Volume 13, Issue 1, Page 104-110, 2024 https://doi.org/10.46810/tdfd.1386233 Research Article

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Screening Some Advanced Upland Cotton (*Gossypium Hirsutum* L.) Genotypes Tolerance Under Water Deficit Stress

Sadettin ÇELİK[1*]

[1]*University of Bingol, Genç vocational school, Department of Forestry, 12500, Genç, Bingol/ Turkey*

Sadettin ÇELİK ORCID No: 0000-0002-8396-4627
**Corresponding author: sadettincelik@bingol.edu.tr*

(Received: 07.11.2023, Accepted: 07.03.2024, Online Publication: 26.03.2024)

Keywords
Drought tolerance,
Cotton,
Cluster,
Morphological traits,
Stress factors

Abstract: Upland cotton is the most widely cultivated among cotton species and is the best natural fiber source for the textile industry. However, abiotic stress, particularly drought stress, adversely affects important cotton planting regions. This study uses phenotypic drought markers to ascertain the tolerances of some advanced cotton lines under the limited irrigation conditions obtained from the drought-tolerant variety development breeding program. The study was conducted according to the completely randomized design with three replications. A total of 16 genotypes were used, with fourteen lines selected from the F_2 segregation stage of the drought-tolerant variety breeding program, and two varieties used as control varieties. Variance analysis (ANOVA) was conducted, and the results revealed highly significant variations between the means ($P<0.01$). The highest root length (RL, 46 cm) was recorded in the Aras 24 genotype, while the lowest was in Aras 28 (15 cm). Aras 40 showed the highest root weight (RW, 0.241 g) value, followed by Aras 24 (0.210 g) and TEX (control 0.206 g). The lowest RW values were seen in Aras 28 (0.086 g) and Aras 29 (0.089 g). Regarding lateral fresh weight (LFW), Aras 28 recorded the lowest (0.019 g), while Aras 24 recorded the highest value (0.088 g). Lastly, Aras 41 showed the highest relative water content (RWC) value of 92%, followed by Aras 38 (89) and TEX (76). In conclusion, if the breeding program is continued with Aras 41, Aras 40, and Aras 24 varieties, it will significantly contribute to the goal.

Bazı Upland Pamuk (*Gossypium hirsutum* L.) Pamuk Genotiplerinin Su Noksanlığı Stresi Altında Toleranslıklarının Görüntülenmesi

Anahtar Kelimeler
Kuraklık toleransı,
Pamuk,
kümelenme,
Morfolojik özellikler,
Stres faktörleri

Öz: Upland pamuğu bütün pamuk türleri arasında en fazla yetiştiriciliği yapılan pamuk türüdür ve tekstil endüstrisi için en iyi doğal lif kaynağıdır. Ancak, özellikle kuraklık stresi gibi abiyotik stres faktörü pamuk yetiştirilen alanları olumsuz yönde etkilemektedir. Bu çalışma, kuraklığa dayanıklı çeşit geliştirme ıslah programından elde edilen bazı ileri pamuk hat ve genotiplerinin kısıntılı sulama koşulları altındaki toleranslarını belirlemek için fenotipik kuraklık markörlerinden yararlanılarak yürütülmüştür. Araştırma tesadüf parselleri deneme desenine göre 3 tekerrürlü olarak yürütülmüştür. Kuraklığa toleranslı çeşit geliştirme programının F_2 segregasyon aşamasından seçilen on dört adet hat, iki çeşit de kontrol olmak üzere 16 genotip kullanılmıştır. Varyans analizi (ANOVA) sonucunda ortalamalar arasındaki farklılıklar önemli bulunmuştur ($P<0.01$). En yüksek kök uzunluğu (RL) Aras 24 genotipinde (46 cm), en düşük kök uzunluğu (RL) değeri ise Aras 28 (15 cm)'de kaydedilmiştir. Aras 40 en yüksek Kök ağırlığı (RW) (0.241 g) değerini göstermiş ve bunu Aras 24 (0.210 g) ve TEX (0.206 g) kontrol çeşidi takip etmiştir. En düşük RW değeri ise Aras 28 (0.086 g) ve Aras 29 (0.089 g) genotiplerinde görülmüştür. Lateral taze ağırlığı (LFW) ise en düşük değer Aras 28 (0.019 g), en yüksek değer Aras 24 (0.088 g) genotipinde kaydedilmiştir. Aras 41 genotipi en yüksek bağıl su içeriği (RWC, 92) değerini göstermiş ve bunu Aras 38 (89) ve TEX (76) kontrol çeşidi takip etmiştir. Sonuç olarak, kuraklığa karşı tolerant çeşit geliştirme ıslah programına Aras 41, Aras 40 ve Aras 24 ile devam edilmesi halinde bu ıslah programının başarıya ulaşmasında önemli katkılar sağlayacağı anlaşılmıştır.

1. INTRODUCTION

Upland cotton (*Gossypium hirsutum* L.) with allotetraploid (2n=4x=52; AADD) genome is one of the most important field crops in the world [1]. Since it is the largest source of natural fiber consumed worldwide, it meets approximately 90% of the world's cotton production [2]. In addition to being an oil plant in terms of oil obtained from its seeds, it also provides raw materials for many industries. It constitutes the primary source of income for millions of people. Due to drought around the world, cotton production in the last five years has decreased from 13,960 million bales to 11,935 million bales, resulting in a decrease in production by approximately 14% [3]. Although the cotton plant (*Gossypium spp*.) has a higher tolerance to abiotic stress factors such as drought than other important industrial field crops, profound decreases are observed in its yield when exposed to drought stress for a long time [4].

High temperatures caused by global warming increase evaporation.". This leads to the induction of water stress in the cotton plant.It causes the cotton plant to enter into water stress. Without water, it becomes imperative to turn to many alternatives. Considering the importance of reducing the expenses of the enterprises, it is seen that the breeding studies to be carried out to increase the tolerance of water stress, which is one of the biggest problems in the cultivation of cotton plants, are of critical importance. Due to the narrow genetic diversity in the Upland cotton species, it is challenging to select varieties for cultivars with high drought stress tolerance [5].

Genetic diversity consists of allelic variations of genes; differences in the plant genome sequence are the result of the reflections in the phenotype as a result of factors such as changes in the gene pool and population of the cotton species [6]. The drought resistance of plants can be followed by morphological characteristics [7]. Saad et al. [8] investigated the relationship between morphophysiological selection and linkage among traits in a study on cotton. The narrowing genetic diversity of Upland cotton in various ways makes it difficult to be successful in intraspecies crossing studies. Studies have shown that the selection of the varieties that will participate in the variety development breeding program as parents among the commercial varieties increases the success of the crossbreeding breeding program to be carried out for the variety breeding since the commercial varieties have high values in terms of agronomic and technological parameters, that is, they have high allele frequency [9].

Since the seedling period is the most sensitive period of cotton, it is a critical period for the following growth stages, when drought stress is most evident [10]. Various parameters are used to evaluate the reactions of cotton genotypes to drought stress during the seedling period. Parameters such as root length (RL), excised leaf water loss, root weight (RW), leaf area (Leaf Area, LA), stomatal conductivity and density, number of lateral roots (LNR), and relative water content (RWC) are the extensively used to assess the drought tolerance of plants [11]. Again, to understand the genetic potential and performance of the plant under drought stress during the seedling period, besides the root system-related parameters, morphological characters [12], cotton seedling period characters [13], seed germination characters [14], and relative growth rate [15]. are used.

This study aims to determine the tolerances of some advanced cotton lines under the deficit irrigation conditions obtained from the drought-tolerant variety development breeding program using morphological drought markers such as germination rate (%) fresh lateral weight (LFW) and Relative Water Content (RWC), Root length (RL), and Root weight (RW). These parameters were used to determine the tolerance level and genotypes of the cotton lines to facilitate the decision regarding which genotype to choose to continue with the breeding program.

2. MATERIAL AND METHOD

2.1. Plant Materials and Climatic Parameters

In the experiment, 14 genotypes provided from STAR SEED company (www.startohum.com) of Upland (*G. hirsutum* L.) cotton genotypes obtained from the F_2 segregation stage of the variety development breeding program against drought were used as plant material. As control varieties, TEX and Beyaz Altın 119 (BA-119) commercial varieties were used, which are not only tolerant to drought stress, but also have wide adaptability, are highly productive, and are intensively planted in the Southeastern Anatolia region, one of the regions where cotton cultivation is done in Turkey.

Table 1. The long-term climatic parameters for all months in the province of Bingol (URL 1, 2022)

Long-term climate data of Bingol province (1927-2021)													
	MONTHS												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Annual**
Average Temperature (°C)	-2.3	-1.1	4.2	10.8	16.3	22.0	26.7	26.5	21.3	14.2	6.8	0.6	12.2
Average Maximum Temperature (°C)	2.3	3.9	9.5	16.7	22.9	29.4	34.6	34.7	29.8	21.6	12.6	5.1	18.6
Average Lowest Temperature (°C)	-5.9	-4.9	-0.1	5.8	10.2	14.7	19.0	18.7	13.6	8.3	2.2	-2.8	6.6
Average Sunbathing Time (hours)	3.4	4.4	4.9	5.5	7.1	9.1	9.4	9.0	8.1	6.1	4.5	3.2	6.2
Average Number of Rainy Days	1.10	1.9	1.12	1.12	1.12	1.3	1.1	1.1	1.3	8.8	1.8	1.10	95.1
Average Monthly Total Rainfall (mm)	140.4	128.7	129.8	116.4	76.2	20.6	7.0	4.2	12.6	66.2	106.1	136.4	944.6
Highest Temperature (°C)	13.3	16.2	22.3	30.3	33.9	38.0	42.0	41.3	37.8	32.1	25.5	22.8	42.0
Lowest Temperature (°C)	-23.2	-21.6	-20.3	-9.2	1.0	3.5	8.8	7.8	4.2	-2.4	-15.0	-25.1	-25.1

2.2. Method

2.1.1. Calculation of germinating percentages

Humus soil taken from forest areas was used to prepare the seedbed. By making three holes in the bottoms of 5-liter pots, the water given to them remained in the root zone, preventing the genotypes from being exposed to physiological drought stress. Drought-tolerant Ba 119 and TEX were used as control cultivars in the experiment, which was established with three replications according to the completely randomized design.

The pots experiment involved thoroughly watering all pots until they reached a field capacity (800 m L-1, utilizing the following formula to determine field capacity.

Field capacity= (Weight of soil at maximum water holding-weight of oven-dried soil)/weight of oven-dried soil) (Junker et al., 2015).

To reach the field capacity, 800 m L^{-1} water was given to the plants during sowing (100% field capacity, Control). 25 days after sowing (DAS), all plants germinated and emerged, drought stress treatments were started to be applied. These treatments included slight drought at 75% field capacity (600 m L^{-1}), moderate drought at 50% field capacity (400 m L^{-1}), and severe drought at 25% field capacity (200 m L^{-1}).

The trial, which was established on Jul 30, 2022, in Turkey-Bingöl Genç (Coordinates: 38 ° 442 58" N and 40 ° 32' 11" E), was terminated on September 20 (52 days).

was terminated. Observations were made for the genotypes' germination percentages (%) 15 days after sowing using the formula mentioned below:

$$\text{Germination percentage (\%)} = \frac{\text{Germinated seeds}}{\text{Total seeds}} x100$$

Measurement of relative water content: Root weight (g) and Lateral fresh weight (g) were directly measured on a precision balance. After the experiment was terminated, the root length (cm) was measured without damaging the roots. The following formula was used to obtain the Relative Water Content (RWC) value:

$$\text{Relative Water Content (RWC)} = \frac{(\text{FW}-\text{DW})}{(\text{TW}-\text{DW})} \text{X}100\ [16]$$

FW is fresh weight, DW is dry weight while TW is Turgid weight.

Fresh weights of 0.6 cm radius discs cut from leaves of each genotype were weighed for turgid weight approximately four days after the last exposure to drought stress. These were placed in Petri dishes filled with distilled water (dH2O), and turgid weights were measured after 4 hours. After this stage, the leaf discs' dry weights were measured at 60 °C in the oven, and the Relative water content (RWC) values were calculated.

2.3. Statistical Analysis

The means of morphological markers that reflect drought stress in phenotype, such as root length (RL), root weight (RW), lateral fresh weight (LFW), and relative water content (RWC), were compared using analysis of variance (ANOVA) to assess the significance of the differences. The means were compared with the Least Significant Difference (LSD) test at the $p<0.05$ significance level. The phylogenetic tree was built using the means of drought-related morphological traits based on the kinship of genotypes using JMP 17.0 (JMP®, Version <17>.

3. RESULTS AND DISCUSSIONS

The majority of breeding programs primarily select cotton with high seed yield and fiber quality under ideal field conditions. The environmental factors effect, which results in low heritability of seed yield and yield parameters [18], however, means that the response to selection is low under inappropriate conditions. Additionally, there are unfavorable correlations between yield and fiber quality traits. In order to simultaneously increase yield and fiber quality, it is crucial to screen cotton for drought tolerance under field conditions. This is because of other abiotic stress factors, such as heat, salt, and biotic stresses, can also have an impact on the results. Although it is more practical to screen a large collection of germplasm at the seedling phase in a greenhouse [19], the true value of a genotype must be evaluated in the field before being used in breeding for drought tolerance. Utilizing a small part of genotypes from one to some cultivars and breeding lines, efforts have been made to develop cotton under drought conditions so far [20, 21, 22].

A large genetic and breeding population for drought tolerance was only briefly evaluated in a few studies [23, 24, 25, 26]. However, research on how drought stress effects cotton yield at the crucial seedling stage is lacking. Therefore, the cotton seeds of advanced genotypes under water-stressed conditions were measured to calculate various sensitivity and tolerance indices to investigate suitable stress resistance indices for screening of cultivars under drought.

As a result of the analysis of variance performed at a 95% confidence level, the differences between genotypes for all phenotypic markers were found to be very significant ($P<0.01$), except for the lateral root weight. Accordingly, while the highest germination rate (%) was recorded in the Aras 30 (90%) genotype, the lowest germination rate was observed in the Aras 24 (40%) genotype.

Figure 1. *G. hirsutum* L. Morphological structure of roots and root lengths

Genotypes Germination, Percent (%), Root Length (RL) seedling stage is lacking. Therefore, cotton seeds of advanced genotypes under water-stressed conditions were measured in order to the highest average root length was Aras 24 (46 cm), while the lowest was Aras 28 (15 cm). Regarding root length, the mean for all the genotypes was 27 cm. A root length above the average was obtained in half of the genotypes. In contrast, a root length close to the average was obtained in the other half (Table 2, Fig. 1). A replication of the Aras 24 genotype resulted in 52 cm of taproot length (Fig. 1). Zahid et al. [27] observed an average Root length of 5.163 cm in their trials in which they investigated the reactions of 23 cotton genotypes to drought stress during the seedling period. They obtained a lower average root length compared to our study because the cotton plant terminated the experiment when it reached the level of 4 true leaves. Studies have shown that the deep root system increases drought tolerance, improves water and nitrogen uptake from the soil, and reduces Axial root and lateral branching, which puts an extra load on the plant under drought stress [28].

Table 2. The means of some drought traits of Upland cotton species

GENO.	GP	RL	RW	LFW	RWC
Aras 21	77ad	32bc	0.132bc	0.024c	0
Aras 22	67ad	27be	0.119c	0.056bc	65ac
Aras 24	40d	46a	0.210ab	0.088ab	42bc
Aras 25	43cd	33bc	0.147bc	0.024c	42bc
Aras 28	87a	15f	0.086c	0.019c	47bc
Aras 29	47bd	24cf	0.089c	0.029bc	42bc
Aras 30	90a	18ef	0.095c	0.056bc	62ac
Aras 36	73ad	19df	0.118c	0.026c	36cd
Aras 37	80ac	29be	0.085c	0.047bc	70ac
Aras 38	83ab	26bf	0.113c	0.048bc	89a
Aras 39	80ac	25cf	0.131bc	0.059bc	68ac
Aras 40	60ad	37ab	0. 241 a	0.084ac	74ac
Aras 41	70ad	30bd	0.159ac	0.036bc	92a
Aras 42	70ad	22cf	0.135bc	0.057bc	41bc
Ba 119	73ab	22cf	0.160ac	0.067bc	62ac
TEX	83ab	29be	0.206ab	0.087ab	76ab
Mean	70 ad	27be	0.13bc	0.079ac	56

The highest root weight was measured in the Aras 40 genotype with an average of 0.241 g, followed by Aras 24 (0.210 g) and control variety TEX (0.206 g). In the experiment, where the mean root weight of all genotypes was measured as 0.13 g, the lowest average root weight was observed in the Aras 28 (0.086 g) and Aras 29 (0.089) genotypes, respectively. Some plants, such as cotton, try to reach deep or distant waters by developing their root systems in the first stages of drought stress.

Developing the root system in field crops is possible not only by root length but also by increasing root weight, number of laterals, and root density per plant. Although the cotton plant develops a root system that is 10 times the length above the ground under drought stress in the first place, it shows that the root system weakens over time under long-term drought stress [29]. In addition, stomata, which close in order to minimize water loss in water deficiency, will reduce the uptake of CO_2, which is a component of photosynthesis, so the rate of photosynthesis decreases and the growth of cotton decreases and as a result, the yield decreases [30]. Riaz et al. [31] conducted a 52-day experiment to monitor drought stress using forward-line genotypes in greenhouse conditions. As a result of the experiment, they stated that the new genotypes' root weight (RFW, Root fresh weight) varied between 0.39 and 0.57 g. Although parallel to our study, their superior root weight can be associated with the stabilization of humidity and temperature under controlled conditions or the frequency of irrigation.

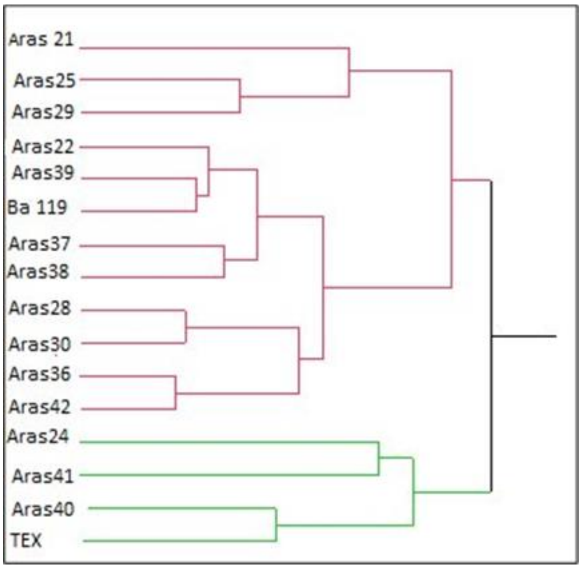

Figure 2. The Phylogenetic Tree of Upland cotton genotypes under drought-stress using JMP 17.0 ver. Software

Genotypes were divided into two main clusters, as seen in Fig. 2. While Aras 24, Aras 40, Aras 41, and control variety TEX were clustered in the first cluster, the remaining genotypes formed a separate cluster. They were divided into two sub-clusters among themselves. Genotypes close to a common ancestor come together in the same cluster, while genotypes far from each other in origin are classified in clusters far from each other.

While the number of lateral roots provides the opportunity to take water and nutrients by spreading over a vast extent in the root area, the weight of the lateral roots tries to increase tolerance to drought stress by storing Glucose ($C_6H_{12}O_6$) compound in the roots when the cotton plant starts to experience water stress. In this study, Aras 24 showed the highest (0.088 g) LFW, followed by TEX with a 0.087 g average.

In the experiment in which the lateral fresh weights mean of the genotypes were calculated as 0.079 g, the lowest average was followed by Aras 28 with an average of 0.19 g and Aras 25 and Aras 21 genotypes with an average of 0.024 g.

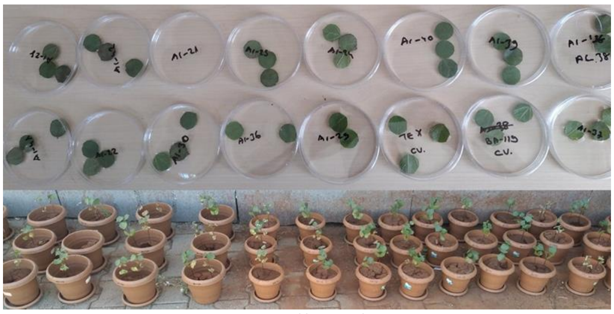

Figure 3. Screening the drought effects of *G. hirsutum* L. Cotton genotypes.

The water content in the leaves is one of the essential drought indicators showing the drought stress tolerance of the cotton plant. Generally, plants with high leaf water content are drought-tolerant [32]. Fig. 3 shows *G. hirsutum* L. cotton genotypes being screened for their drought resistance. Relative Water Content (RWC) content should be mentioned because Aras 21 genotype dries up at the last stage of the limited irrigation application in the experiment. However, while Aras 41 genotype showed the highest value with an RWC of 92 %, Aras 38 (89%) and control variety TEX (76%) followed it. The lowest value was measured in Aras 36 (36%) genotypes, followed by Aras 42 (41%), Aras 24 (42%), and Aras 25 (42%) genotypes. The average RWC values of all genotypes were recorded as 56 % (Table 2). Similar RWC values have been found by Akbar and Hussain [33] and Eid et al.[34]. The RWC value decreases under long-term water stress (Ullah et al., 2012) [35].

4. CONCLUSION

The current study's analysis of morphological parameters such as RW, RL, LFW, and RWC allowed for the conclusion that these indices serve as the most accurate predictors of yield in drought-stressed environments. The genotypes that exhibited high values for the parameters mentioned above were capable of producing high yields under stress environments.

As a result, it has been observed that if the cultivar development breeding program is continued with Aras 41, Aras 40, and Aras 24 varieties, it will significantly contribute to the goal. The genotypes that are promising under drought stress need to be planted in uncontrolled field conditions for a more comprehensive evaluation.

Acknowledgment
I would like to acknowledge to Dr. Sedat SÜLLÜ, who supplied us with seeds from Star SEED company, and Ms. Seda ÇELİK, who helped in the establishment of the experiment and the cultural processes.

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