*Pak. J. Bot.*, 57(6): DOI: http://dx.doi.org/10.30848/PJB2025-6(22)

# EFFECTS OF ABA AND NITRIC OXIDE IN CHICKPEA PLANTS UNDER DROUGHT STRESS

**FUSUN YUREKLI[1] AND OGUZ AYHAN KIRECCI[2*]**

*[1]Inonu University, Faculty of Arts and Science's Department of Biology, Malatya, Türkiye*
*[2]Bitlis Eren University, Hizan Vocational School, Department of Plant and Animal Production, Bitlis, Türkiye*
**Corresponding author Email: kireccioguzayhan@gmail.com*

**Abstract**

In this study, the levels of nitric oxide (NO), proline concentration, lipoxygenase (LOX) activity, malondialdehyde (MDA) level, and total chlorophyll levels were investigated in the leaves of two different chickpea varieties, sensitive (Aksu) and tolerant (Canıtez) to drought stress conditions. To achieve this aim, drought stress was induced by PEG 4000 treatment at the end of day 25. Both drought-stressed and control groups were established for both cultivars. The control group plants were irrigated with Hoagland's culture solution throughout the experiment. Subsets of both chickpea cultivars exposed and unexposed to drought stress were treated with exogenous applications of 100 µM of sodium nitroprusside (SNP, as NO donor), cPTIO [2-(4-carboxyphenyl)-4,4,5,5 tetramethylimidazoline-1-oxyl-3-oxide potassium, as NO scavenger], and Abscisic acid (ABA) for 6 days. The treatments included Control (Hoagland's culture solution), Control + SNP, Control + c-PTIO, Control + ABA, PEG, PEG+SNP, PEG+ c-PTIO, and PEG+ ABA groups. Leaf samples were collected on days 0, 3, and 6 for analysis. All experiments, including germination and growth stages, were conducted under controlled conditions in a plant growth chamber. According to the results obtained, inhibition of nitric oxide synthesis led to increased oxidative stress. Similarly, application of abscisic acid alleviated the adverse effects of drought. Furthermore, prolonged drought stress conditions resulted in more oxidative damage, and both nitric oxide and abscisic acid exhibited positive effects in this scenario as well.

**Key words:** Abscisic acid, Antioxidan enyzmes, Chickpea, Stress, Nitric oxide.

## Introduction

Among environmental factors, drought stress is the most limiting factor for crop yield and quality in economically important plants (Roch *et al*., 2009). Water scarcity influences numerous biochemical and physiological processes in plants, ranging from photosynthesis to protein synthesis (Hu & Schmidhalter, 1998; Vranova *et al*., 2002; Chebab *et al*., 2009). Under these circumstances, drought stress is one of the most common environmental stressors that influence growth and yield and create many physiological, biochemical, and molecular responses in plants. As a meteorological term, drought refers to a long-lasting arid period. It occurs depending on the water-holding capacity of the soil and the evapotranspiration rate of plants in arid periods to cause drought (Ahluwalia *et al*., 2021). (ABA) Abscisic acid, a plant growth regulator, has many physiological effects on the growth and differentiation of highly organized plants. ABA is defined as a precursor for detecting stress and creating a response to drought and other environmental stresses. ABA, at the same time, plays a role in stomatal movement, photosynthesis, and gene expression (Singh & Roychoudhury, 2023). Several studies have indicated that ABA can improve resistance to environmental stress via both external ABA treatment and internal ABA measurements (Yin *et al*., 2004). Drought may cause stomatal closure and elevated biosynthesis of ABA, which is a stress hormone, and drought may even induce the genes to respond to ABA. In the last decade, ABA and stress response genes have been identified through molecular and biochemical studies (Yu & Setter, 2003; Buchanan *et al*., 2005; Poroyko *et al*., 2007).

NO is a signaling molecule that was first identified in mammals. However, studies conducted in 1988 showed that NO also acts as a signaling molecule in plants, and the number of studies on the role of NO in plants has increased (Kolbert *et al*., 2019). Because NO is a free radical, has a small size, short lifetime, easily diffuses from biological membranes, and plays a multifunctional role in plant growth, development, and regulation, plant cell mechanisms have brought NO an important place in the plant science world. Recently, NO has become a critical factor in the tolerance of plants to abiotic stress. The response of NO to environmental stress is also of interest. Previous studies have revealed that NO is an antioxidant molecule that acts as a radical scavenger under abiotic stress. NO has been shown to play an important role in salt, drought, temperature (high and low), UV-B, and heavy metal stress resistance (Xu *et al*., 2011, Siddiqui *et al*., 2010; Fancy *et al*., 2017; Mata-Pe´rez *et al*., 2023). Proline synthesis is a nonspecific response in environments with low water potential; therefore, it is synthesized under water stress as well as salt stress (Ashraf and Harris, 2004). Under normal circumstances, proline accumulation in the cytosol contributes to the osmotic adjustment of the cytoplasm (Parvaiz & Satyawati, 2008). In addition to being an osmotic regulator, proline, which stores the nitrogen and carbon required for recovery and growth after stress, is thought to be an osmotic regulator (Jain *et al*., 2001) and also plays a role in the stabilization of subcellular structures (such as membranes and proteins), catching free radicals, maintaining cellular redox potential (Vijayan, 2009), reducing cytoplasmic acidosis, and maintaining the appropriate NADP+/NADPH ratio in metabolism (Hare & Cress, 1997). One of the major factors in the coordinated response of plants to water deficiency is ABA, which is produced in both roots and leaves exposed to stress. ABA, a stress hormone, plays a dominant role in gene expression during water deficiency; however, NO has attracted attention as a component of the drought signal network in recent years. It has been reported that NO plays a role in reducing ABA during physiological activities, initiating

adaptive responses in stomatal transpiration and water deficiency, and accumulation of NO in guard cells re-regulates actin microfilaments against osmotic stress and regulates vacuoles, inducing stomatal closure (Arasimowicz *et al*., 2009). Additionally, exogenous administration of NO could induce ABA synthesis under water deficiency, which could be reversed by applying c-PTIO as an NO scavenger (Zhang *et al*., 2023).

Chickpeas are an important source of dietary protein and contain substantial quantities of vitamins and minerals (Alajaji *et al*., 2006). Chickpeas (*Cicer arietinum* L.) have significant agricultural and dietary importance worldwide, particularly in Afro-Asian regions. Owing to its carbohydrate and protein content, chickpeas boast superior protein quality compared to other pulses. Although lacking sulfur-containing amino acids, which are essential for protein synthesis, this deficiency can be compensated for by incorporating cereals into daily diets. Despite their low lipid content, chickpeas are abundant in nutritionally valuable unsaturated fatty acids such as linoleic and oleic acids. Sterols, such as β-sitosterol, campesterol, and stigmasterol, also contribute to the nutritional profile of chickpea oil. Essential minerals, such as calcium (Ca), magnesium (Mg), phosphorus (P), and potassium (K) are prevalent in chickpea seeds. Furthermore, chickpeas are rich in vital vitamins, such as riboflavin, niacin, thiamin, folate, and the vitamin A precursor β-carotene. Chickpeas offer numerous potential health benefits and, when combined with other pulses and cereals, may positively impact prevalent human diseases, such as cardiovascular disease (CVD), type 2 diabetes, digestive disorders, and certain cancers. In conclusion, chickpeas have emerged as a crucial pulse crop with a wide array of nutritional and health-promoting advantages (Jukanti *et al*., 2012). Legumes are an important source of nutrition for low-income individuals in many developing countries. In Turkey, legumes are of significant importance to the daily consumption of many families. Considering the total legume production, Turkey ranks among the largest producers globally (Anon., 2023).

The aim of this study was to investigate the adaptive responses of ABA and NO in fighting drought conditions by administering external SNP and ABA to tolerant and sensitive cultivars of chickpeas exposed to drought stress. This study focused on the effects of abscisic acid and NO application on the antioxidant system in plants against drought stress, and it will have major importance in meeting the needs of food based on the increasing population to eliminate or reduce yield loss resulting from drought by NO treatments.

## Material and Methods

**Plant selection, cultivation and applications:** In this study, the seeds used as experimental material were supplied by Gülümser, Azkan, Aksu, Canıtez, Zuhal, Çağatay, Seyitgazi, Damla, Diyar 95 Eskişehir Seed Research Center, and Ankara Registered Seed Certification Center. To identify the most drought-tolerant and sensitive chickpea cultivar, germination percentages were assessed by applying different concentrations of polyethylene glycol PEG 4000 (5%, 10%, 15%, and 20%). Another group was also treated with PEG at different concentrations (5%, 10%, 15%, and 20%) 15 days after the germination. The plants treated with PEG were followed for 10 days. Different doses of SNP (50, 100, 200 and 300, µmol/l) were also applied to seedlings which were separately grouped regarding the morphological changes observed in the plant in the present study, such as leaf roll, change in the colour of leaf, and loss of turgor. MDA contents of samples taken from the leaves of chickpeas were determined at the end of the period. Peroxidation levels of lipids have an important place in evaluating the severity of oxidative stress induced by drought and susceptibility levels of plants. The most convenient doses of SNP and PEG were identified by determining the lipid peroxidation levels of seedlings. SNP treatment was determined as 100 µM and PEG concentration as 10% in the present study based on the preliminary studies we conducted according to literature data as well as the data we obtained from these studies. It was identified as a result of preliminary studies that the most drought-tolerant cultivar among Cicer varieties was Canıtez, and the most sensitive cultivar was Aksu. In the germination experiment, the seeds of cultivars determined as tolerant and sensitive regarding drought stress were subjected to surface sterilization with 10% sodium hypochlorite for 10 minutes before plantation. Following the surface sterilization, seeds were washed with distilled water for 30 minutes. They were then kept to swell in distilled water for 24 hours. Seeds were planted in plastic pots with dimensions of 15 x 20 cm containing perlite at the end of this period. All studies including germination and growth stage were conducted under controlled conditions of the plant growth chamber. The intensity of illumination in the plant growth chamber is 222 µmol $m^{-2}s^{-1}$ on the surface of the plant leaf. 90% of illumination intensity was provided with fluorescent bulbs and 10% with incandescent light bulbs. Plants were grown for 25 days starting from germination. PEG 4000 treatment was administered to induce drought stress at the end of day 25. Both drought and control groups were established for both of the plant cultivars. Plants of the control group were irrigated with Hoagland's culture solution until the end of the experiment. Groups of both chickpea cultivars which were and were not exposed to drought stress were treated with 100 µM of SNP, cPTIO, and ABA exogenously. Treatments were carried out for 6 days. Leaf samples of the group were taken on days 0, 3, and 6 for the analysis. In tolerant and sensitive chickpea plants, experimental groups were generated as follows; Control (Hoagland's culture solution), c-PTIO, ABA, Control + SNP, Control + c-PTIO, Control + ABA, PEG, PEG+SNP, PEG+ c-PTIO, and PEG+ ABA. NO level, proline concentration, LOX activity, MDA level, total chlorophyll were examined using the leaf samples taken in the study.

**Determination of nitric oxide contents:** NO levels in leaf tissues were determined according to Garcia-Mata & Lamattina (2001). Absorbance values of supernatants obtained following the centrifuge process were determined via microplate reader (Molecular Devices Corp., Versamax®) at 550 nm using Cayman Chem. NO assay kit (Catalog no. 780001). Nitric oxide levels were calculated from absorbance values by using a standard curve of nitric oxide.

**Proline assay:** Proline contents of roots and leaves were assayed according to Bates *et al.*, (1973). 0.1 g of leaf samples were filtrated after homogenization with 3% sulfosalicylic acid. The obtained homogenate was kept in water bath at 100°C for 1 hour by adding acid ninhydrin and glacial acetic acid. The mixture was then kept in ice bath until it got cold in order to stop the reaction. Toluene was extracted into the mixture following the cooling process. Toluene was aspired from the liquid phase and absorbance values were read on a spectrophotometer at 520 nm wavelength after the cooling at room temperature. The concentration of proline was calculated with the aid of calibration curve and expressed as µmol proline $g^{-1}$ FW.

**Loc enzyme activity (DPPH):** LOX was measured according to Minguez-Mosquera *et al*., (1993), using 50 mM K-phosphate buffer (pH 6.0) for extraction. The reaction mixture comprised 0.2 $cm^3$ of crude extract and 0.5 mM linoleic acid in a 50 mM K-phosphate buffer (pH 6.0). The enzyme activity was determined by measuring the absorbance increase at 234 nm, applying an extinction coefficient of 25,000 $M^{-1}$ $cm^{-1}$.

**Lipid peroxidation:** For identification of lipid peroxidation, the level of malondialdehyde (MDA) which is the final product of lipid peroxidation was assayed according to Madhava Rao & Sresty (2000). 0.1 g of leaf samples was homogenized with trichloroacetic acid (TCA). The reaction mixture containing thiobarbituric acid (TBA) and TCA was pipetted into supernatant and transferred into tubes after the centrifuge and all test tubes were boiled at 95°C for 30 minutes. The mixture was centrifuged at 12.000 xg for 15 minutes. Absorbance values of the obtained supernatant at 532 and 600 nm were read. MDA concentration was calculated using extinction coefficient ($\epsilon$=155 $mM^{-1}cm^{-1}$).

**Chlorophyll assay:** Chlorophyll was assayed according to Arnon (1949). The samples of fresh leaf (0.25 g) were homogenized within 5ml of 80% acetone. The homogenate was centrifuged at 5000 g at room temperature for 5 minutes. The absorbance of the supernatant was measured in a spectrophotometer at 663, 645, and 450 nm. It was calculated by using the following equations developed by Lichtenthaler (1987) to determine pigment contents.

Chlorophyll a = 12.7 x A663 - 2.69 x A645
Chlorophyll b = 22.9 x A645 - 4.68 x A663
Total chlorophyll = (20.2 x A645) +( 8.02 x A663)

**Statistical analysis**

For statistical analyses, the SPSS 15.0 (SPSS Inc., Chicago, IL, USA) software package was used. The LSD test was employed for comparisons among groups. Results were presented as mean ± SEM (Standard Error of the Mean). The level of statistical significance was considered with respect to p values, and $p<0.05$ was accepted.

**Results**

**The effects of treatment groups on no level in leaf tissue of *Cicer arietinum* L. cv. "Canıtez" and "Aksu" chickpea cultivars:** Nitric oxide level in the leaf tissue of the Canıtez cultivar was determined to increase in all treatments (except for cPTIO) by days compared to control (Table 1). The highest NO level in the Canıtez cultivar was determined as 46.64±3.08 nmol/g FW on day 6 in the PEG+SNP treatment group. According to statistical analysis, the increase between control and treatment groups (excluding PEG+cPTIO) on days 3 and 6 was found to be significant ($p\leq0.05$). In the leaf tissue of the Aksu cultivar, nitric oxide levels increased by days in all treatments. In the Aksu cultivar, the highest NO level was determined as 32.14±0.55 nmol/g FW on day 6 in the PEG+SNP treatment group. As is seen in Table 1, the NO level was found to decrease on days 3 and 6 of the PEG+cPTIO treatment group in Aksu and Canıtez cultivars compared to control and other treatment groups. According to the statistical analysis, the increase observed in PEG+ABA and PEG+SNP treatment groups on days 3 and 6, except for the PEG treatment group on day 6, was found to be significant compared to the control group ($p\leq0.05$).

**The effect of treatment groups on proline level in leaf tissue of *Cicer arietinum* L. cv. "Canıtez" and "Aksu" chickpea cultivars:** As is seen in (Table 2) proline levels increased in both cultivars compared to the control group. It was determined that proline levels were higher in all treatment groups in the tolerant Canıtez cultivar compared to the Aksu cultivar and the highest level was 48.09±0.22 mg/g FW in the PEG+ABA treatment group on day 6. The increase determined on days 3 and 6 compared to control groups was statistically significant ($p<0.05$). Statistically, the 3rd and 6th-day applications of both plants were found to be significant compared to their control groups.

While proline levels of the PEG+ABA group increased approximately 4 times on day 6 compared to day 3 for the tolerant Canıtez cultivar, they increased 2 times in the sensitive Aksu cultivar. In the PEG+SNP group, it increased 2 times in the Canıtez cultivar and about 3 times in the Aksu cultivar. Proline levels decreased by 30% on day 6 in the PEG+cPTIO group of both cultivars compared to day 3 ($p\leq0.05$).

**The effects of treatment groups on lox enzyme activity in leaf tissue of *Cicer arietinum* L. cv. "Canıtez" and "Aksu" chickpea cultivars:** Table 3 showed that LOX enzyme activity results increased in both cultivars by days and also in treatment groups, except for PEG treatment, compared to the control group. The highest increase was identified as 43,45 U/g FW and 31,17 U/g FW in the sensitive Aksu cultivar and tolerant Canıtez cultivar, respectively in the PEG+SNP treatment group on day 6. Statistically, the 3rd day and 6th day values of PEG+ABA and PEG+SNP applications in both plants were found to be significant compared to their control groups.

**The effects of treatment groups on MDA levels in leaf tissue of *Cicer arietinum* L. cv. "Canıtez" and "Aksu" chickpea cultivars:** As is seen in Table 4, the tolerant Canıtez cultivar showed an increase in time and treatment groups compared to the control group. The highest increase was identified as 40.36 umol/gTA in the PEG treatment group on day 6. In the Aksu cultivar, there was an increase in PEG treatment, a decrease in PEG+ABA and SNP treatments, and also an increase in the PEG+cPTIO group on day 3 compared to the control group; whereas, an increase was determined in every treatment group on the day 6 compared to control group. The highest MDA level was observed to be 45.85 umol/g as fresh in the PEG+cPTIO treatment group on day 6. The highest increase of MDA levels was identified in PEG and PEG+cPTIO groups in both of the cultivars.

**Table 1. Changes occurring in NO levels (nmol/g FW) in leaf tissue of *Cicer arietinum* L. cv. "Canıtez" and "Aksu" chickpea cultivars depending on treatment groups and days.**

| Treatment day | Treatment groups | NO Level (AKSU) | NO Level (CANI TEZ) |
|---|---|---|---|
| 0 | Control | 14,45 ± 0,55 | 18,74 ± 0,22 |
| 0 | PEG | 20,66 ± 0,32 | 25,12 ± 0,36 |
| 3 | Control | 14,32 ± 0,21 | 18,47 ± 0,28 |
| 3 | PEG | 19,86 ± 0,12* | 23,17 ± 0,82* |
| 3 | PEG + ABA | 23,78 ± 0,11* | 39,58 ± 0,14* |
| 3 | PEG + SNP | 28,56 ± 0,06* | 41,18 ± 0,29* |
| 3 | PEG + cPTIO | 10,84 ± 0,32* | 16,64 ± 0,41* |
| 6 | Control | 14,93 ± 0,27 | 18,22 ± 0,91 |
| 6 | PEG | 16,78 ± 0,33* | 24,89 ± 0,32* |
| 6 | PEG + ABA | 25,75 ± 0,37* | 42,78 ± 0,71* |
| 6 | PEG + SNP | 32,14 ± 0,26* | 46,64 ± 0,67* |
| 6 | PEG + cPTIO | 11,24 ± 0,19* | 14,82 ± 0,17* |

The results were given as 3 repeated mean ± standard error. (*, $p \leq 0.05$)

**Table 2. Changes occurring in proline levels (nmol/g FW) in leaf tissue of *Cicer arietinum* L. cv. "Canıtez" and "Aksu" chickpea cultivars depending on treatment groups and days.**

| Treatment day | Treatment groups | Proline level (AKSU) | Proline level (CANI TEZ) |
|---|---|---|---|
| 0 | Control | 6.7 ± 0,05 | 8.49 ± 0,09 |
| 0 | PEG | 5.01 ± 0,02 | 7.53 ± 0,07 |
| 3 | Control | 7.7 ± 0,06 | 8,72 ± 0,17 |
| 3 | PEG | 12.05 ± 0,09* | 17.22 ± 0,21* |
| 3 | PEG + ABA | 10.14 ± 0,13* | 12.95 ± 0,36* |
| 3 | PEG + SNP | 9.27 ± 0,19* | 21.5 ± 0,33* |
| 3 | PEG + cPTIO | 30.8 ± 0,39* | 45.31 ± 0,35* |
| 6 | Control | 7.1 ± 0,02 | 8,55 ± 0,85 |
| 6 | PEG | 26.89 ± 0,09* | 38.2 ± 0,28* |
| 6 | PEG + ABA | 23.27 ± 0,03* | 48.09 ± 0,22* |
| 6 | PEG + SNP | 26.85 ± 0,32* | 40.11 ± 0,41* |
| 6 | PEG + cPTIO | 21.51 ± 0,25* | 31.8 ± 0,62* |

The results were given as 3 repeated mean ± standard error. (*, $p \leq 0.05$)

**Table 3. Changes occurring in LOX activity (U/g FW) in leaf tissue of *Cicer arietinum* L. cv. "Canıtez" and "Aksu" chickpea cultivars depending on treatment groups and days.**

| Treatment day | Treatment groups | LOX activity (AKSU) | LOX activity (CANI TEZ) |
|---|---|---|---|
| 0 | Control | 24,16 ± 0,02 | 35,87 ± 0,05 |
| 0 | PEG | 23,97 ± 0,05 | 34,56 ± 0,09 |
| 3 | Control | 24,67 ± 0,03 | 35,96 ± 0,19 |
| 3 | PEG | 23,45 ± 0,09 | 33,78 ± 0,89 |
| 3 | PEG + ABA | 26,12 ± 0,07* | 37,37 ± 0,23* |
| 3 | PEG + SNP | 28,75 ± 0,23* | 39,58 ± 0,35* |
| 3 | PEG + cPTIO | 25,63 ± 0,36 | 36,87 ± 0,22 |
| 6 | Control | 23,88 ± 0,28 | 36,65 ± 0,85 |
| 6 | PEG | 24,68 ± 0,09 | 34,25 ± 0,37 |
| 6 | PEG + ABA | 26,81 ± 0,12* | 38,82 ± 0,76* |
| 6 | PEG + SNP | 31,17 ± 0,33* | 43,45 ± 0,47* |
| 6 | PEG + cPTIO | 26,77 ± 0,21* | 37,17 ± 0,71 |

The results were given as 3 repeated mean ± standard error. (*, $p \leq 0.05$)

**Table 4. Changes occurring in MDA levels (µM/ FW) in leaf tissue of *Cicer arietinum* L. cv. "Canıtez" and "Aksu" chickpea cultivars depending on treatment groups and days.**

| Treatment day | Treatment Groups | MDA Level (AKSU) µM/ FW | MDA Level (CANITEZ) µM/ FW |
|---|---|---|---|
| 0 | Control | 21,88 ± 0,06 | 28,15 ± 0,02 |
| 0 | PEG | 23,17 ± 0,23 | 32,45 ± 0,36 |
| 3 | Control | 22,02 ± 0,05 | 29,01 ± 0,04 |
| 3 | PEG | 33,17 ± 0,32* | 41,82 ± 1,02* |
| 3 | PEG + ABA | 35,09 ± 1,21* | 30,68 ± 0,35 |
| 3 | PEG + SNP | 23,56 ± 1,02* | 30,42 ± 0,56 |
| 3 | PEG + cPTIO | 35,64 ± 1,04* | 41,87 ± 0,65* |
| 6 | Control | 22,14 ± 0,05 | 28,72 ± 0,09 |
| 6 | PEG | 40,36 ± 0,36* | 44,16 ± 0,23* |
| 6 | PEG + ABA | 36,65 ± 1,02* | 34,34 ± 1,02* |
| 6 | PEG + SNP | 24,46 ± 0,69* | 31,18 ± 0,94* |
| 6 | PEG + cPTIO | 38,12 ± 1,25* | 45,85 ± 1,04* |

The results were given as 3 repeated mean ± standard error. (*, $p \leq 0.05$)

**Table 5. Changes occurring in total chlorophyll levels (mg/g FW) in leaf tissue of *Cicer arietinum* L. cv. "Canıtez" and "Aksu" chickpea cultivars depending on treatment groups and days.**

| Treatment day | Treatment groups | Chlorophyll level mg/g FW (AKSU) | Cholorophyl level mg/g FW (CANITEZ) |
|---|---|---|---|
| 0 | Control | 13,19 ± 0,06 | 14,73 ± 0,02 |
| 0 | PEG | 11,72 ± 0,02 | 13,45 ± 0,05 |
| 3 | Control | 13,64 ± 0,09 | 14,72 ± 0,03 |
| 3 | PEG | 13,76 ± 0,03 | 13,53 ± 0,01 |
| 3 | PEG+ABA | 16,29 ± 0,05* | 17,35 ± 0,06* |
| 3 | PEG+SNP | 18,37 ± 0,04* | 19,85 ± 0,09* |
| 3 | PEG+cPTIO | 15,48 ± 0,08* | 16,02 ± 0,02* |
| 6 | Control | 13,56 ± 0,06 | 14,31 ± 0,09 |
| 6 | PEG | 14,82 ± 0,02 | 14,57 ± 0,05 |
| 6 | PEG+ABA | 16,73 ± 0,09* | 18,57 ± 0,08* |
| 6 | PEG+SNP | 20,65 ± 0,06* | 22,73 ± 0,04* |
| 6 | PEG+cPTIO | 16,57 ± 0,05* | 17,06 ± 0,07* |

The results were given as 3 repeated mean ± standard error.

**The effects of treatment groups on chlorophyll levels in leaf tissue of *Cicer arietinum* L. cv. "Canıtez" and "Aksu" chickpea cultivars:** In Canıtez and Aksu cultivars, PEG and PEG+cPTIO treatment groups were found to have decreased depending on time compared to the control group; on the other hand, there was an increase for sensitive Aksu cultivar compared to tolerant Canıtez cultivar in other treatment groups (Table 5). The highest total chlorophyll level was determined to be 20.65±0.06 mg/g TA and 22.73±0.04 mg/g TA in the PEG+SNP treatment group on day 6 in the Aksu cultivar and Canıtez cultivar, respectively. The increase determined in PEG+ABA, PEG+SNP and PEG+c-PTIO treatment groups on days 3 and 6 in both cultivars compared to control groups was statistically significant ($p<0.05$).

## Discussion

It is biologically quite difficult to detect drought stress by the plant, to transmit it and to understand the biochemical and molecular mechanisms of its tolerance. Generally, tolerance to abiotic stress is a very complex system. This is because thanks to stress factors, there are considerably complicated effects between various molecular, biochemical, and physiological events which influence plant growth and development (Bouman *et al.*, 2005, Razmjoo *et al.*, 2008, Jaleel *et al*., 2009). The impacts of drought stress are observed on almost all plants. However, the effect it causes varies from species to species, even within a species (Jaleel *et al*., 2009). Nitric oxide is also stated to involve many developmental stages

in plants as well as in animals and other organisms. It was understood that NO which is a signal molecule had an effect on the regulation and controlling of plant cell functions at every stage of the development (Arasimowicz & Floryszak-Wieczorek, 2007, Leitner *et al.*, 2009, Moreau *et al*., 2010). In a study conducted by Zhao *et al*., (2008) on cane, it was reported that PEG-60000 stimulated NO release and antioxidant activity in stress-tolerant species, did not stimulate in sensitive species and was protective against oxidative damage and increased tolerance to osmotic stress. In addition to all these, it is suggested that the physiological function of NO under stress conditions in plants and its source have not been clarified completely despite intensive studies conducted for a long time (Neill *et al*., 2008, Laspina *et al*., 2005). The effects of NO were also investigated under several abiotic stress conditions such as drought, salinity, and high and low temperature. Reactive oxygen species are released under such stress conditions and numerous oxidative breakdown reactions start within the cells. NO participating in signal transmission pathway under stress conditions is in mutual interaction with active oxygen species. A previous study revealed that NO was protective against oxidative stress resulting from drought in wheat seedlings (Garcia-Mata & Lamattina, 2001). It was found that NO promoting the tolerance to severe drought stress did not function alone, for example, they were functioning together with $H_2O_2$ in stomatal closure in Arabidopsis (Garcia-Mata & Lamattina, 2001). The studies also reported the responses of NO under heat and cold stress. In the study conducted by Silveira *et al*., 2017 on sugarcane genotypes IACSP95-5000 (drought-tolerant) and IACSP97-7065 (drought-sensitive), they presented additional evidence indicating that intracellular NO production increased in case of water deficiency in the leaves of drought tolerant genotype and there was a correlation between NO production and drought tolerance. In the present study, NO levels were identified to increase by days in drought tolerant Canıtez cultivar in PEG and SNP treatments. The decrease in NO levels on day 6 based on drought treatment in the drought-sensitive Aksu cultivar revealed that the response of the plant to drought stress was different from the response by the Canıtez cultivar. As is known, tolerance to drought stress which exists in all plants varies from species to species, even within a species (Jaleel *et al*., 2009). While NO levels increased approximately 1.5 times in the tolerant cultivar compared to control and PEG treatment, approximately 2 times increase was detected in PEG+SNP treatment (Table 1). In the present study, SNP treatment increased drought tolerance based on drought stress treatment, this increase was obvious, particularly in the Canıtez cultivar. In the sensitive cultivar, about 1.5 times increase on day 3 and 2 times increase on day 6 were observed in PEG+SNP treatment compared to PEG treatment. However, when two cultivars were evaluated together; higher levels of NO were identified in SNP treatment in the tolerant Canıtez cultivar depending on PEG treatment compared to the sensitive Aksu cultivar. While there was about a 1.5 times increase in PEG treatment in the tolerant cultivar compared to the control, the sensitive cultivar had a slight increase. The fact that SNP treatment increased NO levels in both cultivars made us think that the positive effect of SNP was distinct in tolerant cultivars. This result of the present study is compatible with the results of Silveira *et al*., (2017) and Zhang (2016). An increase of NO in leaves of drought tolerant genotype introduces additional evidence indicating that there is a correlation between NO production and drought tolerance. The effect of nitric oxide on metabolic and physiological processes depends on the ability to interact and modify multiple targets within plant cells, making it a difficult task to understand the effects on plants (Lamattina *et al.*, 2003). In fact, it should be one of the major aims of NO research in the near future to understand metabolic pathways controlling NO homeostasis in plants. In addition, drought tolerant genotype has higher NO content than drought-sensitive ones. Further studies addressing the long-term responses of plants to water deficiency and NO to modulate both physiological and morphological adaptation in various availability of water should reveal more aspects of this multiple signal molecule in plants.

Proline is a well-known osmoprotectant which accumulates in several plants as a response to applications of numerous biotic and abiotic stress. Even though its contribution to stress physiology has not been cleared up yet, it was suggested to increase tolerance to drought stress by protecting protein cycle mechanisms from stress damage (Rhodes *et al*., 1999; Ali *et al*., 1999; Okuma *et al*., 2000; Sharma & Dubey, 2005; Verbruggen and Hermans 2008; Yang *et al*., 2021). A previous study reported that proline conserved nitrogenase activity in the Glycine max plant (Pedersen *et al*., 1996) during water deficiency stress. SNP treatment provided an increase in the proline content of *Ginkgo biloba* (Qu *et al.*, 2023) and *P. przewalskii* plants exposed to drought (Lei *et al*., 2007). NO was reported to increase the accumulation of free proline induced by drought in *O. sativa* and *T. aestivum* (Hasanuzzaman *et al*., 2018; Dien *et al*., 2019). A study reported that proline levels increased transgenic endogenous NO levels in leaves of *O. sativa* both subjected to foliar SNP treatment and under drought stress (Farooq *et al*., 2009; Cai *et al*., 2015). A recent study revealed that seed priming with SNP significantly alleviated oxidative damage induced by salt stress in *Vigna radiata*, where seed treatment improved the concentration of defence metabolites such as phenolic content, amino acids, carbohydrates, proline content, and antioxidants like CAT, APX, and SOD (Hasanuzzaman *et al*., 2021) In *Satureja hortensis*, a concentration of 200 mM SNP under Cd toxicity exhibited enhanced levels of various biochemical parameters, including chlorophyll content, proline content, and the activity of different antioxidants (Azizi *et al*., 2021). The combination of NO and SA prevented Ni toxicity in *Brassica napus* through the accumulation of proline content, increased chlorophyll concentration, and decreased lipid peroxidation (Kazemi *et al*., 2010). Under high-temperature stress (32, 37, and 42°C), applications of cPTIO, ascorbic acid, and

tetramethyl piperidinooxy were reported to stimulate proline biosynthesis, increase antioxidant enzyme activities, prevent DNA damage, and inhibit chlorophyll degradation in *Vicia faba* plants (Alamri *et al*., 2019). Under salt stress (50 and 100 mM), application of SNP (1, 10, and 100 µM) in Brassica campestris plants has been observed to reduce ROS lipid peroxidation, increase chlorophyll and relative water content, enhance growth rate, and improve photosynthesis (Sami *et al*., 2021). Under drought stress (50% of field capacity), the application of SNP (0, 100, and 200 µmol) in Silybum marianum plants was reported to increase proline content (Zangani *et al*., 2023). In their study, Tan *et al*., (2006) stated that proline content increased depending on the severity of water deficiency and time, and this result and the results of the above researchers also support the results of the present study. In the present study, the tolerant-Canıtez cultivar was determined to accumulate a higher amount of proline as a response to drought compared to the sensitive cultivar (Table 2). In the tolerant cultivar, the proline level increased about 4 times in the PEG treatment group and about 5 times in the PEG+ABA treatment group compared to the control. It was also thought that more proline was synthesized to resist drought in both cultivars by eliminating the NO effect with the addition of NO scavenger because an increase was observed in the PEG+cPTIO treatment group compared to the SNP group in sensitive and tolerant cultivars. Recently, proline has also been indicated to act as an OH scavenger providing a characteristic of protecting cellular membranes against oxidative lipid peroxidation which is an indicator of oxygen damage (Ashraf & Foolad, 2007). Therefore, it is possible to assert that greater accumulation of proline not only acts as osmolite but also protects cells from oxidative damage under drought in Canıtez. Although both NO and proline accumulation seem to be important in drought stress, it is thought that more research is needed on their mutual relationships.

In the study by Zhang *et al*., (2003), LOX activity of *Triticum aestivum* L. (wheat) seeds was reported to decrease after osmotic stress. In their study, Sofo *et al*., 2004 divided olive (*Olea europaea* L.) plants into two groups as 14 stressed control plants (CP) and 36 stressed plants (SP). Control plants (CP) received water in equal amounts transpired during the whole experimental period. In the first 10 days of the experimental period, stressed plants (SP) were exposed to water consumption that was gradually controlled and a daily decrease was applied, which was less than 10% of the total water flow. It was reported that especially leaf tissue is influenced more by water, which led to a distinct increase in LOX activity, and this condition showed a three times increase in LOX activity during severe drought stress compared to control plants. In addition, the highest LOX activities under drought stress were 1.7 and 1.6 times higher than control values, respectively. Sofo *et al*., (2004) suggested that there was a close correlation between the gradual increase of LOX activity and the progress of drought stress conditions. In a study conducted on *Brassica* spp. (*Brassica napus* L. cv. BARI Sharisha 13, *Brassica campestris* L. cv. BARI Sharisha 9 and *Brassica juncea* L. cv. BARI Sharisha 11), plants were exposed to drought stress induced with 15% PEG -6-000 and the samples were taken after 48 hours. The plants were also treated with PEG+ Trehalose. The researchers noted that drought stress decreased fresh and dry and leaf RWC of *Brassica* seedlings, however, B. juncea was more tolerant under drought stress compared to the other two species. Drought stress was also shown to cause an increase in LOX activity and MDA levels in all Brassica seedlings (Alam *et al*., 2014).

In the present study, on the other hand, the result indicating that LOX activity increased against drought stress, particularly in sensitive Aksu cultivars is compatible with the results of Alam *et al*., (2014), Sofo *et al*., (2004) and Aziz *et al*., (1998). Increased LOX activities can be interpreted as causes for lipid peroxidation increased under stress conditions. Because unsaturated fatty acids which are a great majority of phospholipids in cell membranes are the substrate of LOX enzyme, the increase of LOX activity was reported to lead to the breakdown of the lipid composition of the cell membrane and other cellular elements. Therefore, the LOX enzyme plays an important role in the composition of membrane lipids and the entity of the cell membrane (Maalekuu *et al*., 2006). In the present study, lesser enzyme activity was identified in tolerant cultivars than in sensitive cultivars depending on drought stress. Nevertheless, we can say that LOX enzyme activity was higher in the tolerant cultivar, protective effect of exogenously applied SNP was more efficient on both days in especially PEG+SNP treatments in the tolerant cultivar and when MDA results were also considered similar results occurred in other words; cellular components were broken down more, both SNP and ABA treatments were more effective in the tolerant cultivar.

MDA, which is a product of membrane lipid peroxidation, is considered a credible marker and routinely used to evaluate the level of oxidative damage resulting from different stress treatments on plants (Del Rio *et al*., 2005; Mihaljevi´c *et al*., 2021). Hussain *et al*., (2014) detected lower MDA contents in comparative analysis performed on more tolerant cultivars. In the present study, even though an increase was observed for MDA levels of both cultivars compared to the control, PEG+ABA and SNP treatments of sensitive cultivars had similar results with the control group. On day 6, MDA levels decreased in both cultivars in PEG+SNP treatment compared to PEG treatment. Especially MDA levels of tolerant cultivar decreased about 2 times in SNP treatment. The effects of NO on salt stress in wheat plants were researched in a previous study. In the study wheat plants were treated with 150 µM and 300 µM NaCl, 150 µM NaCl+SNP and 300 µM NaCl+SNP. The results of the study revealed that MDA content increased in parallel with concentration of salt stress. MDA content in 150 µM of NaCl+SNP treatment was lower than both the control group and NaCl treatments and MDA content decreased in 300 µM NaCl+SNP treatment (Hasanuzzaman *et al*., 2011). Zhang *et al*., (2014) stated that CCRI-60 could be protected better against oxidative damage under drought stress and the level of MDA produced during peroxidation of membrane lipids could be used as an indicator of oxidative damage in their

study on two cotton cultivars (drought tolerant CCRI-60 and drought-sensitive CCRI-27) and the studies by Del Rio *et al*., (2005), Hussain *et al*., (2014), Zhang *et al*., (2016) were compatible with the present study. In the present investigation, the lower values of MDA in Cansu indicate that at the cellular level, this genotype is better equipped with an efficient free radical quenching system that offers protection against oxidative stress.

Differences in chlorophyll contents were shown to be associated with drought tolerance (Sairam *et al*., 1998). In their study, Rossi *et al*., (2017), Mafakheri *et al*., (2010), Nyachiro *et al*., (2001) and Kpyoarissis *et al*., (1995) reported that total chlorophyll levels decreased based on drought stress. In the present study, total chlorophyll levels decreased based on drought stress on days 3 and 6 in the PEG treatment groups compared to all other groups. The result that chlorophyll levels decreased depending on drought treatment was compatible with the results by Hu *et al*., (2023), Mafakheri *et al*., (2010), Nyachiro *et al*., (2001) and Kpyoarissis *et al*., (1995). Hammad & Ali (2014) found that both chlorophyll levels and photochemical activity decreased based on drought stress, which is compatible with the results of the present study. In the present study, total chlorophyll contents decreased by PEG treatment in both cultivars compared to control increased about 4 times in the sensitive Aksu cultivar and 3 times in the tolerant cultivar by SNP and ABA treatments, and significantly decreased by treatment of cPTIO the NO scavenger. This suggests that SNP and ABA make the plant more drought tolerant under stress conditions and treatment of cPTIO the NO scavenger eliminated the therapeutic effect of SNP.

## Conclusion

The results obtained showed that NO and ABA applications corrected the negative effects of drought stress. In addition, SNP applied as a NO donor had positive effects and the emergence of negative effects with NO scavenger c-PTIO indicated that NO is a molecule that helps antioxidant defense in the plant. Plants are generally exposed to numerous similar factors such as salinity, drought, pollution, heat, and cold during their life spans and their normal growth and development are influenced negatively. Changes occurring in plants under these conditions are defined as stress. Because population density is increasing gradually and arable areas have reduced, it has become substantially important to decrease product loss resulting from stress in our world where nutrition problems might occur in the future. In light of technological advancement developing to this end; it will be a considerably crucial stem to understand defense mechanisms in tolerant plant species, particularly against stress factors for minimizing product loss. Tolerance to all abiotic stressors at the plant and cellular degree is quite complex. This was associated with the complexity of interactions between stress factors and various physiological, biochemical, and molecular events influencing plant development and growth. At present, economically effective technological tools and methods which will facilitate the production of plant species with agricultural importance under stress conditions are not available. However, the development of plants tolerant to environmental stressors is an approach that will aid the increasing needs of developed and developing countries. Development of tolerant plants requires to have knowledge about physiological mechanisms and genetic controls ensuring tolerance at different developmental stages of plants. Comparison of drought tolerance mechanisms in different plant species will be important in terms of understanding the points that regulate possible molecular mechanisms providing drought tolerance. The development of drought-resistant or tolerant cultivars, in addition to plants natural capabilities for drought tolerance, is essential for achieving global food security by balancing population expansion and food demand. Furthermore, comparative research involving various plant species under different stress conditions will provide a better understanding of the potential of SNP to enhance crop improvement and stress tolerance.

## References


Ahluwalia O., P.C. Singh and R. Bhatia. 2021. A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria. *Resour. Environ. Sustain*., 5: 100032.

Alajaji, S.A. and T.A. El-Adawy. 2006. Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods. *J. Food Compos. Anal*., 19(8): 806-812.

Alam, Md., K. Nahar and M. Hasanuzzaman. 2014. Exogenous jasmonic acid modulates the physiology, antioxidant defence and glyoxalase systems in imparting drought stress tolerance in different Brassica species. *Plant Biotechnol. Rep*., 8: 279-293. doi:10.1007/s11816-014-0321-8

Alamri, S.A., M.H. Siddiqui. M.Y. Al-Khaishany, M.N. Khan, H.M. Ali and K.A. Alakeel. 2019. Nitric oxide-mediated cross-talk of proline and heat shock proteins induce thermotolerance in *Vicia faba* L. *EEB*., 161: 290-302. doi:10.1016/j.envexpbot.2018.06.012.

Ali, G., P.S. Srivastava and M. Iqbal. 1999. Proline accumulation, protein pattern and photosynthesis in *Bacopa monniera* regenerants grown under NaCl stress. *Biol. Plant*., 42: 89-95.

Anonymous. 2023. Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/faostat/en/#data/QC.

Arasimowicz-Jelonek, M., J. Floryszak-Wieczorek and J. Kubis. 2009. Involvement of nitric oxide in water stress-induced responses of cucumber roots. *Plant Sci*., 177: 682-690.

Arc, E., M. Galland, B. Godin, G. Cueff and L. Rajjou. 2013. Nitric oxide implication in the control of seed dormancy and germination. *Fron. Plant Sci*., 4: 346. doi: 10.3389/fpls.2013.00346.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol*., 24: 1-15.

Ashraf, M. and P.J.C. Harris. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*., 166: 3-16.

Aziz, A. and F. Larher. 1998. Osmotic stress-induced changes in lipid composition and peroxidation in leaf discs of *Brassica napus* L. *J. Plant Physiol*., 153(5–6): 754-762. doi:10.1016/S0176-1617(98)80231-9.

Azizi, I., B. Esmaielpour and H. Fatemi. 2021. Exogenous nitric oxide on morphological, biochemical, and antioxidant enzyme activity on savory (*Satureja hortensis* L.) plants under cadmium stress. *J. Saudi Soc. Agric. Sci*., 20(6): 417-423. doi:10.1016/j.jssas.2021.05.003.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*., 39: 205-207. doi:10.1007/BF00018060

Bouman, B.A.M., S. Peng, A.R. Castaòeda and R.M. Visperas. 2005. Yield and water use of irrigated rewatering. *Plant Prod. Sci*., 7(4): 406-420. doi:10.1626/pps.7.406

Buchanan, T., J. Johnson and L. Goldberg. 2005. Implementing a five-factor personality inventory for use on the internet. *EJPA*., 21(2): 115-124. doi:10.1027/1015-5759.21.2.115

Cai, W., W. Liu, W.S. Wang, Z.W. Fu, T.T. Han and Y.T. Lu. 2015. Overexpression of rat neurons nitric oxide synthase in rice enhances drought and salt tolerance. *PLoS One*, 10(6): e0131599. doi:10.1371/journal.pone.0131599.

Chehab, H., B. Mechri, F.B, Mariem, M. Hammami, S. Ben Elhadj and M. Braham. 2009. Effect of different irrigation regimes on carbohydrate partitioning in leaves and wood of two table olive cultivars (*Olea europaea* L. cv. Meski and Picholine): *Agric. Water Manag*., 96(2): 293-298.

Del Rio, D., A. Stewart and N. Pellegrini. 2005. A review of recent studies on malondialdehyde as a toxic molecule and biological marker of oxidative stress. *NMCD*., 15: 316-328. doi:10.1016/j.numecd.2005.05.003

Dien, D.C., T. Mochizukiband and T. Yamakawa. 2019. Effect of various drought stresses and subsequent recovery on proline, totalsoluble sugar and starch metabolisms in Rice (*Oryza sativa* L.) varieties. *Plant Prod. Sci*., 22(4): 530-545 https://doi.org/10.1080/1343943X.2019.1647787.

Fancy, N.N., A.K. Bahlmann and G.J. Loake. 2017. Nitric oxide function in plant abiotic stress. *Plant Cell Environ*., 40: 462-472. doi: 10.1111/pce.12707.

Farooq, M., M.A. Basra, A. Wahid and H. Rehman. 2009. Exogenously applied nitric oxide enhances the drought tolerance in fine-grain aromatic rice (*Oryza sativa* L.): *J. Agron. Crop Sci*., 195: 254-261.

Garcia-Mata, C. and L. Lamattina. 2001. Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. *Plant Physiol*., 126: 1196-1204.

Hammad, S.A.R. and O.A.M. Ali. 2014. Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract. *Ann. Agric. Sci*., 59(1): 133-145. doi:10.1016/j.aoas.2014.06.018.

Hare, P.D. and W.A. Cress. 1997. Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regul*., 21: 79-102.

Hasanuzzaman, M., K. Nahar, A. Rahman, M. Inafuku, H. Ok and M. Fujita. 2018. Exogenous nitric oxide donor and arginine provide protection against short-term drought stress in wheat seedlings. *PMBP*., 24: 993-1004. doi: 10.1007/s12298-018-0531-6.

Hasanuzzaman, M., M. Inafuku, K. Nahar, M. Fujita and H. Oku. 2021. Nitric oxide regulates plant growth, physiology, antioxidant defence, and ion homeostasis to confer salt tolerance in the mangrove species, *Kandelia Obovata*. *Antioxidants*. 10(4): 611. doi:10.3390/antiox10040611.

Hasanuzzaman, M., M.A. Hossain and M. Fujita. 2011. Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. *Plant Biotechnol. Rep*., 5: 353-365.

Hu, F., Y. Zhang and J. Guo 2023. Effects of drought stress on photosynthetic physiological characteristics, leaf microstructure, and related gene expression of yellow horn. *Plant Signal. Behav*., 18(1): 2215025.

Hu, Y. and U. Schmidhalter. 1998. Spatial distributions and net deposition rates of mineral elements in the elongating wheat (*Triticum aestivum* L.) leaf under saline soil conditions. *Planta*, 204: 212-219.

Hussain, I., M.A. Ashraf, F. Anwar, R. Rasheed, M. Niaz and A. Wahid. 2014. Biochemical characterization of maize (*Zea mays* L.) for salt. *Plant Biosyst*., 148(5): 1016-1026.

Jain, S., R.C. Verma, L. Murdia, H. Jain and G.P. Sharma. 2011. Optimization of process parameters for osmotic dehydration of papaya cubes. *J.F.S.T*., 48: 211-217. doi:10.1007/s13197-010-0161-7.

Jaleel, A.C., P. Manivannan, A. Wahid, M. Farooq, H.J. Al-Juburi, R. Somasundaram and R. Panneerselvam. 2009. Drought stress in plants: a review on morphological characteristics and pigments composition. *I.J.A.B*., 11(1): 100-105.

Jukanti, A.K., P.M. Gaur, C.L. Gowda and R.N. Chibbar. 2012. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. *Brit. J. Nutrit*., 108: Suppl 1:S11-26. doi: 10.1017/S0007114512000797. PMID: 22916806.

Jyang, J.D., J.Y. Yun, T.H. Zhang and H.L. Zhao. 2006. Presoaking with nitric oxide donor SNP alleviates heat shock damages in mung bean leaf discs. *Bot. Stud*., 47: 129-136.

Kazemi, N., R.A. Khavari-Nejad, H. Fahimi, S. Saadatmand and T. Nejad-Sattari. 2010. Effects of exogenous salicylic acid and nitric oxide on lipid peroxidation and antioxidant enzyme activities in leaves of *Brassica napus* L. under nickel stress. *Sci. Hortic*., 126(3): 402-407. doi:10.1016/j.scienta.2010.07.037.

Kolbert, Z., J.B.Barroso, R. Brouquisse, F.J. Corpas, K.J. Gupta, C. Lindermayr, G.J. Loake, J.M. Palma, M. Petřivalský and D. Wendehenne. 2019. A forty year journey: The generation and roles of NO in plants. *Nitric Oxide*., 93: 53-70. doi: 10.1016/j.niox.2019.09.006.

Kpyoarissis, A., Y. Petropoulou and Y. Manetas. 1995. Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiatae) under Mediterranean field conditions: avoidance of photo inhibitory damage through decreased chlorophyll contents. *J. Exp. Bot*., 46: 1825-1831.

Kumar, R.R., K. Karajol and G.R. Naik. 2011. Effect of polyethylene glycol induced water stress on physiological and biochemical responses in Pigeonpea (*Cajanus cajan* L. Millsp.): *Rec. Res. Sci. Technol*., 3(1): 148-152.

Lamattina, L., C. Garcia-Mata, M. Graziano and G. Pagnussat. 2003. Nitric oxide: the versatility of an extensive signal molecule. *Ann. Rev. Plant Biol*., 54: 109-136.

Laspina, N.V., M.D. Groppa, M.L. Tomaro and M.P. Benavides. 2005. Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. *Plant Sci*., 169: 323-330.

Lei, Y., C. Yin and C. Li. 2007. Adaptive responses of *Populus przewalskii* to drought stress and SNP application. *Acta Physiol. Plant*., 29: 519-526. doi:10.1007/s11738-007-0062-1.

Leitner, M., E. Vandelle, F. Gaupels, D. Bellin and M. Delledonne. 2009. NO signals in the haze: nitric oxide signalling in plant defence. *Curr. Opin. Plant Biol*., 12: 451-458.

Lichtenthaler, H. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol*., 148C: 350-382. 10.1016/0076-6879(87)48036-1.

Maalekuu, B., Y. Elkind, A. Leikin-Frenkel, S. Lurie and E. Fallik. 2006. The relationship between water loss, lipid content, membrane integrity and LOX activity in ripe pepper fruit after storage. *Postharvest Biol. Technol*., 42: 248-255. doi:10.1016/j.postharvbio.2006.06.012

Madhava Rao, K.V. and T.V. Sresty. 2000. Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Sci*., 157(1): 113-128. doi:10.1016/s0168-9452(00)00273-9

Mafakheri, A., A. Siosemardeh, B. Bahramnejad, P.C. Struik and Y. Sohrabi. 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Aust. J. Crop Sci*., 4(8): 580-585.

Mata-Pe´rez, C., I. Sa´nchez-Vicente, N. Arteaga, S. Go´mez-Jime´nez, A. Fuentes-Terro´n, C.S. Oulebsir, M. Calvo-Polanco, C. Oliver and O. Lorenzo. 2023. Functions of nitric oxide-mediated post-translational modifications under abiotic stress. *Fron. Plant Sci*., 14:1158184. https://doi.org/10.3389/fpls.2023.1158184

Mihaljevi´c, I., M.V. Vuleti´c, D. Šimi´c, V.Tomaš, D. Daniela Horvat, M. Josipovi´c, Z. Zduni´c, K. Dugali´c and D. Vukovi´c. 2021. Comparative study of drought stress effects on traditional and modern apple cultivars. *Plants*, 10: 561. https://doi.org/10.3390/plants10030561.

Minguez-Mosquera, M.I. and A. Perez-Galvez. 1998. Study of lability and kinetics of the main carotenoid pigments of red pepper in the deesterification reaction. *J. Agric. Food Chem*., 46: 566-569.

Minguez-Mosquera, M.I. and D. Hornero-Mendez. 1993. Separation and quantification of the carotenoid pigments in red peppers (*Capsicum annuum* L.), paprika, and oleoresin by reverse-phase HPLC. *J. Agric. Food Chem*., 41: 1616-1620.

Moreau, M., C. Lindermayr, J. Durner and D.F. Klessig. 2010. NO synthesis and signaling in plants – where do we stand? *Physiol. Plant*., 138: 372383.

Neidiquele, M.J.T. Silveira, L.F. Hancock, S. Eleni, C.C.M. Fernanda, S. Ione, C.M. Eduardo and V.R. Rafael. 2017. Evidence towards the involvement of nitric oxide in drought tolerance of sugarcane. *Plant Physiol. Biochem*., 115: 354-359. doi:10.1016/j.plaphy.2017.04.011.

Neill, S., J. Bright, R. Desikan, J. Hancock, J. Harrison and I. Wilson. 2008. Nitric oxide evolution and perception. *J. Exp. Bot*., 59: 25-35.

Nyachiro, J.M., K.G. Briggs, J. Hoddinott and A. Flanagan. 2001. Chlorophyll content, chlorophyll fluorescence and water deficit in spring wheat. *Cereal Res. Commun*., 29: 135-142. doi:10.1007/BF03543653.

Okuma, E., K. Soeda, M. Tada and Y. Murata. 2000. Exogenous proline mitigates the inhibition of growth of *Nicotiana tabacum* cultured cells under saline conditions. *Soil Sci. Plant Nutr*., 46: 257-263.

Parvaiz, A. and S. Satyawati. 2008. Salt stress and phyto-biochemical responses of plants-a review. *PSE*., 54: 88-99.

Pedersen, A.L., H.C. Feldner and L. Rosendahl. 1996. Effect of proline on nitrogenase activity in symbiosomes from root nodules of soybean (*Glycine max* L.) subjected to drought stress. *J. Exp. Bot*., 47: 1533-1539.

Poroyko, V., W. Spollen, L. Hejlek, A. Hernandez, M. LeNoble, G. Davis, H. Nguyen, G. Springer, R. Sharp and H. Bohnert. 2007. Comparing regional transcript profiles from maize primary roots under well-watered and low water potential conditions. *J. Exp. Bot*., 58: 279-289.

Qu, Z., Y. Tian, X. Zhou, X. Li, Q. Zhou, X. Wang and S. Dong. 2023. Effects of exogenous sodium nitroprusside spraying on physiological characteristics of soybean leaves at the flowering stage under drought stress. *Plants*, (Basel). 12(8): 1598. doi:10.3390/plants12081598.

Razmjoo, K., P. Heydarizadeh and M.R. Sabzalian. 2008. Effect of salinity and drought stresses on growth parameters and essential oil content of *Matricaria chamomile*. *Int. J. Agric. Biol*., 10: 451-454.

Rhodes, D., P.E. Verslues and R.E. Sharp. 1999. Role of amino acids in abiotic stress resistance. In: (Ed.): Singh, B.K. Plant Amino Acids: *Biochem. Biotechnol*., 319-356: Marcel Dekker.

Roch, J., T. Hewezi, A. Bouniols and L. Gentzbittel. 2009. Real-time PCR monitoring of signal transduction related genes involved in water stress tolerance mechanism of sunflower. *Plant Physiol. Biochem*., 47: 139-145.

Rossi S., P. Burgess, D. Jespersen and B. Huang. 2017. Heat-induced leaf senescence associated with chlorophyll metabolism in bentgrass lines differing in heat tolerance. *Crop Sci*., 57: 169-178. doi: 10.2135/cropsci2016.06.0542

Sairam, R.K., P.S. Deshmukh and D.C. Saxena. 1998. Role of antioxidant systems in wheat cultivars tolerance to water stress. *Biol. Plant*., 41: 387-394.

Sami, F., H. Siddiqui, P. Alam and S. Hayat. 2021. Nitric oxide mitigates the salt-induced oxidative damage in mustard by upregulating the activity of various enzymes. *J. Plant Growth Regul*., 40: 2409-2432. doi:10.1007/s00344-021-10331-4.

Sharma, P. and R.S. Dubey. 2005. Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. *J. Plant Physiol*., 162: 854-864.

Siddiqui, H.M., M.H. Al-Whaibi and M.O. Basalah. 2010. Role of nitric oxide in tolerance of plants to abiotic stress. *Protoplasma*, 248: 447-455. doi:10.1007/s00709-010-0206-9

Silveira, N.M., F.C. Marcos, L. Frungillo, B.B. Moura, A.B. Seabra, I. Salgado and R.V. Ribeiro. 2017. S-nitrosoglutathione spraying improves stomatal conductance, Rubisco activity and antioxidant defense in both leaves and roots of sugarcane plants under water deficit. *Physiol. Plant*., 160(4): 383-395.

Singh, A. and A. Roychoudhury. 2023. Abscisic acid in plants under abiotic stress: crosstalk with major phytohormones. *Plant Cell Rep*., 42(6): 961-974. doi: 10.1007/s00299-023-03013-w.

Singleton, V., R. Orthofer and R. Lamuela-Ravento´s. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: (Ed.): Packer, L. Oxidants and antioxidants, part A. Methods Enzymol. 299: 152-178. New York: Academic Press. Doi: 10.1016/ S0076-6879(99)99017-1.

Sofo, A., B. Dichio, C. Xiloyannis and A. Masia. 2004. Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress. *Physiol. Plant*., 121(1): 58-65. doi:10.1111/j.0031-9317.2004.00294.x.

Tan, Y., Z. Liang, H. Shao and F. Du. 2006. Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among three different genotypes of Radix Astragali at seeding stage. *Colloid. Surf. B. Biointerfaces*., 49(1): 60-65. doi:10.1016/j.colsurfb.2006.02.014

Verbruggen, N. and C. Hermans. 2008. Proline accumulation in plants: a review. *Amino Acids*., 35: 753-759.

Vijayan, K. 2009. Approaches for enhancing salt tolerance in mulberry (*Morus* L): *Plant Omics J*., 2(1): 41-59.

Vranova, E., D., Inze and F. Breusegem. 2002. Signal transduction during oxidative stress. *Expt. Bot*., 372: 1227-1236.

Xu Y.F., J.W. Jin, T.Y. Liu, H. Zhou, T.M. Hu, Z. Wang and M.X. Long. 2011. Regulation function of nitric oxide (NO) in leaves of plant under environmental stress. *Afr. J. of Biotechnol*., 10(70): 15673-15677.

Yang, X., M. Lu, Y. Wang, Y. Wang, Z. Liu and S. Chen. 2021. Response mechanism of plants to drought stress. *Hortic*., 7: 50. https://doi.org/10.3390/horticulturae7030050

Yin, H.H., B.J. Knowlton and B.W. Balleine. 2004. Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *Eur. J. Neurosci*., 19(1): 181-189. doi:10.1111/j.1460-9568.2004.03095.x

Yu, L.X. and T.L. Setter. 2003. Comparative transcriptional profiling of placenta and endosperm in developing maize kernels in response to water deficit. *Plant Physiol*., 131: 568-582.

Zangani, E., H.R. Angourani, B. Andalibi, S.V. Rad and A. Mastinu. 2023. Sodium nitroprusside improves the growth and behavior of the stomata of *Silybum marianum* L. subjected to different degrees of drought. *Life*, 13(4): 875. doi:10.3390/life13040875.

Zhang J., K. Cheng, X. Liu, Z. Dai, L. Zheng and Y. Wang. 2023. Exogenous abscisic acid and sodium nitroprusside regulate flavonoid biosynthesis and photosynthesis of *Nitraria tangutorum* Bobr in alkali stress. *Front. Plant Sci*., 14: 1118984.

Zhang, F., F. Chen, P. Wu, N. Zhang and D. Cui. 2014: Molecular characterization of lipoxygenase genes on chromosome 4BS in Chinese bread wheat (*Triticum aestivum* L.): *Theor. Appl. Genet*., 128: 1467-1479. doi:10.1007/s00122-015-2518-9

Zhang, H., W. Shen and L. Xu. 2003. Effects of nitric oxide on the germination of wheat seeds and its reactive oxygen species metabolisms under osmotic stress. *Acta Bot. Sin*., 45(8): 901-905. CBA:364799.

Zhang, L., X. Li, X. Li, Z. Wei, M. Han, L. Zhang and B. Li. 2016. Exogenous nitric oxide protects against drought-induced oxidative stress in Malus rootstocks. *Turk. J. Bot*., 40(1): 1-10. doi:10.3906/bot-1407-311196-1204.

Zhang, X.L., X.F. Jia, B. Yu, Y. Gao and J.G. Bai. 2011. Exogenous hydrogen peroxide influences antioxidant enzyme activity and lipid peroxidation in cucumber leaves at low light. *Sci. Hort*., 129: 565-662.

Zhao, L., J. He, X. Wang and L. Zhang. 2008. Nitric oxide protects against polyethylene glycol-induced oxidative damage in two ecotypes of reed suspension cultures. *J. Plant Physiol*., 165: 182-191.

(Received for publication 31st July 2024)