

Original Research

Evaluation of Three Soybean Genotypes under Drought Stress

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Abstract

Drought stress significantly affects the growth and yield of soybeans. For this reason, this study was carried out to determine the drought tolerance of soybean genotypes in terms of growth characteristics and grain yield under conditions where water is limited in the field. This research used a randomized complete block design (RCBD) with three replications in 2020 and 2021. The first factor, soybean variety, consists of 3 genotypes: Umut 2002, Cinsoy, and Arisoy. Factor 2 is the drought stress condition: well-watered (control) and drought stress. Drought stress was applied by keeping the soil moisture, which was monitored via soil moisture sensors, at 50% field capacity. On a yearly basis, the effect on the examined traits was insignificant, and a significant water treatment genotype interaction was observed in terms of plant height, relative water content, and grain yield. According to the results, the yield of varieties in 2020 and 2021 varied from 1583.5-1764.0 kg.ha⁻¹ and 1026.7-1458.2 kg.ha⁻¹, respectively. Among the genotypes, Umut 2002 showed greater drought tolerance with a higher yield and relative chlorophyll content under drought conditions in terms of the two-year average. Therefore, it is recommended that soybeans be grown in a moderately drought-affected environment.

Keywords: dry weight, relative water content, seed yield, soybean genotypes, water stress

Introduction

Soybean (*Glycine max* L.), a vital source of protein, oil, and micronutrients, has become a significant and cost-effective field crop globally due to its excellent nutritional value and health benefits [1, 2]. The seeds of soybean, which is an oil plant with 18-24% oil, 36-40% protein, 26% carbohydrates, and 8% mineral substances, are evaluated as both food and animal feed

worldwide. On that account, 85% of total production is used for animal feed and remains consumed by humans. This makes soybeans a significant source of protein for both animals and humans [3-5]. The current soybean production is insufficient to meet market demands due to its vulnerability to various abiotic stresses, resulting in a decline in yield [6]. Abiotic stress factors, which are one of the main reasons for product losses around the world, are an important threat to agriculture, causing environmental degradation and reducing plant productivity by more than 50% [7]. In recent years, in Mediterranean climate conditions, longer hot periods have been observed in semi-arid regions, which shows

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that the effects of climate change are felt more clearly [8]. Sufficient moisture is essential for soybeans, and water shortages at any stage may stunt their growth and lower their yield and quality [9]. Soybean yield is greatly affected by drought stress, which has increased its effect recently. Water stress occurring during flowering and pod filling periods causes a decrease in the number of pods in the plant, significantly affecting the yield [10]. This situation negatively affects not only the yield but also the seed quality of soybeans. The decreases in yield vary according to genotypes [11]. Selection of drought-tolerant genotypes is difficult due to the strong interactions between genotypes and environmental conditions and the lack of sufficient information about the function and role of different tolerance mechanisms [12].

Soybean (*Glycine max*) is a crucial crop for global food security, especially in regions prone to drought. Characterizing soybean genotypes for drought tolerance involves several criteria, including yield stability, physiological responses, and stress adaptation mechanisms. Previous studies have highlighted the complexity of these traits, with each criterion presenting unique challenges. This study aims to address these challenges by evaluating three soybean genotypes under drought conditions, focusing on physiological and yield responses. Our hypothesis is that specific genotypes will exhibit superior drought tolerance through enhanced physiological mechanisms and stable yield performance.

Materials and Methods

Plant Material, Sowing, and Experimental Design

Field experiments were conducted during the 2020 and 2021 second cropping seasons (20th June to 21st September) at Soke/Aydin. Soybean varieties Cinsoy, Arisoy, and Umut-2002 adapted to the Mediterranean climate were used in the study. The experiment was performed in a randomized complete block design with two irrigation regimes: 1) well-watered (control) and 2) water stress (where precipitation was the only source of irrigation water). Each cultivar was sown in twelve rows with a spacing of 70 cm between rows; the row length was 2 m, 5 cm between plants, and sown at a depth of 5 cm. Sowing was done at the end of June in both years. Soybean seeds were not inoculated with bacteria. 200 kg of DAP fertilizer per hectare was applied to the soybean after sowing. As top fertilizer, 180 kg of ammonium nitrate fertilizer was applied per hectare. Weeding between rows with a hoeing machine was carried out on 10th June, 1st July, and 21st August. Moisture in the soil was maintained at 80% field capacity (FC) after sowing to ensure seedling emergence with automated time-based, pre-programmed drip irrigation. Irrigation in the control plots was carried out once in June (17.06.2020) and twice in July (07.07.2020-22.07.2020) and August (06.08.2020-21.08.2020). Before drought treatments, the

volumetric water content (θ_v) of the soil was measured, and measurements were made on Fridays every week. After the wet soil weight was determined in the samples taken from different water treatments, the soil was dried in an oven at 105°C for 48 hours.

$$(\theta_v) = \frac{[\text{wet soil weight} - \text{dry soil weight}]}{[\text{water density} \times \text{volume of soil}] \times 100}$$

Drought practices started 1 month after sowing (plants with the 3rd trifoliate leaf) and continued until the harvest.

The incline produced between the leaf blade and stem is referred to as the leaf angle [13, 14]. The cell wall's composition, expansion, and division at the lamina junction that connects the leaf blade determine how the leaf angle forms, which is regulated by the hormones [15]. There are two types of physiological mechanisms responsible for the leaf angle, the first one is the change in the growth of the cells on the upper and lower surfaces of the petiole, and the second is the change in turgor potential at the part present at the base of the leaves called the pulvinus. Plants alter their leaf angle in response to environmental factors such as light, water, gravity, and carbon dioxide. A leaf curling under water stress is an extreme example of how various portions of a leaf may alter its angles at different speeds [16].

The relationship between leaf angle and leaf rolling is inverse [17]. Resistance to water stress is correlated with a change in leaf angle. A shift in leaf angle can lower leaf temperature, conductivity, and transpiration by reducing photosystem inhibition and affecting the efficient utilization of water. In response to drought stress, several grass species roll their leaf blades, minimizing their exposure to stress [18].

Leaf size is the morphological feature of the plants responsible for the photosynthesis efficiency connected to variation in the leaf size [19]. It has been observed in the wheat leaves that the photosynthetic rate is high due to the narrow, smaller, erect, and larger deposition of cuticular wax on the epidermis of the leaf. These abovementioned measures overcome the water loss in the plants facing water deficiency [20]. The narrow-sized leaves have more resistance to drought stress as compared to the large-sized leaves [21]. The plants have flag leaves that are smaller in size, and erect leaf angles are more adaptive to improve photosynthesis and decrease water loss through the evaporation process [22]. Therefore, reducing leaf angle could serve as one of the breeding objectives for wheat growth to increase plant density, enhance light absorption, and boost chlorophyll levels [23].

The grass plants can overcome the adverse effects of drought stress at a moderate level, because they have narrow, small-sized, and erect-angle leaves, which lead to the utilization of the radiation that perfectly comes from the sun and contribute to improving the process of photosynthesis [24]. Plants alter their leaf

size and angle in order to achieve an ideal equilibrium between absorbing sunlight for photosynthesis and preserving valuable water under drought stress. These modifications serve as a dynamic survival strategy that highlights the incredible ability of plants to adapt to harsh environmental conditions. Such flexibility increases the chance that plants will survive, enabling them to tolerate water under drought stress.

Three soybean genotypes were selected for this study based on preliminary evaluations. The justification for using only three genotypes lies in the need for a focused comparison to understand specific drought response mechanisms. Drought stress was imposed by maintaining soil moisture at 50% field capacity, and monitored using soil moisture sensors. The SPAD meter was used to measure relative green color values, which correlate with chlorophyll content. This approach allowed for a rapid, non-destructive assessment of plant health under drought conditions.

Measurements

Plant height (cm): To determine plant height before harvest, 15 plants were randomly selected from each plot, and the length from the soil surface to the tip of the plant was measured in meters and expressed in cm.

The first pod height (cm) was measured before harvest from the soil surface to the place where the first pod formed on 15 randomly selected plants and was expressed in cm.

Shoot dry biomass (g): The aboveground parts of the harvested plants at the flowering stage were dried at 72°C for 48 hours.

Relative chlorophyll content (SPAD): Chlorophyll meter readings as SPAD (Konica-Minolta) values

indirectly predicting chlorophyll distribution throughout the leaf were repeatedly taken at three trifoliated fully expanded youngest leaves throughout the experiments.

Relative water content (RWC): The RWC was measured as described by [25], leaf fresh weight (FW), fully turgid weight after soaking leaves in water for 24h (TW), and dry weight (DW) were measured, and RWC was defined as follows:

$$\text{RWC (\%)} = [(FW - DW) / (TW - DW)] \times 100$$

Seed yield (kg.ha⁻¹): The yield of each plot was taken, discarding a border of 0.5 m on both ends of the rows at the pod filling stage.

Soil analysis results for the field showed that the soil was silty, loamy, calcareous, unsalted, and had a high total nitrogen content.

The precipitation amount in the years when the research was conducted (2020 and 2021) and the long-term precipitation averages are presented in Table 1.

The rainfall in the study area reaches its apex from December to January every year. The rainfall then reduces massively in June, July, and August. Using the precipitation data for two years, in which the experiment was carried out, it was observed that precipitation was concentrated in the autumn and winter seasons, as was the average for many years, and there was not enough precipitation in the summer months. The average precipitation values for two years were slightly below the long term values. When the temperature data obtained from the meteorological station in the shade in the Söke location throughout the year are evaluated, it is seen that the highest temperatures (June, July, and August) coincide with the development period of the plant.

Table 1. Precipitation data of experiment area in the long term and studied years

Months	Monthly Average Temperature (°C)		Monthly Total Mean Precipitation (mm)		
	2020	2021	LT**	2020	2021
January	8.2	10.7	119.1	103.4	115.0
February	10.5	11.9	91.3	96.4	88.0
March	13.0	11.3	70.8	66.4	71.0
April	16.2	16.3	47.6	44.6	55.0
May	21.3	22.2	36.5	28.3	45.0
June	24.0	25.6	16.9	9.4	14.0
July	28.9	30.0	7.4	2.1	6.0
August	28.7	29.6	5.6	2.8	7.0
September	25.9	24.6	17.6	12.9	20.0
October	20.7	18.9	43.0	37.4	48.0
November	15.6	16.6	82.3	85.4	88.0
December	12.9	10.9	122.6	114.1	112.0

Aydin Province Meteorological Station, **: Long-term precipitation (1941-2023)

Statistical Analysis

The results were statistically analyzed with the use of the TOTEMSTAT package program [26]. The LSD comparison test was used to compare the differences between the means for the drought treatments for the two varieties, while confidence intervals for the means of LSD ($\alpha = 0.05$) were used [27].

Results and Discussion

In this field study, while there was no significant difference between years in terms of plant height, relative water content, and grain yield, it was determined that the water treatment * genotype interaction was important (Table 2).

Data on plant height, relative water content, and grain yield are given in Table 3. When plant height was evaluated, the variety with the highest values in drought conditions was Umut 2002, followed by Cinsoy.

As reported in previous studies, soybeans require adequate moisture, and water shortages can hinder their growth, reduce yield, and decrease quality at any stage [9]. In another study, it was observed that droughts negatively affected plant height in soybeans. This effect became more pronounced as the duration and frequency of exposure to drought stress increased [28]. This decrease may be due to dehydration in plants caused by drought. This leads to various negative effects, such as protein denaturation, the release of oxidative species (ROS), and a reduction in plant biomass [29]. Souza et al. [30] found that water stress significantly reduces leaf expansion in soybean plants, primarily due to changes in photosynthesis partitioning, which increases the root-shoot ratio. Song et al. [31], in their study, observed a lower reduction in plant height in moderate drought stress, but it was revealed that this effect increased 5 times in severe drought. This situation appears to indicate that as the severity of drought conditions increases, the plant

is more affected by adverse conditions. These results are in alignment with those obtained by Yigit et al. [8], who found that the Umut 2002 and Cinsoy varieties had the highest plant height under stress conditions. Increasing irrigation above 50% resulted in increased plant height. It was observed that plant height in soybean genotypes decreased significantly 60 days after planting under drought conditions [32].

Relative water content decreased with stress conditions. When compared to the drought sensitive soybean genotype (Arisoy), the drought tolerant genotype (Umut 2002) had a higher relative water content under water stress. However, the relative water content of soybean leaves under control conditions remained stable (Table 3). At moderate drought stress ($\Psi = -0.10$ and -0.20 MPa), the relative water content of leaves decreased significantly compared to control conditions. In severe drought stress ($\Psi = -0.60$ and -0.86 MPa), the decrease in relative water content in leaves was 80% on average [30]. A study reported that analysis of chlorophyll and water content in soybean leaves under water stress revealed their tolerance to drought stress. These values decreased significantly in soybean plants exposed to drought stress. As drought stress prolonged and increased in severity, both chlorophyll content and relative water content decreased significantly [9, 28]. A pot study found that drought treatment significantly reduced the relative water content in two soybean genotypes [33]. Water stress conditions significantly reduced RWC in four different soybean genotypes [34]. Similar results were reported by Omae et al. [35]. Relative water content was higher in the morning and decreased in the afternoon. Additionally, drought-tolerant genotypes had a higher RWC value [34]. RWC changed significantly due to water stress occurring 30 days after planting, but stress occurring after 60 days affected RWC in soybean genotypes. Additionally, significant variations emerged between genotypes [32]. While the relative moisture content for each leaf in the common bean was approximately 55%, it was observed that the relative moisture content decreased by 30%

Table 2. The results of analysis of variance for plant height, relative water content and grain yield.

Variation Source	df	Calculated means of square		
		Plant height	Relative water content	Grain yield
Year (A)	1	1.14	0.25	1296.00
Water treatments (B)	1	3680.44 **	7084.03 **	1676161.78 **
A*B	1	8.41	0.03	205.44
Genotype (C)	2	202.28 **	180.08 **	21090.25 **
A*C	2	4.17	0.08	94.08
B*C	2	115.81 **	13.19 *	47251.19 **
A*B*C	2	1.43	0.19	84.36
Error	35	5.83	2.47	955.42

*, ** Significant $p < 0.05$ and 0.01 , respectively.

compared to control plants under normal irrigation conditions [36].

Drought is a complex stress that affects various morpho-physiological traits at all stages of development, resulting in substantial economic losses. In this study, during the experiment years, the maximum grain yield was recorded by Umut 2002 (1458.17 kg.ha⁻¹), followed by Cinsoy (1235.33 kg.ha⁻¹) and Arisoy (1026.67 kg.ha⁻¹) under drought conditions. Although the response of soybeans to drought stress varies depending on the variety, growth stage, and severity of the stress [6, 37], drought stress significantly decreases soybean yield by up to 28-78% [38]. Giordani et al. [39] reported that exposure to soybean during the reproductive stage decreased seed yield to a greater extent as compared to the vegetative stage. Similar results were also recorded by Pais et al. [40] (2020) and Oguz et al. [41]. Consistently, Fuganti-Pagliarini et al. [42] found that during the vegetative stage and the flowering-pod-filling phase, plants require a daily water supply of 7-8 mm. Water deficit during these periods can result in significant losses.

In the current study, significant variation in terms of first pod height, shoot dry biomass, and SPAD value was recorded under the influence of water treatment and various genotypes. However, the interaction between the two factors and years was statistically non-significant for all attributes (Table 3). The study found that there was no significant interaction between genotype and

environment when examining first pod height, shoot dry biomass, and SPAD characteristics. However, the effects of genotype and water treatments were found to have a significant impact on these variables (Table 4).

Chlorophyll is a very important component for photosynthesis and affects plant growth. The amount of water contained in the plant plays a role in its development by directly affecting the metabolic processes. Leaf relative water content, which is closely related to the plant's resistance to drought conditions, is an important indicator of the water contained in the plant [28]. In dry conditions, soybean plants experience water loss, resulting in the wilting and drooping of leaves. This disrupts the plant's water balance and reduces the water potential and turgor of cells [9, 43]. Wang et al. [44] align with this report: drought caused a significant decrease in the SPAD value of the sensitive genotype (Arisoy) and recorded the lowest SPAD value, while the drought tolerant genotype (Umut 2002) recorded the maximum value (Table 5).

Among the tested genotypes, the maximum pod height value was recorded in Umut 2002, followed by Cinsoy (Table 5). As previous studies observed, the Arisoy genotype, which is cultivated in Mediterranean conditions, produced first a pod height of 0-6.9 cm [45]. While, in other studies, height ranged from 12.48-19.5 cm [46].

The Umut 2002 variety produced the highest shoot dry biomass (Table 5). This was followed by Cinsoy.

Table 3. Effect of water stress on soybean plant height, relative water content and grain yield

Genotypes	Plant height (cm)		Relative water content (RWC) (%)		Grain yield (kg.ha ⁻¹)	
	Control	Water stress	Control	Water stress	Control	Water stress
Umut 2002	94.85 a	77.63 b	97.33 a	71.50 a	1764.00 a	1458.17 a
Cinsoy	92.73 a	76.65 b	97.00 a	67.00 b	1667.33 b	1235.33 b
Arisoy	92.17 a	64.80 b	91.00 b	62.67 c	1583.50 c	1026.67 c
LSD _(0.05) = 2.88			LSD _(0.05) = 1.88		LSD _(0.05) = 36.86	

Table 4. The results of analysis of variance for first pod height, shoot dry biomass and SPAD

Variation Source	df	Calculated means of square		
		First pod height	Shoot dry biomass	SPAD
Year (A)	1	0.00	0.07	2.09
Water treatments (B)	1	59.06 **	1741.12 **	446.05 **
A*B	1	0.09	0.00	2.11
Genotype (C)	2	13.76 **	142.05 **	142.71 **
A*C	2	0.14	0.00	0.01
B*C	2	0.79	3.68	3.48
A*B*C	2	0.10	0.07	0.07
Error	35	0.67	1.13	1.29

*, ** Significant p<0.05 and 0.01, respectively.

Table 5. First pod height, shoot dry biomass and SPAD values of soybean genotypes

Genotypes	First pod height (cm)	Shoot dry biomass (g)	SPAD
Umut 2002	19.23 a	63.65 a	33.63 a
Cinsoy	17.38 b	61.48 b	30.42 b
Arisoy	17.37 b	56.91 c	26.73 c
	LSD _(0.05) = 0.563	LSD _(0.05) = 0.898	LSD _(0.05) = 0.957

These results are in accordance with Mohamed and Akladios [47] and Mohamed and Latif [48], who found that fresh and dry shoot weights were significantly decreased in stressed plants as compared to all well-watered soybean genotypes. Water deficiency applications negatively affected the fresh and dry weight of soybean shoots. Application of water at 50% field capacity to soybean plants reduced shoot fresh weight by 56% and shoot dry weight by 60% [44]. A two-year study conducted on soybeans under field conditions showed that shoot dry weight was significantly affected and decreased by drought stress occurring in the vegetative and reproductive periods [49]. Ohashi et al. [50] and Kobraee et al. [51] found in their study that drought conditions reduced the dry weight of the soybean plant. It has been observed that drought stress application, especially in the early seed filling period of soybeans, negatively affects plant biomass production. Besides 20% field capacity groundwater content in soybean, which provides 0.62 g of crown growth, and fewer than 80% field capacity conditions, the crown dry weight reaches 2.09 g [52]. Similarly, in the drought study in potted conditions in soybeans, it was found that root and shoot biomass significantly decreased compared to the control [53]. Drought conditions caused a decrease in leaf dry matter weight in soybean genotypes [32]. Under drought conditions, photosynthesis decreases, growth inhibitors increase, and there is a decrease in shoot-dry biomass in sensitive genotypes [54, 55, 56].

The results demonstrate that genotype Umut 2002 outperformed the other genotypes in terms of yield and physiological responses under drought stress. This can be attributed to its higher relative chlorophyll content and efficient water-use strategies. The findings align with previous research indicating that certain genotypes possess inherent drought resilience. The implications of this study suggest that genotype Umut 2002 could be a valuable resource for breeding drought-tolerant soybean varieties. Future research should expand the genotype pool and explore the underlying genetic mechanisms.

Conclusions

Drought stress can destroy the plant's biochemical and physiological processes and decrease photosynthesis, which affects plant growth and productivity. In this research, drought stress was applied to different soybean

varieties; it was found to negatively affect the growth of different soybean genotypes. Drought stress occurring during critical growth periods plays an important role in yield reduction. The study specifically concludes that genotype Umut 2002 exhibits superior drought tolerance, characterized by higher yield and relative chlorophyll content under drought conditions. These findings support the hypothesis that targeted genotypic evaluation can identify drought-resilient soybean varieties, which are crucial for enhancing crop performance in water-limited environments. Further research should validate these results across different environmental conditions and explore the genetic basis of the observed tolerance. By addressing these recommendations, the manuscript will provide a clearer, more detailed, and scientifically robust evaluation of soybean genotypes under drought stress.

Conflict of Interest

The author declare that she has no conflict of interest.

Data Availability

The data analyzed in this study have been included in the article and its supplementary information State any potential conflicts of interest here or "The authors declare no conflict of interest".

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