

## Effect of Drought Acclimation on Drought Stress Resistance in Okra Seedlings

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### Abstract

In this study, the effects of drought acclimation were investigated in okra (*Abelmoschus esculentus* L.) during seedling period. For this purpose, four different irrigation applications were applied as non-acclimated and non-stressed (NA), drought acclimated and non-stressed (DA), non-acclimated and drought stressed (NAS), drought acclimated and drought stressed (DAS). The effects of the treatments on the parameters such as plant height, stem diameter, plant fresh weight, plant dry weight, root fresh weight, root dry weight, leaf area, leaf relative water content (LRWC), chlorophyll reading value (SPAD), chlorophyll content (a, b and total chlorophyll), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content, malondialdehyde (MDA) content and antioxidant enzyme activity (CAT, POD and SOD) of okra seedlings were studied. Drought stress (NAS) had a negative effect on plant growth parameters, however, the damage caused by DAS was lower. In addition, plant height, stem diameter, plant fresh weight, plant dry weight, root fresh weight, root dry weight, leaf area, LRWC, SPAD, and chlorophyll content were at the highest level with DA. There was an increase in H<sub>2</sub>O<sub>2</sub>, MDA and antioxidant enzyme activity with drought stress (NAS), and the increase in these parameters with DAS was lower than NAS application. In the study, it was determined that drought accumulation applied in the seedling period of okra can increase the tolerance against drought stress as well as its positive effect on plant growth under normal conditions.

**Keywords:** drought, tolerance, okra, plant growth

### Bamya Fidelerinde Kuraklık Aklımasyonunun Kuraklık Stresi Direncine Etkisi

#### Öz

Bu çalışmada bamyada (*Abelmoschus esculentus* L.) fide döneminde kuraklık aklımasyonunun etkileri araştırılmıştır. Bu amaçla, aklımasyon yapılmamış ve stressiz (NA), aklımasyon yapılmış ve stressiz (DA), aklımasyon yapılmamış ve kuraklık stresli (NAS), aklımasyon yapılmış ve kuraklık stresli (DAS) olmak üzere dört farklı sulama uygulaması yapılmıştır. Uygulamaların bamya fidelerinde bitki boyu, gövde çapı, bitki taze ağırlığı, bitki kuru ağırlığı, kök taze ağırlığı, kök kuru ağırlığı, yaprak alanı, yaprak bağıl su içeriği (LRWC), klorofil değeri (SPAD), klorofil içeriği (a, b ve toplam klorofil), hidrojen peroksit (H<sub>2</sub>O<sub>2</sub>) içeriği, malondialdehit (MDA) içeriği ve antioksidan enzim aktivitesi (CAT, POD ve SOD) gibi parametreler üzerine etkisi incelenmiştir. Kuraklık stresi (NAS), bitki büyüme parametreleri üzerinde olumsuz bir etkiye sahipken, DAS'ın neden olduğu hasar daha düşük olmuştur. Ayrıca bitki boyu, gövde çapı, bitki taze ağırlığı, bitki kuru ağırlığı, kök taze ağırlığı, kök kuru ağırlığı, yaprak alanı, LRWC, SPAD ve klorofil içeriği DA ile en yüksek düzeyde olmuştur. Kuraklık stresi (NAS) ile H<sub>2</sub>O<sub>2</sub>, MDA ve antioksidan enzim aktivitesinde artış olmuş, DAS ile bu parametrelerdeki artış NAS uygulamasına göre daha düşük olmuştur. Çalışmada bamyada fide döneminde uygulanan kuraklık aklımasyonunun, normal koşullarda bitki büyümesine olumlu etkisinin yanı sıra kuraklık stresine karşı toleransı artırabileceği belirlenmiştir.

**Anahtar Kelimeler:** kuraklık, tolerans, bamya, bitki büyümesi

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## 1. Introduction

Drought is one of the most important abiotic stress factors in agricultural production and negatively affects plant growth especially in arid and semi-arid regions of the world [1]. Drought, which can be defined as water scarcity caused by a temporary imbalance in the amount of moisture in a region, is actually a normal and recurrent climatic event [2]. However, increasing temperatures and decreasing precipitation as a result of global climate change have increased the frequency and impact of drought [3]. In addition, factors such as low precipitation, salinity, high and low temperatures, high light intensity also cause drought. Even though there is enough water in the soil, if the plants cannot take this water, stress called physiological drought occurs [4, 5]. Drought is the most critical threat to food security in the world. The reduction of the world's water supply is likely to worsen future food demand due to a rapidly growing population [6].

With a lack of water in the plant, a decrease in photosynthesis occurs, and a regression in vegetative growth occurs [7]. In addition, the stem and leaves of the plant are more sensitive to water stress than the roots, and in the first stage, the plant slows down the stem elongation to reach more water and increases root development. In ongoing drought conditions, both stem and root growth stop, and the number of leaves, leaf area decreases, and leaf shedding occurs in the future [8]. In addition to the changes in metabolism such as cell division and cell elongation in plants, drought causes loss of turgor, deterioration in enzyme activities and decreased photosynthesis efficiency in plants [9, 10]. The effect of drought varies according to the severity and duration of the stress and the response of the plants [11].

In the survival of plants in drought conditions or resistance to drought, responses occur in the plant through avoidance (preventing or delaying the reduction of the water potential of the protoplasm) or tolerance (the ability of the protoplasm to dry out without being damaged) [12]. Thus, plants develop a set of adaptive responses to overcome drought stress. During drought stress, plant water management occurs by both increasing water intake and reducing water loss. Differences occur in the formation of the effects of drought stress on the plant, and it is known that some plants gain the ability to increase stress resistance after being exposed to low levels of stress (acclimation). Adaptation or acclimation to stress varies depending on both the plant genotype and the environment [13]. In nature, plants often experience cycles of water deficiency stress. It is stated that mild water stress in the early period of seedling growth provides resistance to severe water stress in the later growth and development period [14]. Khanna-Chopra and Selote [15] stated that mild drought stress at the seedling stage preserves water relations and antioxidant defense in leaves, making the plant more adaptable to drought.

Okra (*A. esculentus*) is one of the most important vegetable species preferred for production and consumption. Okra is the most well-known plant of the Malvaceae family. It is consumed as boiled, fried and salad in meals. It is produced commercially and evaluated as canned, in brine, fresh and dry. It is an important dietary vegetable and is rich in protein, vitamins and fiber [16, 17]. Okra is a hot climate vegetable and because it is sensitive to cold, the plant cannot develop well in cool regions. It shows good growth at temperature of optimum 25 °C [17]. Irrigation is important in okra cultivation, and is mostly carried out in the form of flood irrigation in our country. Okra plant's need for water during the flowering and fruit formation period is higher than other periods [16, 17].

One of the most important effects of global warming on earth is the decrease in water resources and drought. The decrease in water resources adversely affects plant life, reduces quality and yield and creates a danger in plant production. For this reason, in agricultural production, more emphasis is placed on maintaining plant cultivation in drought conditions, mitigating damage or increasing stress tolerance by taking various measures.

In this study, it was determined how the deficit irrigation application, which is expressed as adaptation to drought stress in the seedling stage, will provide an effect against the water stress that the plant will encounter in the future. Although there are many studies on the determination of drought stress tolerance mechanisms in okra, there is a lack of research examining drought adaptation or acclimation and determining whether the plant gains drought stress tolerance in this way.

## 2. Material and Methods

This study was carried out as a pot experiment in a semi-controlled greenhouse in 2022. In the study, okra (*A. esculentus* L. cv. Sultani) was used as plant material. Okra seeds were sown in a peat: perlite mixture (2:1, v:v) in viols, then when the seedlings had 2-3 true leaves, the seedlings were transferred to 2.5 L pots with mixture of soil: sand: peat (2:1:1, v:v:v), with one seedling in each pot. Pots were randomly placed on the benches according to a randomized plot design.

In order to determine the amount of water to be given, the field capacity of the medium was determined according to the pot volumes. The amount of water given to the pots was calculated according to the field capacity of the control pots. For this, the field capacity was determined according to the volume-based moisture values measured with a portable moisture meter every 2 or 3 days in the morning. After transplanting the seedlings were first irrigated according to the field capacity. The seedlings were grouped into four treatments, namely, non-acclimated and non-stressed (NA-100% of field capacity), drought acclimated and non-stressed (DA-80% of field capacity and then 100% of field capacity), non-acclimated and drought stressed (NAS-100% of field capacity and then 40% of field capacity), drought acclimated and drought stressed (DAS-80% of field capacity and then 40% of field capacity). Acclimation was done in the first two weeks after transplanting.

The study was completed 45 days after the start of the deficit irrigation application. At the end of the experiment, measurements such as plant height (cm), stem diameter (mm), plant fresh weight (g), plant dry weight (g), root fresh weight (g), root dry weight (g) were made to determine the effects of water restriction application. In addition, the chlorophyll reading value of optimally sized leaves was determined with the SPAD-502 chlorophyll meter.

Leaf discs from 2 randomly selected plants for LRWC were weighed immediately and their fresh weights were determined (FW). After weighing, the discs were kept in containers containing some pure water for 24 hours. Later, the excess water on the discs was wiped off with the help of blotting paper and weighed again, and their turgor weights were determined (TW). Then, these discs were dried in an oven set at 72 °C for 48 hours and weighed again and their dry weights were determined (DW). Leaf relative water content (LRWC) is calculated according to the following formula [18].

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

The chlorophyll content of the plants was determined spectrophotometrically as  $\text{mg g}^{-1}$  fresh weight at 645 and 663 nm wavelengths. The chlorophyll a, chlorophyll b and total chlorophyll contents were calculated according to the method of Lichtenthaler and Wellburn [19] and Wellburn [20].

The total leaf area of plants was determined by the leaf area meter (CI-202 Portable Laser Leaf Area Meter by CID Bio-Science, USA).

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) content and lipid peroxidation (malondialdehyde-MDA) were determined according to Velikova et al. [21] and Sahin et al. [22]. Antioxidant enzyme (CAT, SOD and POD) analyzes were performed on fresh leaf samples according to methods of Angelini et al. [23], Abedi and Pakniyat [24], Gong et al. [25], Agarwal and Pandey [26] and Yordanova et al. [27].

In the study, randomized plots experimental design, a total of 72 plants were used, with 3 replications and 6 plants in each replication. The data obtained as a result of the study were subjected to variance analysis using the SPSS program and Duncan multiple comparison test was performed [28].

### **3. Results and Discussion**

The effects of the treatments on plant fresh weight, plant dry weight, root fresh weight and root dry weight were statistically significant ( $p < 0.001$ ). Drought stress (NAS) caused the greatest decrease in plant fresh weight (62%) compared to DA. However, there was a 51% reduction in DAS application. According to the results obtained, the highest plant dry weight was obtained in DA application, while the lowest plant dry weight with drought stress (NAS). Compared to NA, the dry weight of plants decreased by 43% with NAS and 25% with DAS application (Table 1).

It was determined that the highest root fresh weight in okra seedlings was obtained in DA application, while the lowest root fresh weight was found in NAS application. Root fresh weight decreased by 41% with NAS and 26% with DAS. There was a 12% decrease in root dry weight in NAS application compared to NA (Table 1).

**Table 1.** Effect of drought acclimation on plant fresh weight, plant dry weight, root fresh weight and root dry weight in okra seedling

Treatments	Plant fresh weight (g)	Plant dry weight (g)	Root fresh weight (g)	Root dry weight (g)
NA	12.79 b	1.45 b	3.10 b	0.17 b
DA	14.70 a	1.81 a	3.31 a	0.21 a
NAS	4.80 d	0.82 d	1.81 d	0.15 c
DAS	6.17 c	1.09 c	2.28 c	0.19 a

NA: non-acclimation, DA: drought acclimation, NAS: non-acclimation and drought stress, DAS: drought acclimation and drought stress There is no statistically ( $p < 0.001$ ) difference between the averages of the applications indicated with the same letter according to Duncan multiple comparison test.

The effects of applications on LRWC, plant height, stem diameter and leaf area of okra seedlings were found to be statistically significant (Table 2). When the LRWC values in okra seedlings were examined, the highest LRWC was determined in NA, DA and DAS applications, and the lowest in NAS application. According to the results of the research, the highest plant height in okra seedlings was in NA and DA applications, and the lowest plant height was in NAS application. Plant height decreased by 28% with NAS and 21% with DAS compared to NA. According to the results, it was determined that the highest stem diameter in okra seedlings was in DA application, the lowest stem diameter was in NAS application, and there was a 20% decrease when compared to NA. The highest leaf area in okra seedlings was obtained from NA and DA applications, and the lowest leaf area was in NAS application. Compared with NA, the leaf area decreased by 55% with NAS application and 50% with DAS application.

**Table 2.** Effect of drought acclimation on LRWC, plant height, stem diameter and leaf area in okra seedling

Treatments	LRWC	Plant height (cm)	Stem diameter (mm)	Leaf area (cm <sup>2</sup> )
NA	64.26 a	34.50 a	3.77 b	174.81 a
DA	61.83 a	34.67 a	3.96 a	180.41 a
NAS	53.72 b	24.84 c	3.01 d	78.47 c
DAS	60.94 a	27.38 b	3.35 c	89.29 b

NA: non-acclimation, DA: drought acclimation, NAS: non-acclimation and drought stress, DAS: drought acclimation and drought stress There is no statistically ( $p < 0.001$ ) difference between the averages of the applications indicated with the same letter according to Duncan multiple comparison test.

The effects of applications on SPAD value, chlorophyll a, chlorophyll b and total chlorophyll amount were found to be statistically significant (Table 3). In the study, the highest SPAD value was determined in NAS application, and the lowest was determined in NA and DA applications. Chlorophyll a, chlorophyll b total chlorophyll content in okra was lowest in NAS application, and these parameters decreased by 5%, 2% and 4%, respectively, in NAS application compared to NA.

**Table 3.** Effect of drought acclimation on chlorophyll reading value (SPAD), chlorophyll a, chlorophyll b and total chlorophyll content in okra seedling

Treatments	Chlorophyll reading value (SPAD)	Chlorophyll a (mg g <sup>-1</sup> )	Chlorophyll b (mg g <sup>-1</sup> )	Total Chlorophyll (mg g <sup>-1</sup> )
NA	37.00 b	5.64 a	2.96 b	8.60 b
DA	37.37 b	5.69 a	3.68 a	9.32 a
NAS	44.07 a	5.33 b	2.89 b	8.29 b
DAS	42.73 ab	5.59 a	3.61 a	9.21 a

NA: non-acclimation, DA: drought acclimation, NAS: non-acclimation and drought stress, DAS: drought acclimation and drought stress There is no statistically ( $p < 0.001$ ) difference between the averages of the applications indicated with the same letter according to Duncan multiple comparison test.

The results of the study showed that the effects of applications on H<sub>2</sub>O<sub>2</sub> and MDA content, and CAT, POD and SOD enzyme activity were statistically significant ( $p < 0.001$ ) (Table 4). It was determined that the highest amount of H<sub>2</sub>O<sub>2</sub> and MDA in okra seedlings was in NAS application and the lowest in NA application. According to the analysis results, CAT, POD and SOD activity in okra seedlings were highest in NAS application and lowest in NA application. The increase in the DAS application was lower than the NAS application.

**Table 4.** Effect of drought acclimation on H<sub>2</sub>O<sub>2</sub> and MDA content, and CAT, POD and SOD activity in okra seedling

Treatments	H <sub>2</sub> O <sub>2</sub> (mmol kg <sup>-1</sup> )	MDA (mmol kg <sup>-1</sup> )	CAT (eu g <sup>-1</sup> )	POD (eu g <sup>-1</sup> )	SOD (eu g <sup>-1</sup> )
NA	16.09 d	7.55 d	125.32 d	2210.10 c	768.28 d
DA	24.02 c	12.77 c	368.06 c	2927.52 c	1068.54 c
NAS	46.37 a	43.52 a	742.00 a	14383.98 a	2273.05 a
DAS	37.25 b	21.23 b	450.16 b	12832.01 b	1894.79 b

NA: non-acclimation, DA: drought acclimation, NAS: non-acclimation and drought stress, DAS: drought acclimation and drought stress There is no statistically ( $p < 0.001$ ) difference between the averages of the applications indicated with the same letter according to Duncan multiple comparison test.

In this study, the effects of drought stress and drought stress acclimation on some morphological, physiological and biochemical properties in okra were investigated. Drought stress decreases CO<sub>2</sub> assimilation by decreasing stomatal conductivity in the plant. Drought results in decreased stem elongation and root development, degradation of photosynthetic pigments and reduced gas exchange, resulting in decreased plant growth and productivity. In addition, drought disrupts plant water relations and reduces water use efficiency, causes the production of active oxygen species and increases antioxidant systems [10, 29]. Exposure to low levels of stress (acclimation) in some plants increases stress tolerance, and the plant species and environmental factors are effective in this situation [13]. Acclimation is associated with mechanisms such as membrane composition, accumulation of various osmo-protectants, gene expression, increase in ABA and antioxidant levels [30].

It was determined that there were significant decreases in plant fresh weight, plant dry weight, root fresh weight, root dry weight, plant height, stem diameter and leaf area in okra seedlings with drought stress (Table 1 and 2). Similarly, in a study conducted in okra, 3 different irrigation levels (control, 1/3 field capacity and 2/3 field capacity) were investigated and it was determined that leaf area, fresh weight and dry weight decreased significantly under stress compared to control [31]. This damage caused by drought in seedling growth of okra may also affect the subsequent growth and development of the plant. Amin and Mahmood [32] stated in their study that drought stress reduced germination and seedling growth in okra.

However, drought acclimation had a positive effect on plant fresh weight, plant dry weight, root fresh weight, root dry weight, plant height, stem diameter and leaf area, and there was a significant increase in these parameters with drought acclimation compared to drought stress in non-acclimation plants in this study (Table 1 and 2). Xu et al. [33] stated that biomass or leaf area is less than control in plants exposed to prolonged or severe drought, and the improvement in plant growth after re-watering may depend on pre-drought intensity and duration. Acclimated wheat plants maintained higher root relative water content under severe water stress conditions than the non-acclimatized plant [14, 34].

LRWC and chlorophyll content of okra seedlings decreased with drought stress, while  $H_2O_2$ , MDA and proline content, and CAT, POD and SOD activities increased (Table 2, 3 and 4). Drought stress increases the accumulation of reactive oxygen species (ROS) containing singlet oxygen ( $^1O_2$ ), superoxide ( $O_2^{\cdot -}$ ), hydroxyl and  $H_2O_2$  in the plant, causing a negative effect on antioxidant metabolism and thus cell peroxidation damage occurs [33, 35]. In another study, it was determined that LRWC, leaf chlorophyll content, biomass accumulation and growth of okra decreased with increasing drought [36]. With the promotion of peroxidation by drought stress, the related antioxidant system is activated to eliminate the damage of ROS in the plant [33]. Amin et al. [31] reported that lipid peroxidation, sugar, proline content and ion leakage increase in plants with drought stress, and also that stress affects the photosynthetic process in okra plants with a significant decrease in photosynthetic pigment content. Also, Kusvuran [37] investigated that the effect of gradually reducing irrigation water in 4 days (100%, 75%, 50% and 25%) in 8 okra genotypes. The researcher stated that drought-resistant genotypes exhibited a better protection mechanism against oxidative damage by maintaining higher hereditary and induced antioxidant enzyme activity than sensitive genotypes.

Drought stress in okra reveals its negative effect on plant growth by changing the plant biochemical content and negatively affecting photosynthesis. However, drought acclimation had a curative effect on these parameters (LRWC, chlorophyll content,  $H_2O_2$ , MDA, proline content, CAT, POD and SOD activities) (Table 2, 3 and 4). In another study, it was determined that the effect of drought on okra was more severe in plants exposed to a 10-day water deficiency at 25% field capacity during the vegetative and reproductive stages. Similarly, it was determined that plants exposed to water deficiency only in the vegetative stage recovered quickly, and after recovery the plants produced more chlorophyll than the control [36]. Acclimation is important in the adaptive response to stress and enabling it to retain higher water content in the root tissue. It was reported that acclimation has provided an important role in gaining resistance, especially the higher water holding ability during dehydration [14]. Drought acclimation reduced leaf wilting, induced thicker cuticular layer and more open stomata of potato plant compared to drought stress directly under stress [13]. Selote et al. [38] determined in their study that drought acclimation in wheat caused a 2-fold increase in superoxide radical accumulation in leaves and roots without significant membrane damage.

Various studies have been conducted on the effects of drought stress on plant growth and yield in okra by earlier researchers, and results have been given regarding the effects of both cultivars and irrigation level in these studies. However, no study has been found on the effect of acclimatization to drought stress, which is the subject of this study, on okra. The details of how the okra seedlings will react in case of a mild water restriction at the beginning and severe water restriction afterward are still not clear. It was stated that post-drought re-irrigation provides recoveries in plant growth and photosynthesis, plant growth continues through stomatal opening and peroxidation reduction, and recovery from re-irrigation mostly depends on pre-drought intensity, duration and species [33].

#### 4. Conclusion

In this study, the effects of drought acclimation on some morphological, physiological and biochemical contents of okra seedling period were investigated. It was determined that the damage to the plants exposed to severe drought applied after drought acclimation was lower than the plants that were not acclimated but severely drought applied. In the study, it is thought that drought acclimation in the seedling period of okra may have an effect on increasing the tolerance to stress in the plant and this effect may continue in the next period of plant development.

#### Ethics in Publishing

There are no ethical issues regarding the publication of this study.

#### Author Contributions

ME and EY designed the experiment, UT, EY and EY conducted the experiment, ME and EY analyzed the results and all author wrote the manuscript.

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