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Evaluation of Elite Southern Maturity Soybean Breeding Lines for Drought-Tolerant Traits

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ABSTRACT

Breeding efforts in soybean [*Glycine max* (L.) Merr.] have addressed the challenge of water-limited yields by incorporating parental stocks which exhibit drought-tolerant traits. Multiple cycles of empirical selection for improved yielding ability in water-deficient field environments have produced new generations of adapted breeding lines. However, the impact of this selection process on specific putative drought-tolerant traits is unknown. The objective of this study was to determine if breeders' selection of 10 elite lines for high seed yield under dry conditions is associated with the presence of physiological expression of three putative drought-tolerant traits: (i) limited transpiration rate under high vapor pressure deficit (VPD), (ii) early decrease in transpiration rate with soil drying, and (iii) drought-tolerant N_2 fixation. Greenhouse experiments were undertaken to characterize each genotypes for their phenotype of each of these three traits. Unlike most soybean cultivars, 9 of the 10 elite lines expressed a limited transpiration rate under elevated VPD. The VPD at which transpiration rate became limited was 1.9 kPa or less. There was no difference among genotypes in the threshold for decline in transpiration rate with soil drying, although all genotypes expressed high thresholds indicating an ability to conserve soil water. All lines expressed drought tolerance in their N_2 fixation rates, which was superior to that commonly observed in soybean. This study demonstrated that mating of parents that expressed a drought trait and multiple rounds of progeny selection based on improved yield under water-limited conditions resulted in the elite lines expressing improved drought traits.

Drought is one of the greatest threats to crop profitability. Not surprisingly, circumventing this problem is a priority for agriculturalists (Lauer et al., 2012). In recent years, researchers have pursued the promising approach of intentionally breeding new crop genotypes which increase yields under drought conditions. In soybean, the first drought-tolerant soybean type was identified in the 1980s (Purcell and Specht, 2004), and several genotypes are now poised for release as drought-tolerant cultivars. However, there is no clear understanding of the underlying basis for this progress. Thus, there is no guidance at present as to how, when, or where these improved breeding lines might be used to best advantage in future breeding efforts. The many interacting physiological attributes that govern drought response, as well as a large number of controlling genes (Hufstetler et al., 2007; Charlson et al., 2009; Abdel-Haleem et al., 2011, 2012; Ries et al., 2012) indicate that additional progress in achieving drought tolerance would likely be aided by such specific guidance.

An important advance in resolving physiological traits to enhance drought tolerance has been the identification of soybean genotypes expressing the delayed-wilting or "slow-wilting" phenotype. Sloane et al. (1990) investigated the slow-wilting genotype PI 416937 (USDA-ARS National Genetic Resources Program, 2012a), which was collected from Japan. However, they were unable to offer a clear resolution for the basis of the advantage of PI 416937 under drought. More recently, PI 416937 has been shown to have limited transpiration rates when the vapor pressure deficit (VPD) of the atmosphere exceeds about 2 kPa (Fletcher et al., 2007; Sinclair et al., 2008). Nearly all other soybean genotypes studied thus far show a continuing increase in transpiration rate with increasing VPD (Sadok and Sinclair, 2009). Therefore, most soybean genotypes lose larger amounts of water at high VPD in contrast to PI 416937. Limiting water use, especially early in the growing season, will allow more water to be available later in the growing season to complete crop growth, and potentially increase crop yield (Sinclair et al., 2010).

In addition to the limited transpiration trait of PI 416937, the slow-wilting genotype PI 471938 (USDA-ARS National Genetic Resources Program, 2012b), which was collected in Nepal, has been found to have N_2 fixation that is extremely tolerant to soil drying (Devi and Sinclair, 2013). In nearly all soybean genotypes, even moderate soil dehydration to a "fraction of transpirable soil water" (FTSW) of 0.4 to 0.5 results

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Abbreviations: ARA, acetylene reduction activity; BP, breakpoint; FTSW, fraction of transpirable soil water; NARA, normalized acetylene reduction activity; NTR; normalized transpiration rate; TR, transpiration rate; VPD, vapor pressure deficit in the initiation of decreased rates of N_2 fixation (Weisz et al., 1985; Sinclair, 1986; Marino et al., 2007). The threshold for the decrease in N_2 fixation with soil drying of PI 471938 is at a FTSW substantially lower than most genotypes (about 0.23, Devi and Sinclair, 2013). Nitrogen fixation tolerance to drought is a rare soybean trait, but has been identified in the cultivar Jackson (Sall and Sinclair, 1991;Johnson, 1958) and eight plant introductions, including PI 227557 (USDA-ARS National Genetic Resources Program, 2012c), identified from a screening of about 3500 exotic soybean accessions (Sinclair et al., 2000). Line PI 227557 (Geden Shirazu) is an important ancestor in Japanese soybean breeding (Zhou et al., 2000) and appears in the ancestry of the recently developed U.S. soybean germplasm release N6202 (Carter et al., 2010). The cultivar Jackson derives three-eighths of its pedigree from the Japanese landrace Tokyo (USDA-ARS National Genetic Resources Program, 2012d), which is unusual for a U.S. cultivar, because most of the ancestry of U.S. cultivars is derived from China and Korea rather than Japan (Carter et al. (2004). Tokyo is believed to be the source of the drought-tolerant N_2 fixation trait discovered in Jackson. Although both PI 227557 and Tokyo are from Japan, the genetic relationship between the two is unknown.

A third putative drought trait that has not yet been linked to slow-wilting genotypes is an early decrease in stomata conductance with soil drying, which results in soil water conservation. Water conservation earlier in soil drying slows the progress in the development of drought and can help the crop avoid severe drought. The response to soil drying can be defined by the FTSW remaining at the point where a decrease in transpiration rate is initiated. Commonly the FTSW thresholds for the decrease in transpiration rate for most crop plants occur in the FTSW range of 0.25 to 0.40 (Sadras and Milroy, 1996). While large variability in the threshold has been identified in many crop species (Bhatnagar-Mathur et al., 2007; Devi et al., 2009; Schmidt et al., 2011; Devi and Sincliar, 2011; Gholipoor et al., 2012), limited variation has been observed among soybean germplasm. Hufstetler et al. (2007) identified genetic variability among soybean genotypes when the experiment was performed using a very sandy soil, which has been shown to accentuate genotypic differences (Sinclair et al., 1998), but neither PI 416937 nor PI 471938 exhibited unusually high FTSW thresholds.

Sinclair et al. (2010) examined the potential benefit of each of the three drought traits described above on soybean yields across the United States using a mechanistic simulation model. The model results showed that N_2 fixation drought tolerance had the most positive benefit across the United States with yield increases in 85% or more of the years in virtually all locations where soybean is currently grown. The next most beneficial trait was the limited-transpiration trait under high VPD resulting in yield increases in 80% or more of the years in all commercial soybean regions. The early decrease in transpiration rate with soil drying resulted in yield increases in about 70% or more of the years in most locations. Comparison in amount of yield gain associated with each of these three traits also showed a ranking with the largest yield increases due to the N_2 fixation drought-tolerance trait, then the limited-transpiration trait with high VPD, and the least from the transpiration decrease with early soil drying. Interestingly, the N_2 fixation drought-tolerance trait also commonly showed substantial yield benefits even in years assumed to be "wet".

The objective of this work was to investigate the expression of each of the three drought traits in 10 elite southern breeding lines that were developed through multiple breeding cycles of empirical selection for improved yield performance under drought conditions. The favorable yields of these lines in water-deficient growing conditions make them candidates for germplasm or cultivar release, and for parental stock in new breeding efforts. This study was to determine if the original mating of a parent with a drought-tolerance trait and subsequent selection of progeny lines based on empirical yield selection as compared to standard commercial checks resulted in the identification of high-yielding genotypes expressing one or more of the three drought-tolerance traits. A set of experiments was undertaken to resolve possible differences among genotypes in their response in transpiration rate over a range of VPD. A second set of experiments was done to compare the soil water content at which transpiration rate began to decrease with soil drying and at which acetylene reduction activity began to decrease.

MATERIALS AND METHODS

Genotypes

In the southern United States, soybean breeding programs conducted by the University of Arkansas, Fayetteville, and the USDA-ARS in Raleigh, NC, have been empirically developing drought-tolerant genotypes for more than 20 yr. These efforts have involved the traditional approach of mating parents suspected of drought-tolerant characteristics and evaluating progeny lines under field conditions. The initial screens were for agronomic traits such as robust plants, absence of diseases, suitable flowering time, and no pod shattering. Finally, yield capacity was observed over a range of water-limited environments, generally compared with high-yielding commercial check cultivars. For this study, 10 genotypes were selected from the two programs for physiological evaluation based on their identification as generally having higher yields over a range of water-limited conditions, rather than their yields when there was little or no water limitation. Several of these lines are now under final evaluation for consideration of germplasm or cultivar release. The "R" lines were developed in Arkansas and the "N" lines were developed in North Carolina (Table 1).

In Arkansas, much of the selection was done at the University of Arkansas Rice Research and Extension Center at Stuttgart in east central Arkansas. The selection of progeny lines was generally initiated on rows of F4:5 lines. About 15% lines were visually selected based for evaluation of their yield potential. Further, selection for high yield in water-limited environments led to the identification of the six genotypes included in this study (Table 1).

In North Carolina, the initial selections and identifications of the breeding lines used in this study were accomplished under drought conditions at the North Carolina State University Sandhills Research Station in southwestern North Carolina. Four advanced lines were included in this study (Table 1). Genotypes N05-7432 and N06-7194 exhibits the slow-wilting trait (T. Carter, personal communication, 2014). Genotype

Table 1. Maturity group and pedigree of 10 elite soybean breeding lines selected for improved yield and agronomic performance under drought-stressed conditions. Antecedents Jackson, its progeny 93705-35-1, Plant Introduction (PI) 227557, and PI 471938 were identified previously as having N_2 fixation drought tolerance. Antecedents PI 416937, its progeny N93-110-6, PI 471938, its progeny N98-7265, and NTCPR94-5157 were identified previously as slow wilting during drought stress. Antecedent PI 416937 was identified previously as having limited transpiration rate under high vapor pressure deficit conditions, although antecedent PI 471938 did not. Antecedent NTCPR94-5157 has not been evaluated for the presence of physiological mechanisms associated with drought tolerance. The ranking from greater tolerance to less tolerance among the 10 lines for each of the two physiological traits are included in the table. At "t" on the ranking indicates a tie between two lines.

					N_2 Fixation drought tolerance		Limited transpiration
Genotype identification	Maturity Group	Parental pedigree†	Parental antecedents‡	Rank	Antecedent with trait	Rank	Antecedent with trait
R02-1325	V	93705-35-1 × PI 227557	93705-35-1 = Jackson × KS 4895	1	50% PI 227557 25% Jackson	8	No known source
N05-7432	VIII	N7002 × N98-7265	N7002 = N7001 × Cook N98-7265 = Hutcheson × PI 471938	2	25% PI 471938	3	12.5% PI 416937
R05-5559	V	UA4805 × PI 471938	UA4805 = Hartz 5545 × KS 4895	3	50% PI 471938	linear	No known source
N04-9646	VII	Boggs × NTCPR94-5157	Boggs = G81-152 × Coker 6738 NTCPR94-5157 = Davis × N73-1102 N73-1102 = Tracy × Ransom	4	No known source	4	No known source
N06-7194	VIII	N98-7265 × N93-110-6	N98-7265 = Hutcheson × PI 471938 N93-110-6 = Young × PI 416937	5	25% PI 471938	5	25% PI 416937
R09-1069	V	R01-52F × Ozark	R01-52F = R93-5455 × Minsoy R93-5455 = Jackson-derived line × PI 416937 Ozark = Holladay × DP 415	6	6.25% Jackson	1t	12.5% PI 416937
N06-7543	VII	NC-Roy × N8001	NC-Roy = Holladay x Brim N8001 = N7001 x Cook	7	No known source	7	12.5% PI 416937
R07-5235	V	R01-52F × R02-6268F	R01-52F = R93-5455 x Minsoy R93-5455 = Jackson-derived line × PI 416937 R02-6268F = KS 4895 x Jackson	8	31.25% Jackson	9	12.5% PI 416937
R09-1121	V	R01-888F × R05-5559	R01-888F = Jackson × KS 4895 R05-5559 = UA 4805 x PI 471938	9	25% Jackson 25% PI 471938	1t	No known source
R07-7044	V	Lonoke × NTCPR94-5157	Lonoke = Manokin × Asgrow 6297 NTCPR94-5157 = Davis x N73-1102 N73-1102 = Tracy × Ransom	10	No known source	6	No known source

† N7001, N7002, and N8001 are high-yielding cultivars derived from PI 416937, but typically do not exhibit the "slow-wilting" trait.

NC-Roy is a "fast-wilting" cultivar with typically yields well under well-watered conditions.

Soybean cultivars UA4805, Boggs, Ozark, and Lonoke are adapted to the southern United States and are not known as drought tolerant.

‡ Cultivars Brim, Cook, Holladay, Hutcheson, Hartz 5545, KS4895, Ransom, and Tracy are adapted to the southern United States and are not known as drought tolerant.

N04-9646 exhibits the slow wilting trait, but usually only under extreme drought conditions. Genotype N06-7543 does not exhibit the slow wilting trait, but tends to yield well under drought conditions. Genotype N05-7432 was an interesting exception since it tended to have high yield under a wide range of environmental conditions (Gillen and Shelton, 2013).

Transpiration Response to Vapor Pressure Deficit

Transpiration response to VPD was measured by adapting an approach used by Devi et al. (2010a) with peanut (*Arachis hypogaea* L). Plants were grown in pots made of polyvinyl chloride pipe (100 mm diam., 180 mm tall) fitted with a toilet flange to allow easy attachment of a VPD chamber during measurement. The pots were filled with Gardenplus top soil (#92432, Lowes Inc., NorthWilkesboro, NC), containing 14–6.1–10 N–P–K fertilizer. Three seeds were sown per pot and *Bradyrhizobium* (Nitragin, Inc., Brookfield, WI) were added. The plants were grown in a greenhouse (Phytotron, North Carolina State University, Raleigh, 35°46′ N; 78°39′ W) in which the air temperature of the greenhouse was regulated at 31 d/26°C night. After 1 wk, each pot was thinned to one plant. Plants were maintained in a well-watered condition.

For each VPD test, 4 wk after sowing 12 pots were moved to a walk-in growth chamber (31°C) the day before the transpiration measurements were to begin. The photon flux density in the growth chamber was 550 μmol m^{-2} s^{-1} at plant level. The 12 pots included four replications for each of three genotypes. The genotypes in each test were randomly selected among the 10 genotypes. Duplicate sets of measurements were obtained for each plant in three VPD ranges on two successive days. Experiment 1 which included measurements of all 10 genotypes was conducted from 23 to 31 May 2012. Measurements were repeated in Exp. 2 from 2 to 12 July 2012.

Each plant was prepared for measurement by enclosing it in a 340-mm diam., 21-L transparent food container (Cambro Manufacturing, Huntington Beach, CA), which was attached to the toilet flange of the pot. The connection between the humidity chamber and the toilet flange was not sealed to allow continuous air flow through the chamber. Each humidity chamber was fitted with a 12-V, 76-mm-diam. computer box fan (Northern Tool and Equipment, Burnsville, MN) to continuously stir the air inside the chamber. A humidity/temperature data logger (Lascar Electronics, Erie, PA) was mounted through the side wall of each humidity chamber to record the environment in each chamber.

Transpiration response to VPD was measured using the approach described by Fletcher et al. (2007). Various humidity levels were achieved in the chambers by flowing air at different volume rates and sources to balance the humidifying effect of the transpiring leaves. For each test of plants, the plants were subjected to three VPD levels: low (0–1.5 kPa), medium (1.5–2.5 kPa), and high (2.5–3.7 kPa). On each day, measurements were started at the lowest VPD and then the medium VPD and finally the highest VPD. This sequence was selected to avoid any recovery that might be needed if stomata closure was induced by exposure to the high VPD treatment. Chambers were allowed to stabilize for 30 min after introducing each humidity treatment. Transpiration rate (TR) was taken as the difference in weights between the start and end of each measurement period. The VPD for each chamber was calculated on the basis of temperature and relative humidity during each observation period. After completing measurements on the first day, the pots were watered to dripping so that they were fully watered for the second day of measurement. Following measurements on the second day, the plants were harvested. Leaves were separated from the stem and leaf area was measured using a leaf area meter (LI-1300, Licor, Lincoln, NE). The TR was expressed as water-loss rate divided by plant leaf area.

The experimental results for each genotype resulted in 48 data (two experiments × 2 d × three VPD treatments × four replications). All the data of each genotype were first examined by attempting to fit the data to a two-segment linear regression using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA). The outputs of a successful regression fit to the two-segment model were the coefficients defining two intersecting linear regressions, where BP is the breakpoint VPD between the two segments.

$$\text{If VPD} < \text{BP, TR} = \text{slope 1} \times \text{VPD} + \text{intercept 1}$$

$$\text{If VPD} \geq \text{BP, TR} = \text{slope 2} \times \text{VPD} + \text{intercept 2}$$

The slopes of the two linear regressions (slope1 and slope2) were statistically compared to determine if they differed significantly ($p < 0.05$). If the slopes differed, the two-segment linear regression was retained. When the slopes were not significantly different, a simple linear regression was applied to all the data. The VPD breakpoint values, which were an output of the regression, were compared among genotypes for their significance level using Tukey's method.

Transpiration Rate and Nitrogen Fixation Response to Soil Drying

The response to soil drying of transpiration rate and N_2 fixation rate was measured using the technique described by Devi and Sinclair (2013) in their study of PI 471938. The plants were grown in pots (1.4 L) constructed from 10-cm diam. polyvinyl chloride pipes and filled with sandy loam soil (69% sand, 18% silt, and 13% clay). The pots were made with a bottom end cap into which an opening was drilled to allow water drainage and to provide an entry port for gas during N_2 fixation measurements. A toilet flange was attached to the top of the pot so that a lid could be easily attached to seal the pot during N_2 fixation measurements. Three seeds were sown in each pot and treated with *Bradyrhizobium* (Nitragin, Inc., Brookfield, WI). Each pot was thinned to one plant after 1 wk of sowing. All plants were initially grown under well-watered conditions in a greenhouse (28°C day/night Temp.). The drought experiment was started approximately 30 d after sowing.

The measurement of daily N_2 fixation rates is labor intensive and only 40 pots could be tested at one time. Therefore, the 10 genotypes were split between two experiments: genotypes N05-7432, R05-5559, R07-5235, R09-1069, and R09-1121 in the first and N06-7194, N04-9646, N06-7543, R02-1325, and R07-7044 in the second experiment. The first experiment was sown on 27 July 2012 and the plants with four to five fully expanded leaves were subjected to soil drying from 29 Aug. to 14 Sept. 2012. The second experiment was sown on 20 Aug. 2012 and the dry-down experiment was conducted from 18 Sept. to 2 Oct. 2012.

The eight pots allocated to each genotype were divided at the initiation of the dry-down experiments between three well-watered plants and five drought-stressed plants. The day before beginning the experiment, all pots were fully watered and allowed to drain overnight. Early the following morning, the pots were sealed with the two-piece lids and the pots were weighed for initial weight. The lid prevented soil evaporation and allowed a closed flow system for the introduction of acetylene into the pots. The pots were weighed daily and the difference in weight between days was calculated as TR. The well-watered plants were irrigated daily to return soil moisture to 100 g below initial weight. The drying pots were watered if necessary to maintain the net daily loss of water at no more than 70 g (approximately 10% of the initial transpirable soil water in the pots). This protocol allowed the drying cycle to extend over about 2 wk to more closely match the duration of field drying conditions.

The TR data were analyzed by first calculating for each drying pot on each day the ratio of its TR and the mean TR of the three well-watered plants of the same genotype. To minimize variability due to plant size a second normalization was done for each plant. The transpiration ratio of the drying pots on each day was divided by the mean transpiration ratio of that same plant during the first 3 d of the experiment when the plant was still under well-watered conditions. This ratio was called normalized transpiration ratio (NTR) and by definition had a value during the wet phase of the dry-down cycle for each plant centered on a value of 1.0.

A flow-through acetylene reduction assay was performed each day for each pot during the drying treatment to monitor N_2 fixation activity (Sall and Sinclair, 1991; Devi et al., 2010b). Each afternoon, a 1:9 mixture of acetylene/air was continuously introduced to the pot at 1 L min^{-1}. After flowing the gas through the pots for 15 min to achieve a steady-state output, three replicate samples of the gas mixture leaving each pot was collected in 100 cm^3 insulin syringes. Immediately after all samples were collected, which required about 5 min, the pots were flushed for at least 1 h with only air to remove any residual acetylene and ethylene. The ethylene concentration of the gas samples was measured by injecting the gas samples into a gas chromatograph fitted with a flame-ionization detector. Normalized acetylene reduction activity (NARA) for each drought-stressed plant on each day was calculated similar to the NTR calculation. That is, an acetylene reduction activity (ARA) ratio was calculated for each pot and then the NARA was calculated by dividing the daily ratio by the mean ARA value on the first 3 d of the experiment when the plant was still under well-watered condition.

Measurements were discontinued for each plant when it reached a NTR value of 0.1 or less. The difference between the initial weight and the final weight was the total transpirable soil water in each pot. The FTSW was calculated for each day based on daily measurements of pot weight. For each genotype, the results of NTR and NARA for the five plants were combined for regression with respect to FTSW. The data was analyzed by using the two-segment regression analysis of Graph Prism 5.0 to obtain the FTSW threshold values for decrease in both NTR and NARA with drying soil.

RESULTS

Transpiration Response to Vapor Pressure Deficit

Similar to past studies with soybean (Fletcher et al., 2007; Sinclair et al., 2008), these 10 soybean genotypes showed two distinct patterns in the response of TR to increasing VPD. Genotype R05-5559 (Fig. 1b) had a linear increase in TR with increasing VPD. On the other hand, N05-7432 exhibited a breakpoint in transpiration rate above which there was little or no increase in TR with further increases in VPD (Fig. 1a). Unlike most previous studies, there was a clear preponderance of expression of a VPD breakpoint rather than a linear response among the 10 genotypes since only R05-5559 exhibited the linear increase (Table 2). The two-segment regression proved to be a good fit for those genotypes expressing a breakpoint with the R^2 ranging from 0.72 to 0.91.

For the nine genotypes with VPD breakpoints, the VPD of the breakpoint values ranged from 1.41 kPa for R09-1069 and R09-1121 to 1.94 kPa for R07-5235. Based on their significant levels shown by Tukey's model; lines R02-1325 and R07-5235 had significantly higher VPD breakpoints than the rest of the genotypes except for N06-7543 and R07-7044.

Transpiration Rate Response to Soil Drying

The change in TR with soil drying showed the common two-segment pattern (data not shown). At high soil water content, TR of water-deficit treated plants was equivalent to the well-watered plants. However, with further soil drying a threshold was reached below which TR decreased linearly with decreasing FTSW. The two-segment regression represented these results well with R^2 for the genotypes ranging from 0.82 to 0.95 (Table 3). The threshold obtained from the regression analysis ranged from 0.47 to 0.55 among genotypes. There was no significant difference ($p < 0.05$) in the thresholds among genotypes.

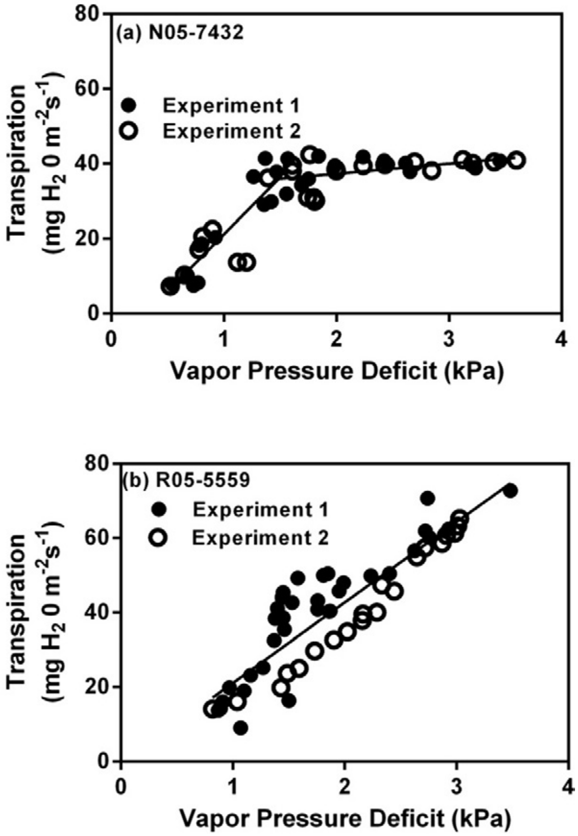

Fig. 1. Transpiration rate of two soybean genotypes (a) N05-7432 and (b) R05-5559 at different levels of vapor pressure deficit (kPa). Results from the two sets of experiments for each genotype are distinguished by the open and closed circles. Data in panel (a) was fitted with a two-segment linear regression and in panel (b) was fitted with a single linear regression.

Nitrogen Fixation Rate Response to Soil Drying

The two-segment regression model described well the response of NARA to decreasing FTSW as illustrated by R02-1325 and R07-7044 in Fig. 2. Across all genotypes the R^2

Table 2. Transpiration rate (mg H_2O m^{-2} s^{-1}) response of 10 soybean breeding lines to increasing vapor pressure deficit (VPD). Except for R05-5559, all genotypes were fitted with a two-segment linear regression model. Slope1 is the slope of the regression at lower VPD and Slope2 is the regression at high VPD above the breakpoint. The breakpoint is the intersection between the two linear regression segments. The results for R05-5559 are from a single linear regression for all the data. Breakpoints of genotypes followed by the same letter are not significantly different from each other based on Tukeys model. Genotypes are arranged based on their breakpoint values.

Breeding line	Slope 1 ± SE	Breakpoint (X_o) ± SE	Slope 2 ± SE	R^2
R09-1121	42.6 ± 7.23	1.41 ± 0.11a	–1.72 ± 4.10	0.81
R09-1069	37.7 ± 7.21	1.41 ± 0.17a	8.95 ± 4.28	0.83
N05-7432	31.4 ± 4.91	1.47 ± 0.10a	3.66 ± 1.13	0.76
N04-9646	26.5 ± 2.78	1.48 ± 0.06a	0.27 ± 1.29	0.8
N06-7194	41.6 ± 5.11	1.52 ± 0.07a	1.96 ± 2.66	0.82
R07-7044	37.5 ± 3.47	1.74 ± 0.08ab	1.28 ± 3.08	0.81
N06-7543	38.5 ± 1.70	1.80 ± 0.05ab	–7.10 ± 2.62	0.91
R02-1325	34.9 ± 5.00	1.88 ± 0.13b	–2.47 ± 3.59	0.76
R07-5235	25.3 ± 3.69	1.94 ± 0.12b	–7.76 ± 5.25	0.72
R05-5559	21.6 ± 1.45	no breakpoint	na†	0.80

† na: not applicable.

Table 3. The fraction of transpirable soil water (FTSW) threshold at which the decline of transpiration rate (TR) is initiated with soil drying. Confidence limits for the FTSW-threshold values and their R^2 values are presented. Genotypes with same alphabetical letter for their FTSW threshold TR values are not significantly different ($p < 0.05$) from each other based on their confidence intervals. Genotypes are ranked according to their FTSW threshold for TR values.

Breeding line	FTSW threshold for TR	Confidence limit	R^2
N05-7432	0.47a	0.43–0.55	0.93
N06-7543	0.47a	0.45–0.57	0.91
N04-9646	0.48a	0.42–0.55	0.95
N06-7194	0.50a	0.45–0.57	0.93
R07-5235	0.51a	0.45–0.58	0.93
R05-5559	0.52a	0.41–0.63	0.83
R02-1325	0.53a	0.44–0.58	0.82
R07-7044	0.55a	0.46–0.59	0.86
R09-1069	0.55a	0.47–0.59	0.85
R09-1121	0.55a	0.46–0.58	0.85

values ranged from 0.85 to 0.91 (Table 4). The range of threshold values of FTSW at which ARA started to decrease for the 10 genotypes was from 0.25 for R02-1325 to 0.37 for R07-7044. Differences among genotypes for the FTSW threshold values of NARA were based on the confidence intervals of the thresholds. The thresholds for genotypes R02-1325, N05-7432, N04-9646, R05-5559, and N06-7194 were significantly less ($p < 0.05$) than for R07-7044 and R09-1121 (Table 4).

DISCUSSION

Three physiological traits contributing to drought tolerance were examined in 10 elite southern soybean genotypes that had been empirically selected in breeding programs for improved yield in water-deficit environments. To fully understand the drought tolerance capacities of these genotypes, it was necessary to document the behavior of specific physiological traits. These results offered some insights about the physiology of these genotypes.

The fact that 9 of these 10 genotypes selected for high yield under water-limited environments expressed a breakpoint in TR to increasing VPD (Table 3) confirms that this trait is likely to be highly desirable trait for drought conditions. These results differ from the previous observation in which nearly all tested genotypes did not express the VPD breakpoint phenotype (Sadok and Sinclair, 2009). This circumstantial evidence indicates that selection for the expression of a VPD breakpoint could be a major contributor to drought tolerance. The one exception to the breakpoint phenotype was R05-5559, which was found to have an especially low threshold in its N_2 fixation activity with soil drying.

Table 4. The fraction of transpirable soil water (FTSW) threshold at which the decline of acetylene reduction activity (ARA) is initiated with soil drying. Confidence limits for the FTSW-threshold values and their R^2 values are presented. Threshold values with the same letter are not significantly different ($p < 0.05$) from each other based on their confidence intervals. Genotypes are ordered based on their FTSW threshold for ARA values.

Breeding line	FTSW threshold for ARA	Confidence limit	R^2
R02-1325	0.25a	0.22–0.28	0.90
N05-7432	0.27a	0.24–0.30	0.88
N04-9646	0.28a	0.25–0.30	0.91
R05-5559	0.28a	0.23–0.30	0.85
N06-7194	0.30a	0.24–0.32	0.88
R09-1069	0.32ab	0.29–0.36	0.89
N06-7543	0.33ab	0.30–0.38	0.91
R07-5235	0.35ab	0.30–0.40	0.85
R09-1121	0.36b	0.32–0.40	0.90
R07-7044	0.37b	0.33–0.43	0.92

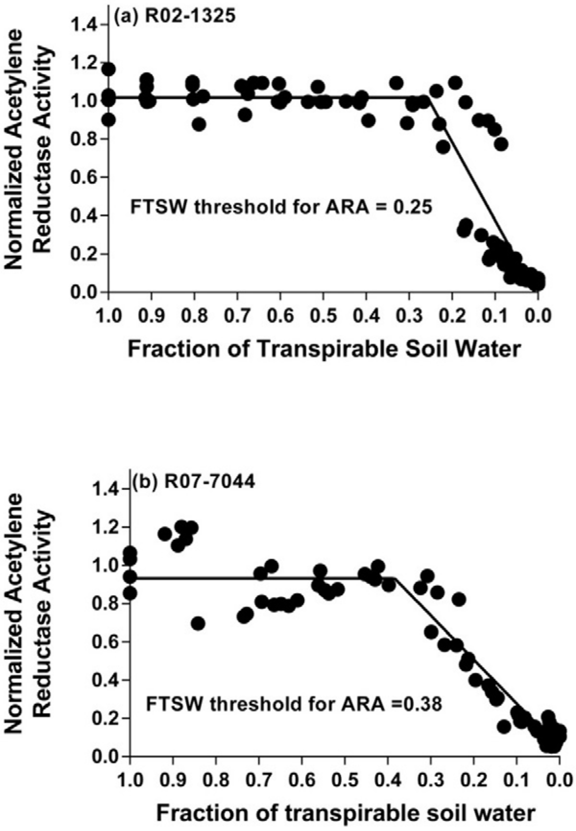

Fig. 2. Normalized values of Acetylene Reduction Activity (ARA) of two soybean genotypes (a) R02-1325 and (b) R07-7044 plotted against fraction of transpirable soil water (FTSW). Solid lines in each figure are the regression results from the two-segment linear analysis.

Among the genotypes expressing a VPD breakpoint, four genotypes had a higher threshold of 1.74 to 1.88 kPa as compared to five genotypes with thresholds in the range of 1.41 to 1.52 kPa. However, the breakpoints of all nine genotypes are all less than that previously reported value of about 2.1 kPa for PI 416937 (Fletcher et al., 2007; Sinclair et al., 2008). Therefore, these results indicate that selection for high yield under water-limited conditions favored those genotypes with especially low VPD breakpoint. The lower threshold allows for greater water conservation, especially early in the season, so that greater amounts of soil water are available for grain filling.

The most desired genotype in locations where growing seasons vary between low and high rainfall years, however, may not be one with a very low VPD breakpoint. A very low VPD breakpoint will result in frequent limitations on leaf gas exchange including photosynthesis rate. In this case, the frequent loss in photosynthesis activity may not be compensated later in the growing season by the soil water conserved water as a result of the low VPD breakpoint. In the simulations of Sinclair et al. (2010), where an advantage of the limited-transpiration trait was found, the assumed VPD breakpoint was 2.0 kPa. An assessment is required to determine the optimum VPD breakpoint for each geographical region, but it may well be that the four genotypes with somewhat higher VPD breakpoints may be better selections in many locations in the United States.

Among the 10 genotypes, 5 had PI 416937 in their pedigree (Table 1). Somewhat surprisingly, four of the genotypes that were found to express the limited-transpiration trait did not have PI 416937 in their pedigree. These results indicate the genetic basis for expression of the limited-transpiration trait remains ambiguous. In fact, the four genotypes that may have the more desirable higher VPD breakpoints either had no (R07-7044 and R02-1325) or a small (N06-7543 and R07-5325) component of PI 416937 in their pedigree.

The measurement of plant response to drying soil did not demonstrate any significant difference in the threshold for the decline in NTR among the 10 genotypes (Table 3). While it appears this trait offered no physiological basis for differentiating these genotypes, the thresholds observed in these experiments (0.47–0.55 FTSW) were greater than those commonly reported. Hufstetler et al. (2007) reported thresholds for soybean genotypes in the range of 0.19 to 0.29. However, their experiments were done on a very sandy soil which has been shown to skew the results towards low thresholds (Sinclair et al., 1998). Seversike et al. (2013) found the breakpoint in the range of 0.2 to 0.3 for two *G. max* genotypes as compared to as high as 0.6 to 0.7 for *G. soja* when tested at 25°C. In any case, all 10 genotypes expressed the desirable high FTSW threshold for the decline in NTR, which would result in soil water conservation at the initiation of soil water deficit conditions.

Nitrogen fixation response to soil drying resulted in differences among genotypes (Table 4). Five genotypes (R02-1325, N05-7432, N04-9646, R05-5559, N06-7194) with the lowest NARA thresholds had thresholds at 0.25 to 0.30 FTSW, which is substantially superior to the common threshold range of 0.4 to 0.5 FTSW. While the thresholds of these elite lines are not as low as exhibited by the eight exotic germplasm identified by Sinclair et al. (2000) in their screen of 3500 lines, which were as low as 0.11, the five elite lines with the lowest NARA thresholds have threshold that are substantial improved over commonly observed thresholds. The low NARA thresholds of the five genotypes could certainly result in yield advantages over lines with higher thresholds (Sinclair et al., 2010).

The genotype with the lowest NARA threshold (0.25 FTSW), R02-1325, had PI 227557 as a parent (Table 1). PI 227557 was identified by Sinclair et al. (2000) as one of the selections from the screen of 3500 lines, which was originally found to have a threshold of 0.22 FTSW. The advantage of PI 227557 as a parent of R02-1325 may have been augmented by also having Jackson as a grandparent.

Among the other four genotypes with especially low NARA threshold, three (N05-7432, R05-5559, and N06-7194) had PI 471938 in their pedigree (Table 1). Devi and Sinclair (2013) reported that PI 471938 expressed a low threshold for N_2 fixation on drying soil. Among the five genotypes with the higher NARA threshold, two had no known source of N_2 fixation drought tolerance and two had only Jackson as the source of the trait. These results indicate that PI 227557 and PI 471938 may be the more effective parents than Jackson in transferring N_2 fixation drought tolerance to high-yielding progeny.

Overall, these results highlight physiological traits that are associated with increased soybean yields under water-limited conditions. All genotypes expressed a high threshold for the decrease in TR with soil drying allowing for substantial soil water conservation. Further, all but one of the genotypes had a breakpoint in TR in response to increasing VPD allowing additional soil water conservation. The exception in the response to VPD was R05-5559, which may have balanced the sustained plant water loss at high VPD with substantial tolerance in N_2 fixation to soil drying. Nitrogen fixation tolerance to drought was especially high in five genotypes (R02-1325, N05-7432, N04-9646, R05-5559, N06-7194). Given the greater contribution of N_2 fixation drought tolerance to yield increase over the limited-transpiration trait shown in the simulation analysis for the United States by Sinclair et al. (2010), these five genotypes might be especially attractive for further study.

IMPLICATIONS FOR BREEDING

The discovery that selection for improved yield can result in a correlated response in drought-related physiological traits indicates that commercial breeders interested in drought tolerance should carefully consider the genetic make-up of their breeding populations. The genetic base of elite U.S. soybean breeding programs is narrow, and may well lack the genetic variability needed for maximum breeding progress in seed yield under drought conditions. Thus, it is important to include parental stocks which carry drought tolerance traits. Genotype R02-1325 offers an interesting combination of drought-related physiological traits. It had the lowest NARA breakpoint of all genotypes (0.25 FTSW) and a modest VPD threshold (1.88 kPa). This combination of physiological traits may position this genotype well as having greater potential for yield increase over a range of drought-stressed environments. On the other hand, N05-7432 may be especially desirable for drier environments where the high N_2 fixation tolerance and the low threshold for the limited-transpiration trait could contribute to superior yield performance. It also exhibits the slow wilting trait. N05-7432 is among the highest-yielding maturity group VIII breeding lines in the USDA Uniform Soybean Tests (Gillen and Shelton, 2013). Of course, the actual yield level of all genotypes under field conditions is influenced by the specific weather scenario in each growing season and physiological traits other than the drought-tolerance traits examined in this study.

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