Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 12(4): 2014 - 2026, 2022
Journal of the Institute of Science and Technology, 12(4): 2014 - 2026, 2022
ISSN: 2146-0574, eISSN: 2536-4618
Biology **DOI: 10.21597/jist.1149099**
Research Article
Received: 26.07.2022 **Accepted: 26.09.2022**

To Cite: Yetissin F, Kardeş I, 2022. Acetone O-(4-chlorophenylsulfonyl)Oxime as an Agent Alleviating the Adverse Effects of Drought Stress in Maize. Journal of the Institute of Science and Technology, 12(4): 2014 - 2026.

Acetone O-(4-chlorophenylsulfonyl)Oxime as an Agent Alleviating the Adverse Effects of Drought Stress in Maize

Fuat YETİŞSİN[1*], İnci KARDEŞ[2]

ABSTRACT: The negative effects of drought stress, which is the most effective type of stress on the yield loss of crops with a rate of 26% among abiotic stresses, are increasing day by day with global warming. The purpose of the study is to find out if Acetone O-(4 chlorophenylsulfonyl) oxime (AO) has positive effects on the metabolism of maize seedlings under drought or not. The following experimental setup was established: 18 hours distilled water Control (C), 6 hours 0.66 mM AO+12 hours distilled water (AO), 6 hours distilled water+12 hours 3% PEG (D), and 6 hours 0.66 mM AO+12 hours 3% PEG (AO+D). While ABA content decreased in AO application compared to control, it was determined that ABA decreased in AO+D application compared to D. While a difference could not be determined between AO by control and between D by AO+D applications on RWC content, it was observed that stress significantly reduced in RWC. It was determined that AO increased the Photosynthetic pigment content in the AO+D compared to the D. It was determined that AO reduced MDA and H_2O_2 content by regulating the activities of antioxidant system enzymes. It was observed that the proline content increased in AO application compared to control and in AO+D application compared to D. While ASA content decreased in AO application compared to control, it was determined that ASA content increased in AO+D application compared to D. Significant fluctuations in the contents of phenolic substances were determined. As a result, the pre-application of AO to maize under drought stress may prevent the formation of radicals, and this situation is thought to be due to the antioxidative properties of AO.

Keywords: Antioxidant system, water stress, zea mays, abscisic acid, phenolics

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This study was produced from İnci KARDEŞ's Master's thesis.

INTRODUCTION

As of the abiotic stress factors, drought stress is one of the most devastating factors on the growth, development, and productivity of plants. Its dramatic effects are considered to increase further in the future (Nadeem et al., 2019). The relevant stress factor constitutes 26% of natural stress factors. As the in case of drought stress faced, the plants exhibit critical morphological, biochemical, and Physiological plasticity as a response to the stress for their survival. Those responses are regarded as an active plant defense mechanism (Levitt, 1980). Plants protect and maintain their cellular structure through an orchestrated antioxidant system, viz. enzymatic and non-enzymatic compounds (Gill and Tuteja, 2010; Cramer et al., 2011).

Drought stress can cause moderate water loss causing stomata closure and restriction of gas exchange, and complete disruption of the cell structure and metabolic activities, so as a result, vegetative tissue in most plants cannot enter the process of recovery when the water ratio decrease below 30% (Smirnoff, 1993). As a common environmental stress factor that affects plant growth and yield, drought causes several molecular, biochemical, and physiological responses in plants. Consequently, plants can adapt to limited environmental conditions and develop some tolerance mechanisms (Arora et al., 2002). Water scarcity is firstly sensed in the roots of plants, and the abscisic acid (ABA) produced by roots regulates the closure of the stomata by moving to the leaves to prevent water loss (Pei et al., 2000). Pre-applications of various plant metabolites have been made to alleviate the undesirable effects of abiotic stresses (Ahmad et al., 2013). Pre-application of hormones and metabolites can be an efficient and cost-effective way to increase plants' abiotic stress tolerance (He et al., 2009; Hamdia and Shaddad, 2010).

In plants, oximes are chemical compounds that contain nitrogen and have very important functions, which are formed as a result of secondary metabolism (Sørensen et al. 2018). Since various organic sulfonate derivatives such as AO show anticancer, antimicrobial, apoptosis inducer, enzyme inhibition, chelating, and anti-inflammatory biological properties, the interest in these compounds is increasing day by day (Kendre et el. 2019; Kanabar et al. 2020; Taslimi et al. 2021; Su et al. 2021; Yetişsin and Kardeş, 2021; Korkmaz and Bursal, 2022; Şenkardeş et al. 2022). It can be said that Acetone O-(4-chlorophenylsulfonyl)oxime (AO), a sulfonated derivative of acetone oxime, can provide the attachment of radicals due to the atoms it has and the double bonds in its structure. Since electronegative or electropositive groups in the molecular structure are known to increase the stability of radicals, the electron-withdrawing -Cl group in the structure of AO has the feature of increasing the stability of radicals (Korkmaz and Duran, 2021; Korkmaz, 2021a, 2021b).

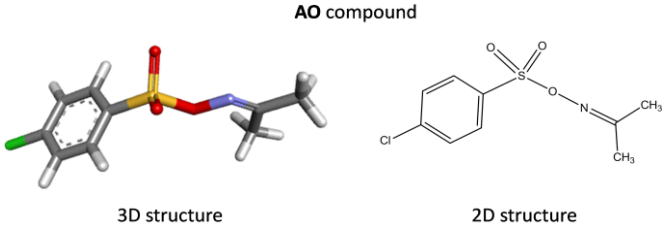

Figure 1. Structure of acetone O-(4-chlorophenylsulfonyl) oxime (AO)

Maize (*Zea mays L.*), a significant grain product grown across the world, is produced and consumed the most in China after the USA (Gale et al., 2014; Ashraf et al. 2016). Maize production, which is an important component of global food security, is on the rise because of the increasing demand in various industries (Rosegrant et al., 2012). Maize is very sensitive to water deficiency and is cultivated in large areas in both hemispheres (Haarhoff and Swanepoel, 2018). Drought stress not

only causes a decline in photosynthetic rate and relative water content in the maize plant, but also causes cellular drying, protein oxidation, free radical formation, impaired enzymatic activity, and lipid peroxidation (Hussain et al., 2019).

Along with the study, we hypothesized that the potential radical formation would be buffered/stabilized through an exogenous application of AO due to the chemical structure. In this regard, the protective effects of AO were assayed against drought stress in maize through an array of physiological and biochemical attributes. To our best knowledge, the effects of AO was for the first time examined for its potential effects against drought stress.

MATERIALS AND METHODS

Plant Materials and Treatments

Maize seeds, ADA-523 were sown into 10 pots with soil. Six seeds were sown in each pot and seedlings were left for a 4-week-cultivation in the growth chamber under a relative humidity of 60±5%, a light intensity of 400 µmol $m^{-2}s^{-1}$, a temperature of 25±2 ˚C, and a photoperiod of 16/8 hours. AO concentration was identified according to the preliminary test in which the measurement of the contents of MDA and H_2O_2 was carried out at 0.66 mM of AO. The results of drought stress experiments in previous studies have shown that 2% -10% PEG concentrations can be sufficient. In this study, it was decided that it would be more appropriate to use a PEG concentration of 3% as the root system of the plants is cut. The experiments were carried out in a randomized block design with 3 replications. Four seedlings were cut (2 cm above the ground level), put in the glass tubes with distilled water, and left in the tubes for one hour to decrease the effect of the cut on the seedlings. Then, they subjected the treatments of AO and PEG solutions, (0.66 mM and 3%, respectively). The treatment groups of the study were as follows: 18-hour distilled water Control (C), first 6-hour AO+ then 12-hour distilled water (AO), first 6-hour distilled water + then 12-hour PEG (D), and first 6-hour AO+ then 12-hour PEG (AO+D) (In many previous studies by our group, it was determined that 12 hours was suitable for drought and 6 hours was suitable for pre-application). After seedlings were subjected to these treatments, a liquid nitrogen treatment was carried out on them and then they were kept in a fridge at -20 ˚C until the following parameters were measured.

Determining relative water content (RWC)

RWC was determined using the following formula reported by Barrs and Weatherley, (1962):

RWC (%) = [(FW - DW)/(TW - DW)] x 100,

where FW refers to fresh weight, DW to dry weight, and TW to Turgid weight.

Determining the contents of chlorophyll and carotenoids

Carotenoid and chlorophyll contents of the maize leaves were determined using the technique reported by Arnon (1949). Briefly, 0.25 g of fresh leaves were subjected to homogenization with acetone (5 ml, 80%). The homogenate was centrifuged for five minutes at 5000 rmp at room temperature. The supernatant absorbance was measured at 450, 645, and 663 nm (Nicolet evolution 100, Thermo Scientific, USA). The determination of pigment contents was done using the equations reported by Lichtenthaler (1987).

Determining of malondialdehyde (MDA) content

The levels of lipid peroxidation were measured based on the MDA contents by the method proposed by

Heath and Packer (1968). The fresh maize leaves (0.5 g) were subjected to homogenization using a pestle and mortar in trichloroacetic acid solvent (0.1%). The mix was left for centrifugation for 5 minutes at 15000 rpm and then, TBA (thiobarbituric acid, 4 ml, 0.5%) in 20% TCA was transferred

to the supernatant (1 ml). The supernatant absorbance was measured at 600 and 532 nm. The difference between the absorbance values at 532 and 600 nm was calculated. The resulting value was used in the formula (A = E.c.1) to compute the MDA concentration (Ɛ =155 mM^{-1} cm^{-1}).

Determining of hydrogen peroxide (H_2O_2) content

H_2O_2 contents were determined according to the method reported by Velikova et al. (2000). The fresh leaves (0.25 g) were subjected to homogenization by crushing with charcoal (0.1 g) in TCA (5 ml, 0.1 %). The mix was centrifuged for 15 minutes at 15 000 rpm and 4°C. Later, the supernatant (1000 µl) was put into a glass cuvette and mixed with 1 M KI (1500 µl) and phosphate buffer (10mM, pH 7.0). The solution absorbance was measured at 390 nm.

Determining the antioxidant enzyme activities

Determining the SOD (Superoxide Dismutase) activity

SOD (EC 1.15.1.1) activities were measured using the method reported by Beauchamp and Fridovich (1971). The reaction was started by adding a mixture of 50 µl plant extract, 0.1 mM EDTA, 50 mM potassium phosphate buffer (pH 7.0), and 13 mM NBT (nitro blue tetrazolium) to 2 µM riboflavin. The mix was subjected to "white light" at 375 µmol m^{-2} s^{-1} for 10 minutes. The absorbance was measured at 560 nm.

Determining the GPX (Guaiacol peroxidase) activity

GPX (EC 1.11.1.7) activities were measured in line with the method reported by Urbanek et al. (1991). The 2 ml reaction mixture of 100 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 5mM guaiacol, 15 mM H_2O_2, and 50 µl enzyme extract was measured at 470 nm for 1 minute to determine the enzyme activity. The GPX activities were computed using the extinction coefficient (ε=26.6 $mM^{-1}cm^{-1}$).

Determining the CAT (Catalase) activity

CAT (EC 1.11.1.6) activities were determined using the method reported by Aebi (1983). The enzyme activity was determined by means of measuring 1 mL mix that contains 20 µl enzyme extract, 30 mM H_2O_2, and 50 mM potassium phosphate buffer (pH 7.0) at 240 nm for 5 minutes. The enzyme activities were computed using the extinction coefficient (ε=39.4 $mM^{-1}cm^{-1}$) for H_2O_2.

Determining the APX (Ascorbate Peroxidase) activity

APX (EC 1.11.1.11) activities were determined relative to the decrease in the absorbance at 290 nm (Nakano & Asada 1981). The enzyme activities were determined by the measurement of 1mL reaction mix that contains 20µl enzyme extract, 5 mM H_2O_2, 250 µM ASC (ascorbate), and 50 mM potassium phosphate buffer (pH 7.0). The enzyme activities were computed using the extinction coefficient (ε=2.8 $mM.1cm^{-1}$) for ASC at 290 nm.

Determining of proline content

For quantification of proline content, 0.2 g of fresh leaves was subjected to homogenization in 10 mL sulphosalicylic acid (3%). After the mix was filtered, it was centrifuged for 5 minutes at 22 ºC and 5000 rpm. The supernatant (1 mL) was mixed with ninhydrin (1mL) and acetic acid (1 mL). Then, the mix was left for incubation for one hour at 100 ºC and cooled in ice. Later on, 3 mL of toluene was transferred onto the samples, and then the mix was vortexed. The samples were transferred into tubes and subjected to centrifugation for 5 minutes at 4000 rpm. The supernatant was put into the cuvette, and the optical density of the toluene solution was measured using a spectrophotometer at 520 nm (Bates et al., 1973). The results were expressed in µg g^{-1} fresh weight.

Determining of ABA, ASA and phenolic compounds contents

To determine the amount of abscisic acid (ABA), ascorbic acid (ASA) and phenolic contents by HPLC, the last concentrations of abscisic acid, ascorbic acid, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, transp-coumaric acid, myricetin, apigenin, kaempferol, catechol, rosmarinic acid and cinnamic acid standards were weighed to obtain solutions with a concentration of 10 mg mL^{-1}. Then, 1% acetonitrile and acetic acid were added (at a rate of 1/9, respectively) to the standards, and methanol was added at the same rates to prepare stock standards. The stock standards were used for the calibration curve after they were diluted as 10, 25, 50, 75, and 100 µg mL^{-1} (Tapan, 2016). The concentration of maize leaf extracts was diluted as 20 mg/mL by using the solutions that were used in the standard. The extracts were loaded to an HPLC by filtering with a 0.45 µm membrane filter. HPLC analysis was performed by using the Agilent Technologies 1260 Infinity II HPLC (Agilent, USA). The HPLC configuration was as follows: a G7130A column furnace (28°C), a 1260 Quat Pump VL pump with a flow rate of 1.0 mL/minutes, a 1260 DAD WR detector (at 272, 280, and 310 nm), and a 1260 Vial sampler (20 µL injected). ACE 5 C18 (250 x 4.6 mm) was used as the analytical column for the analysis.

Statistical analysis

The experiments were designed in randomized blocks with 3 repetitions. SPSS (v.17, SPSS Inc., U.S.A.) was used to analyze the data. Duncan's Multiple Range Test was applied to determine the statistical significance. Statistical significance was set at $P<0.05$ in all the analyses.

RESULTS AND DISCUSSION

Pigment Contents

The leaves' chlorophyll content was found to be statistically significantly higher in AO alone treatment than in the others (Figure 2). AO+D treatment significantly increased chlorophyll content compared to drought treatments. AO+D treatment was observed to yield the significantly highest carotenoid content. The control treatment had the lowest carotenoid content. Carotenoid content of D treatment was higher than AO application and lower than AO+D application. Drought stress decreases the contents of total carotenoid and chlorophyll in soybean under drought stress (Basal et al., 2020). Mishra et al., (2020) asserted that the total chlorophyll contents significantly decreased in maize seedlings subjected to drought stress. In the present study, we found that total chlorophyll of AO alone treatment was significantly higher than other treatments, also AO+D treatment prevented degeneration of chlorophyll compared to drought. As for carotenoid contents, drought treatments increased carotenoid contents compared to control. Conspicuously, seedlings treated with AO+D were found to have a better carotenoid content compared to drought application. Results obtained from seedlings under drought stress showed that AO had a protective effect on the contents of both carotenoid and chlorophyll.

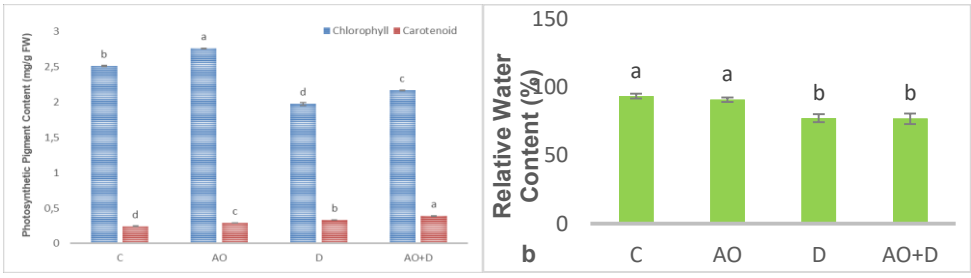

Figure 2. The effect of AO application on RWC and photosynthetic pigment content under drought stress conditions. The difference between the bars with the same letters on the columns is insignificant ($P < 0.05$)

Relative water content (RWC)

The maize leaf RWC was given in Figure 2b. Drought alone treatment significantly decreased water content in comparison to control and solo treatment of AO ($p<0.05$). A significant difference between C and AO, and also between D and AO+D treatments in terms of water content was not determined. Drought stress causes a decrease in the RWC content (Hussain et al., 2019). Elbasan et al., (2020) noted that *Oryza sativa* exposed to drought stress yielded a decreased water content, and the application of scandium improved the water content. In the current study, while RWC was the highest at the control and AO alone application, drought and AO+D treatments reduced RWC compared to control and AO alone. However, a statistically significant difference could not be found between control and AO alone, and also between drought and AO+D.

Hydrogen peroxide (H_2O_2) Content

Figure 3a shows the hydrogen peroxide contents. AO treatment was found to have the least effect on H_2O_2 formation compared to the control, drought, and AO+D treated seedlings. Conspicuously, the significantly highest hydrogen peroxide content was observed in the groups subjected to drought. AO+D application significantly alleviated the phytotoxic effect of H_2O_2 compared to drought application.

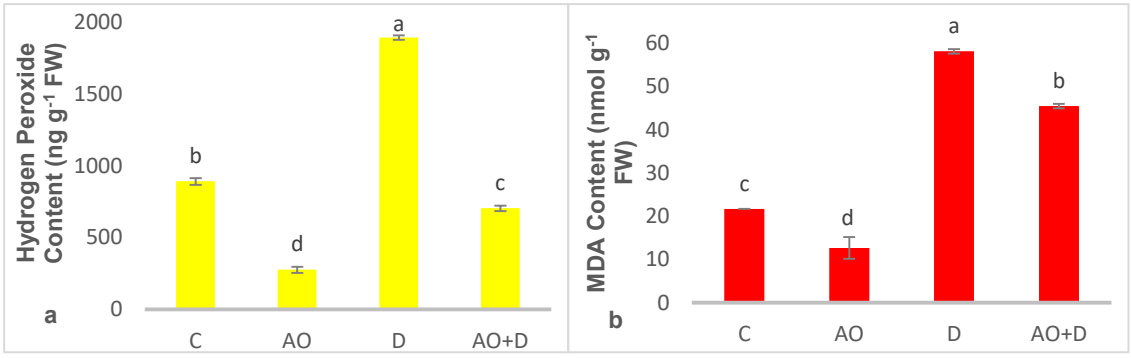

Figure 3. The effect of AO application on MDA content and hydrogen peroxide content under drought stress conditions. The difference between the bars with the same letters on the columns is insignificant ($P < 0.05$)

Malondialdehyde (MDA) content

The effects of AO on the membrane damage were examined through MDA analysis (Figure 3b). The drought treatment was found to yield the highest MDA content, while the AO treatment was found to yield the lowest MDA content. A significant MDA formation decrease was observed in the AO+D treatment compared to the drought treatment. MDA is known to be a product of lipid peroxidation that occurs as a result of ROS accumulation in stressed plants (Mittler, 2002). It was stated that MDA content increased in *Brassica rapa* plants under the conditions of drought stress, and a statistically significant decrease occurred in MDA content with salicylic acid pre-application (Lee et al. 2019). In the current study, AO application reduced the level of MDA formation compared to control. Similarly; The fact that AO+D application significantly reduced MDA content compared to D suggests that AO alleviates the negative effects of stress on the membrane system. In a study carried on *Oryza sativa* seeds, Lui et al. (2019), stated that drought stress caused an increase in the MDA content, similar to our study. The decrease in MDA content in AO+D application compared to drought application and the accompanying decrease in H_2O_2 content indicates that the AO substance contributes significantly to the scavenging of ROS.

Antioxidant enzyme activities

SOD enzyme activity

The seedlings subjected to drought showed a considerably increased SOD activity than those not subjected to drought. The seedlings subjected to AO were found to show the higher SOD activity compared to own controls (Figure 4a).

GPX enzyme activity

AO application was found to cause a significant increase in GPX activity compared to all other applications. AO+D treated seedlings decreased GPX activity level compared to drought (Figure 4b). AO+D applied seedlings had the lowest GPX activity compared to the control application.

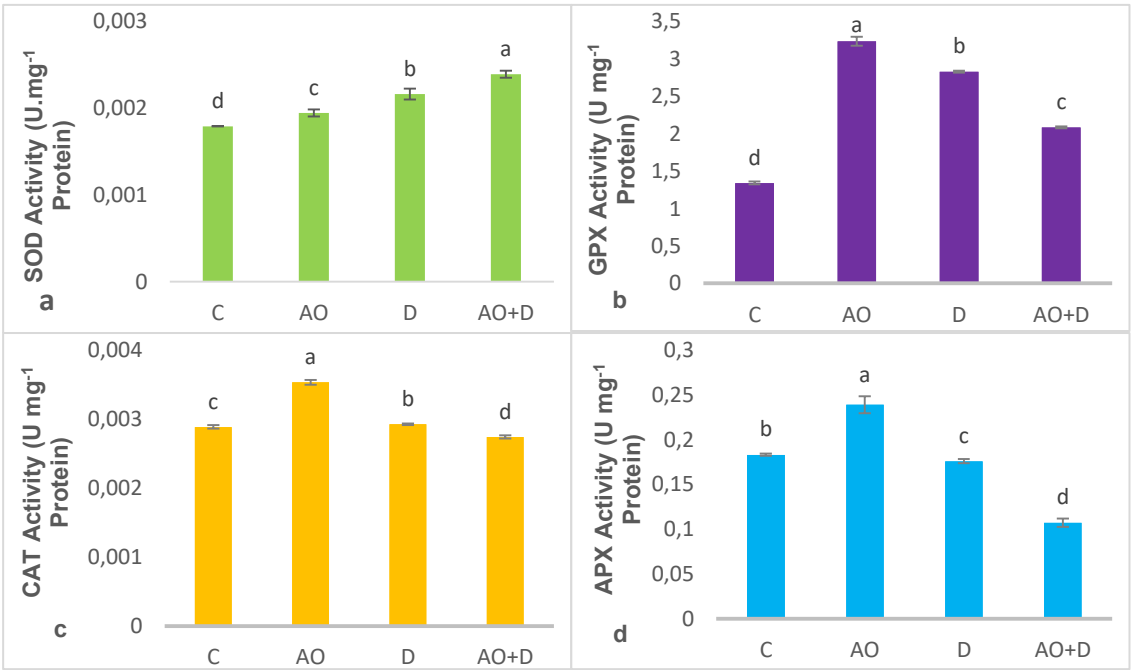

Figure 4. The effect of AO application on the activities of CAT, GPX, APX, and SOD under drought stress conditions. The difference between the bars with the same letters on the columns is insignificant ($P < 0.05$)

CAT enzyme activity

AO+D treatment was found to cause the lowest CAT activity compared to all other treatments. Seedlings exposure to AO alone has the highest CAT activity. Seedlings exposed to drought increased CAT activity compared to control (Figure 4c).

APX enzyme activity

The AO+D was found to cause the lowest APX activity in the seedlings among all the treatments. On the other hand, the treatment of AO alone yielded the highest APX activity. The drought-treated seedlings were found to have a statistically significantly lower APX activity than those in the treatment groups of control and AO alone (Figure 4d).

Drought induces a redox reaction and causes the formation of H_2O_2 and superoxide (O_2•) (Hossain et al., 2019; Lee et al., 2019). The hydroxyl radicals (•OH) are formed by the Haber-Weiss reaction, that is, the reaction of superoxide (O_2 •-) and H_2O_2. Increased drought stress can induce Fenton reaction, causing oxidative damage (Smirnoff 1993). This mechanism might explain the H_2O_2 accumulation observed in drought-treated seedlings (Figure 3a). Moreover, the SOD, APX, GPX, and CAT activities were found to be higher in these experimental groups compared to others (Figures 4a, 4b, 4c, and 4d). The reason for this situation may be that the ascorbic acid content is higher in drought application compared to control (Figure 6B). In a study conducted on *Amarantus tricolor* leaf, it was

noted that drought stress application increased the ASA content significantly (Sarker and Oba, 2018). Drought stress could lead to oxidative stress in plants and could consequently cause an increase in the antioxidant responses because of the increase in the production of oxygen free radicals that are highly toxic (Hossain et al., 2020). Additionally, the AO alone treatment was found to yield the lowest level of H_2O_2 (Figure 3a).

Abscisic acid (ABA) content

Abscisic acid (ABA), which is considered a sesquiterpene from isoprenoid metabolites, is a phytohormone that has a serious relationship with stress. It was determined that the seedlings exposed to drought had significantly the highest ABA content. Importantly, the ABA content of AO+D-treated seedlings was lower than that of drought treated seedlings. In addition, it is a remarkable result that the ABA content of the seedlings treated with only AO was statistically significantly reduced compared to the control (Figure 5). Abscisic acid (ABA) is an important isoprenoid phytohormone responsible for activating drought resistance (Gai et al., 2020). The fact that the content of ABA, a phytohormone with important functions in plant metabolism, decreased in AO+D application compared to drought application, indicates that AO application alleviates the adverse effects of stress. Lui et al. (2019) stated that ABA content increased in *Oryza sativa* seeds under drought stress conditions. In this study, it was determined that the ABA content of seedlings treated with AO+D was higher than seedlings under drought stress conditions (Figure 5). AO application before drought stress (AO+D) seems to indicate that AO activates metabolic defense mechanisms more strongly due to its metabolic effect.

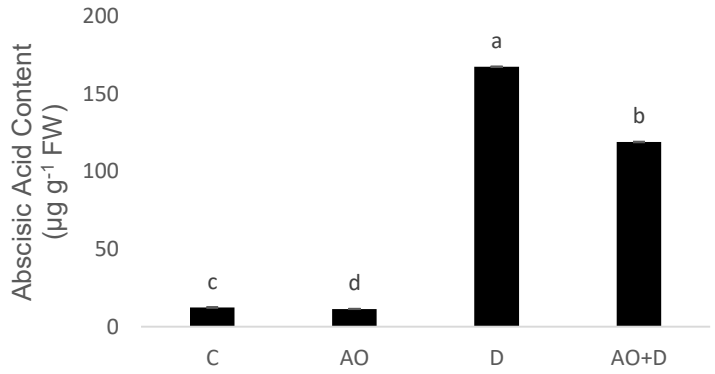

Figure 5. The effect of AO application on ABA contents under drought stress conditions. The difference between the bars with the same letters on the columns is insignificant ($P < 0.05$)

Proline content

Figure 6A shows the proline contents of the maize leaves. AO application increased significantly proline content compared to control. Also, the application of AO+D was found to cause a higher proline production in the seedlings compared to all other applications. Drought application increased significantly the proline production compared to control treatments. Kadıoglu et al., (2002) stated that the proline level in the leaf increased to provide osmotic adjustment in *C. setosa* plant under drought stress depending on the degree of stress. In this study, it was found that AO alone application had an increasing effect of proline content on proline formation compared to control. In addition, it can be said that the higher level of proline content was determined in AO+D application compared to D application, and AO pre-application to maize seedlings under drought stress contributed to alleviating the adverse effects of stress by contributing to osmotic regulation and reducing the rate of ROS formation. Similar to our study, proline content increased in the leaves and roots of *Glycyrrhiza glabra L*. plants subjected to drought stress (Hosseini et al. 2018).

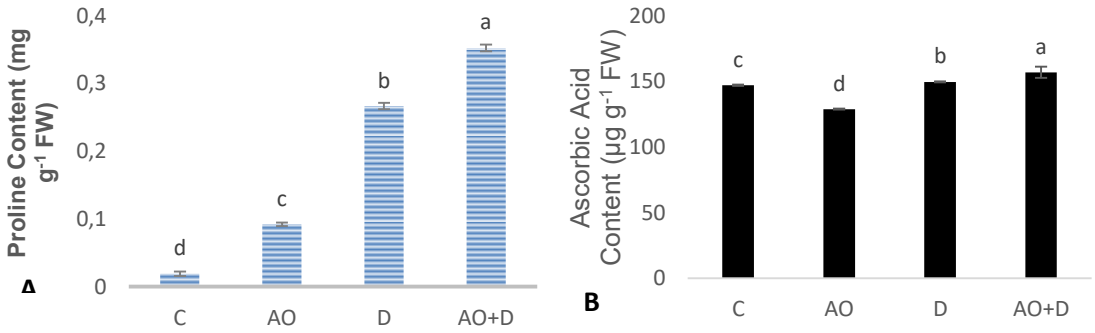

Figure 6. The effect of AO application on proline and ASA contents under drought stress conditions. The difference between the bars with the same letters on the columns is insignificant ($P < 0.05$)

Ascorbic acid (ASA) content

A significant fluctuation was observed in the content of ascorbic acid (ASA), which is one of the important non-enzymatic components of the antioxidant system. Conspicuously, the ASA content of seedlings exposed to AO+D was significantly higher than drought treatment. The AO treatment had the lowest ASA content, as the application of AO alone reduced the ASA content compared to the control (Figure 6B). Conti et al. (2022), in a study they conducted with 12 different tomato cultivars exposed to drought stress, stated that drought stress caused an increase in the ASA content of some cultivars while a decrease in some cultivars. The fact that ASA content was found to be the highest in AO+D application in the current study can be considered as one of the important signs that AO has positive effects on the antioxidant system of maize seedlings under drought stress. In a study on the external spraying of ASA on the leaves of a drought-stressed pepper plant, it was reported that the ASA content increased significantly as a result of the application of ASA to the peppers under drought stress (Khazaei and Estaji, 2020).

Content of phenolic substances

It was determined that the total phenolic content of maize seedlings exposed to drought stress increased significantly compared to the control. The fact that the total phenolic content of AO+D application decreased significantly compared to the D application and was close to control, suggests that AO pre-application causes a decrease in the amount of phenolic substances by encouraging interaction with the antioxidant system and makes a significant contribution to plant defense. The lowest trans-p-coumaric acid content was observed in AO application, while the highest content was determined in AO+D application. A significant increase was observed in the D treatment compared to the control. There was no statistically significant difference between control and AO+D treatments. The lowest Myricetin content was observed in the AO application, while the highest content was determined in the control and AO+D application. A significant decrease was observed in D application compared to control. The lowest content of 4-Hydroxybenzoic acid was observed in the AO+D application, while the highest content was determined in the D application. A significant decrease was observed in the AO application compared to the control. While 3,4-Dihydroxybenzoic acid content increased in drought application compared to control, a significant decrease was determined when compared with AO+D application. While the apigenin content decreased in the drought application compared to the control, a significant increase was detected when compared to the AO+D application. A significant increase was noted in AO application compared to drought and control. In terms of kaempferol content, a significant increase was determined in drought application compared to AO+D application. The highest Catechol content was observed in the control application, while the lowest content was determined in AO. A significant decrease was determined in drought application

compared to AO+D application. Although there was no significant fluctuation in Cinnamic acid content, it was observed that drought treatment significantly increased Cinnamic acid content compared to control. However, no statistical difference could be determined between D and AO+D applications. While a significant increase was observed in rosmarinic acid content with AO application compared to the control, a significant increase was observed in the AO+D application compared to the control. The content of phenolic substances showed statistically significant fluctuations depending on the applications (Table 1). Phenolic compounds provide H^+ to free radicals and eliminate their harmful effects. An increase in the amount of phenolic compounds is observed under stress conditions (Kaya and Artuvan, 2016). In parallel with this study, it was determined that the total phenolic content of maize seedlings exposed to drought stress increased significantly compared to the control. The fact that the total phenolic content of AO+D application decreased significantly compared to the D application and was close to control, suggests that AO pre-application causes a decrease in the amount of phenolic substances by encouraging interaction with the antioxidant system and makes a significant contribution to plant defense. It has been stated that the pretreatment of phenolics-rich extracts under abiotic stress conditions helps plants against the adverse effects of stress on cell components by scavenging radicals (Afzal et al., 2022).

Table 1. The effects of AO treatment on phenolic compound amounts under drought stress conditions (µg g^{-1} FW). The difference between same-letter values is insignificant ($P< 0.05$).

Phenolic substances	C	AO	D	AO+D
trans-p-coumaric acid	17.242±0.004^{c}	16.53±0.04^{d}	19.9±0.19^{b}	20.83±0.005^{a}
Myricetin	44.16±0.5^{a}	30.42±0.9^{c}	41.64±0.6^{b}	44.54±0.5^{a}
4-Hydroxybenzoic acid	35.33±0.15^{b}	28.84±0.08^{c}	55.0±0.05^{a}	11.51±0.12^{d}
3,4-Dihydroxybenzoic acid	15.29±0.05^{d}	16.27±0.09^{c}	25.61±0.13^{b}	32.17±0.27^{a}
Apigenin	17.42±0.22^{c}	23.70±0.19^{b}	10.15±0.03^{d}	27.48±0.03^{a}
Kaempferol	N/A	10.07±0.02^{b}	12.31±0.008^{a}	9.93±0.004^{b}
Catechol	31.82±0.03^{a}	6.26±0.25^{d}	22.30±0.01^{b}	11.60±0.03^{c}
Cinnamic acid	8.08±0.03^{b}	7.59±0.002^{b}	9.51±0.03^{a}	9.64±0.03^{a}
Rosmarinic acid	6.21±0.004^{c}	6.94±0.002^{b}	N/A	7.48±0.06^{a}
Total phenolic compound	185.552	146.62	203.42	175.18

CONCLUSION

When all the findings of the current study, which aims to examine whether AO pre-application on maize seedlings under drought stress has positive effects on the physiological and biochemical parameters of the plants, are evaluated as a whole; it was determined that AO pre-application contributed to the decrease of H_2O_2 level by affecting the antioxidant system and phenolic content in order to alleviate the adverse effects of drought stress, and in this case, it caused a decrease in MDA content. The effects of this improvement were demonstrated through both the photosynthetic pigment contents and the stabilization of the ABA content, which is one of the most important indicators of the general condition of the system. This can be explained by the fact that AO may be a metabolic regulator and have high antioxidant effects. As a result, maize is an important industrial agricultural product in energy production as well as being consumed as a food product. Although it has been shown by the current study that AO pre-application to maize seedlings under drought stress alleviates the adverse effects of stress on biochemical parameters, it is obvious that it should be supported by further studies to reduce yield losses with field studies.

ACKNOWLEDGEMENTS

This study was funded by Committee of Scientific Research Projects, Muş Alparslan University (Grant No: MŞÜ-BAP-20-TBMY-4902-02). Thanks to Ph.D. Adem KORKMAZ, who synthesized the AO compound and the Sakarya Maize Research Institute, Turkey, that supplied the maize seeds, cultivar ADA-523.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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