



# Drought Tolerance in Soybean (*Glycine max* L. Merr.) Genotypes during the Flowering Stage of Development

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

**Aims:** To access the responses of 22 soybean genotypes under drought stress during the flowering stage of development.

**Study Design:** A completely randomized experimental design (CRD) was adopted in this study. Five seeds of each of the 22 soybean genotypes were planted in 12-liter (L) plastic buckets (with holes at the bottom) containing sandy loam soil. There was one genotype per pot with three replications. The setup was repeated to represent experimental (drought-stressed (DS) and control (well-watered (WW) groups.

**Place and Duration of Study:** Teaching and Research Farm of the School of Agriculture, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana in 2019.

**Methodology:** Twenty-one exotic soybean genotypes and a local variety were assessed for their responses to drought stress at the flowering stage of development using pot experiment in the greenhouse. One group (experimental) was exposed to drought stress by withholding water for 15

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days whilst the other group (control) was watered regularly at three-day intervals. Data collected were the number of leaves, plant height, stem girth, leaf area, and number of flowers, at 5, 10, and 15 days after treatment (DAT) and relative leaf water content at 15 DAT. The number of days to permanent wilting of the genotypes was also recorded.

**Results:** Drought stress reduced the number of leaves and plant height significantly ( $P$ -values = 0.033, 0.000) and ( $P$ -values = 0.004, 0.000) at 10 and 15 days after drought treatment respectively. Also, stem girth, leaf area, and number of flowers were significantly reduced at all sampling dates ( $P$ -value = 0.002, 0.000, 0.000), ( $P$ -values = 0.004, 0.000, 0.000) and ( $P$ -values = 0.009, 0.000, 0.000) respectively. At 15 DAT, drought significantly ( $P < 0.001$ ) reduced relative leaf water content. Genotypes TGX-1989-11F and TGX-1987-62F were the first and last to wilt at 7 days and 16 days after rewatering respectively.

**Conclusion:** The pot screening method revealed that the number of leaves, plant height, stem girth, leaf area, number of flowers, relative leaf water content, and days to permanent wilting differed significantly ( $P = 0.05$ ) at 15 days of drought exposure.

*Keywords: Soybean genotypes; drought tolerance; flowering stage; climate change.*

## 1. INTRODUCTION

World food security is faced with the threat posed by water scarcity, a phenomenon known as drought and the most significant among the abiotic stresses. Drought events are predicted to become more frequent and severe due to global environmental changes, and water scarcity in semi-arid areas will likely worsen [1]. Everyone including men, women, boys, and girls admitted that drought was the cause of food insecurity in a survey conducted in Somalia [2]. They further reported that to cope with the ever-increasing food insecurity, they resorted to rationing of food intake [2]. To obtain good yields, an important crop such as soybean needs enough water availability during its growing cycles [3]. Drought stress, on the other hand, produces more than just harm to plants. To compensate for the damage and losses caused by drought stress, soybean plants might display progressive physico-biochemical growth compensation or overcompensation in terms of metabolism and growth and development after a particular level of dehydration and for a short period following rehydration [4-6].

Drought stress can drastically reduce chlorophyll a, b, and total chlorophyll levels [7]. Drought damage is mainly ascribed to the inhibition and disruption of photosynthesis, which is the essential process for sustaining plant growth and recovering from drought [8]. This damage can occur at any stage of soybean growth [8]. Plants employ compensation as a key self-regulation mechanism to guard against environmental shocks or damage. It is also an important physiological reference for successful water

control in plants and a signal of extremely water-efficient agriculture [9,10]. For many years, researchers have screened genotypes for drought tolerance using photosynthetic-related traits, especially chlorophyll content [11]. Plant growth and development are governed by the synchronization of multiple endogenous hormones and physio-biochemical processes. Endogenous hormones are the most important regulators throughout the plant's life cycle, and they play a role in stress signal transduction when plants are challenged. These hormones control plant physio-biochemical metabolism, growth, and development by opposing endogenous hormone regulation to improve plant tolerance to a harsh environment [12,4]. Plant drought resistance is characterized by osmoregulation, in which the quantity of malondialdehyde (MDA), a result of membrane lipid peroxidation that happens when plants are stressed, can reflect the degree of cell membrane damage [13,12]. The antioxidant system can actively adapt, maintain, and eliminate reactive oxygen species (ROS). When soybeans are faced with water stress, for example, the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) frequently rise, as does the MDA level [14]. The study aimed at screening for drought tolerance in soybeans by assessing their responses to drought stress. Twenty-two (22) soybean genotypes were screened for their drought tolerance levels at the flowering stage of development at 5, 10, and 15 days of drought exposure using pot experiment. Screening for drought tolerance is a necessity in the era of climate change to help alleviate the challenges posed by food insecurity.

## 2. MATERIALS AND METHODS

### 2.1 Study Site

The study was carried out in a greenhouse at the School of Agriculture Technology Village, of the College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana. The area is located on latitude 05° 08' N and longitude 01° 20' W and is characterized by a coastal savannah agro-ecology and Acrisol soil type [15].

### 2.2 Experimental Materials and Sources

Twenty-one out of the 22 soybean genotypes used in this study were obtained from the International Institute of Tropical Agriculture (IITA). The remaining genotype, ANIDASO, is a local check from the Crop Research Institute of the Council for Scientific and Industrial Research, Ghana.

### 2.3 Experimental Design and Plant Culture

A completely randomized experimental design (CRD) was adopted in this study. Five seeds for each of the 22 soybean genotypes were planted in 12-L plastic buckets (with holes at the bottom) containing sandy loam soil, one genotype per pot with three replications in the greenhouse. The setup was repeated to represent experimental (drought-stressed (DS) and control (well-watered (WW) groups.

After emergence, thinning was carried out leaving one plant per pot. Hand weeding was also done the first week after planting and subsequently when necessary. A total of one

hundred and thirty-two (132) buckets were used for the drought screening at the flowering stage with its corresponding number of plants. Sixty-six (66) of the pots were watered with 1500 ml of water every three days until 48 days after planting (48 DAP) when water was withdrawn. The remaining 66 pots were watered throughout the experiment to serve as the control. The drought-stressed plants were rewatered after 15 DAT and monitored till the day of permanent wilting. The soybean genotypes were ranked based on the relative percentage of each parameter determined by the ratio of individual performance of a genotype under drought stress to that of the well-watered as described by Abdou Razakou [16]. The soybean genotype which recorded the highest value of relative percentage was termed most tolerant, while the most susceptible was the one with the lowest value.

### 2.4 Ranking Soybean Genotypes Based on the Relative Percentages of Six Growth and Yield Parameters

The 22 soybean genotypes were ranked according to their tolerance level to water stress. The scoring was done in such a way that genotypes with the highest total score value of relative percentage were scored number twenty-two (22), followed by subsequent scores (21, 20) to the lowest relative percentage [16].

In the greenhouse it was adopted that any genotype with a total (summation) relative percentage value from 103-132 for the growth and yield parameters may be considered highly tolerant, from 73-102 may be considered moderately tolerant, and lastly susceptible if less than 73.

**Table 1. Soybean genotypes used for the study**

S/N.	Genotype	S/N	Genotype.
1	TGX-1990-114FN	12	TGX-1990-40F
2	TGX-1993-4FN	13	TGX-1988-5E
3	TGX-1989-75FN	14	ANIDASO
4	TGX-1987-62F	15	TGX-1990-52F
5	TGX-1990-78F	16	TGX-1990-95F
6	TGX-1989-48FN	17	TGX-1990-46F
7	TGX-1985-10E	18	TGX-1990-57F
8	TGX-1989-11F	19	TGX-1989-49FN
9	TGX-1990-21F	20	TGX-1990-55F
10	TGX-1990-110F	21	TGX-1989-68FN
11	TGX-1989-45F	22	TGX-1989-40F

Source: The authors

The scale made was as follows:

103 - 132: highly tolerant

73 - 102: moderately tolerant

< 73: susceptible

## 2.5 Data Collection and Analysis

Data were collected at 5, 10, and 15 DAT, and three plants were selected from each treatment. Parameters measured were the number of leaves, plant height, stem girth, leaf area, number of flowers, relative leaf water content, and number of days to permanent wilting. The total number of leaves was counted and recorded at each sampling date. Plant height was measured from the base of the stem just above the soil surface to the collar of the latest fully expanded leaf with a meter rule. The stem girth was measured with a digital vernier caliper at 1.5 cm above the soil surface. The leaf area was estimated by measuring the widest part of the leaf as the width [17] and measuring the length of the leaf along the midrib up to the tip of the leaf with a ruler. The leaf area was then calculated as follows:

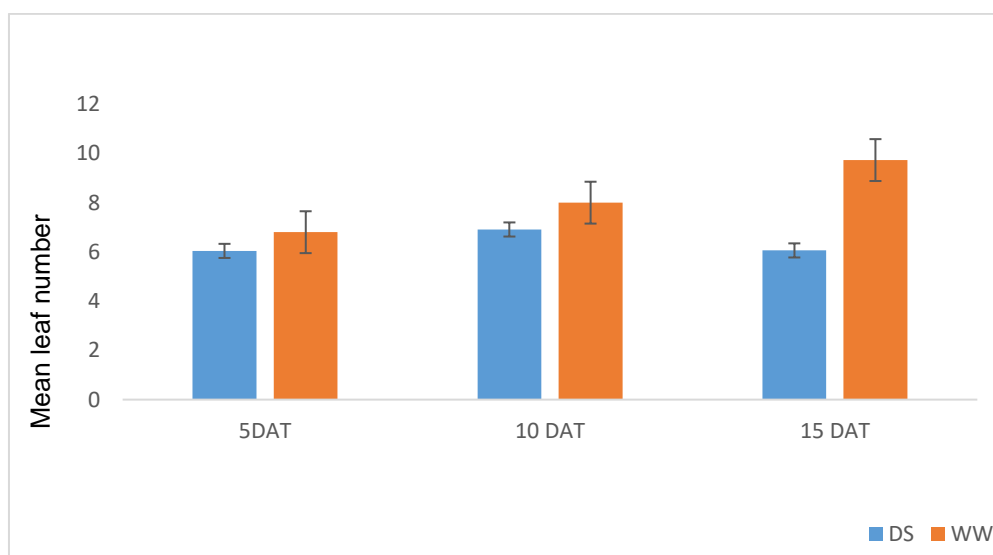
Leaf area (cm<sup>2</sup>) = 0.75 x length (cm) x width (cm). The number of flowers was also counted and recorded. The relative leaf water content was recorded for each soybean genotype at 15 DAT. The relative leaf water content was measured by taking 0.1g fresh leaves (Wf), soaking them in distilled water for 12 h, weighing

saturated (Wt), then putting them into an oven at 105 °C for 10-15 minutes and then drying them to a constant weight (Wd) at 80°C [18]. The relative leaf water content was computed as  $RWC (\%) = (Wf - Wd)/(Wt - Wd) \times 100$ . The number of days to permanent wilting was counted for each soybean genotype after rewatering at 15 DAT. Days to permanent wilting were recorded for each replicate of a genotype and the average days to permanent wilting were calculated. Growth and yield data were analyzed using analyses of variance (ANOVA) in MINITAB software version 18. Individual means of drought-stressed genotypes were compared to their corresponding well-watered in a One-way ANOVA to determine differences in treatment means.

## 3. RESULTS AND DISCUSSION

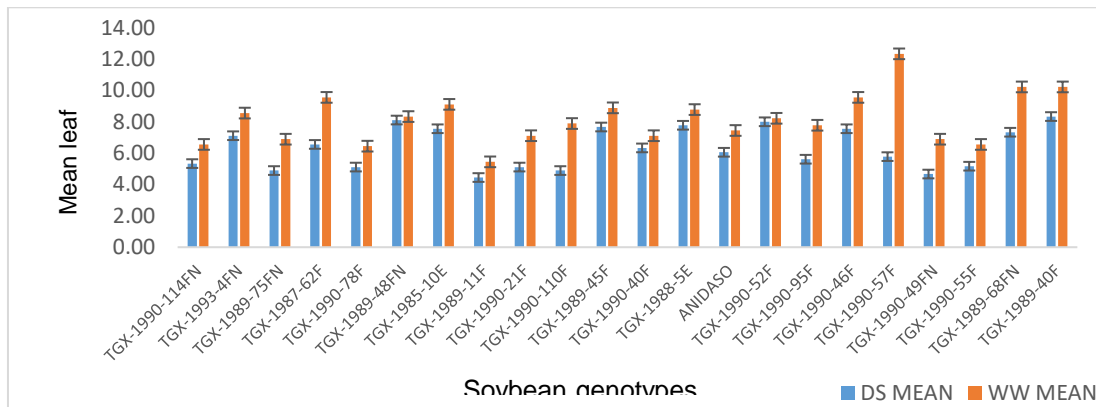
### 3.1 Number of Leaves

Drought-stressed (DS) and control (WW) plants had similar numbers of leaves of 6.04 and 6.80, respectively, (Fig. 1a). Drought stress reduced the number of leaves significantly ( $p < 0.05$ , P-values = 0.033, 0.000) at 10 DAT and 15 DAT. The highest mean number of leaves 8.3 and 12.33 were recorded for TGX-1989-40F and TGX-1990-57F for the DS and WW groups respectively (Fig. 1b). Upon comparison of the mean number of leaves for all the sampling dates of the drought-stressed plants to their control, drought reduced the number of leaves at the flowering stage by 22.50 %



**Fig. 1a. Mean leaf number among drought-stressed (DS) and control (WW) plants of soybean at the flowering stage at 5, 10, and 15 DAT. Values are means of three replicates**

Source: Calculated and collated according to statistical software



**Fig. 1b. Mean number of leaves among drought-stressed (DS) and control (WW) plants of soybean**

Source: Calculated and collated according to statistical software

### 3.2 Plant Height

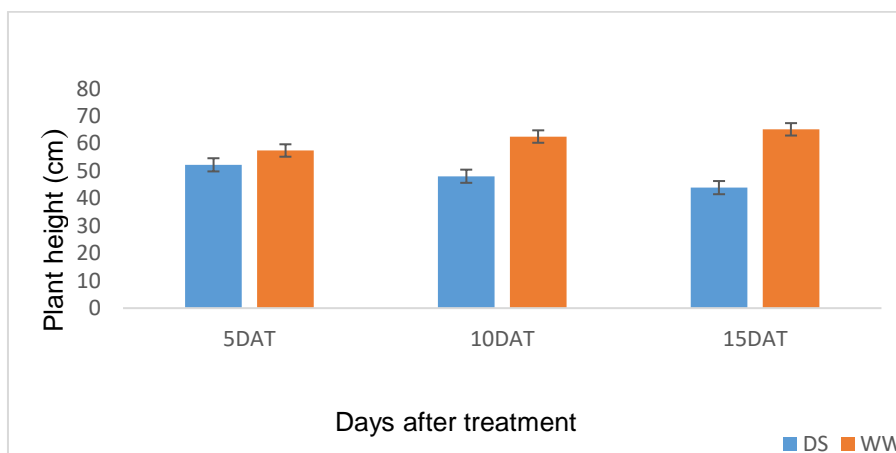
Generally, drought stress affected plant height (Fig. 2a). It reduced plant height significantly ( $P$ -values = 0.004, 0.000) at 10 and 15 DAT. (Fig. 2a). TGX-1990-57F recorded the highest plant height of 77.54 cm and 104.11 cm for both the drought-stressed and the control groups respectively (Fig. 2b). Upon comparison of the mean plant height for all the sampling dates of the drought-stressed plants to their control, drought stress reduced plant height by approximately, 22.1 %.

### 3.3 Stem Girth

At 5, 10, and 15 DAT, stem girth was reduced significantly ( $P$ -values = 0.002, 0.000, and .000 respectively) (Fig. 3a). Thus, drought stress affected stem girth negatively. The highest stem

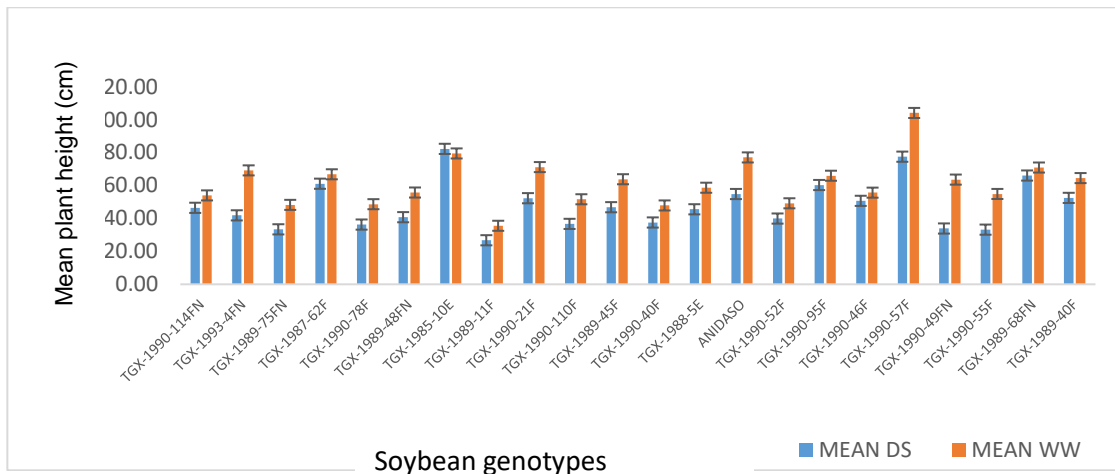
girth of 2.74 mm and 3.51 mm for the DS and WW groups respectively were recorded in genotype TGX-1989-40F (Fig. 3b). Upon comparison of the mean stem girth for all the sampling dates of the drought-stressed plants to their control, stem girth was reduced by drought stress at approximately 21.7%.

Leaf area was reduced significantly at all sampling dates (Fig. 4a) ( $P$ -values = 0.004, 0.000 and 0.000) respectively. The mean leaf area for the DS and WW groups were 9.73 cm<sup>2</sup> and 14.30 cm<sup>2</sup> respectively. Genotypes TGX-1990-78F and TGX-1990-49FN had the highest mean leaf area of approximately 13.43 cm<sup>2</sup> and 18.27 cm<sup>2</sup> among the DS and WW groups respectively (Fig. 4b). Upon comparison of the mean leaf area for all the sampling dates of the drought-stressed plants to their control, drought reduced leaf area by approximately 31.97 %.

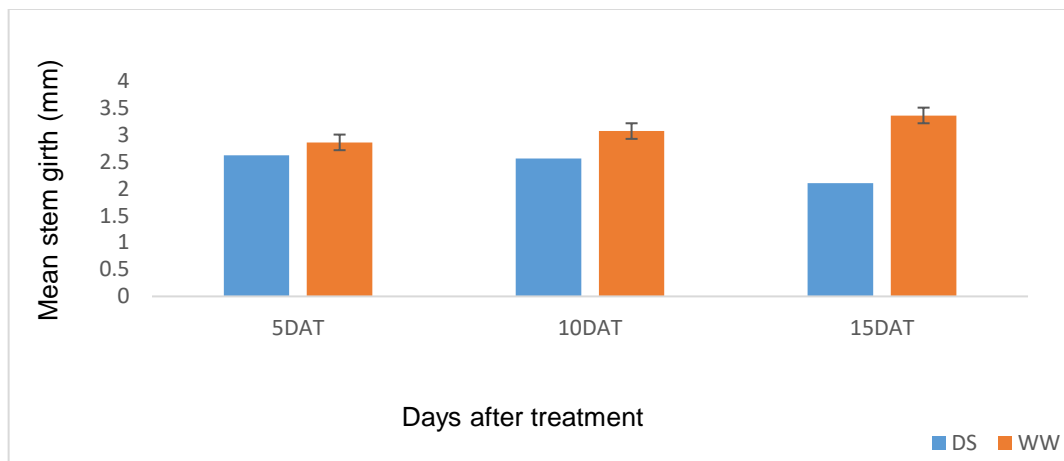


**Fig. 2a. Mean plant height among drought-stressed (DS) and control (WW) plants of soybean at the flowering stage at 5, 10, and 15 DAT. Values are means of three replicates**

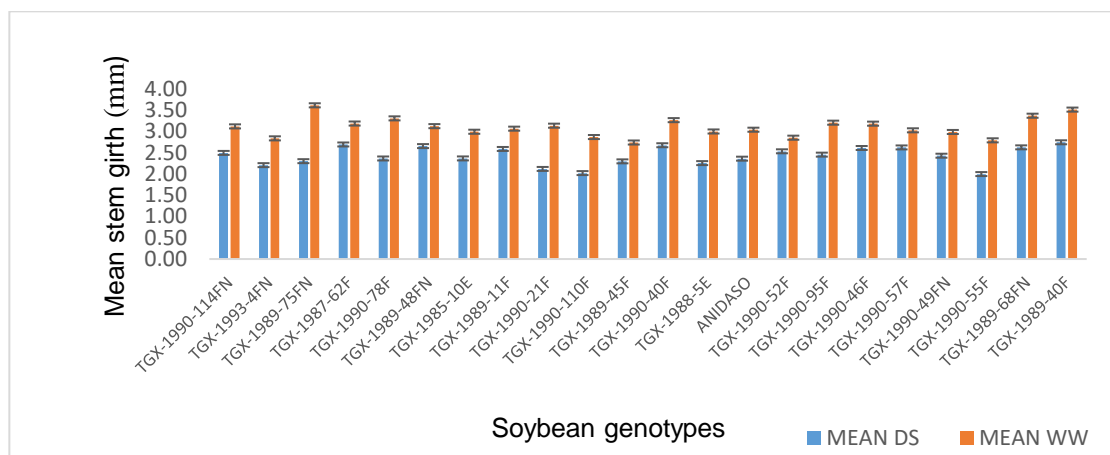
Source: Calculated and collated according to statistical software



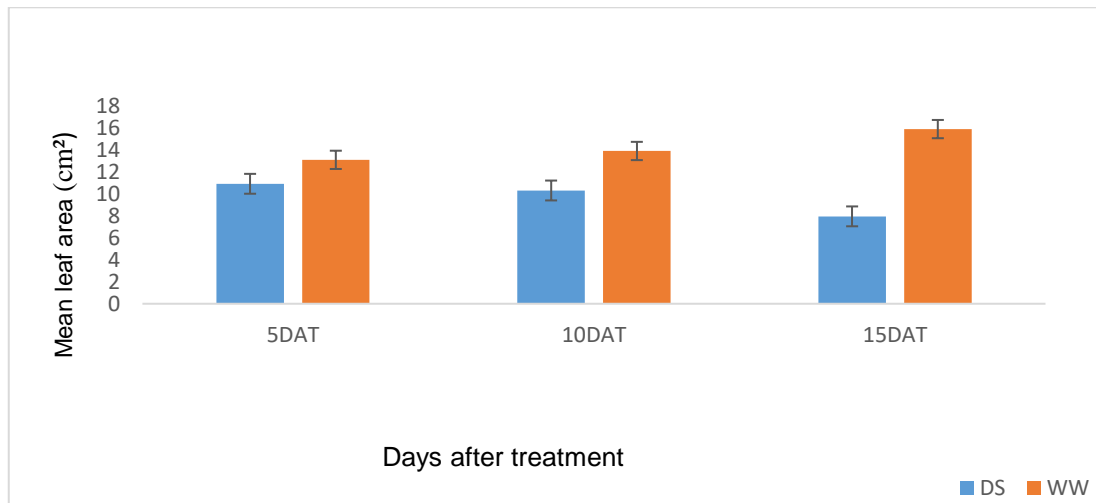
**Fig. 2b. Mean plant height among drought-stressed (DS) and control (WW) plants of soybean**  
 Source: Calculated and collated according to statistical software



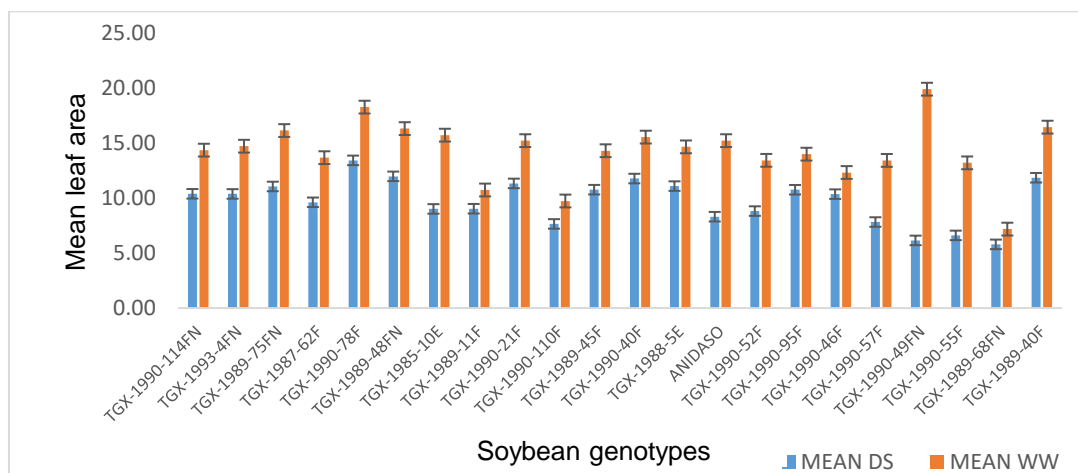
**Fig. 3a. Mean stem girth among drought-stressed (DS) and control (WW) plants of soybean at the flowering stage at 5, 10, and 15 DAT. Values are means of three replicates**  
 Source: Calculated and collated according to statistical software



**Fig. 3b. Mean stem girth among drought-stressed (DS) and control (WW) plants of soybean**  
 Leaf area  
 Source: Calculated and collated according to statistical software



**Fig. 4a. Mean leaf area among drought-stressed (DS) and control (WW) plants of soybean at 5, 10, and 15 DAT. Values are means of three replicates**  
 Source: Calculated and collated according to statistical software



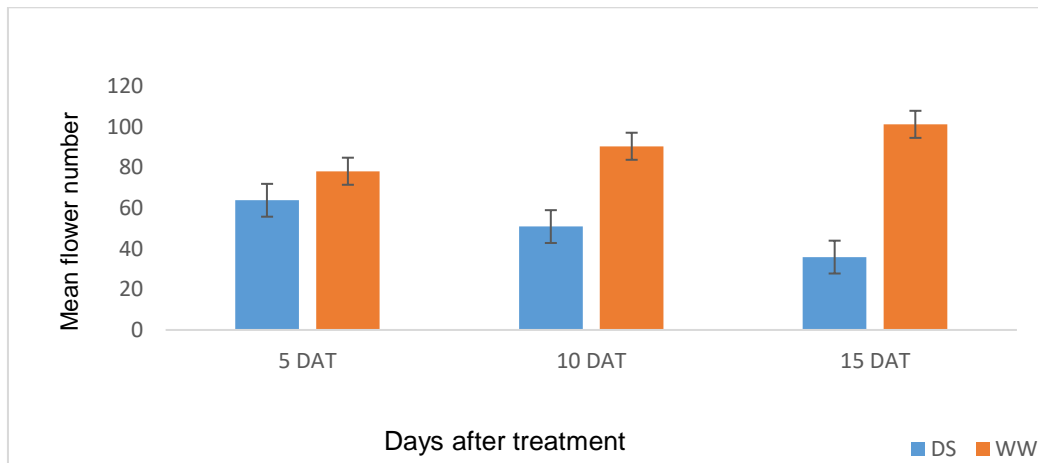
**Fig. 4b. Mean leaf area among drought-stressed (DS) and control (WW) plants of soybean. Number of flowers**  
 Source: Calculated and collated according to statistical software

Number of flowers was significantly ( $P$ -values = 0.009, 0.000, 0.000) reduced by drought stress at all sampling dates (5, 10, and 15 DAT) (Fig. 5a). Among the DS group, genotype TGX-1990-78F recorded the highest mean flower number of 84.00 (Fig. 5b). Genotype TGX-1990-78F again had the highest mean flower number (131.00) among the WW group). Upon comparison of the mean number of flowers for all the sampling dates of the drought-stressed plants to their control drought stress reduced number of flowers by approximately 44.1%.

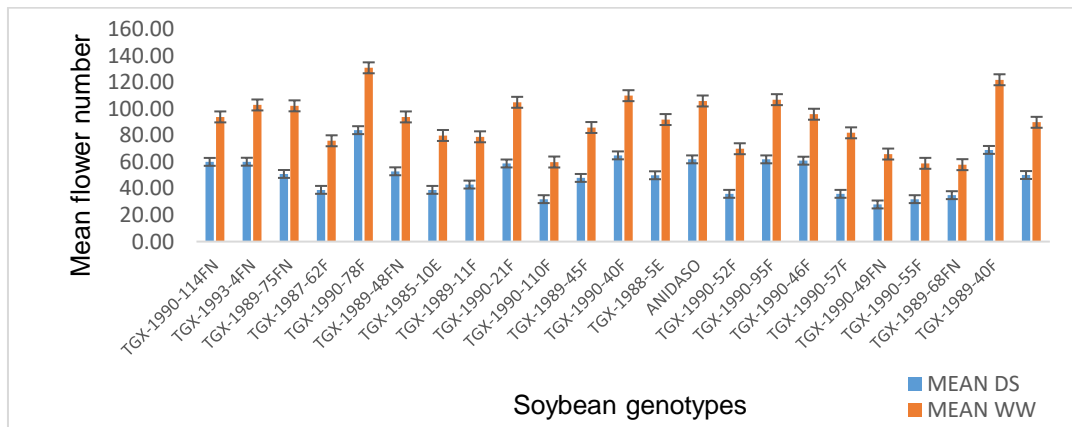
### 3.4 Relative Leaf Water Content (RLWC)

The relative leaf water content was highly significantly ( $P < 0.001$ ) reduced by drought

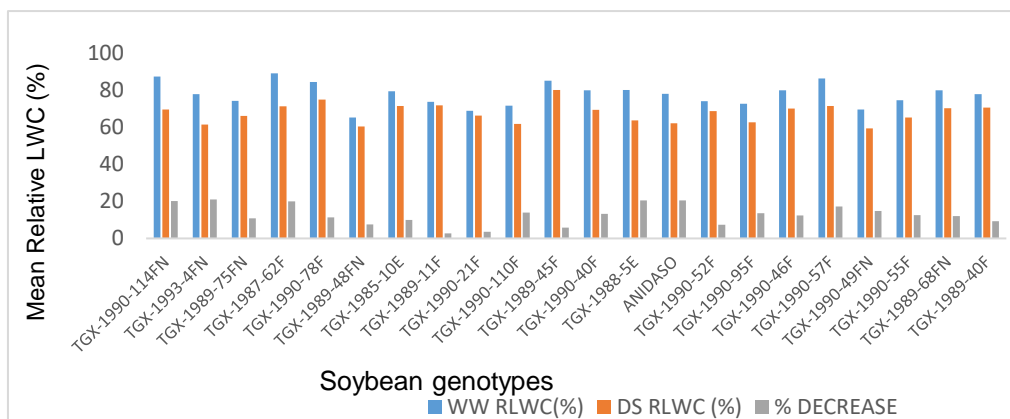
stress at 15 DAT (Fig. 6). The mean RLWC for the DS and WW plants were 70.5 % and 77.6 %. Among the genotypes under drought stress conditions (DS), TGX-1989-45F had the highest relative leaf water content of 80.0 %. However, TGX-1987-62F had the highest relative leaf water content of 89.0 % among the WW plants (Fig. 6). The percentage decrease in RLWC caused by drought in TGX-1989-45F was 5.89 % as compared to 20 % in TGX-1987-62F. TGX-1990-49FN and TGX-1989-48FN had the lowest RLWC among the DS and the WW plants respectively. On average, drought stress reduced relative leaf water content by 9.1 %.



**Fig. 5a. Mean flower number among drought-stressed (DS) and control (WW) plants of soybean at 5, 10, and 15 DAT. Values are means of three replicates**  
 Source: Calculated and collated according to statistical software

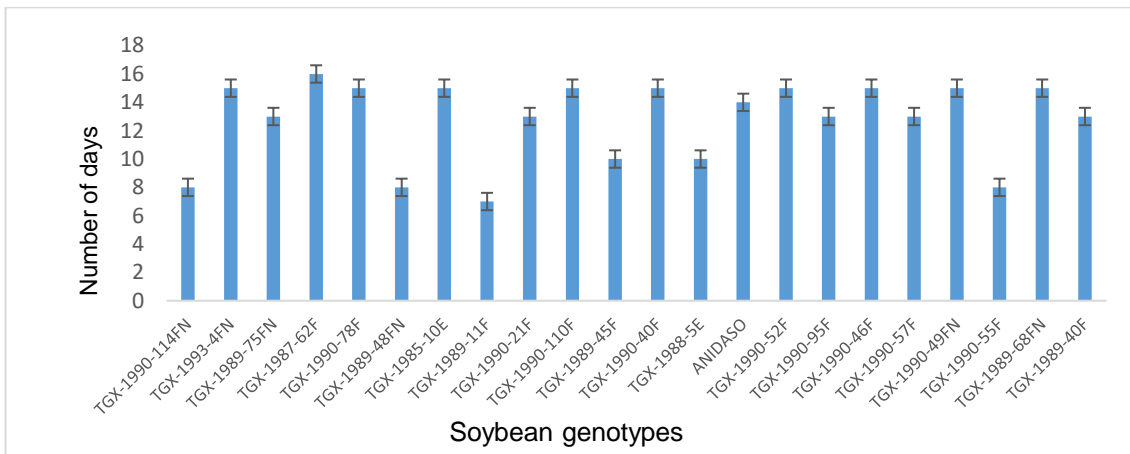


**Fig. 5b. Mean flower number among drought-stressed (DS) and control (WW) plants of soybean**  
 Source: Calculated and collated according to statistical software.

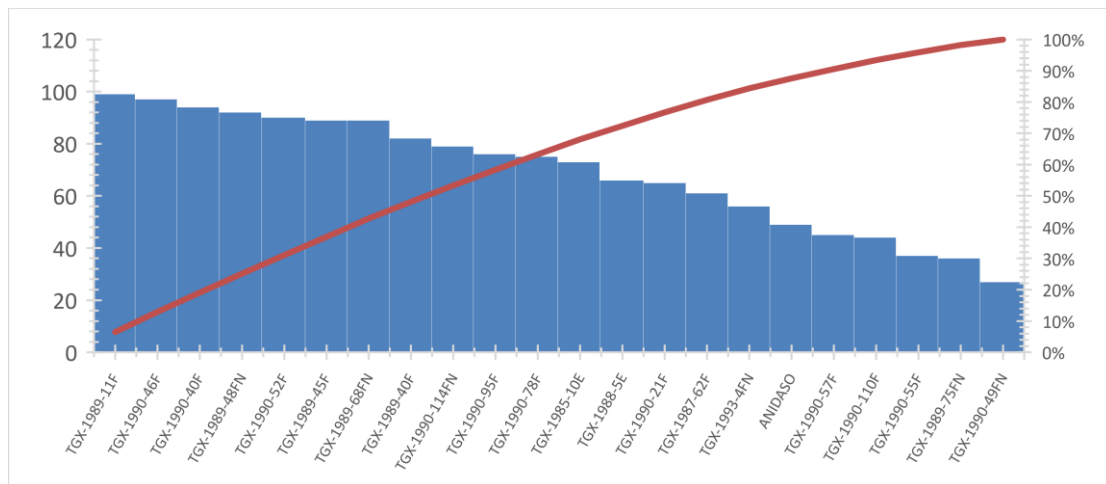


**Fig. 6. Mean RLWC of drought-stressed and control plants of soybean at the flowering stage at 15 DAT. Values are means of three replicates**  
 Source: Calculated and collated according to statistical software.





**Fig. 7. The mean number of days to permanent wilting after rewatering of 22 soybean genotypes at the flowering stage exposed to 15 days of drought stress**  
 Source: Calculated and collated according to statistical software.



**Fig. 8. Scores and rankings of soybean genotypes based on their relative performance in the five growth and yield parameters at the flowering stage; MT: Moderately tolerant (12), S: Susceptible (10)**  
 Source: Calculated and collated according to statistical software

### 3.5 Days to Permanent Wilting

Genotype TGX-1989-11F was the first to wilt permanently at 7 days after rewatering whilst it took genotype TGX-1987-62F, 16 days (the highest number of days) to permanent wilting (Fig. 7). TGX-1990-114FN, TGX-1989-48FN, TGX-1989-11F, and TGX-1990-55F wilted permanently before 10 days whilst TGX-1989-45F and TGX-1988-5E wilted permanently at 10 days after rewatering. There was an approximately 56.3 % decrease in the number of days to permanent wilting between the longest surviving plant and the shortest one to permanent wilting.

### 3.6 Scores and Rankings of Soybean Genotypes Based on their Relative Performance Based on the Five Growth and Yield Parameters

The scores and rankings of soybean genotypes at the flowering stage are presented in Fig. 8. Ten (10) genotypes scored between 0 and 72 and were therefore labeled as drought-susceptible. Twelve (12) genotypes had total scores between 72 and 102 and were also ranked as moderately tolerant to drought stress (Fig. 8). None of the soybean genotypes were found to be highly tolerant to drought stress at the flowering stage. In descending order, the first

three best performers were TGX-1989-11F, TGX-1990-46F, and TGX-1990-40F. The first three medium performers in descending order were TGX-1990-52F, TGX=1989-68FN, and TGX-1989-45F. However, genotypes TGX-1988-5E, TGX-1990-21F, and TGX-1987-62F were the top three worst performers in descending order.

## 4. DISCUSSION

Plant morphological characteristics directly reflect the growth and development of crops [19]. Plant height is one of the most important indicators of plant growth and development, and it reflects the growth rate. The leaf area is also an important parameter related to the amount of light energy captured by crops, and therefore it directly affects photosynthesis, transpiration, and the final yield.

### 4.1 Number of Leaves and Leaf Area

The results obtained for the number of leaves and leaf area imply that the drought effect on leaf initiation and expansion was very critical. Therefore, TGX-1989-40F, TGX-1990-57F, and TGX-1990-78F which performed well even under drought conditions, possessed the characteristics for adaptation to a water-limited environment. Other scientists have reported similar results to the current study. Basal and Szabo confirmed in their studies that comparing the partially and fully-irrigated groups, respectively, to the nonirrigated counterpart, irrigation increased the Leaf area index (LAI) by 8.3 and 14.9% of the soybean plants [20]. This was due to the decreased production of new leaves, increased leaf shedding, and decreased average leaf size. Leaf production and leaf expansion were therefore the two phenomena most sensitive to water stress [19]. Sun et al. have also reported that continuous drought stress had a greater effect on the leaf area of winter wheat reducing it by 30 % when compared to the control [21] They also emphasized that different levels of water stress applied on chickpea and soybean plants showed that stress conditions have a more devastating effect in soybean as compared to chickpea.

### 4.2 Plant Height

The drought effect on plant height in this study aligned with an observation made by Elsalahy and Reckling. They reported a decrease in soybean plant height caused by drought stress both at the vegetative and flowering stages [22].

That being said, it is possible that, regardless of the stage of plant growth, the processes of cell division and expansion were significantly impacted by dry conditions, resulting in shorter plants [23,24]. From Licht and Archontoulis's perspective, soybean plants have shorter plant height and smaller leaves as a result of lack of water, nutrient availability, and nutrient uptake [25].

### 4.3 Stem girth

Statistically drought stress significantly ( $P = .05$ ) reduced stem girth at all sampling dates. The present result is in agreement with the findings of Du and his colleagues. Their research revealed that soybean stem, root, and seed biomass was reduced by drought stress during the reproductive stages [26] and a lower biomass allocation to the seed as compared to the control, which ultimately resulted in a decrease in seed weight [26]. Under drought stress, plants lose a significant amount of their yield and their ability to produce and mobilize assimilates to develop seeds. In another study by Gallardo et al, stem growth rate (SGR) was found to be most sensitive to drought during the early growth stages whilst maximum daily stem shrinkage (MDS) was at the late growth stage [27]. Shrinking expansion of the stem occurs in response to changing water potential in the xylem [28-30]. More daily shrinkage of the stem under water-stressed conditions hinders the growth of the stem [31].

### 4.4 Number of flowers

The reduction in number of flowers was apparent and significant ( $P = .05$ ) at all sampling dates (5, 10, and 15 DAT). This is in agreement with work done by other scientists. For example, Lenssen also observed that soybean plants were most sensitive to intense drought and prolonged stress during the flowering and early pod-fill growth stages [32]. He also mentioned that drought can cause floral abortion, reduced number of pods, cause production of fewer seeds per pod and reduced seed size. Drought stress can significantly reduce or stop nitrogen fixation, disrupting seed development [32].

### 4.5 Relative Leaf Water Content (RLWC)

Statistically, drought stress significantly ( $P < 0.05$ ) reduced the RLWC of the genotypes after 15 days of drought exposure. This is in agreement with results from Chowdhury et al.

They reported that water stress significantly reduced the relative leaf water content of three soybean genotypes at different growth stages due to higher evaporation resulting from increased temperature and light intensity [33]. They concluded that plants grown under water-stress conditions had a lower RWC than those grown under non-stress conditions [33]. According to Lugojan and Ciulca, leaf relative water content is an important indicator of water status in plants. It shows whether the water supply to the leaf tissue is balanced to the rate of transpiration [34].

#### 4.6 Days to Permanent Wilting

TGX-1987-62F and TGX-1989-11F recorded the highest (16) and lowest (7) number of days to wilting respectively (Fig. 7). TGX-1987-62F probably had an inherent adaptation to wilting compared to other genotypes. The mean number of days to permanent wilting after 15 DAT was 12.77 (Fig. 7). Eleven of the genotypes wilted permanently by the end of two weeks (14 days) (Fig. 7). The remaining seven genotypes wilted permanently by the end of day 16. Six of those seven genotypes wilted at day 15 with the earlier mentioned genotype TGX-1987-62F wilting permanently at day 16 after rewatering. Statistically, drought significantly reduced the number of days to wilting. Novak and Lipiec have reported that plants respond to drought stress in the form of hydraulic signaling, that is, decreased root growth, water uptake, water potential, turgidity, and leaf expansion [35]. Drought stress reduces water potential and transpiration rate thereby reducing cell turgor and relative water content damaging the plant cell (wilting). This condition was seen after a few days in the susceptible genotypes especially, TGX-1989-40F, TGX-1989-11F, and TGX-1990-114FN. According to Ribas-Carbo and other researchers, drought stress decreases the photosynthesis rate in soybean plants by 40 % and 70 % in mild and severe stress conditions respectively [36]. Flexas and colleagues also reiterated that plant growth, plant survival, and yield are determined by the downregulation and inhibition of photosynthesis under water stress [37].

#### 4.7 Scoring and Ranking of Soybean Genotypes

The 15 days drought treatment at the flowering stage scored and ranked the soybean genotypes as highly drought-tolerant (T), moderately drought tolerant (MT), and drought-susceptible

(S) (Fig. 8). After the drought screening, none of the 22 soybean genotypes was ranked as T. Twelve (54.5 %) were MT and the remaining 10 (45.5 %) were S (Fig. 8). The MT group had a total relative percentage score ranging from 73 to 99. The genotype with the highest total score, TGX-1989-11F had a score of 97.1 % of the maximum total score of 102. On the other hand, the genotype with the lowest total score among the MT group was TGX-1985-10E. It scored 91.7 % of the maximum total score of 102. The genotype with the highest total score, TGX-1988-5E scored 91.7 % of the maximum total score of 72. However, TGX-1990-49FN recorded the lowest total score of 27 and 37.5 % of the maximum total score. The soybean genotypes that were ranked MT probably experienced a moderately damaging effect from the drought stress on their growth and yield parameters. Drought-susceptible (S) genotypes however might have suffered highly damaging effects from the drought stress on their growth and yield parameters.

### 5. CONCLUSION

Drought stress reduced the number of leaves, plant height, stem girth, leaf area, number of flowers, relative leaf water content, and days to permanent wilting among the soybean genotypes. The number of leaves, stem girth, leaf area, and number of flowers were the most sensitive to drought stress. The drought effect was dependent on the period of exposure, the longer the period, the more intense the deleterious effect. The pot experiment revealed 12 (54.5 %) of the soybean genotypes to be moderately drought-tolerant (MT)(TGX-1989-11F, TGX-1990-46F, TGX-1990-40F, TGX-1989-48FN, TGX-1990-52F, TGX-1989-45F, TGX-1989-68FN, TGX-1990-114FN, TGX-1990-95F, TGX-1990-78F, TGX-1985-10E, TGX-1989-40F) and 10 (45.5 %) to be drought susceptible after 15 days of drought exposure.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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