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Research Article

The Trait Of Early Stomatal Closure In Soybeans Is Inherited By The Ellis N09-13890 Population.

Shekoofa*, Rictoria, Dripa Dakal, Usha Poudel, Santalone.

Department of Plant Sciences, University of Tennessee, 2505 E.J. Chapman Dr., Knoxville, TN 37996, USA.

Abstract

Crop growth and yield are decreased by drought conditions, which cause a variety of physiological and morphological changes in crops. Finding and choosing genotypes that are most suited to restricted water availability under particular environmental conditions can be a useful tactic to lessen the detrimental effects of drought stress on soybean (Glycine max L. Merr.) production. This study used a population of recombinant inbred lines (RILs) produced from a cross between N09-13890 and Ellis to evaluate the inheritance of early stomatal closure traits in soybeans. Using a dry-down experiment, thirty soybean lines were exposed to increasing levels of water-deficit stress. The experiment was carried out in a controlled setting at the University of Tennessee's West Tennessee Research and Education Center (WTREC) in Jackson, Tennessee, between June and November 2022.The early stomatal closure thresholds of several soybean lines were found to differ significantly in this investigation. The 30 tested lines' fraction of transpirable soil water (FTSW) thresholds varied from 0.18 to 0.80, where a decrease in transpiration with soil drying was noted. The FTSW threshold values for nearly 65% of the RILs ranged from 0.41 to 0.80. These findings, which show inheritance, support the high level of early stomatal closure trait expression in progeny lines during cultivar development under water-deficit stress conditions. Thus, identifying the differences in genotypes of water use and their response to water-deficit stress conditions can provide a foundation for selecting new cultivars that are best adapted to arid and semi-arid agricultural production systems.

Keywords : *Fraction of transpirable soil water (FTSW); Recombinant inbred lines (RILs) soybean;Water-deficit stress.*

INTRODUCTION

Extreme weather conditions like droughts, erratic rainfall patterns, and rising temperatures—all of which are significant environmental stressors—limit crop growth and yield [1,2]. One of the most significant physiological factors affecting soybeans' (Glycine max L. Merr.) growth, productivity, and metabolism is water-deficit stress [3]. A major legume crop with enormous economic importance, soybean production is heavily reliant on ideal irrigation or rainfall [4–6].Drought is the main factor reducing soybean yields in the Southeastern United States [4–6]. Therefore, methods that increase plants' resistance to stressors are required to guarantee continuous productivity in both present and future climates. Transpiration rate (TR) response to increasing soil drying is one possible characteristic that may give soybeans drought tolerance [7–11]. Reduced water uptake and increased water loss through transpiration are the outcomes of anatomical, physiological, and biochemical changes that occur in plants under water deficit stress. However, in response, they trigger physiological processes like reduced transpiration rate and early stomatal closure [12,13].

The cumulative rate of photosynthesis and water consumption during the growing season are greatly influenced by stomatal conductance, which is essential for controlling plant CO_2 uptake and water loss [14]. Therefore, a promising physiological characteristic for creating drought-resistant soybean genotypes is early reductions in stomatal conductance under drought conditions. Previous studies have shown that plants with low transpiration rate partially close their stomata under water-deficit stress conditions, conserving more water in the soil for the later stages of growth [15]. Several physiological traits such as transpiration rate, leaf expansion, leaf epidermal conductance, leaf area, and photosynthesis have been examined in previous studies to evaluate drought tolerance in soybean [11,16].

***Corresponding Author:** Shekoofa, Department of Plant Sciences, University of Tennessee, 2505 E.J. Chapman Dr., Knoxville, TN 37996, USA.
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The fraction of transpirable soil water (FTSW) threshold is the point at which plants respond to soil drying out by reducing their rate of transpiration. Significant sensitivity to early soil drying is shown by the higher FTSW threshold values, suggesting the possibility of better drought tolerance and soil water conservation [17]. The majority of crop plants, including soybeans, have FTSW thresholds for a drop in transpiration rate between 0.25 and 0.40, according to Sadras and Milroy's research [18]. Studies have revealed notable differences in the FTSW threshold between various crops and species, though.

Devi et al., for instance, examined the transpiration response of 17 peanut genotypes to drying soil and discovered a broad range of FTSW threshold values, from 0.22 to 0.71 [8]. The FTSW threshold values of 24 soybean genotypes cultivated in two distinct potting media—sandy soil (80% sand) and earth potting mix—varyed significantly, ranging from 0.22 to 0.29 and 0.19 to 0.27, respectively, according to another study [19]. Additionally, it was discovered that the ancestral soybean (Glycine soja) growing at two different temperatures had an impact on the FTSW threshold value; threshold values of 0.28 and 0.67 were noted at 30 and 25 °C, respectively [20]. It is essential to carefully choose suitable parents for crossing in order to screen for drought tolerance traits. Ellis [21], a drought-tolerant soybean cultivar created by the University of Tennessee, Agricultural Experiment Station, TN, is one example. According to a prior study, Ellis expressed a FTSW threshold between 0.59 and 0.69, which means that in response to increasing soil drying, the plant begins to close its stomata and lower the transpiration rate [11]. In addition, Ellis showed delayed wilting under water deficit conditions in the field [22]. Another line, N09-13890, is a slow wilting line with a high water use efficiency, biomass, and seed yield but has reduced root development with more resources allocated for shoot growth [23].

The identification of important genes controlling drought tolerance is now feasible due to the recent release of the complete soybean genome sequence and genome-wide expression profiling data.However, the main requirements for cultivar development are the discovery and application of drought-tolerant traits as well as the creation of appropriate screening methods [24].This study set out to evaluate the performance of 30 recombinant inbred lines (RILs), which were produced by crossing Ellis and N09-13890 soybean lines, under conditions of water deficit stress. Finding the most promising RILs based on their transpiration response to increasing soil drying and examining the inheritance of early stomatal closure, a drought tolerance trait, were the objectives of the study.

RESULTS

The TR trait's expression

This study's main goal was to evaluate 30 RILs' transpiration rate response. With reference to the early stomatal closure trait during soil dry-down, we postulated that some of the RILs might possess drought tolerance traits inherited from their parental lines (Ellis and N09-13890). The findings showed that the RILs' FTSW thresholds varied significantly, ranging from 0.18 to 0.80 (Table 1). A number of other RILs exceeded Ellis' FTSW threshold, which ranged from 0.61 to 0.70. The FTSW value of several lines was higher than that of Ellis, their parent line. Approximately 13% of the RILs had FTSW threshold values between 0.71 and 0.80, indicating that the combination of the two parental lines had a positive impact on the expression of drought tolerance traits in their offspring (Figure 1). The FTSW threshold values for nearly 65% of the Ellis × N09-13890 tested population fell between 0.41 and 0.80 (Figure 1). The two-segment linear regressions with an R2 coefficient ranging from 0.83 to 0.99 (Table 1) showed that all lines showed changes in NTR as the soil dried. This suggested that the transpiration rate response among RILs could be accurately predicted by progressive soil drying. Based on their 95% CIs, the FTSW threshold values for NTR were compared across soybean lines (Table 1).The two lines with the highest and lowest FTSW threshold values, 19-31_13 and 19-31_24, respectively, were 0.80 and 0.18 (Figure 2). Lines with higher FTSW threshold values were found to have lower transpiration rates and the earliest stomatal closure.

The Vapor Pressure Deficit (VPD) and Transpiration Rate Response

Lines took varying amounts of time to reach the drying down cycle's endpoint, and the experiment's duration varied among the three sets (Table 2 and Figure 3). The environmental factors, like temperature and VPD, during each set of experiments were probably the cause of these variations. When FTSW threshold values were reached in set 1, the average VPD was approximately 3.0 kPa. The corresponding kPaVPD rates for sets 2 and 3 were 2.0 and 1.3, respectively. There was a significant difference in the VPD between set 1 and the other two sets, according to Tukey's HSD test ($p < 0.05$) of the average for each of the three experiments (Figure 3).Because set 2 had lower evaporative demand rates than set 1 of the experiment, the drying down time was longer for the majority of lines. Furthermore, compared to the other two sets, all of the lines in set 3 had lower FTSW threshold values.

DISCUSSION

To deal with water-deficit stress, plants have evolved sophisticated defenses that include alterations in gene expression, osmotic adjustment, stomatal regulation, and root architecture [25]. Numerous environmental factors, such as temperature, humidity, soil type, and nutrient availability, affect the expression of these mechanisms, which are controlled by a complex network of genes [26, 27]. Plant water loss responses under water deficit conditions have been evaluated extensively in recent years using the fraction of transpirable soil water (FTSW) threshold responses to transpiration rate [10,11,28]. This study's goal was to evaluate the performance of 30 recombinant inbred lines (RILs) produced by crossing Ellis and N09-13890 soybean lines in the presence of water deficit. During progressive soil water drying experiments, we looked into the line's FTSW threshold values for the drop in NTR. The range of thresholds among 30 RILs varied significantly between experiments (Table 1).

More than half of the lines, according to their FTSW values, had a FTSW threshold that was either equal to or higher than Ellis, the parental line that was used to develop the population [11,22]. These results suggest that the progeny benefited from the combination of genes inherited from both parental lines, while the N09-13890 parent helped to express limited transpiration rate (TRlim), another trait related to drought tolerance [23]. Thus, a thorough comprehension of the physiological underpinnings of stomatal regulation is necessary. Transpiration efficiency (TE), a crucial metric that is genetically controlled, can lower water loss in environments with limited water supplies [24].

Long-term water deficit stress negatively impacts photosynthetic metabolism in plants by restricting stomatal activity [29]. During photosynthesis, stomatal closure lowers the amount of CO2 needed for the mesophyll's carboxylation process. Although this may lower photosynthetic metabolism rates, it also aids in water conservation and transpiration rate reduction, both of which are critical for crop survival and productivity during protracted drought. Stomatal conductance is influenced by the quantity, size, opening, and configuration of stomata on the leaf surface [16]. According to one earlier study, water-stressed soybean cultivars had low stomatal densities [3], and the different genotypes' stomatal densities varied significantly.

The results of the current study showed that the transpiration rate response of soybean RILs under water-deficit stress varies by genotype. The findings showed that when average VPD increased during the time when FTSW threshold values were reached, some lines responded more quickly than others to conserve water. The range of FTSW threshold values (Table 1) was greater in the experiment with higher VPD rates (sets 1 and 2) (Figure 3) than in set 3. In some soybean lines with the limited transpiration trait, the high VPD causes stomatal closure, which conserves soil water [9,30]. The high percentage of RILs (i.e., 65%) (Figure 1) placed in 0.41 to 0.80 FTSW threshold values category in the progeny lines suggests that it is possible to develop drought-resistant soybean lines from crossing drought tolerant lines with expression of varying drought tolerance traits [11,22,23]. Thus, these results are encouraging in that crossing in a breeding program with a parent (i.e., Ellis) that expressed both early stomatal closure under soil drying as well as air drying, high VPD.

These results showed that soybean RILs had genotypic variation. The findings highlight how crucial it is to take genotypic variation into account when choosing drought-resistant soybean lines with regard to transpiration rate response, particularly for production in a variety of environmental settings. Breeders could create more water-efficient lines that can sustain or boost yield in a variety of environmental circumstances by focusing on stomatal characteristics that vary among lines.

RESOURCES AND PROCEDURES

Plant Composition and Growth State

At the East Tennessee Research and Education Center (ETREC) in Knoxville, Tennessee, a cross between Ellis as the male and N09-13890 as the female resulted in a population of recombinant inbred lines (RILs) in 2019. The F2 seeds were sown at ETREC in 2020, while the F1 seeds were cultivated under lights at the USDA Tropical Agricultural Experiment Station in Isabela, Puerto Rico. The F3 seeds were harvested by picking one pod per plant and grown in an unlighted nursery at 3rd Millennial Genetics in Santa Isabel, Puerto Rico, during the winter of 2020–2021.About 300 single F4 plants were chosen to grow the F4:5 in 2022 after the following seed was harvested using single pod descent after maturity. The F4 seeds were then planted at ETREC in the summer of 2021. Thirty lines were selected at random from among them, and in a controlled setting at the University of Tennessee's West Tennessee Research and Education Center (WTREC) in Jackson, Tennessee, their transpiration rate response to progressive soil drying was examined.

In 3.8-liter pots filled with a soil mixture consisting of 50% sand and 50% Lexington silt loam (fine-silty, mixed, active, thermal Ultic Hapludalf), the soybean lines were cultivated in a greenhouse. Plants were trimmed to one per pot once the unifoliate leaf appeared. Pots were fertilized with 200 mL of 0.075% v/v liquid fertilizer (0-10-10,N-P2O5-K2O,GH Inc., Sebastopol, CA, USA) 13 and 20 days after planting. USB data loggers (Lascar Electronics Ltd., Erie, PA, USA) were used to record the greenhouse's temperature and relative humidity every five minutes. The vapor pressure deficit (VPD, kPa) was computed using the recorded air temperature and relative humidity data. The quantity of vapor that can be held in the air until it reaches saturation point at the same temperature is known as the vapor pressure deficit. The difference between the saturation vapor pressure and the actual vapor pressure can be used to compute this variable [10].

First, the saturation vapor pressure (SVP, kPa) was calculated:

$$SVP = 0.6108 \times \exp(T/(T + 237.3) \times 17.2694)$$

T: degrees Celsius.

Then, VPD, kPa:

$$VPD = SVP \times (1 - RH/100)$$

To maintain a schedule of 15 hours for the day and 9 hours for the night, artificial lighting was added to the natural solar radiation [11]. The plants were kept in a well-watered state throughout the first pretreatment phase and were dispersed along the greenhouse tables in a fully randomized fashion. Each line had seven replicate pots, with the exception of lines 19–31_15, which were removed from the experiment due to extremely poor germination. From June through November 2022, the experiments were carried.

Gradual Drying of the Soil

A soil dry-down experiment was started for each of the three sets of experiments once the plants had four fully grown trifoliate leaves. All of the pots were irrigated until they began to drip in the afternoon and left to drain overnight prior to starting the dry-down experiment.Two clear plastic bags were placed over the pots the following morning, and a plastic tube was placed between the plant's base and the bag to enable watering throughout the trial [22]. A plastic twist tie was used to fasten the bag and plastic tube to the stem's base (Figure S1). As soon as the pots were covered, they were weighed, and the initial weight was noted.Between 1200 and 1400 Central Standard Time (CST), daily pot weights were recorded in order to calculate the gravimetric water loss from transpiration. As explained by Devi et al. [8], the daily transpiration rate (TR) was computed as the variation in weight of each pot over the course of several days.

Three pots of each line were designated as well-watered (WW) and four pots as water-deficit stressed (DD) following the first three days of daily TR measurements. The normalized transpiration rate (NTR) was computed using the WW plants as a reference. By replenishing the daily water loss, the well-watered plants were kept 100 g below the initial saturated pot weight. At a rate of no more than 70 g per day, the soil in the remaining four pots was gradually dried. According to Shekoofa et al. [10], the DD plants were only watered if the daily loss exceeded 70 g day^{-1}. Following the experiment, the difference between the pot's initial and final weights was used to determine the total amount of transpirable soil water that each pot's plants could use.The fraction of transpirable soil water (FTSW) for each pot in the DD treatment each day was used to represent the soil water availability for line comparison. The formula below illustrates how FTSW was determined by dividing the difference between daily and final.

Numerous studies have successfully used transpirable soil water as the foundation for comparing how plants react to soil drying under various circumstances [7, 8].The method outlined by Ray and Sinclair [7] was used to analyze the transpiration data.The daily ratio of the TR of each drought-stressed pot divided by the average TR for the well-watered plants in each line was computed in order to reduce the impact of varying plant sizes, which result in notable variations in daily TR across days.A second normalization was then performed to make comparing soybean lines easier.By dividing the daily transpiration rate by the mean transpiration rate of the same plant over the first three days of the experiment, when the soil still had a high water content, this normalization was accomplished. Known as the normalized transpiration rate (NTR), this ratio had a value of 1.0 for each plant at the start of the dry-down cycle. When all of the DD plants achieved an NTR value less than 0.10—the endpoint of the transpirable soil water—the experiment was over.

Analysis of Statistics

Plotting the relationship between NTR and FTSW using a two-segment linear regression analysis was the statistical method used. GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA) was used to create the final graphs. We can use this software to find the FTSW threshold between the two segments at which the NTR decline started. Tukey's honest significant difference (HSD) test ($p < 0.05$) was used to separate treatment means (JMP, version Pro 16, SAS Institute Inc., Cary, NC, USA).

CONCLUSIONS

We can infer from this set of experiments that the FTSW threshold values for nearly 65% of the RILs fell between 0.41 and 0.80. These findings corroborate Shekoofa et al. (2017) [17]'s earlier finding that limited transpiration was expressed in progeny lines at a comparatively high frequency. Therefore, in a breeding program, crossing parents that exhibit drought-tolerant traits (such as limited transpiration and early stomatal closure) may result in inheritance with a sizable percentage of RILs expressing a high FTSW threshold that falls within a range that is advantageous for achieving drought tolerance in the field.

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