Bilge International Journal of Science and Technology Research
ISSN: 2651-401X
e-ISSN: 2651-4028
Research Article
2024, Volume: 8, Issue: 1, 27-37
Received: 17.01.2024; Accepted: 13.03.2024
DOI: 10.30516/bilgesci.1421304

Humic Acid Mitigates Drought Stress in Tomato

Esra Aytaç[1], Halime Özdamar Ünlü[2*], İbrahim Ertan Erkan[1], Ufuk Çelikkol Akçay[1]

Abstract: Drought stress, one of the most important abiotic stresses, severely limits global crop production. To increase tolerance for this stress, environmentally friendly practices are emphasized. Humic acid, one of the most important natural biostimulants, has positive effects on plant growth and yield. Recently, it has also been reported to play an important role in resistance to various abiotic stresses. However, many physiological and molecular mechanisms by which humic acid confers drought resistance have not been fully elucidated. Therefore, the effects of humic acid application (3 ml L^{-1}) on different morphological and physiological stress indicators and some antioxidative enzyme gene expressions of tomato seedlings under drought stress conditions were investigated in this study. It was found that drought stress decreased the shoot fresh/dry weight, root fresh/dry weight, shoot and root length, chlorophyll content and relative water content of plants by 67%, 56%, 31%, 38%, 22%, 20%, 15% and 25%, respectively. Humic acid application significantly increased these parameters, while reducing ion leakage, MDA, and proline levels. The antioxidant enzyme gene expression of tomato seedlings under drought conditions showed no significant difference in *SOD* and *APX* gene expression, whereas *CAT* gene expression increased and *GR* gene expression decreased with humic acid application. Our results showed that humic acid application interacted with stress-related antioxidant enzyme gene expression and may be effective in reducing drought stress.

Keywords: Drought stress, Humic acid, Tomato, Gene expression

[1]**Address:** Isparta University of Applied Sciences, Faculty of Agriculture, Department of Agricultural Biotechnology, 32260, Isparta, Türkiye
[2]**Address:** Isparta University of Applied Sciences, Faculty of Agriculture, Department of Horticulture, 32260, Isparta, Türkiye

***Corresponding author**: halimeunlu@isparta.edu.tr

Citation: Aytaç, E., Ünlü, H. Ö., Turan, T., Erkan, İ. E., Akçay, U. Ç., (2024). Humic Acid Mitigates Drought Stress in Tomato. Bilge International Journal of Science and Technology Research, 8(1): 27-37.

1. INTRODUCTION

The agricultural sector worldwide is facing a variety of stresses that are causing major losses in crop productivity and compromising the sustainability of agriculture. Abiotic stresses are known as the most important environmental factors influencing agricultural productivity worldwide, and alone or in combination, they produce excessive amounts of reactive oxygen species (ROS), leading to disruption of redox homeostasis and oxidative stress, which affect plant physiology (Gupta et al., 2022).

Abiotic stresses include pH, high soil salinity, extreme temperature, and drought stress. Drought stress is the most catastrophic stress factor, with severe effects on the yield stability of crops (Manna et al., 2021). Therefore, there is a requirement for a more complete understanding of the responses of plants to abiotic stress and to develop stress tolerant plants and/or practices to improve plant stress tolerance (Alcázar et al., 2020; Manna et al., 2021).

The development of drought-tolerant crop varieties has been possible through genetic engineering, conventional and molecular breeding techniques. However, these processes are laborious and time-consuming, and regulatory concerns about genetically modified crops have prevented their widespread acceptance (Joshi et al., 2020). As an alternative to these methods, the use of biostimulants is one of the most promising strategies for alleviating drought stress (Calvo et al., 2014).

Humic acid is one of the most valuable biostimulants that can be applied externally to increase plant resistance to stress (Arslan et al., 2021). Various field experiments and experimental findings have shown that humic matter can alleviate the effects of abiotic stress (Canellas et al., 2020). Humic acid, which is derived from plant or animal waste, functions as a hormone-like compound, actively promotes the growth and development of plants, and provides protection against abiotic stresses (Arslan et al., 2021).

Humic acid supports plant growth under drought stress by enhancing osmotic adjustment, antioxidant capacity, and photosynthesis (Shen et al., 2020a). It has been suggested that humic acid can improve the hydro-physical properties of soils and increase the drought tolerance of plants, but the underlying molecular process is not yet known (Chen et al., 2022).

Tomatoes are one of the most economically important and widely grown crops in the world (Ansari et al., 2023). With a wide range of health benefits, antioxidant and anti-cancer properties, they are also important products for human well-being (Yadav et al., 2023). As the world's second-most important horticultural crop, both in terms of yield and consumption, tomatoes are also challenged by drought. Tomato plants are affected negatively by drought stress in several biochemical, morphological, physiological, and genetic ways. This not only reduces fruit quality and seed production but also causes significant yield losses (Islam et al., 2023). Therefore, this study was carried out to investigate the effects of humic acid on various morphological and physiological stress indicators and some antioxidative enzyme gene expressions of tomato seedlings under drought stress conditions.

2. MATERIAL AND METHOD

2.1. Plant material and treatments

This research was planned according to a randomised experimental design with 3 replicates and 10 plants in each replicate. Kayra F_1 tomato cultivar (Anamas Seeds, Antalya) seeds were sown in 400 ml polypropylene containers containing sterile perlite. Every two days, irrigation was carried out using Hoagland's solution (Hoagland and Arnon, 1950). Plants were grown for 15 days in a growth chamber with 50% humidity at 24˚C in a 16h light/8h dark cycle. The control treatment had no application other than irrigation. Humic acid (TKİ HÜMAS) was sprayed on the leaves at the rate of 3 ml/L in humic acid treatment. The drought stress started at the end of the 15th day and the water potential of 0 was reached on the 3rd day. 7 days after drought stress samples for analysis have been collected.

2.2. Growth parameters

In order to determine dry weight, the tissues of the shoots and roots were separated, weighed, and dried at 60˚C for 48 hours, then weiged again.

2.3. Relative water content (RWC)

RWC was determined in accordance with the formula given in Smart and Bingham (1974). Accordingly, RWC (%) = (Fresh weight-Dry weight)/ (Turgid weight-Dry weight) X 100. The turgid weight was measured by soaking the leaves in distilled water for 24 hours at room temperature.

2.4. Determination of proline content

The proline content was determined by applying the Bates et al. (1973) method. For this, 0.3g of sample was ground in liquid nitrogen and dissolved by adding 1ml 3% sulphosalicylic acid. 0.1 ml of this mixture was taken and centrifuged. Ninhydrin (0.2 ml), 96% acetic acid (2 ml) and 3% sulphosalicylic acid (0.1 ml) were added. The mixture was kept at 96˚C for 1 hour. After the addition of 1 ml of toluene, centrifugation was repeated and the absorbance of the supernatant obtained was measured at 520 nm with a spectrophotometer.

2.5. Determination of membrane damage

To assess the membrane damage caused by drought stress, malondialdehyde (MDA) levels and membrane electrolyte leakage were determined.

Membrane damage resulting from lipid peroxidation was determined by using the Ohkawa et al. (1979) method to estimate MDA levels. Liquid nitrogen was used to homogenize 0.2 g of the sample, and 1 ml of 5% trichloroacetic acid (TCA) was added. After centrifugation, 0.5% thiobarbituric acid (TBA) was added to the same volume of 20% TCA and kept at 96˚C for 25 minutes. After cooling the samples on ice, the absorbance values were determined at 532 nm. Non-specific absorbance values were read at 600 nm and subtracted from the initial absorbance values.

Electrolyte leakage was determined using the method described in Nanjo et al. (1999). According to this method, 6 leaves were kept in test tubes containing 0.4 M mannitol for 3 hours with shaking, and the electrical conductivity was determined as C1. After 15 minutes in boiling water, the samples were cooled to room temperature and C2 was read. This C2 value has been calculated using the leakage dependent conductivity formula [(C1/C2) X 100]. For electrical conductivity, a Thermo Scientific Orion 013016MD MD 2 condactivimeter probe was used, which can measure in the range 0.01-300 mSs/cm.

2.6. Chlorophyll content determination

The Spad-502 Plus chlorophyll meter was used to measure the amount of chlorophyll content in tomato leaves. Measurements were taken at different points on the leaves of each plant and the results were expressed in SPAD.

2.7. Gene expression analysis

In the study, total RNA was first isolated (Qiagen Rneasy Plant Mini Kit, Qiagen USA) to be used in the semi-quantitative RT-PCR method. The cDNA was then

synthesized (VitaScript cDNA synthesis kit, Procomcure Biotech Austria). PCR amplification was performed with primers specific for four different antioxidant system enzymes, *FeSOD*, *CAT2*, *GR1* and *APX1* genes, prepared using the Primer Premier program (PREMIER Biosoft International, USA). The NCBI Gene Bank reference sequence codes of the genes analyzed in this study are NM_001313769.1, NM_001247257.2, NM_001321393.1, and NM_001247853.2 respectively. The *EF-1* (elongation factor 1 alpha) gene with reference sequence code X14449.1 was used as an internal control in the study.

2.8. RNA isolation

RNA was isolated from tomato leaves using Qiagen RNeasy Plant Mini Kits based on guanidine isothiocyanate lysis and silica membrane purification. Total RNA amounts were determined spectrophotometrically using Nanodrop 2000. The quality of total RNA was determined by separating and visualizing it using 2% agarose gel electrophoresis.

2.9. RT-PCR (Semi-quantitative Reverse Transcription-PCR)

From the RNA molecules obtained, cDNA was synthesized using the VitaScript cDNA synthesis kit with oligodT primers. Primers specific for the genes studied were designed using PrimerPremier 5.0, CA, USA, and PCR amplification was performed using the primers that gave the most appropriate amplification conditions. The bands obtained were separated on a 0.8% agarose gel and visualized using the Biolab UV Tech gel imaging system. The imaged bands were analyzed using ImageJ software developed by the National Institute of Health (NIH) to reveal differences in gene expression levels. The cycle steps and times for PCR analyses are shown in Table 1.

Table 1. PCR step times and cycles

Cycle Step	Temperature	Time	Number of cycles
Initial denaturation	95 ^{0}C	5 min	
Denaturation	95 ^{0}C	1 min	
Annealing	-	45 s	28
Extension	72 ^{0}C	45 s	
Final extension	72 ^{0}C	10 min	

2.10. Data statistical analysis

The Minitab (17) Inc. was used to perform an analysis of variance on the study's data. Using the Tukey test, the differences between the significant means were indicated by distinct letters.

3. RESULTS AND DISCUSSIONS

3.1. Effects of humic acid on the growth parameters

To determine the effects of humic acid on the growth parameters of the tomato plant, measurements of root fresh/dry weight, shoot fresh/dry weight, shoot and root length were carried out and the morphological effects are given in the table below. The effect of the treatments on all these parameters was significant (P<0.05).

Table 2. Effects of treatments on growth parameters

Treatments	Root Fresh Weight (g)	Root Dry Weight (g)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Shoot Length (cm)	Root Length (cm)
Control	0.52 ±0.01^b	0.034 ±0.00^b	2.20 ±0.02^b	0.16 ±0.00^b	17.72 ±0.36^b	18.28 ±0.26^b
Humic Acid	0.64 ±0.00^a	0.046 ±0.00^a	2.44 ±0.04^a	0.17 ±0.00^a	19.06 ±0.16^a	19.04 ±0.50^a
Drought Stress	0.36 ±0.01^d	0.021 ±0.00^d	0.72 ±0.01^d	0.07 ±0.00^d	13.88 ±0.18^d	14.61 ±0.14^d
Drought Stress + Humic Acid	0.45 ±0.01^c	0.030 ±0.00^c	1.08 ±0.00^c	0.11 ±0.00^c	15.09 ±0.04^c	16.80 ±0.22^c

Note: Differences between values shown with different letters are significant at P<0.05 level.

The results of the study showed that shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of tomato plants decreased by 67%, 56.2%, 30.7% and 38.2% respectively when comparing the control and drought-stressed groups of plants (Table 2). Altunlu (2011) in tomato, Kılıçaslan Caşka (2019) in bean, Avşaroğlu (2015) in watermelon, Faaek (2018) in strawberry and Sadak (2018) in pepper reported that drought stress reduced the fresh-dry weights of plant roots and shoots. Shoot fresh-dry weights were found to decrease under drought stress in tomato (Kıran et al., 2014; Zhou et al., 2017; Alp, 2017) and eggplant (Kıran et al., 2016). All these reports support the findings that shoot fresh-dry weight, root fresh-dry weight values decreased with drought stress compared to the control in our study. The stage of development of the plant during the periods of water limitation also varies according to the growth development of the plant and the effect of physiological characteristics (Farooq et al., 2009). Plant growth under water stress is very variable, depending on the duration of the drought. When drought stress begins, the plant increases root growth to access water. In addition, with prolonged drought stress, shoot and root development

stops and leaf area and number of leaves decrease (Anjum et al., 2011).

In the study, drought-stressed tomato plants showed a 21.6% reduction in shoot length and a 20% reduction in root length compared to control plants. Güzel (2006) and Altunlu (2011) reported that shoot and root length decreased in tomato, Ecem (2010) in maize and Avşaroğlu (2015) in watermelon because of drought stress application. Kuşvuran et al. (2020) and Parveen et al. (2019) reported that shoot length decreased with drought stress in pepper and tomato plants, respectively. These results are parallel to our findings.

Humic substances are heterogeneous, large organic complexes that are composed of the components of humus. They play an important role in soil aeration, long-term water retention in the soil, anion and cation exchange, and the chelation of mineral elements (Pettit, 2004). At the same time, it is stated that the effect of humic acids on plant germination and growth, expansion and elongation of root cells, oxygen uptake, respiration, photosynthesis is positive, and they show hormone-like growth (Vaughan, 1985; Garcia et al., 1992; Dell'Amico et al., 1994).

In the study, it was determined that humic acid application increased shoot fresh weight by 10%, shoot dry weight by 6.2%, root fresh weight by 23%, root dry weight by 35.2%, shoot length by 7% and root length by 4.1% in tomato plants (Table 2). Humic acid application has been reported to increase shoot/root fresh and dry weights in tomato (Aksoy, 2019; Khan et al., 2020; Ural, 2020) and strawberry plants (Doğan, 2018). Ashraf and Raddy (2014) found that humic acid application resulted in an increase in root fresh weight in eggplant and tomato plants. In addition, Yaman (2016) in strawberry plants and Qin and Leskovar (2020) in pepper, tomato, watermelon and lettuce plants found that root fresh and dry weights increased as a result of humic acid application. Similarly, Maibodi et al. (2015) reported that humic acid application in grass (*Lolium perenne* L.) resulted in an increase in shoot fresh/dry weight and length, and root fresh weight and length. Humic acid was found to increase root and shoot length in pepper plants by Aslanpay (2011) and in maize plants by Güngör (2018). In addition, Kocamanoğlu (2018) found that the shoot length of purslane and Kalyoncu (2013) found that the root length of mung bean increased with humic acid application. These reports support our findings.

In our study, humic acid treatment against drought stress increased shoot fresh weight by 50%, shoot dry weight by 57%, root fresh weight by 25%, root dry weight by 43%, shoot length by 9% and root length by 15% in tomato plants compared to plants under drought stress. Similar results have been reported for root and shoot lengths in basil and cumin plants (Haghighi et al., 2012). Similar reports have been shown for shoot fresh and dry weights in maize plants (Kaya et al., 2020) and root fresh and dry weights in melon plants (Kıran et al., 2019).

3.2. Effects of humic acid on physiological stress indicators

Physiological stress indicators (MDA, ion leakage, chlorophyll, proline, relative water content) were studied to determine the effects of humic acid on tomato plants under drought stress. As shown in Table 3, the effect of treatments on all these parameters was found to be significant ($P<0.05$).

Table 3. Effects of treatments on physiological stress indicators

TREATMENTS	MDA (nmol/g)	Ion leakage (%)	Chlorophyll (SPAD)	Proline (µmol/g)	RWC (%)
Control	7.27 ±0.11^{d}	8.89 ±0.13^{b}	43.88 ±0.09^{b}	24.78 ±0.46^{c}	74.55 ±0.97^{b}
Humic Acid	7.58 ±0.09^{c}	8.16 ±0.14^{c}	44.75 ±0.14^{a}	22.48 ±0.66^{d}	81.28 ±0.65^{a}
Drought Stress	21.93 ±0.10^{a}	11.78 ±0.15^{a}	37.19 ±0.10^{d}	156.35 ±0.40^{a}	56.20 ±0.35^{d}
Drought Stress + Humic Acid	14.06 ±0.08^{b}	9.19 ±0.17^{b}	41.38 ±0.19^{c}	60.06 ±0.32^{b}	68.45 ±0.45^{c}

Note: Differences between values shown with different letters are significant at $P<0.05$ level.

MDA is a commonly used indicator of oxidative lipid damage and its concentration varies depending on stress (Davey et al., 2005). The osmotic potential of plants is reduced to keep the water content of plants stable during drought stress, and the change that occurs in the plant during drought stress also affects MDA levels. As a result, it has been reported that drought stress influences MDA levels and that the amount of MDA increases with the increase in cell damage (Kayabaşı, 2011). When comparing plants under drought stress with plants in the control group, a 201.6% increase in MDA was observed (Table 3). Similar results have been determined by different researchers in tomato (Alp and Kabay, 2017), grapevine (Koç, 2020), bean (Kabay and Şensoy, 2016), and pepper (Sadak, 2018; Kuşvuran et al., 2020). Compared to drought-stressed plants, plant MDA was reduced by 35.8% when humic acid was applied during drought stress. This positive effect of humic acid was also found in wheat (Arslan, 2018) and in melon (Kıran et al., 2019).

Oxidative stress on the membrane causes an increase in ion leakage during drought stress (Assaha et al., 2016). In this respect, it is believed that one of the best physiological markers of drought stress tolerance is ion leakage, which is an indicator of the stability and integrity of the cell membrane (Kocheva et al., 2004; Bat et al., 2020). Determination of ion leakage is a method used to determine the relationship between environmental stress and growth,

development and genotypic changes in membrane integrity (Bat et al., 2020). Our study showed that drought stress caused a 32.5% increase in ion leakage in tomato plants. Similar situations were reported by Çetin (2018) in wheat, by Can (2017) in cotton and by Ecem (2010) in maize. Also, ion leakage was reduced by 21.9% compared to drought-stressed plants when humic acid was applied under drought-stressed conditions. Like our findings, Abdelaal et al. (2018) reported the positive effect of humic acid onion leakage in barley plants.

Chlorophyll content in leaves is one of the most important factors influencing the efficiency of photosynthesis in plants. It has been reported that significant differences in chlorophyll pigment content occur in plants exposed to drought stress. It has been reported that significant differences in chlorophyll pigment content occur in plants exposed to drought stress (Güzel, 2006). The decrease in chlorophyll content in plants under drought stress may be due to the degradation of chlorophyll (Christ et al., 2014) or changes in enzymatic activities involved in chlorophyll synthesis that slow or inhibit chlorophyll synthesis (Cotrina Cabello et al., 2023). In our study, drought stress caused a 15.2% decrease in leaf chlorophyll content compared to the control. Drought stress was also found to have a negative effect on chlorophyll content in grape (Geçene, 2020) and strawberry plants (Faaek, 2018). In addition, humic acid application to drought-stressed plants was found to increase leaf chlorophyll levels by 11.2% compared to plants under drought stress conditions. Similar findings to our results were reported by Haider et al. (2014) in maize and Korkmaz (2018) in strawberry.

Proline is an essential amino acid that supports plant development and metabolism under abiotic stress conditions. It acts as an antioxidant defence molecule, a molecular chaperone, a signalling molecule that scavenges ROS and activates specific gene functions essential for the plant to recover from stress due to its metal chelating properties. In order to reduce oxidative damage and repair cell structures, plant cells produce a high level of proline, which helps to maintain cellular homeostasis, osmotic adjustment, water uptake, and redox balance (Ghosh et al., 2022). Proline can act as a stress tolerance enhancer, antioxidant, osmolyte and signalling molecule in plants (Kılıç, 2020). Drought stress increased the proline content of the plants by 530.9% compared to the control plants. Different researchers have reported similar results to our findings in Pistacia genotypes (Aljemaa, 2020), soybean (Kayabaşı, 2011) and tomato plants (Sanchez-Radriguez et al., 2010; Khan et al., 2015; Parveen et al., 2019). Humic acid application under drought stress conditions reduced proline levels by 61.5% compared to plants under drought stress conditions. Khorasoninejad et al. (2018) reported similar results in echinacea (*Echinecea purpurea*).

Relative water content (RWC), which is the water retention capacity of tissues, is also an indicator of cell membrane stability and tissue structural integrity (Celikkol Akcay and Okudan, 2023). It is a mechanism that helps regulate the water balance in tissues to protect the plant from stress factors (Bat et al., 2020). Drought stress reduced the relative water content of tomato plants compared to control plants. Similar results have been determined by different researchers in pepper (Cengiz, 2017; Yaban, 2018) and tomato (Altunlu, 2011; Zhou et al., 2017). The positive effect of humic acid on relative water content was observed when plants under drought stress were compared with plants treated with humic acid. Indeed, this positive effect of humic acid was demonstrated in a study on okra plants by Barzegar et al. (2016).

3.3. Effects of humic acid on *EF-1*, *FeSOD*, *APX1*, *CAT2*, and *GR1* antioxidant enzyme genes expression

The gene expressions of FeSOD, APX1, CAT2 and GR1 antioxidant defense enzymes were studied to reveal the effects of drought stress, humic acid and humic acid + drought stress treatments in tomato plants, and *EF-1* was selected as a homebox gene to elucidate these gene expressions. Between treatments, the expression of the *EF-1* gene remained largely the same. This is an indication that EF-1 is a suitable internal control and that the vital activities of the plants continue under the treatments (Figure 1).

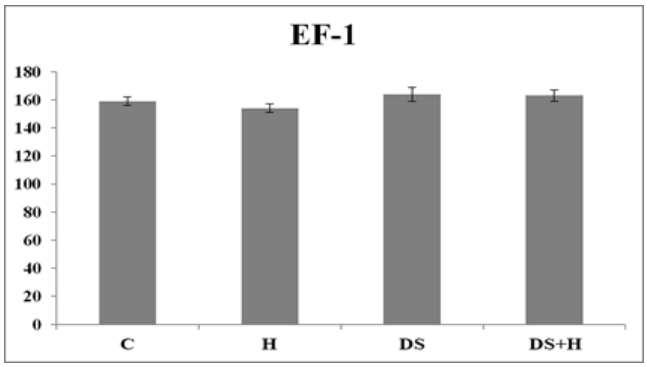

Figure 1. Semi-quantitative *EF-1* gene expression of tomato plants under treatments (C: Control, H: Humic acid, DS: Drought stress, DS+H: Drought stress+Humic acid)

In the study, when *FeSOD* gene expression levels in tomato plants were analyzed according to treatment, it was found that they varied between 0-91 (Figure 2). However, the highest values were observed in the control, drought stress +humic acid and drought stress treatments, while the lowest value was observed in the humic acid treatment. Drought stress causes the ROS formation such as hydrogen peroxide (H_2O_2) and superoxide (O_2). To scavenge ROS, stressed plants activate both enzymatic and non-enzymatic antioxidants and restore cellular redox homeostasis to reduce oxidative stress. Superoxide dismutases (SODs), which are antioxidant enzymes, are very important attendants of antioxidant biocatalysts through the dismutation of O2- to H2O2. In this way, they increase the tolerance of the plants to stress (Saibi and Brini, 2018). *FeSOD* gene expression levels of tomato plants under drought stress showed a 26.3% decrease compared to the control treatment. Kireçci (2012) in wheat and Çalık (2016) in chickpea reported that drought stress caused a decrease in SOD enzyme activity. It was found that applying humic acid reduced *FeSOD* gene expression levels in tomato plants by 100% compared to the control. Haghighi and Teixeira Da Silva (2013) reported that humic acid application decreased SOD enzyme activity in tomato

compared to control plants. The application of humic acid to tomato plants under drought stress increased the expression of the *FeSOD* gene. Kıran et al. (2019) and Kaya et al. (2020) reported similar results in melon and maize plants, respectively.

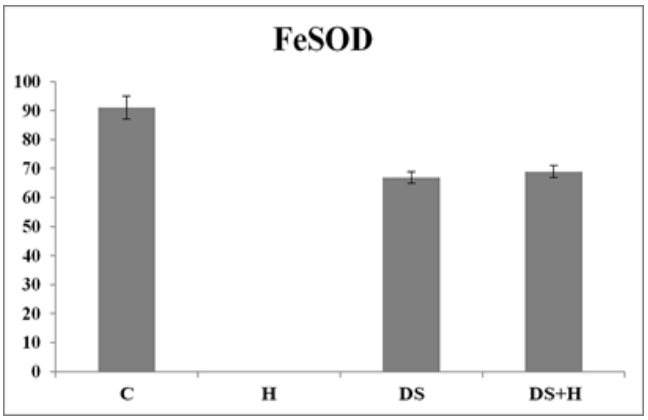

Figure 2. Semi-quantitative *FeSOD* gene expression of tomato plants under treatments (C: Control, H: Humic acid, DS: Drought stress, DS+H: Drought stress+Humic acid)

When *APX1* gene expression levels were analyzed, the lowest level (0) was observed in the humic acid treatment and the highest level (166) in the drought stress treatment (Figure 3). Ascorbate peroxidase (APX), an enzymatic antioxidant, metabolises stress-induced ROS such as H_2O_2 and controls their potential effects on cellular metabolism and function. APX has a high affinity for H_2O_2 and appears to be an important parameter in the complete destruction of H_2O_2 using ascorbate (AsA) as a specific electron donor in organelles including mitochondria, chloroplasts, peroxisomes, and cytosol (Anjum et al., 2016). *APX1* gene expression in the control plants showed close levels compared to the drought-stressed plants. Similar results to our findings were found in tomato (Aydın, 2015; Alp, 2017; Raja et al., 2020), pepper (Yaban and Kabay, 2019; Kuşvuran et al., 2020) and grapevine (Koç, 2020). Humic acid application reduced *APX1* gene expression by 100% compared to the control plants. Dinler et al. (2016) reported that applying fulvic acid reduced APX1 enzyme activity in soybean plants compared to control group. This statement is in parallel with our findings. Our results showed that there was a 0.6% decrease in *APX1* gene expression because of humic acid application to plants under drought stress. Tartoura (2010) in wheat plants and Aguiar et al. (2016) in sugarcane reported that humic acid application to plants under drought stress reduced APX1 enzyme activity.

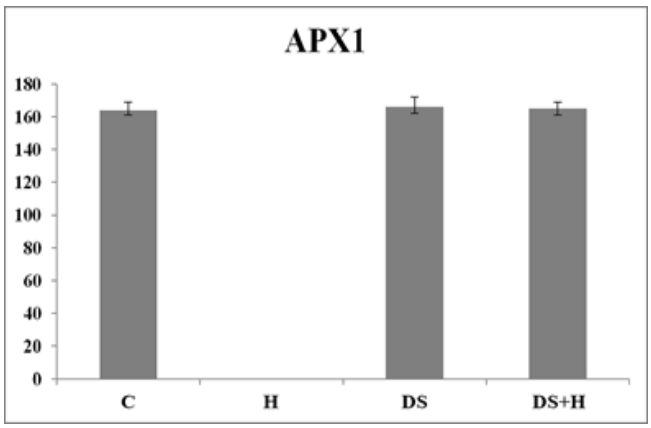

Figure 3. Semi-quantitative *APX1* gene expression of tomato plants under treatments (C: Control, H: Humic acid, DS: Drought stress, DS+H: Drought stress+Humic acid)

CAT2 gene expression levels were found to vary between 0 and 138 (Figure 4). While the highest value was obtained from drought stress + humic acid, the lowest value was obtained from humic acid treatment. Superoxide dismutase in plant cells forms the first line of defence against ROS (Ighodaro and Akinloye, 2018), catalysing the superoxide radical to molecular oxygen and hydrogen peroxide. This hydrogen peroxide is then removed by catalase (CAT) (Young and Woodside, 2001). In our study, *CAT2* gene expression was found to decrease by 46.1% in drought-stressed plants compared to control. Some researchers reported that drought stress caused a decrease in CAT enzyme activity in tomato (Gökçe Gündüzer, 2015) and wheat (Yediyıldız, 2008; Baltacıer, 2019) plants. There was a 100% decrease in *CAT* gene expression in tomato plants treated with humic acid compared to control plants. Shen et al. (2020b), in their study on millet plants, found that applying humic acid reduced CAT enzyme activity compared to control plants. In addition, Bijanzadeh et al. (2021) found that applications of humic acid and jasmonic acid to wheat plants reduced CAT enzyme activity compared to control plants. These reports support our findings. Also, compared to plants treated with drought stress + humic acid, it was observed that *CAT* gene expression increased by 97.1% in plants under drought stress conditions. Similar results to our findings were found in melon plants by Kıran et al. (2019).

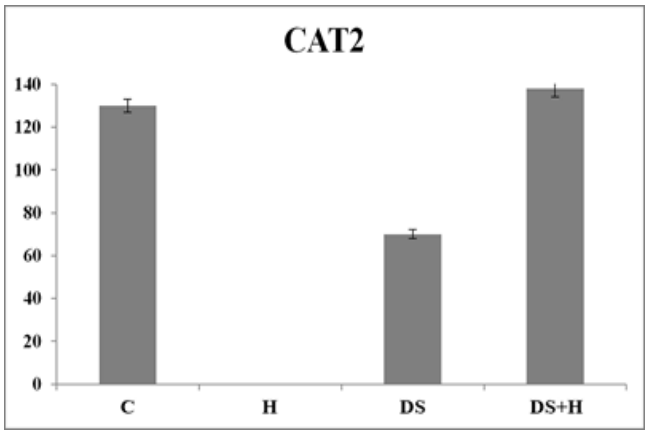

Figure 4. Semi-quantitative *CAT2* gene expression of tomato plants under treatments (C: Control, H: Humic acid, DS: Drought stress, DS+H: Drought stress+Humic acid)

GR1 gene expression values were lowest in the humic acid treatment (13) and highest in the drought stress (143) (Figure 5). Glutathione reductase (GR) is one of the important antioxidant enzymes that help protect cells against ROS and their reaction products. GR is a NAD(P)H-dependent antioxidant and plays a role in maintaining the reduced glutathione (GSH) and thiol pools in cells. The differential regulation of GR in plants suggests that it is an important component of the plant defence system (Gill et al., 2013). Compared to the control treatment, drought stress increased *GR1* gene expression levels by 10.8%. Taşğın et al. (2017), Çancıoğlu (2014), Çetinkaya (2013) and Özkur (2010) reported an increase in GR enzyme activity with drought stress. Also, our study shows that humic acid application reduced *GR1* gene expression by 89.9% in comparison to drought-stressed tomato plants. Oktay Yiğit (2018) in wheat and Ural (2020) in tomato, found that humic acid applications reduced GR1 enzyme activity compared to control groups. In addition, the expression of the *GR1* gene was decreased by 11.8% when humic acid was applied to plants under drought stress. Tartoura (2010) found a similar situation in wheat plants and reported that this situation may vary depending on applications and doses.

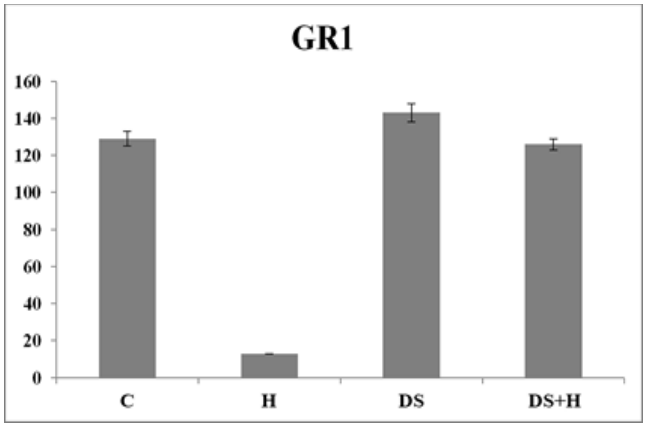

Figure 5. Semi-quantitative *GR1* gene expression of tomato plants under treatments (C: Control, H: Humic acid, DS: Drought stress, DS+H: Drought stress+Humic acid)

4. CONCLUSIONS

Application of humic acid to plants under drought stress resulted in an increase in shoot fresh-dry, root fresh-dry, shoot and root length, chlorophyll and relative water content values. It was also found that MDA, ion leakage and proline levels decreased when humic acid was applied to plants under drought stress. In addition, humic acid application to plants under drought stress did not cause a significant change in *SOD* and *APX* gene expression levels, while it caused an increase in *CAT* gene expression and a decrease in *GR* gene expression. These results indicate that humic acid applications may be effective in reducing the negative effects of drought stress, particularly by increasing *CAT* gene expression. Also, the fact that all the antioxidant enzyme gene expressions disappeared only under the humic acid treatment or were at the lowest level compared to all the treatments, suggests that humic acid positively affects the general physiological and metabolic responses of tomato plants.

Acknowledgements
This study is derived from Esra Aytaç's master's thesis.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: E.A., H.Ö.Ü., U.Ç.A; Investigation: E.A., H.Ö.Ü., U.Ç.A; Material and Methodology: E.A., H.Ö.Ü., U.Ç.A, İ.E.E; Supervision: H.Ö.Ü., U.Ç.A; Visualization: H.Ö.Ü., U.Ç.A; Writing-Original Draft: E.A., H.Ö.Ü; Writing-review & Editing: E.A., H.Ö.Ü; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Abdelaal, K.A.A., Hafez, Y.M., El-Afry, M.M., Tantawy, D.S., Alshaal, T. (2018). Effect of some osmoregulators on photosynthesis, lipid peroxidation, antioxidative capacity, and productivity of barley (Hordeum vulgare L.) under water deficit stress. Environmental Science and Pollution Research, 25, 30199-30211. https://doi.org/10.1007/S11356-018-3023-X

Aguiar, N.O., Medici, L.O., Olivares, F.L., Dobbss, L.B., Torres-Netto, A., Silva, S.F., Novotny, E.H., Canellas, L.P. (2016). Metabolic profile and antioxidant responses during drought stress recovery in sugarcane treated with humic acids and endophytic diazotrophic bacteria. Annals of Applied Biology, 168, 203-213.

Aksoy, E. (2019). The Effects of Humic Acid on Plant Development, Yield, Some Quality and Biochemical Properties on Tomato Grown in Salty Conditions. (Master's Thesis, Harran University Graduate School of Natural and Applied Sciences)

Alcázar, R., Bueno, M., Tiburcio, A.F. (2020). Polyamines: Small amines with large effects on plant abiotic stress tolerance. Cells, 9(11), 2373. https://doi.org/10.3390/cells9112373

Aljemaa, B.A. (2020). Determination of Morphological, Physiological and Biochemical Reactions of Some Pistacia Genotypes in Drought Stress. (Ph.D. Thesis, Selçuk University Graduate School of Natural and Applied Science)

Alp, Y., Kabay, T. (2017). Kuraklık stresinin yerli ve ticari domates çeşitlerinde bazı fizyolojik parametreler üzerine etkileri. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 22(2), 86-96.

Alp, Y. (2017). The Effect of Drought Stress on Early Plant Development in Some Native and Commercial Tomato Genotypes. (Master's Thesis, Yüzüncü Yıl University Graduate School of Applied and Natural Sciences)

Altunlu, H. (2011). The Effects of Grafting Against Drought Stresse in Tomatoes. (Ph.D. Thesis, Ege University Graduate School of Applied and Natural Sciences)

Anjum, S.A., Xie, X., Wang, L., Saleem, M.F., Man, C., Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stres. African Journal of Agricultural Research, 6, 2026-2032.

Anjum, N.A., Sharma, P., Gill, S.S., Hasanuzzaman, M., Khan, E.A., Kachhap, K., Tuteja, N. (2016). Catalase and ascorbate peroxidase—representative H2O2-detoxifying heme enzymes in plants. Environmental science and pollution research, 23, 19002-19029. https://doi.org/10.1007/s11356-016-7309-6

Ansari, M., Ahmed, S., Abbasi, A., Hamad, N.A., Ali, H.M., Khan, M.T., Haq, I.U., Zaman, Q. (2023). Green synthesized silver nanoparticles: a novel approach for the enhanced growth and yield of tomato against early blight disease. Microorganisms, 11(4), 886. https://doi.org/10.3390/microorganisms11040886

Arslan, E. (2018). The Effect of Drought Stress and Humic Acid on Mrna Expression Levels of Some Genes and DNA Conformation Changes in Bread Wheat (Triticum aestivum L.). (Ph.D. Thesis, Atatürk University Graduate School of Applied and Natural Sciences)

Arslan, E., Agar, G., Aydin, M. (2021). Humic acid as a biostimulant in improving drought tolerance in wheat: The expression patterns of drought-related genes. Plant Molecular Biology Reporter, 39(3), 508-519. https://doi.org/10.1155/2016/3747501

Ashraf, O., Raddy, M.M. (2014). Effect of humic acid as an additive to growing media to enhance the production of eggplant and tomato transplants. Journal of Horticultural Science & Biotechnology, 89(3), 237-244.

Aslanpay, B. (2011). The Effects of Arbuskular Mycorrhizal Fungus (AMF) and Humic Acid on The Growth of Pepper (Capsicum annum L.) Plant And Root Rot Disease Caused By Phytophthora Capsici Leonian (Master's Thesis, Yüzüncü Yıl University Graduate School of Applied and Natural Sciences)

Assaha, D.V.M., Liu, L., Ueda, A., Nagaoka, T., Saneoka, H. (2016). Triveni Enterprises. Journal of Environmental Biology, 37, 107-114.

Avşaroğlu, Z.Z. (2015). The Effects of Nitric Oxide Applications on the Growth of Local and Wild Types of Watermelon Genotypes Under Drought Conditions. (Master's Thesis, Harran University Institute of Natural and Applied Sciences)

Aydın, M. (2015). Investigation of the Effects of Toxic Boron with Drought Stress on Tomato. (Master's Thesis, Fatih University the Graduate School of Sciences and Engineering)

Baltacıer, G. (2019). Effects of Rosa Canina L. Extract on Antioxidant Activity in Two Wheat Varieties Under Drought Stress. (Master's Thesis, Çanakkale Onsekiz Mart University Graduate School of Natural and Applied Sciences)

Barzegar, T., Moradi, P., Nikbakht, J., Ghahremani, Z. (2016). Physiological response of okra cv. Kano to foliar application of putrescine and humic acid under water deficit stress. International Journal of Horticultural Science and Technology, 3(2), 187-197. https://doi.org/10.22059/İjhst.2017.213448.147

Bat, M., Tunçtürk, R., Tunçtürk, M. (2020). Ekinezya (Echinacea purpurea L.) bitkisinde kuraklık stresi ve deniz yosunu uygulamalarının bazı fizyolojik parametreler üzerine etkisi. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 23(1), 99-107. https://doi.org/10.18016/Ksutarimdoga.Vi.535210

Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39, 205-207.

Bijanzadeh, E., Emam, Y., Pessarakli, M. (2021). Biochemical responses of water-stressed triticale (X triticosecale wittmack) to humic acid and jasmonic acid. Journal of Plant Nutrition, 44(2), 252-269. https://doi.org/10.1080/01904167.2020.1806312

Calvo, P., Nelson, L., Kloepper, J.W. (2014). Agricultural uses of plant biostimulants. Plant Soil, 383, 3-41. https://doi.org/10.1007/s11104-014-2131-8

Can, D. (2017). Determination of The Effects of Deficit Irrigation on Quality, Yield and Yield Components of Some Cotton Variety in Amik Plain Conditions. (Yüksek Lisans Tezi, Harran University Graduate School of Natural and Applied Sciences)

Canellas, L.P., Canellas, N.O., da S. Irineu, L.E.S., Olivares, F.L., Piccolo, A. (2020). Plant chemical priming by humic acids. Chemical and Biological Technologies in Agriculture, 7, 1-17. https://doi.org/10.1186/s40538-020-00178-4

Celikkol Akcay, U., Okudan, N. (2023). Exogenous serotonin improves drought and salt tolerance in tomato seedlings. Plant Growth Regulation, 101, 239-249. https://doi.org/10.1007/s10725-023-01016-x

Cengiz, A. (2017). Determination of the Relationship Between Drought Stress and Nitric Oxide in Pepper Plant. (Master's Thesis, Yüzüncü Yıl University Graduate School of Applied and Natural Sciences)

Chen, Q., Qu, Z., Ma, G., Wang, W., Dai, J., Zhang, M., Wei, Z., Liu, Z. (2022). Humic acid modulates growth, photosynthesis, hormone and osmolytes system of maize under drought conditions. Agricultural Water Management, 263, 107447. https://doi.org/10.1016/j.agwat.2021.107447

Christ, B., Egert, A., Suessenbacher, I., Kraeutler, B., Bartels, D., Peters, S., Hoertensteiner, S. (2014). Water deficit induces chlorophyll degradation via the 'PAO/phyllobilin'pathway in leaves of homoio-(Craterostigma pumilum) and poikilochlorophyllous (X erophyta viscosa) resurrection plants. Plant, Cell & Environment, 37(11), 2521-2531. https://doi.org/10.1111/pce.12308

Cotrina Cabello, G. G., Ruiz Rodriguez, A., Husnain Gondal, A., Areche, F. O., Flores, D. D. C., Astete, J. A. Q., & Cruz Nieto, D. D. (2023). Plant adaptability to climate change and drought stress for crop growth and production. CABI Reviews, (2023), 1-9. https://doi.org/10.1079/cabireviews.2023.0004

Çalık, B. (2016). The Antioxidant Responces Against to Drought Stress and Orobanche Crenata İnfection on Chickpea (Cicer Arietinum L.) Varieties Which Have Known Diferent Drought Tolerances. (Master's Thesis, Çanakkale Onsekiz Mart University Graduate School of Natural and Applied Sciences)

Çancıoğlu, E. (2014). Investigation of The Effects Carnitine on Reactive Oxygen Species Signalling and Antioxidant System Under Drought Stress. (Master's Thesis, Ege University Graduate School of Natural and Applied Sciences)

Çetin, F. (2018). Investigation of Physiological and Biochemical Parameters of Turkish Wheat Cultivars with Different Ploidy Level Under Drought Stress Conditions. (Master's Thesis, Kastamonu University Graduate School of Natural and Applied Sciences)

Çetinkaya, C. (2013). Physiological Effects of Drought and Rtreatments in Some Strawberry Cultivars. (Master's Thesis, Uludağ University Graduate School of Natural and Applied Sciences)

Davey, M.W., Stals, E., Panis, B., Keulemans, J., Swennen, R.L. (2005). High-throughput determination of malondialdehyde in plant tissues. Analytical biochemistry, 347(2), 201-207. https://doi.org/10.1016/j.ab.2005.09.041

Dell'Amico, C., Masciandaro, G., Ganni, A., Ceccanti, B., Garcia, C., Hernandez, T., Costa, F. (1994). Effects of specific humic fractions on plant growth in humic substances. In: The global environment and implications on human health, ed. Senesi, N. And Miano, T.M., 563-566, Elsevier Science, Amsterdam, The Netherlands.

Dinler, B.S., Gündüzer, E., Tekinay, T. (2016). Pre-treatment of fulvic acid plays a stimülant role in protection of soybean (Glycine max L.) leaves against heat and salt stress. Acta Biologica Cracoviensia Series Botanica, 58(1), 29-41. https://doi.org/10.1515/abcsb-2016-0002

Doğan, M. (2018). Investigation on the Effects of Humic Acid and Silicon Under Cadmium Stress in Strawberry. (Master's Thesis, Harran University Graduate School of Natural and Applied Sciences)

Ecem, N. (2010). Investigation of the Effect of Drought Stress in Different Maize (Zea Mays L.) Cultivars and Lines. (Master's Thesis, Sakarya University Graduate School of Applied and Natural Sciences)

Faaek, M.F.F. (2018). Effects of Drought Stress in Some Strawberry Cultivars. (Master's Thesis, Selçuk University Graduate School of Applied and Natural Sciences)

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development, 29, 185-212.

Garcia, C., Hernandez, T., Costa, F., Ceccanti, B., Dell'Amico, C. (1992). Characterization of the organic fractions of an uncomposted and composted sewage sludge by isoelectric focusing and gel-filtration. Biology and Fertility of Soils, 13, 112-118.

Geçene, İ. (2020). Determination of Tolerance to Drought Stress Infoxy Grape (Vitis labrusca L.) by Peg Application in Vitro Conditions. (Master's Thesis, Ordu University Institute of Natural and Applied Sciences)

Gill, S.S., Anjum, N.A., Hasanuzzaman, M., Gill, R., Trivedi, D.K., Ahmad, I., Tuteja, N. (2013). Glutathione and glutathione reductase: a boon in disguise for plant abiotic stress defense operations. Plant Physiology and Biochemistry, 70, 204-212. https://doi.org/10.1016/j.plaphy.2013.05.032

Ghosh, U.K., Islam, M.N., Siddiqui, M.N., Cao, X., Khan, M.A.R. (2022). Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms. Plant Biology, 24(2), 227-239. https://doi.org/10.1111/plb.13363

Gökçe Gündüzer, E. (2015). Determination of Catalase Gene mRNA Expression Level with Real Time PCR in Tomato (Solanum lycopersicum L.) Plant Grown Under Salt and Drought Stress. (Ph.D. Thesis, Ankara University Graduate School of Natural and Applied Sciences)

Gupta, A., Bano, A., Rai, S., Mishra, R., Singh, M., Sharma, S., Pathak, N. (2022). Mechanistic insights of plant-microbe interaction towards drought and salinity stress in plants for enhancing the agriculture productivity. Plant Stress, 4, 100073. https://doi.org/10.1016/j.stress.2022.100073

Güngör, K. (2018). Effect of Humic Acid Application on Root Growth and Nutrient Elements Uptake of Maize (Zea mays L.) plant. (Master's Thesis, Ankara University Graduate School of Natural and Applied Sciences)

Güzel, A. (2006). Investigation of Effects of Abscisic Acid (ABA) and Calcium (Ca2+) on the Several Physiological and Growth Parameters in Tomato Plants Exposed to Drouhgt Stress. (Master's Thesis, Mersin University Graduate School of Applied and Natural Sciences)

Haghighi, M., Teixeira Da Silva, J.A. (2013). Amendment of hydroponic nutrient solution with humic acid and glutamic acid in tomato (Lycopersicon esculentum Mill.) culture, Soil Science and Plant Nutrition, 59(4), 642-648. https://doi.org/10.1080/00380768.2013.809599

Haghighi, M., Teixeira Da Silva, J.A., Mozafariyan, M., Roustaii, F. (2012). Humic acid affects the germination of basil and cumin and alleviates the negative impacts of salinity and drought stress. Global Science Books, Medicinal and Aromatic Plant Science and Biotechnology, 6(1), 63-67.

Haider, G., Koyro, H.W., Azam, F., Steffens, D., Müller, C., Kammann, C. (2014). Biochar but not humic acid product amendment affected maize yields via improving plant-soil moisture relations. Plant and soil, 395, 141-157. https://doi.org/10.1007/S11104-014-2294-3

Hoagland, D.R., Arnon, D.I. (1950). The water-culture method for growing plants without soil circular.

California Agricultural Experiment Station, 347(2), 32.

Ighodaro, O.M., Akinloye, O.A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria journal of medicine, 54(4), 287-293. https://doi.org/10.1016/j.ajme.2017.09.001

Islam, M.R., Islam, R., Dutta, T., Jhelom, F.R. (2023). Genetic and microbial insights into drought stress alleviation in tomato (Solanum lycopersicum L.). Biology and Life Sciences Forum, 27(1), 22. https://doi.org/10.3390/IECAG2023-15223

Joshi, R.K., Bharat, S.S., Mishra, R. (2020). Engineering drought tolerance in plants through CRISPR/Cas genome editing. 3 Biotech, 10(9), 400. https://doi.org/10.1007/s13205-020-02390-3

Kabay, T., Şensoy, S. (2016). Kuraklık stresinin bazı fasulye genotiplerinde oluşturduğu enzim, klorofil ve iyon değişimleri. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 26(3), 380-395.

Kalyoncu, Ö. (2013). The Effect of Humic Acid on Growth and Ion Uptake of Mung Bean (Vigna radiata (L.) R. Wilczek) Grown Under Salt Stress. (Master's Thesis, Marmara University Graduate School of Applied and Natural Sciences)

Kaya, C., Şenbayram, M., Akram, N.A., Ashraf, M., Alyemeni, M.N., Ahmad, P. (2020). Sulfur-enriched leonardite and humic acid soil amendments enhance tolerance to drought and phosphorus deficiency stress in maize (Zea mays L.). Scientific Reports, 10, 6432. https://doi.org/10.1038/S41598-020-62669-6

Kayabaşı, S. (2011). Drought Stress in The Soybean (Glyncine max. L.) With Some Physiogical Parameters Investigation of Accumulations Proline. (Master's Thesis, Harran University Graduate School of Natural and Applied Sciences)

Khan, M.A., Asaf, S., Khan, A.L., Jan, R., Kang, S., Kim, K., Lee, I. (2020). Extending thermotolerance to tomato seedlings by inoculation with sa1 isolate of Bacillus cereus and comparison with exogenous humic acid application. Plos ONE, 15(4), E0232228. https://doi.org/10.1371/Journal. Pone.0232228

Khan, S.H., Khan, A., Litaf, U., Shah, A.S., Khan, M.A., Bilal, M., Ali, M.U. (2015). Effect of drought stress on tomato cv. bombino. Journal of Food Processing and Technology, 6(7), 1000465. https://doi.org/10.4172/2157-7110.1000465

Khorasaninejad, S., Ahmadabadi, A.A., Hemmati, K. (2018). The effect of humic acid on leaf morphophysiological and phytochemical properties of Echinacea purpurea L. under water deficit stress. Scientia Horticulturae, 239, 314-323. https://doi.org/10.1016/J.Scienta.2018.03.015

Kılıç, B. (2020). Investigation of the Effects of Proline Pre-treatment on Germination of Black Pine Seeds Under Drought Stress. (Master's Thesis, Artvin Çoruh University Graduate School of Natural and Applied Sciences)

Kılıçaslan Caşka, S. (2019). The Effect of Drought Stress on Plant Growth, Some Physiological and Biochemical Properties of Bean. (Master's Thesis, Atatürk University Graduate School of Natural and Applied Sciences)

Kıran, S., Kuşvuran, Ş., Özkay, F., Ellialtıoğlu, Ş.Ş. (2016). Tuza tolerant ve hassas patlıcan genotiplerinin kuraklık stresi koşullarında bazı morfolojik özelliklerinde meydana gelen değişimler. Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi, 21(2), 130-138.

Kıran, S., Furtana, G.B., Talhouni, M., Ellialtıoğlu, Ş.Ş. (2019). Drought stress mitigation with humic acid in two Cucumis melo L. Genotypes differ in their drought tolerance. Bragantia, Campinas, 78(4), 490-497. https://doi.org/10.1590/1678-4499.20190057

Kıran, S., Özkay, F., Kuşvuran, Ş., Ellialtıoğlu, Ş.Ş. (2014). Tuz stresine tolerans seviyesi farklı domates genotiplerinin kuraklık stresi koşullarında bazı özelliklerinde meydana gelen değişimler. Journal of Agricultural Faculty of Gaziosmanpaşa University, 31(3), 41-48.

Kireçci, O.A. (2012). The Investigation of Some Signal Transduction Biomolecules in Triticum aestivum L. (Wheat Plant) Exposed to Drougt Stress. (Ph.D. Thesis, Inonu University Graduate School of Natural and Applied Sciences)

Kocamanoğlu, Ç. (2018). The Effect of Growing Media and Humic Acid Treatments on Some Yield Properties of Purslane (Portulaca oleracea L.) (Master's Thesis, Ordu University Institute of Natural and Applied Sciences)

Kocheva, K., Lambrev, P., Georgiev, G., Goltsev, V., Karabaliev, M. (2004). Evaluation of chlorophyll fluorescence and membrane injury in the leaves of barley cultivars under osmotic stress. Bioelectrochemistry, 63(1), 121-124. https://doi.org/10.1016/j.bioelechem.2003.09.020

Koç, M. (2020). Physiological Biochemical and Molecular Responses of Some Grape Varieties Against Drought Stress. (Ph.D. Thesis, Tokat Gaziosmanpasa University Graduate School of Natural and Applied Sciences)

Korkmaz, K. (2018). Investigation on the Effects of Humic Acid and Silicon Under Water Stress in Strawberry. (Master's Thesis, Harran University Graduate School of Natural and Applied Sciences)

Kuşvuran, Ş., Kıran, S.U., Altuntaş, Ö. (2020). The morphological, physiological and biochemical effects of drought in different pepper genotypes. Food Science and Technology, 8(6), 1359-1368. https://doi.org/10.24925/Turjaf.V8i6.1359-1368.3375

Maibodi, N.D.H., Kafi, M., Nikbakht, A., Rejali, F. (2015). Effect of foliar applications of humic acid on growth, visual quality, nutrients content and root parameters of perennial ryegrass (Lolium perenne L.). Journal of Plant Nutrition, 38(2), 224-236. https://doi.org/10.1080/01904167.2014.939759

Manna, M., Thakur, T., Chirom, O., Mandlik, R., Deshmukh, R., Salvi, P. (2021). Transcription factors as key molecular target to strengthen the drought stress tolerance in plants. Physiologia Plantarum, 172(2), 847-868. https://doi.org/10.1111/ppl.13268

Nanjo, T., Kobayashi, M.Y., Yoshiba, Y., Kakubar, K., Yamaguchi, S.Y., Shinozaki, K. (1999). Antisense

suppression of the proline degradation improves 123 tolerance to freezing and salinity in Arabidopsis thaliana. Febs Letters, 461(3), 205-210.

Ohkawa, H., Ohishi, N., Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2), 351-358

Oktay Yiğit, Ö. (2018). Effect of Humic Acid on Some Genes in Wheat Seeds in Salinity Temperature Stresses. (Master's Thesis, Mustafa Kemal University Graduate School of Applied and Natural Sciences)

Özkur, Ö. (2010). Antioxidant Defence System in Capparis ovata Plants Under Drought Stress. (Ph.D. Thesis, Ege University Graduate School of Applied and Natural Sciences)

Parveen, A., Rai, G.K., Mushtaq, M., Singh, M., Rai, P.K., Rai, S.K., Kundoo, A.A. (2019). Deciphering the morphological, physiological and biochemical mechanism associated with drought stress tolerance in tomato genotypes. International Journal of Current Microbiology and Applied Sciences, 8(5), 227-255. https://doi.org/10.20546/İjcmas.2019.805.028

Pettit, R. E. (2004). Organic matter, humus, humate, humic acid, fulvic acid and humin: their importance in soil fertility and plant health. CTI Research, 10, 1-7.

Qin, K., Leskovar, D.I. (2020). Humic substances improve vegetable seedling quality and post-transplant yield performance under stress conditions. Agriculture Journal, 10, 254. https://doi.org/10.3390/Agriculture10070254

Raja, V., Qadir, S.U., Alyemeni, M.N., Ahmad, P. (2020). Impact of drought and heat stress individually and in combination on physio-biochemical parameters, antioxidant responses and gene expression in Solanum lycopersicum. 3 Biotech, 10, 208. https://doi.org/10.1007/S13205-020-02206-4

Sadak, A. (2018). The Effect of PGPR on Pepper Seedlings Under Drought Stress. (Master's Thesis, Yüzüncü Yıl University Graduate School of Applied and Natural Sciences)

Saibi, W., Brini, F. (2018). Superoxide dismutase (SOD) and abiotic stress tolerance in plants: An overview. In Superoxide Dismutase: Structure, Synthesis and Applications. (pp. 101-142)

Sanchez-Rodriguez, E., Rubio-Wilhelmi, M., Cervilla, L.M., Blasco, B., Rios, J.J., Rosales, M.A., Romero, L., Ruiz, J.M. (2010). Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant Science, 178, 30-40.

Shen, J., Guo, M., Wang, Y., Yuan, X., Dong, S., Song, X., Guo, P. (2020a). An investigation into the beneficial effects and molecular mechanisms of humic acid on foxtail millet under drought conditions. Plos ONE, 15(6), E0234029. https://doi.org/10.1371/Journal.Pone.0234029

Shen, J., Guo, M.J., Wang, Y.G., Yuan, X.Y., Wen, Y.Y., Song, X.E., & Guo, P.Y. (2020b). Humic acid improves the physiological and photosynthetic characteristics of millet seedlings under drought stress. Plant Signaling & Behavior, 15(8), 1774212. https://doi.org/10.1080/15592324.2020.1774212

Smart, R.E., Bingham, G.E. (1974). Rapid estimates of relative water content. Plant Physiology, 53, 258-260.

Tartoura, K.A.H. (2010). Alleviation of oxidative stress induced by drought through application of compost in wheat (Triticum aestivum L.) plants. American-Eurasian Journal of Agricultural & Environmental Sciences, 9(2), 208-216.

Taşğın, E., Nadaroğlu, H., Adıgüzel, A., Baltacı, M.Ö., Sönmez, Z. (2017). Soya bitkisindeki glutatyon redüktaz aktivitesi ve mrna seviyesinin kuraklık stresinde salisilik asit ile değişimi. Apjes, 5(2), 48-52.

Ural, C. (2020). The Effect of Humic Acid on Some Physiological Parameters and Gene Expressions in Tomato (Solanum lycopersicum) Under Salt Stress. (Master's Thesis, Isparta University of Applied Sciences the Institute of Graduate Education)

Vaughan, D. (1985). Effect of humic substances on metabolic processes in plants. In Humic Substances Effects on Soil and Plants, 54-77.

Yaban, İ., Kabay, T. (2019). Kuraklık stresinin urfa biberinde iyon klorofil ve enzim içerikleri üzerine etkisi. Toprak Su Dergisi, 8(1), 11-17. https://doi.org/10.21657/Topraksu.544650

Yaban, İ. (2018). The Effect of Drought Stress on Some Landraces and Varieties of Urfa Pepper. (Master's Thesis, Yüzüncü Yıl University Graduate School of Applied and Natural Sciences)

Yadav, P., Ansari, M.W., Kaula, B.C., Rao, Y.R., Al Meselmani, M., Siddiqui, Z.H., Kumar, S.B., Rani, V., Sarkar, A., Rakwal, R., Gill, S.S., Tuteja, N. (2023). Regulation of ethylene metabolism in tomato under salinity stress involving linkages with important physiological signaling pathways. Plant Science, 334, 111736. https://doi.org/10.1016/j.plantsci.2023.111736

Yaman, M. (2016). Effects of Humic Acid, Amino Acid, Hormones and Micro Elements Applications on Quality and Yield at Strawberry Seedling. (Master's Thesis, Erciyes University Graduate School of Natural and Applied Sciences)

Yediyıldız, A.G. (2008). Application of Drought and Salt Stresses on Two Wheat Cultivars (Triticum aestivum L. cv. Bayraktar and Atay 85) for Monitoring the Activity of Antioxidative Enzymes. (Master's Thesis, Erciyes University Graduate School of Natural and Applied Sciences)

Young, I. S., Woodside, J. V. (2001). Antioxidants in health and disease. Journal of clinical pathology, 54(3), 176-186.

Zhou, R., Yu, X., Ottosen, C., Rosenqvist, E., Zhao, L., Wang, Y., Yu, W., Zhao, T., Wu, Z. (2017). Drought stress had a predominant effect overheat stress on three tomato cultivars subjected to combined stress. BMC Plant Biology, 17(24), 1-13. https://doi.org/10.1186/S12870-017-0974-X