1988), sorghum (Muchow and Sinclair, 1989), and wheat (Araus et al.,1991). Rawson and Clarke (1988) noted that cuticular resistance to water loss for a genotype changes rather than remaining constant depending on the vapor pressure deficit (VPD) even though the direction of the response is unpredictable with both positive (Jordan et al., 1984) and negative correlations (Moreshet, 1970; Dube et al., 1975) being reported in the literature.

Sinclair and Ludlow (1986) characterized three phases in which plants respond to declining soil water. Stage I is the period in which water is freely available from the soil and both stomatal conductance and water vapor loss are at high levels. Thus, the environmental conditions surrounding the shoot determine the rate of plant water loss. Stage II commences when the rate of water uptake from the soil can no longer equal the potential transpiration rate. This diminished rate of soil water supply is thought to be a consequence of declining soil hydraulic conductivity as the volumetric water content drops. As a result, stomatal conductance declines which establishes the transpiration rate at approximately the same rate as soil water uptake and achieves the maintenance of the water balance of the plant. Stage III begins when stomata have lost their ability to compensate for the declining rate of uptake from the soil, and stomatal conductance reaches its minimum. Since stomata have closed, water loss from the leaf is determined by g_e and the vapor pressure difference between the leaves and the air. Sustained water balance of the plant during Stage III depends on the presence of sufficiently small g_e values so that the low rate of soil water uptake can restore the water loss. Stage III continues until the critical relative water content is reached and plants die due to leaf desiccation. Two types of leaf responses can occur during severe water deficit: (1) all leaves may be retained by the plant until their water content decreases nearly uniformly down to the lethal relative relative water content (RWC_l), or (2) water may be lost progressively from leaves to the RWC_l and these leaves are then shed sequentially as the drought continues until all or nearly all of the leaves have been shed, and the plant dies (Sinclair, 2000).

The duration of plant survival of four grain legumes during Stage III was consistent for the most part with lower g_e imparting higher dehydration tolerance (Sinclair and Ludlow, 1986). Soybean survived for the least amount of time (2 d) after reaching the end-point of transpirable soil water (relative transpiration (RT) = 0.1)) while the other three species survived for longer periods. Black gram survived for 14 d, pigeonpea for 18 d, and cowpea for 24 d. Dehydration tolerance was defined as the water potential and relative water content of the youngest fully expanded leaf on leguminous plants when all of the other fully expanded leaves had died (Sinclair and Ludlow, 1986). Dehydration tolerance of leaves from the four grain legumes during Stage III was reported by the authors with the following rank: pigeonpea>cowpea>black gram>soybean with pigeonpea having the greatest dehydration tolerance and soybean having the least. These rankings were the same for lowest (pigeonpea) to highest g_e (soybean). Thus, of the four grain legumes, soybean had the highest g_e and lowest dehydration tolerance. Finding genetic variation in soybean for reduced g_e might permit breeders to increase the dehydration tolerance of elite cultivars.

The goal of this work was to quantify genetic variation for g_e among 23 soybean breeding lines, plant introductions, and cultivars chosen in consultation with Dr. T.E. Carter (USDA-ARS, Raleigh, NC), based on the results of prior field screening and putative water use characteristics using leaf gas-exchange measurement techniques. In addition, concurrent measurements of WUE on these same lines provided the opportunity to determine if there was a correlation between g_e and WUE.

MATERIALS AND METHODS

Growth Conditions

All experiments were conducted between June 2002 and January 2003 at The Univ. of Georgia in Athens, GA (33.9º N, 88.3º W). Soybean plants were grown in glasshouses in Brown Earth potting mix (Craven, Inc., Commerce, GA). The 23 soybean plant introductions, cultivars, and breeding lines (genotypes) used in this experiment are shown in Table 3.1.

Seeds were sown four to a pot and fertilized weekly with 20-20-20 (N-P-K) fertilizer plus micronutrients (Miller Greenhouse Special, Miller Chemical and Fertilizer Co. Corp., Hanover, PA). Pots were 2.5-L white plastic food containers (Berry Plastics Corporation, Evansville, IN) with four drainage holes added to each. Plants were maintained in well-watered and fertilized conditions for 30 d prior to data collection. Six replications were planted sequentially. Within each replication, genotypes were randomized in terms of their location on the greenhouse bench.

Air temperatures were maintained at 27 ± 4º C during the day and 20 ± 2º C during the night. Photoperiod was extended to 16 h with overhead 400-W metal halide lamps that produced supplemental photosynthetic photon flux density (PPFD) of approximately 230 µmol m^{-2} s^{-1} at the tops of plants.

g_e Measurements

Epidermal conductance measurements were taken at approximately 30 d after sowing (DAS) with two LI- 6400 gas exchange systems (LI-COR, Inc., Lincoln, NE). The 23 genotypes were placed in a dark room for ≥ 36 hours prior to the start of gas exchange measurements. This dark adaptation period was sufficient to cause significant stomatal closure and prevent circadian rhythms such as stomatal opening in response to time of day (i.e. daybreak) according to preliminary experiments (H. Earl, unpublished data).

To facilitate data collection during the measurements, a dim source of green light was added to the room. Two low output (25 W) bulbs (Philips Lighting Company, Somerset, NJ) were used. Measured PAR from these bulbs in the room ranged from 0-1 µmol m^{-2} s^{-1}. Frechilla et al. (2000) found that 120 µmol m^{-2} s^{-1} of green light caused only a slight increase in stomatal aperture (0.3 ± 0.2 µm) in contrast to the same amount of red light which caused an increase of 1.6 ± 0.1 µm; therefore, 0-1 µmol m^{-2} s^{-1} was considered to be a negligible amount of radiation from green light for this experiment.

Two LI-6400 gas exchange systems were used to monitor g_e after the dark adaptation period. On each measurement day, the water and CO_2 infra-red gas analyzers of the LI-6400s were calibrated using a verified CO_2 standard and a dew point generator. Measurements were made with the instruments' light sources and temperature controllers deactivated. Ambient air was drawn from within the laboratory and passed through a 4-L buffer volume. Flow rate of air through the sample chamber was maintained at 200 µmol s^{-1}. After a leaf was installed in the chamber, gas exchange was allowed to equilibrate until a graph of the water vapor concentration differential between the sample and reference paths stabilized. Then, data points were logged at 10-s intervals for 120 s, providing 12 sequential measurements of g_e for each leaf, which were then averaged to get the g_e estimate for that leaf (Fig. 3.1). For each plant, these measurements were made on both the youngest fully expanded main stem leaf (YFEL) and three nodes below this leaf (YFEL-3). The middle leaflet of the trifoliolate was measured in each case.

Data Analysis

Values of g_e were compared across genotypes as well as leaf position using PROC MIXED (SAS Institute, Cary, NC). Due to missing data from broken or otherwise injured plants, LSMeans were calculated for g_e values. To determine whether an association was present between g_e and WUE values determined for these same 23 genotypes in a previous experiment, correlation analysis in SAS was performed on the 23 genotype means (see Chapter 2).

RESULTS AND DISCUSSION

Significant differences were found between genotypes for minimum g_e values ($P = .0003$) across leaf positions, but there was no difference in g_e values based on leaf position (upper vs. lower), and no genotype x leaf position interactions were present (Table 3.2). ‘Fendou 34’ had the highest average g_e value (18.5 mmol m^{-2} s^{-1}) while N98-7265 had the lowest (8.60 mmol m^{-2} s^{-1}). In Table 3.2, the first genotypes with sufficiently low g_e to differ from Fendou 34 were ‘Haskell’ and G2120 while the first genotype with sufficiently high g_e to differ significantly from N98-7265 was ‘Tokyo’.

Previously, Paje et al. (1988) found a range in g_e of 17.1 to 31.5 mmol m^{-2} s^{-1} (data converted from mm s^{-1}) among the first 40 soybean accessions they tested and a range of 10.3 to 23.9 mmol m^{-2} s^{-1} in the next 34 accessions they screened. One genotype that was represented in their work and in the current work was G2120. While we found a g_e value of 12.3 mmol m^{-2} s^{-1}

for G2120, Paje et al. found a value of 18.2 mmol m^{-2} s^{-1} in their first experiment and 11.4 mmol m^{-2} s^{-1} in their second which if averaged (14.8) gives a value that is slightly higher than 12.3. Interestingly, G2120 was the most stable genotype across environments of the six accessions tested by Paje et al. in 1988.

The relationship between the 23 genotypes for their mean g_e and WUE values is demonstrated in Figure 3.2. While several authors have established that variation for g_e (see introduction) and WUE (see previous chapter) exist in crop species independently, we are not aware of any literature that attempts to correlate these two traits. In the current work, a negative association was found between g_e and WUE for all 23 genotypes ($r = -0.559$), and an even stronger association was found when Fendou 34, an obvious outlier, was removed ($r = -0.736$) which was determined to be an outlier with a studentized residual > |2|. Thus, lower g_e was strongly correlated to higher WUE even though the plants from the g_e experiment and the WUE experiment had different culture systems and stress histories (see previous chapter). The relationship between g_e and WUE does not imply causality; that is, lower g_e values do not necessarily cause higher WUE values.

Future work in this area of research might include examining the causes of variation in g_e in soybean and the evaluation of low g_e in screening tests as a reliable predictor for drought resistance of different genotypes. Several theories have been proposed to explain the variation of g_e, including the quantity of cuticular wax (Clark and Levitt, 1956; Blum, 1975), quality of wax such as the structure and chemical composition (Rama Das et al., 1979), and stomatal density (Muchow and Sinclair, 1989). In sorghum, Muchow and Sinclair (1989) found that increased g_e was highly correlated to increased stomatal density ($r^2 = 0.816$). They hypothesized that at minimum stomatal apertures water loss from the cuticle above guard cell teichodes (holes in external cell walls of guard cells) becomes a significant source of leaf water loss. However, Araus et al. (1991), found conflicting results in durum wheat (*Triticum turgidum* var *durum* L.). They found no significant correlation between g_e and total stomatal density or between g_e and either adaxial or abaxial stomatal density in the fourth leaf, first node leaf, or flag leaf. After attributing differences in g_e partly to changes in environment and partly to species differences, Paje et al. (1988) conducted an experiment to test whether responses in stomatal density to environmental change in soybean were associated with environmentally induced changes in g_e. In the six soybean accessions tested, they found that differences in g_e in response to differences in environmental conditions were not associated with stomatal density. Determining whether stomatal density is constitutively correlated to g_e in soybean could yield interesting insight into epidermal conductance variation.

Other future work might include using lower g_e values as a screening tool to predict drought resistance between these genotypes. A necessary requirement for this trait is that the environmental, leaf-age induced, or random variability within a test be much smaller than the inherited differences between genotypes (Kersteins, 1996). Additionally, genotypes are not tested in low water availability environments until after the trait and yield potential have been combined (Ludlow and Muchow, 1990), and breeders tend to avoid any mechanistic trait that lacks definitive association with yield (Specht et al., 2001). To assess the value of the g_e trait for yield in soybean, breeders could use near isogenic lines that contrast in their expression of g_e (Richards, 1988). Further work could uncover inheritance of g_e and its position in the soybean genome. Perhaps it will be found on a shared QTL with water use efficiency since a solid correlation was found between the two traits.

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Table 3.1. Maturity Groups and putative drought response characteristics of the 23 soybean genotypes.

Genotype	Maturity Group	Putative drought characteristics/comments†
Fendou 34	IV	Slow wilter from China
Holladay	V	Adapted to high yield environments
Hutcheson	V	Very stable over environments
PI471938	V	Slow wilter from Nepal, high yield under stress
PI 407859-2	V	Slow wilter from Korea
N98-7265	V	High yield; progeny of PI471938
N94-7784	V	Slow wilter from Egypt; all US pedigree
PI416937	V	Slow wilter from Japan, parent of QTL population
Dillon	VI	Adapted to high yield
N94-7589	VI	Slow wilter; progeny of PI416937
Young	VI	Parent of QTL population
Boggs	VI	Stable over environments; common check
Tokyo	VII	Source of N_2 fixation; possible tolerance to drought
Benning	VII	Commercial Cultivar
Jackson	VII	N_2 fixation; tolerant of drought
Haskell	VII	Commercial Cultivar
N95-7424	VII	Fast grow off
N90-7199	VII	High yield; progeny of PI416937; released as N7001
N96-6809	VII	Very high yield; grandchild of PI416937
Cook	VIII	Very high yield
N97-9765	VIII	Very slow wilter; grandchild of PI416937
N96-7031	VIII	Very slow wilter; grandchild of PI416937
G2120	IX	Indonesian type with possible unusual leaf properties or heat tolerance

† Personal communication, T.E. Carter.

Table 3.2. Mean leaf epidermal conductance (g_e) for the 23 soybean genotypes. Measurements averaged across two leaf positions per plant. N = 6 plants.

Genotype	g_e
	(mmol m^{-2} s^{-1})
Fendou 34	18.5
PI416937	17.0
PI 407859-2	14.9
Tokyo	14.6
N97-9765	13.7
N90-7199	13.2
N96-7031	13.0
N95-7424	12.9
Haskell	12.3
G2120	12.3
PI471938	11.5
Cook	11.4
Holladay	11.3
Jackson	11.2
N94-7589	11.2
Young	10.8
Boggs	10.5
N94-7784	10.4
N96-6809	10.3
Dillon	10.3
Benning	10.0
Hutcheson	9.90
N98-7265	8.60
LSD $_{(0.05)}$ = 5.67	

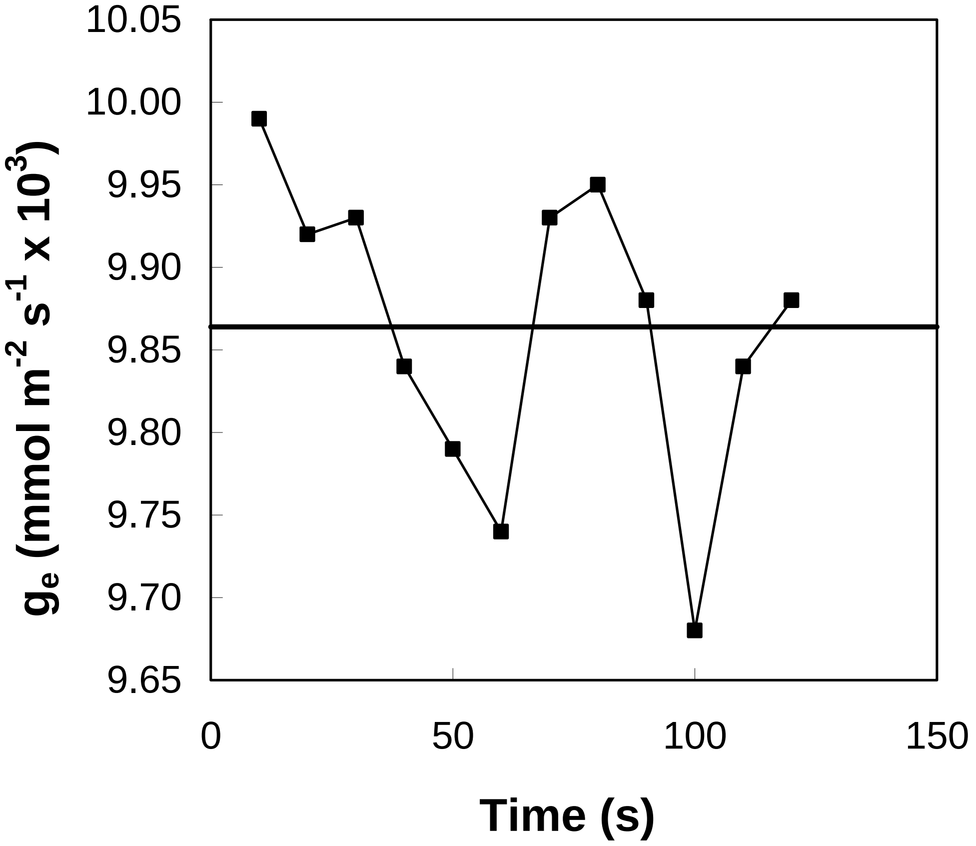

Figure 3.1. Epidermal conductance (g_e) measurements for a typical soybean leaf (PI471938, Upper leaf) in the dark taken 10 s apart. Mean value of 12 readings was 9.86 mmol m^{-2} s^{-1}.

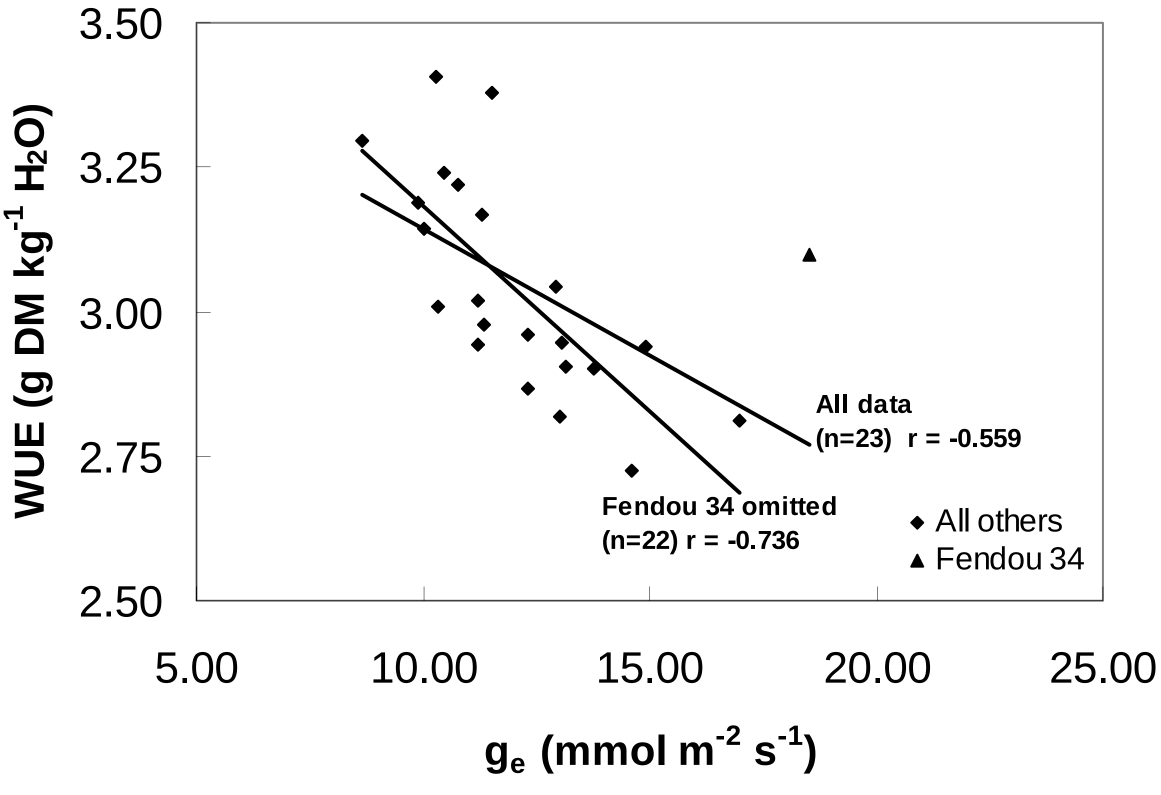

Figure 3.2. Relationship between soybean LSMean WUE and LSMean g_e values for the 23 genotypes (6 reps and 2 leaf positions). Each data point represents a different genotype. Pearson correlation coefficients significant at $P = 0.0055$ with $n = 23$ and $P < 0.0001$ with $n = 22$.

CHAPTER 4

SUMMARY

Considerable variation was found among soybean genotypes for vegetative stage WUE, while small but significant variation was found for down regulation of whole plant water use during drought stress. The variation between Group 1 genotypes was 21.4% for WUE, 32.3% for $FTSW_C$, and insignificant for recovery from stress as measured by NTR while the variation in Group 2 between genotypes was 19.1% for WUE, 45.2% for $FTSW_C$, 114.3% on Recovery Day 1 for NTR, and 61.8% on Recovery Day 2 for NTR. When Groups 1 and 2 were combined, the variation across genotypes for WUE was 25.4%. Significant differences were found between genotypes for minimum epidermal conductance (g_e) values ($P = 0.0003$) across leaf positions, but there was no difference in g_e values based on leaf position (upper vs. lower), and no genotype x leaf position interactions were present. Fendou 34 had the highest average g_e value (18.5 mmol m^{-2} s^{-1}) while N98-7265 had the lowest (8.60 mmol m^{-2} s^{-1}). In the present work, a significant negative correlation was found between g_e and WUE among the 23 genotypes ($r = -0.559$), and an even stronger correlation was found when Fendou 34 was removed ($r = -0.736$) which was determined to be an outlier with a studentized residual $> |2|$. Thus, lower g_e was strongly correlated to higher WUE even though the plants from the g_e experiment and the WUE experiment had different culture systems and stress histories. The results from the two experiments demonstrate that all of our objectives were met since genetic variability was found in the 23 genotypes for the three traits examined WUE, NTR (whole plant transpiration in response to drying soil), and g_e, and a correlation between g_e and WUE was established.