ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 14, Issue, 10, pp.22434-22441, October, 2022
DOI: https://doi.org/10.24941/ijcr.44103.10.2022

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EFFECT OF EXOGENOUS NITRIC OXIDE DONOR ON OXIDATIVE STRESS IN BASIL (*OCIMUM BASILICUM* L.) UNDER DROUGHT STRESS

Aishah A. Somali[1], Manal El-Zohri[1,2,*] and Sameera O. Bafeel[1]

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21488, Saudi Arabia
[2]Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut 71516, Egypt

ARTICLE INFO

Article History:
Received 20th July, 2022
Received in revised form
17th August, 2022
Accepted 29th September, 2022
Published online 19th October, 2022

Key words:

Basil; Sodium Nitroprusside; Water stress; Osmoregulators, Antioxidants; Antioxidant Enzymes.

**Corresponding Author:*
Manal El-Zohri

ABSTRACT

This study investigated the efficiency of sodium nitroprusside (SNP) foliar spraying on basil (*Ocimum basilucum*) growth and oxidative stress alleviation under drought conditions. Basil plant subjected to four water levels (100, 75, 50 and 25% FC), at the same time leaves were sprayed with 100, 150 and 250 µM SNP. The results demonstrate that basil growth significantly decreased under drought stress, while SNP spraying, with all studied concentrations, significantly enhanced plant growth under drought conditions. Out of three SNP concentration tested, 150 µM proved to be the optimal treatment for ameliorating drought stress. Chlorophyll a, chlorophyll b and carotenoids content increased under drought conditions. Application of SNP at all studied treatments significantly improved chlorophyll a and carotenoid content mainly under moderate and severe drought condition (50, 25% FC). Proline concentration in the basil leaves significantly decreased under drought condition while SNP application alleviated osmatic stress. As indicators of drought stress, H_2O_2 and MDA content increased. SNP application alleviate drought stress by reducing H_2O_2 and MDA content. The beneficial effect of SNP was apparent in plants defensive state, where the concentration of ascorbic acid, oxidized glutathione and the activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) showed higher levels compered to untreated stressed control. Under all drought levels, the most pronounced improvement achieved for SOD activity (3 folds) by applying 250 µM SNP, for APX activity (24 folds) at150 µM SNP and for GR activity (2 folds) at 100 µM higher than untreated stressed plants. Therefore, foliar usage of SNP at 100, 150 and 250 µM might be suggested as an efficient way for enhancing basil tolerance to drought stress.

Citation: ***Aishah A. Somali, Manal El-Zohri and Sameera O. Bafeel.*** **2022.** "Effect of exogenous nitric oxide donor on oxidative stress in basil (*Ocimum basilicum* L.) under drought stress*". International Journal of Current Research*, 14, (10), 22434-22441.

INTRODUCTION

Drought stress is the most prevalent environmental factor limiting crop productivity (FAO, 2020). Climate models have predicted increased severity and frequency of drought under the ongoing global climate change scenarios (Walter *et al*., 2011). It is a multidimensional stress where its severity depends on many factors such as occurrence and distribution of rainfall, evaporative demands and moisture storage capacity of soils (Gogoi and Tripathi, 2019). Because the world's water supply is limiting, future food demand for rapidly increasing population pressures is likely to further aggravate the effects of drought (Somerville and Briscoe, 2001). Furthermore, drought impairs the effect of the other stresses to which plants are submitted (abiotic or biotic) such as salt and cold stresses (Cruz de Carvalho, 2008). Drought severely affects plant growth and development with substantial reductions in crop growth rate and biomass accumulation (Farooq *et al*., 2012). However, the effects of drought stress vary with the crop, stage of crop as well as with the local environmental conditions (Okunlola *et al*., 2017). Many studies demonstrated that, drought affects the seed germination, vegetative and reproductive growth as well as maturity stages of a crop at different scale depending upon the frequency and duration of drought stress (Okunlola *et al*., 2017). Plants have ability to tolerate drought stress with an array of morphological, physiological, and biochemical adaptations (Bohnert *et al*., 1995). These adaptive traits involve maintenance of cell turgor through osmotic adjustment and cellular elasticity, and increasing protoplasmic resistance. In this regard, some plants could achieve higher tissue water status by maintaining the water uptake through increased rooting, hydraulic conductance, etc. under drought stress (Basu *et al*., 2016). Other plants use water effectively through reduced loss of water by reducing transpiration rate, radiation absorption, etc. (Blatt, 2000). If prolonged over to a certain extent, drought stress will inevitably result in oxidative damage due to the over production of reactive oxygen species (Wang *et al*., 2019). Reactive oxygen species (ROS) are continuously produced in plants as byproducts of various metabolic pathways that are localized in different cellular compartments. There are basically four forms of cellular ROS, singlet oxygen ($^1O_2$), superoxide radical (O^2), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\cdot}$) (Cruz de Carvalho., 2008). All ROS are extremely harmful to organisms at high concentrations (Sharma *et al*., 2012). When the level of ROS exceeds the defense mechanisms, a cell is said to be in a state of

oxidative stress (Sharma *et al*., 2012). Plants primarily deal with oxidative stress via an endogenous defensive mechanism consisting of different enzymatic (SOD; CAT; APX; GR; etc.) and nonenzymatic (ascorbic acid, AsA; glutathione, GSH; phenolic acids; alkaloids; flavonoids; carotenoids, etc.) antioxidants (Kaur *et al*., 2019). In plant cells, the antioxidant defense system and ROS accumulation uphold a steady-state balance (Hasanuzzaman *et al*., 2012). Maintaining an optimum ROS level in the cell enables proper redox biology reactions and the regulation of numerous processes essential for plants such as growth and development (Mittler 2017). This intermediate level is maintained by the balance between ROS production and ROS scavenging (Hasanuzzaman *et al*., 2019). However, during stress conditions, over generation of ROS demolishes the equilibrium and causes cellular damage by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death (PCD) pathway and ultimately leading to death of the cells (Mishra *et al*., 2011). Nitric oxide (NO) is a redox, gaseous, highly reactive nitrogen species produced in living cells under normal as well as biotic and abiotic stress conditions. When the concentration of ROS becomes toxic to a plant, NO may act as a detoxifier and minimize any detrimental effects (Lipton *et al*., 1993). Nitric oxide also has a role in respiratory function, namely electron transport pathways in mitochondria, where it modulates ROS thereby activating defense mechanisms through enhanced antioxidant production in plants exposed to various abiotic stresses (Zottini *et al*., 2002). Additionally, exogenous supply of NO leads to activation of antioxidant enzymes, especially SOD, and restricts O^2 and lipid oxidation and organic radicals (Shi *et al*., 2007). NO was also shown to be an endogenous modulator of several plant hormones, in addition to inhibiting the induced programmed cell death and aiding in stomatal function in several plant species such as *Arabidopsis*, wheat, and pea (Bright *et al*., 2006). Moreover, NO possesses several additional properties favorable to activity as a signaling messenger during unfavorable or multiple stress conditions such as the presence of free radical, small size redox molecules, neutral, and easily diffusible through a cell membrane, all these make it a very significant agent to act as a dynamic molecule (Domingos *et al*., 2015).

Basil (*Ocimum basilicum* L.), Family Lamiaceae, is a very important medicinal plant and culinary herb marketed fresh, dried, or frozen (Loughrin and Kasperbauer, 2001). is one of the best known genera for its medicinal properties and economically important aromatic oils (Rastogi *et al*., 2014). It is native to Asia, Africa, South America, and the Mediterranean but widely cultivated in many countries (Labra *et al*., 2004). The leaves and flowers are used for medicinal and culinary purposes such as curing headaches, coughs, diarrhea and kidney malfunctions (Georgiadou *et al*., 2018). In the extracts of these plants, a number of phenolic compounds with strong antioxidant activity have been found (Georgiadou *et al*., 2018). These antioxidant qualities have the ability to maintain health and prevent from coronary heart disease and cancer (Javanmardi *et al*., 2003). The *O. basilicum* essential oils exhibited a wide and varying array of chemical compounds, that showed antimicrobial activity. Aroma compounds are also extracted from *O. basilicum* and used in a wide variety of products such as cosmetics and natural flavors (Loughrin and Kasperbauer, 2001). Although plants adopt physiological, biochemical, and molecular strategies to cope up drought stress, these natural adaptation strategies are not sufficient to fulfil the targeted outputs (Dey *et al*., 2021).Variations in endogenous NO levels and or exogenous NO application has shown to regulate abiotic stress resistance suggesting that this approach may contribute in enhancing crop production under stress conditions (Siddiqui *et al*., 2011).Therefore, this study focus in improving drought tolerance of basil plant using foliar sparing with NO donor as a practical method for improving plant productivity under water stress conditions.

MATERIALS AND METHODS

Experimental design and treatments: *Ocimum basilicum* (basil) seeds sown in plastic pots filled with 3 Kg homogenously mixed sand:clay soil (2:1), under natural light condition in the greenhouse of King Abdulaziz University. The pots divided into two sets; one set did not treated with sodium nitroprusside (SNP) donor, while the other sprayed with SNP (100, 150 and 250 µM) dissolved in deionized water. For drought stress conditions, each set subdivided into four subsets, these subsets irrigated with tap water at 100% FC (control), 75%, 50% and 25% FC respectively as mild, moderate and severe drought conditions. Drought and SNP treatments started after the growth of the fourth true leave. The experiment was carried out in a Complete Randomized Design (CRD) with 3 replicates. At the end of the experimental period (March to May, 2019), plant samples were collected and transferred immediately to the laboratory for analysis. Shoot and root fresh and dry weights were determined. For all assays, plant samples were frozen immediately in liquid nitrogen then stored at -80° C for further analysis.

Growth parameter

Fresh and dry weight of Shoots and Roots: The samples were washed with distilled water and gently dried by tissue paper. A freshly harvested shoots and roots were weighted and recorded. Then the samples were wrapped in foil paper and kept in oven-dried by JSON-100 Natural Convection Oven at 70°C until constant weight of each sample was reached (48 hours), to determine the dry weight.

Photosynthetic pigments: Chlorophylls and Carotenoids were extracted in 95% ethyl alcohol and measured using UV-VIS Spectroscopy according to Hiscox and Israelstam (1979) with some modifications by Su *et al*. (2010). The absorbance readings were measured using spectrophotometry with a Lamda 25 UV-Vis spectrophotometer at wavelengths of 663, 644, and 452 nm.

Proline Determination: Proline wase extracted and determined in basil leaves as described by Bates *et al*. (1973). About 0.6 g leaves samples were homogenized in 1.5 ml 3% sulphosalicylic acid followed by centrifugation at 10,000 rpm for 10 min. One ml of the supernatant was mixed with 1 ml ninhydrin reagent (250 mg ninhydrin, 20 ml glacial acetic acid, 130 ml 6 M phosphoric acid, dissolved under shaking and slight heating) and boiled in water bath for 1 hour. The developed color was extracted in 2 ml toluene and measured colourimetrically at 520 nm against toluene.

Lipid peroxidation: The level of lipid peroxidation in plant leaves was determined as 2-toberhituric acid (TBA) reactive metabolites, i.e. malondialdehyde (MDA) by (Narwal *et al*, 2009). Briefly, 0.45g tissue sample was homogenized in 2.5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10.000 rpm for 5 min. To 1 ml aliquot of the supernatant 4 mil of 20% TCA containing 0.5% TBA were added. The mixture was heated at 95℃ for 30 min and cooled immediately on an ice-bath. The mixture was centrifuged at 10,000 rpm 15 min. and the absorbance of the supernatant was monitored at 532 mm.

H_2O_2 Determination: The levels of H_2O_2 content in basil leaves were colourimetrically measured by a modified method of Mukherjee and Choudhuri (1983). (0.1g leaf samples were extracted with cold acetone. An aliquot 3 ml of the extracted solution was mixed with 1 ml of 0.5 g titanium dioxide in 5 ml H_2SO_4, heated gently until fumes of sulfuric acid appear, after that cooled. Then ambitiously diluted to about 100 ml with distilled water and filtered. To 1ml of this clear filtrate, 3ml of the extracted solution was added. The intensity of yellow color of the supernatant was measured at 415nm.

Antioxidants: Fresh leaves samples (0.5 g) were ground in 6 ml 5 % (W/V) metaphosphoric acid on ice bath and then centrifuged at 13,000 g for 20 min. Then, the supernatant was used to determine the contents of ascorbic acid, oxidize and total glutathione (Anderson *et al*., 1992; Yan *et al*., 2010).

Ascorbic Acid Determination: According to Mukerjee and Choudhuri (1983) to 0.2 ml of plant extract, 0.8 ml of 10% TCA was added. After vigorous shaking, the tubes kept in an ice bath for 5 min., then

centrifuged at 3000 rpm for another 5 min. 0.5 ml of the extract was diluted to 2.0 ml using double-distilled water, and after 0.2 ml of diluted Folin reagent (commercially prepared Folin-Ciocaiteu reagent of 2 M concentration diluted 10-fold with double-distilled water) was added to the extract, the tubes were vigorously shaken. After 10 min. the absorbance of the blue colour developed was measured at 760 nm.

Total Glutathione Determination: Total glutathione content of basil leaves was determined according to the procedure of Adams and Liyanage (1991) with minor modifications. Based on enzymatic recycling, GSH is oxidized by 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and reduced by NADPH in the presence of GR, and GSH content is evaluated by the rate of absorption change at 412 nm of 2-nitro-5-thiobenzoic acid (NTB) generated from the reduction of DTNB (Anderson 1985). In brief, GR (Sigma-Aldrich, St Louis, MO, USA), DTNB, and NADPH were pre-diluted with reaction buffer (0.5 M potassium phosphate buffer, pH 7.5). Leaves extract (200 µL) was mixed with 500µL reaction buffer, 100 µL of GR and 100 µL of DTNB (30 mg mL). The reaction was started by adding 100 µL of NADPH (2 mg mL); after mixing, the rate of absorption changes at 412 nm.

Oxidized Glutathione Determination: Oxidized glutathione (GSSG) concentration was determined after Rahman *et al*. (2006) with some modifications. Briefly, 400 µL of leaves extract was mixed with 500 µL reaction buffer then 8 µL of 2-vinylpyridine and kept for 1 hour at room temperature. Then, the reaction was started by adding 100 µL of GR, 100 µL of DTNB (30 mg mL) and100 µL of NADPH (2 mg mL); after mixing, the absorption change was followed at 412 nm.

Activity of antioxidant enzymes: Enzyme extraction will be prepared according to Cakmak and Marschner (1992). 0.5 g of leaves tissues was grounded to a fine powder in liquid (N_2). After that it will be homogenized in 5 ml of 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM Methylene diamine tetra acetic acid (EDTA) and 0.1 g polyvinyl pyrrolidone (PVP). The homogenate will be centrifuged at 18,000 rpm for 10 min at 4 ºC and the supernatants will be collected and used for the assays of enzymes activity.

Superoxide dismutase (SOD, EC 1.15.1.1): Superoxide dismutase (SOD) activity was measured by following the autooxidation of epinephrine (adenochrome) as described by Cakmak and Marschner (1992). Enzyme activity was measured in a final volume of 2 ml of the reaction medium containing 25 mM of sodium carbonate buffer (pH 10.2), 200 µl 0.5 mM EDTA and 100 µl enzyme extracte. The reaction was started by addition of 100 µl of 15 mM epinephrine (dissolved in 10 mM HCl, pH 2.4). Autoxidation of epinephrine was determined at A480 nm.

Catalase (CAT, EC 1.11.1.6): Catalase activity will be assayed spectrophotometrically by monitoring the change in A240due to the decreased absorption of H_2O_2 (Zhang and Kirkham, 1996). The reaction medium contained 50 mM potassium phosphate buffer (pH 7), and 500 µl of enzyme extract in a 3 ml volume. The reaction was initiated by addition of 100 µl of 10 mM H_2O_2.

Ascorbte peroxidase (APX, EC 1.11.11.11): Ascorbte peroxidase activity will be determined according to Zhang and Kirkham (1996). The rate of hydrogen peroxide-dependent oxidation of ascorbic acid was determined in a reaction mixture contained 50 mM potassium phosphate buffer (pH 7). 5 mM H_2O_2, 0.1 mM Na_2-ETDA, 0.5 mM ascorbic acid and 50 µl enzyme extract. The oxidation rate of ascorbic acid was estimated from the decrease in absorbance at 290 nm.

Glutathione reductase (GR, EC 1.6.4.2): Glutathione reductase activity was determined at 25 °C following oxidation of NADPH at 340 nm in 1ml of reaction mixture containing 100 mM potassium phosphate buffer (pH 7.8), 2 mM EDTA, 0.2 mM NADPH and 0.5 mM GSSG (Rao *et al*., 1996).

Statistical Analysis: The data were analyzed using the statistical software SPSS. A two-way analysis of variance (ANOVA) was performed to examine the effects of the studied water levels, SNP treatments, and their interactions upon all investigated traits. Significant differences between treatments ($p<0.05$) were confirmed using Bonferroni multiple comparison test. All values were expressed with their standard error (SE) as a mean value of three replicates.

RESULTS

Growth parameter: As shown in Figure (1) and Figure (2) plant growth significantly decreased in response to drought stress. At severe drought conditions (25% FC), shoot FW and DW decreased by bout (45.47%) and (42.11%) lower than their corresponding unstressed controls (Fig.1A,B).

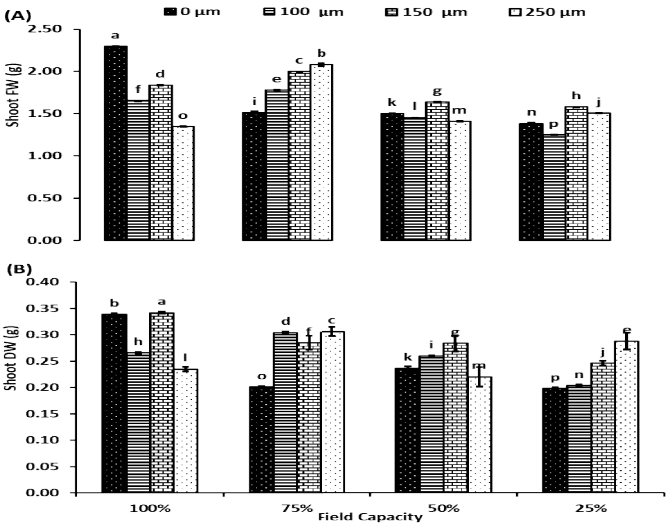
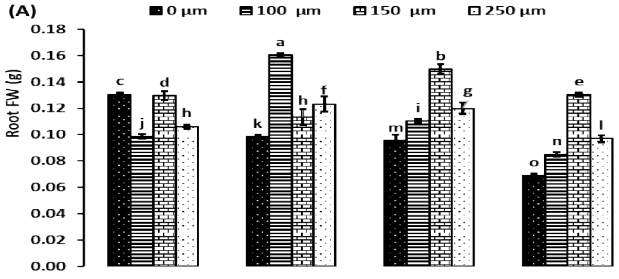


Figure 1. Shoot biomass, (A) fresh weight (FW) and (B) dry weight (DW) of basil plant as affected by foliar spraying with different concentrations of SNP under different levels of drought stress. Each point represents a mean value of three replicates (n = 3) with vertical bars representing standard error of the mean. Bars with different letters indicate a significant difference (p < 0.05) between SNP treatments at all studied water levels as determined by two-way ANOVA and Bonferroni multiple comparison test.

However foliar spraying with the studied concentrations of SNP (100, 150 and 250 µM) significantly increased plant growth under drought conditions. Shoot fresh and dry weight in addition to root dry weight significantly enhanced by increasing SNP concertation under mild drought condition (Fig. 1A, B and Fig. 2B). The most pronounced enhanced achieved under mild drought conditions (75% FC) by applying 250 µMSNP where, shoot fresh weight increased by about 37.48 %, shoot dry weight increased by about 51.74%, root fresh weight increased by about 25.51%, root dry weight increased by about 122.22% more than untreated stressed control (Fig.1 and Fig.2). In the same context, root fresh weight significantly increased by increasing SNP concentration under all investigated drought conditions (Fig. 2A).



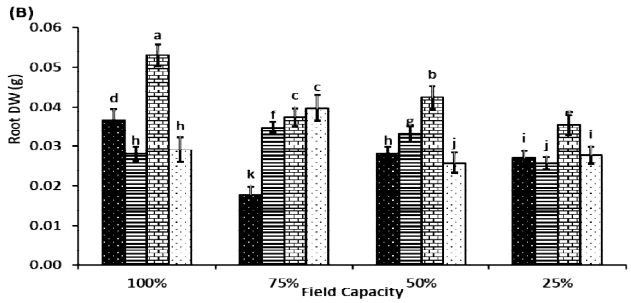


Figure 2. Root biomass, (A) fresh weight (FW), (B) dry weight (DW) of basil plant as affected by foliar spraying with different concentrations of SNP under different levels of drought stress. Each point represents a mean value of three replicates (n = 3) with vertical bars representing standard error of the mean. Bars with different letters indicate a significant difference ($p < 0.05$) between SNP treatments at all studied water levels as determined by two-way ANOVA and Bonferroni multiple comparison test.

At moderate and severe drought conditions (50, 25% FC) adding 150 µM of SNP significantly enhanced shoot fresh weight by about 9.18 % and 14.08, shoot dry weight by about 20.42% and 23.73%, root fresh weight by about 57.89% and 88.41%, root dry weight by about 50.00% and 29.63% respectively higher than untreated stressed control (Fig.1 and Fig.2).

Plant pigments: At presented in Figure (3), chlorophyll a, chlorophyll b and carotenoids slightly increased by increasing drought conditions. Foliar spraying with SNP at all studied concentrations (100,150 and 250 µM) under mild and moderate drought conditions (75, 50% FC) significantly decreased chlorophyll b by about 62.66% and 32.96% lower than untreated stressed control (Fig. 3B). Moreover, under moderate and severe drought conditions (50, 25% FC) applying SNP at all studied treatments significantly improved chlorophyll a by about 55.68% and 37.32%, carotenoid by about 126.41% and 86.51% higher than untreated stress control (Fig. 3A and C).

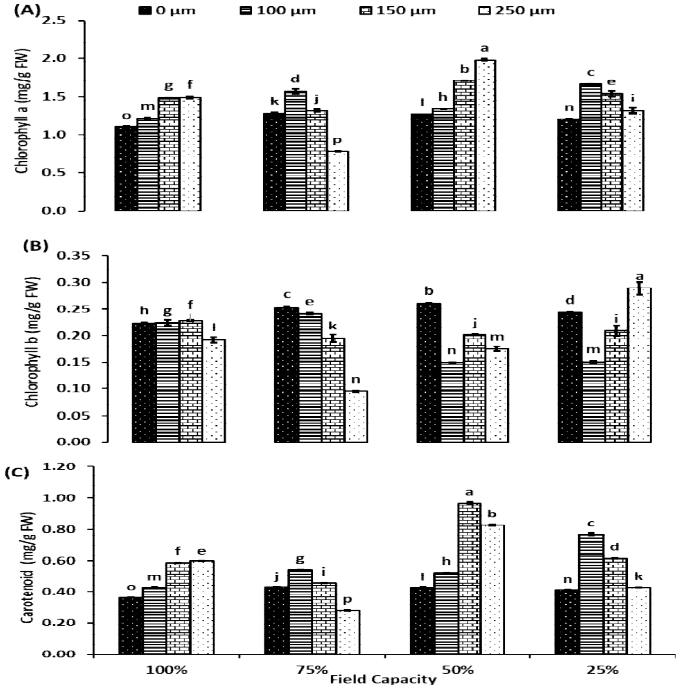


Figure 3. plant pigments, (A) chlorophyll a, (B) chlorophyll b, (C) carotenoids in basil leaves as affected by foliar spraying with different concentrations of SNP under different levels of drought stress. Each point represents a mean value of three replicates (n = 3) with vertical bars representing the standard error of the mean. Bars with different letters indicate a significant difference (p < 0.05) between SNP treatments at all studied water levels as determined by two-way ANOVA and Bonferroni multiple comparison test

Adding 250 µM of SNP at moderate drought conditions (50% FC) significantly increased chlorophyll a by about 55.68% higher than untreated stressed control while applying 150 µM of NO increased carotenoid by about 126.41% higher than untreated control (Fig.3A & C).

Stress Indicators: MDA and H_2O_2 concentration significantly increased while proline concentration in the leaves significantly decreased under drought stress conditions. foliar spraying with SNP at all studied treatment (100,150 and 250 µM) significantly enhanced proline concentrations in the leaves under drought conditions by about 76.13%, 182.14% and 66.06% higher than untreated stress control (Fig.4 A). However, most of the studied SNP treatment significantly decreased MDA concentration by about 60.90%, 76.10%, 61.55% and H_2O_2 concentration by about 32.57%, 3.67% and 55.04% lower than stressed control (Fig. 4B and C).

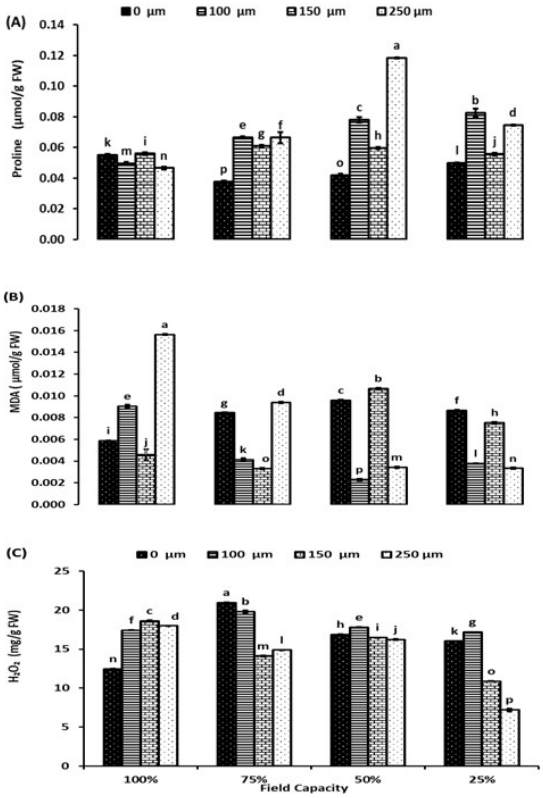


Figure 4. stress indicators, (A) proline fresh weight (FW) (B) malondialdehyde (MDA) and (C) hydrogen peroxide (H_2O_2) concentration in basil leaves as affected by foliar spraying with different concentrations of SNP under different levels of drought stress. Each point represents a mean value of three replicates (n = 3) with vertical bars representing the standard error of the mean. Bars with different letters indicate a significant difference (p < 0.05) between SNP treatments at all studied water levels as determined by two-way ANOVA and Bonferroni multiple comparison test.

Antioxidants: As displayed in Figure (5) AsA and GSSG concentration significantly decreased by increasing drought stress level while GSH concentration significantly increased. At moderate and severe drought condition foliar spraying with NO at all studied treatments (100,150 and 250 µM) significantly improved AsA concentrations by about 34.17% and 33.41% higher than the untreated stress control (Fig.5 A). Applying SNP at all studied treatments under moderate drought conditions (50%FC) significantly increased GSSG concentrations by about 332.5% higher than stressed control (Fig.5 B). GSH concentration significantly decreased after adding SNP at all studied treatment by about 46.74%, 53.24% and 50.67% respectively lower than untreated stress control (Fig.5 C).

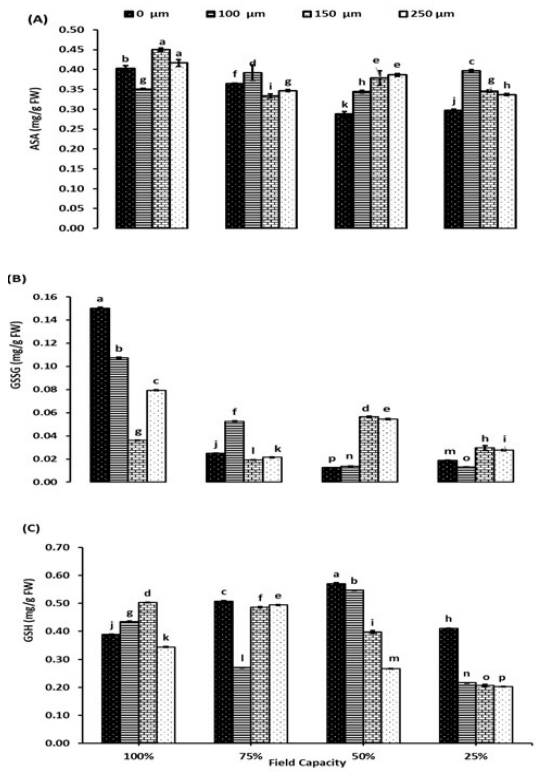


Figure 5. Antioxidants, (A) Ascorbic Acid (AsA), (B) oxidized Glutathione (GSSG) and (C) reduced Glutathione (GSH) in basil leaves as affected by foliar spraying with different concentrations of SNP under different levels of drought stress. Each point represents a mean value of three replicates (n = 3) with vertical bars representing the standard error of the mean. Bars with different letters indicate a significant difference (p < 0.05) between SNP treatments at all studied water levels as determined by two-way ANOVA and Bonferroni multiple comparison test.

Antioxidant enzyme: As shown in Figure(6) and Figure (7), Antioxidants enzyme activity significantly decreased by increasing drought level .At moderate and severe drought conditions (50, 25%FC)foliar spraying with SNP at all studied treatment (100,150 and 250 µM) significantly improved SOD and APX activity by about 3folds, 6.5 folds, 24 folds and 2.5 folds subsequently higher than untreated stress control (Fig.6A and Fig.7A).Adding 100 µM of SNP under severe drought conditions (25%FC) significantly enhanced SOD, CAT, APX and GR activity by about 617.53%, 226.32%,

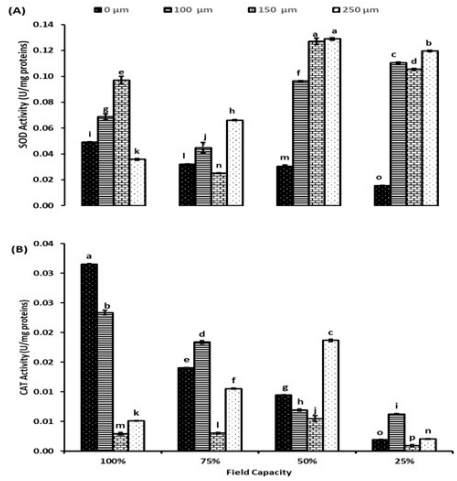


Figure 6. Antioxidant enzymes, (A) superoxide dismutase (SOD), (B) catalase (CAT) activity in basil leaves as affected by foliar spraying with different concentrations of SNP under different levels of drought stress. Each point represents a mean value of three replicates (n = 3) with vertical bars representing the standard error of the mean. Bars with different letters indicate a significant difference (p < 0.05) between SNP treatments at all studied water levels as determined by two-way ANOVA and Bonferroni multiple comparison test.

202.92% and 14.66% higher than stressed control (Fig.6and Fig.7). The most obvious improvement achieved at all studied drought level by adding 250 µM of SNP where SOD activity increased by about 3 folds and applying 150 µM of SNP where APX activity increased by about 24 folds and adding 100 µM of SNP where GR activity enhanced by about 112.42% higher than untreated control (Fig. 6A and Fig.7).

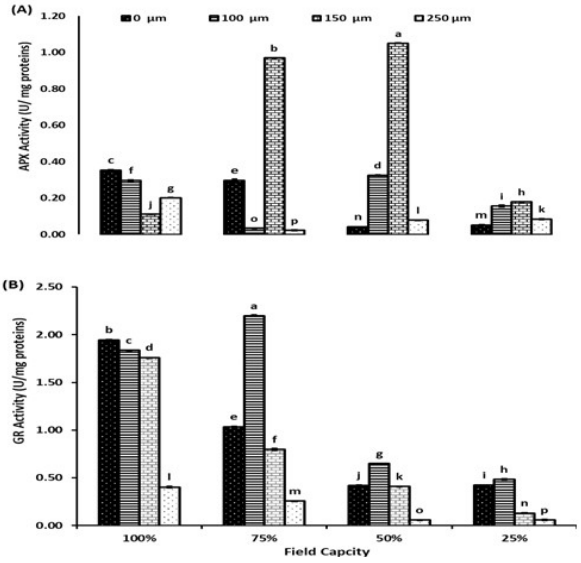


Figure 7. Antioxidant enzymes, (A) ascorbate peroxidase (APX) and (B) glutathione reductase (GR) activity in basil leaves as affected by foliar spraying with different concentrations of SNP under different levels of drought stress. Each point represents a mean value of three replicates (n = 3) with vertical bars representing the standard error of the mean. Bars with different letters indicate a significant difference (p < 0.05) between SNP treatments at all studied water levels as determined by two-way ANOVA and Bonferroni multiple comparison test.

DISCUSSION

Nitric oxide (NO) is a small, diatomic molecule with high-permeability therefor readily diffuses both in the hydrophilic space of the cytoplasm and in the lipid portion of the membrane (Arasimowicz and Floryszak-Wieczorek, 2007). Nitric oxide, with its high lipophilic nature, is easily released from membranes and acts as an intracellular and intercellular messenger in many physiological reactions including growth and development (Corpas *et al*., 2011), stomatal movement (Neill *et al*., 2003), seed germination (Sírovˇa et al., 2011), flowering (Khurana, 2011), respiratory metabolism and aging (Procháˇzkovˇa and Wilhelmovˇa 2011), biochemical interactions and responses to biotic and abiotic stresses (Hajihashemi *et al*., 2021).In this study, drought stress significantly decreased basil plant growth in term of shoot and root fresh and dry weight. However, exogenous application of SNP at all studied concentration (100, 150 and 250 µM) significantly alleviated plant growth under drought conditions.150 µM of SNP was more effective in inducing drought tolerance mainly under moderate and severe drought conditions (50 and 25% FC). In accordance with our results, foliar spraying with 100 and 150 µMSNP promoted plant biomass of hulless barley and garlic under water stress conditions (Gan *et al*., 2015; Astaneh *et al*., 2022). Under stressful conditions, the photosynthetic rate in cultivated plants may be decreased due to the destruction of chlorophyll. However, the correlation between plant stress tolerance and the pigment concentration of its leaves is not always evident (Khan *et al*., 2009). There is a contradiction concerning the data of chlorophyll accumulation; Both increase and decease of chlorophyll concentration under stress conditions have been recorded (Pirzad *et al*., 2011). In the current investigation, chlorophyll a, chlorophyll b and carotenoids slightly increased by increasing drought conditions in basil plants.

Moreover, under moderate and severe drought conditions (50, 25% FC) applying SNP at all studied treatments (100, 150 and 250 µM) significantly improved chlorophyll a and carotenoid content. In the same context Leite *et al*. (2019) revealed that chlorophylls content increased under water stress in SNP treated and untreated plants compared to well-watered plants. However, foliar spraying with SNP at all studied concentrations under mild and moderate drought conditions (75, 50% FC) significantly decreased chlorophyll b content in basil plant in our study. According to Santos (2004) and Eckardt (2009), the elevation in chlorophyll content in the process of pigment degradation could be due to the conversion of chlorophyll b to chlorophyll a. Proline is one of the major osmoprotectants (Ahluwalia *et al*., 2021). It has a variety of roles like maintaining cell turgor, stabilizing sub-cellular structures like proteins, enzymes, cell membranes and lipids and acting as a cytosolic pH buffer (Ahluwalia *et al*., 2021). Proline also acts as a source of organic carbon, nitrogen, and energy during the stress recovery stage, thereby increasing the overall growth of the plant (Proch´azkov´a *et al*., 2014; Barnawal *et al*., 2019). In the current study, proline concentration in basil leaves significantly decreased under drought stress conditions. While foliar spraying with SNP at all studied treatment significantly enhanced proline concentrations in the leave under drought conditions. It was proved that due to drought stress, intake of NO and its transport to leaves is reduced, as a consequence of which proline synthesis decreases (Etesami and Maheshwari, 2018). According to Arasimowicz-Jelonek *et al*. (2009) supplementation of drought-affected seedlings with NO significantly increased the Proline content, which further influence osmoregulation and water status restoration, increasing the leaf RWC of wheat seedlings (Hasanuzzaman *et al*., 2018).

One of the stress indicators in plant membranes is the production of malondialdehyde (MDA), which is considered as an indicator of lipid peroxidation (Astaneh *et al*., 2022).H_2O_2 level is also characteristic feature of oxidative stress and categorized as stress indicator (Sahay *et al*., 2019). It has been shown that exogenous SNP ameliorates the oxidative stress induced by a range of abiotic conditions (Farooq *et al*., 2009). In our study, as consequence of drought stress the content of H_2O_2 and MDA in basil increased. However, exogenous SNP application alleviates drought stress by reducing H_2O_2 and MDA content.SNP application significantly decreased H_2O_2 and MDA content under drought stress in Hulless barley also as indicated by Gan *et al*. (2015). Nitric oxide has antioxidant properties and acts as a signal in the activation of antioxidant enzymes under environmental stress (Mazid *et al*., 2011). NO directly or indirectly triggers expression of many redox-regulated genes (Astaneh *et al*., 2022). It reacts with lipid radicals thus preventing lipid oxidation, exerting a protective effect by scavenging superoxide radical and formation of proxy nitrite that can be neutralized by other cellular processes. Plant possesses several antioxidant defense system consisting of enzymatic and non-enzymatic component that normally keep ROS in balance within the cell (Akladious and Mohamed 2016). For instant, AsA is the major redox regulatory antioxidant and able to detoxify ROS by direct scavenging, acting as substrate in the ascorbate glutathione (AsA–GSH) cycle (Apel and Hirt 2004). The results of the present study showed that drought stress decreased the content of AsA, oxidized glutathione (GSSG) and increased reduced glutathione (GSH) in basil leaves. However, application of SNP at all studied treatments improved AsA content. Applying SNP at all studied treatments under moderate drought conditions (50%FC) significantly increased GSSG concentration. However, GSH concentration significantly reduced after adding SNP at all studied treatments. Hasanuzzaman *et al*. (2018) reported the AsA content decreases in wheat seedlings under drought stress. Addition of SNP to drought-stress seedlings enhanced APX, activity, which restored the AsA level (Hasanuzzaman *et al*., 2018). Similar roles of SNP in enhancing elements of the AsA–GSH cycle have been demonstrated by Hasanuzzaman *et al*. (2011). Glutathione is a non-protein, sulfhydryl-bearing antioxidant with an essential role in stress protection, including drought tolerance (Nahar *et al*. 2015). On contrary with this study, the level of GSSG was markedly increased by drought and reduced in response of NO treatment in wheat (Hasanuzzaman *et al*., 2018). The participation of GSH in the ROS scavenging process triggers its conversion to GSSG; thus, GSSG levels increase in the drought exposed seedlings. The results of the current study showed that antioxidant enzymes activity significantly decreased in basil leaves by increasing drought level. Under all drought levels, the most pronounced improvement achieved for SOD activity by applying 250 µM SNP, for APX activity at150 µM SNP and for GR activity at 100 µM. Exogenous SNP application stimulated the activation of antioxidant enzymes (SOD, CAT, GPX, APX, and GR) under water deficit conditions in previous studies (Boogar *et al*., 2014; Hasanuzzaman *et al*., 2018; Majeed *et al*., 2020).Superoxide dismutase is the first line enzymatic antioxidant that plays a crucial role by scavenging first ROS radical i.e., $O_2^{-\bullet}$(superoxide anion radical), and convert it into comparatively less toxic ROS (H_2O_2) (Sahay *et al*., 2019). Catalase may act in line after SOD dismutation of $O_2^{-\bullet}$, thus eliminates the H_2O_2 by converting it into H_2O and O_2(Sahay *et al*., 2019). Ascorbate peroxidase performs the same function as CAT does. As a central component of AsA-Glu cycle, APX enzyme uses two molecules of AsA to scavenge H_2O_2, and convert into H_2O along with DHA (Sahay *et al*., 2019). Glutathione reductase reductase is the flavo-protein based enzymatic antioxidant enzyme involved in H_2O_2 removal through the AsA-Glucycle. The main function of GR is to catalyze a reaction using NADPH and converts GSSG to GSH, and maintain GSH level in plant cells (Gill and Tuteja 2010; Li *et al*. 2014).

CONCLUSION

Based on the finding of this research, the usage of sodium nitroprusside as NO doner significantly ameliorate drought stress damage in basil plants. The results showed that the studied plant growth parameters were improved by the application of SNP under stress conditions. SNP treatment at all studied concentration (100, 150 and 250 µM) enhanced chlorophyll and carotenoids in basil. Proline concentration in basil plant significantly increased duo to the application of SNP at all studied concentrations. Exogenous SNP application alleviates drought stress by reducing H_2O_2 and MDA concentrations. The concentration of ascorbic acid, oxidized glutathione and the activity of superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase enzymes showed higher levels in SNP treated basil plants compered to untreated stressed control. Therefore, for improving basil tolerance to drought stress foliar spraying of SNP at 100, 150 and 250 µM is recommended as an efficient approach.

Conflict of Interest statement: The authors declare no conflict of interest.

REFERENCES

Adams, DO., Liyanage, C. 1991. Modification of an enzymatic glutathione assay for determination of total glutathione in grapevine tissues. Am J Enol Vitic., 42:137-140.

Ahluwalia, O., Singh, PC., Bhatia, R. 2021. A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria. Resources, Environment and Sustainability, *5*:100032.

Akladious, SA., Mohamed, HI. 2017. Physiological role of exogenous nitric oxide in improving performance, yield and some biochemical aspects of sunflower plant under zinc stress. Acta Biol. Hung., 68(1):101-114.

Anderson, JV., Chevone, BI., Hess, JL. 1992. Seasonal variation in the antioxidant system of eastern white pine needles. Plant Physiol., 98:501-508.

Anderson, ME. 1985. Determination of glutathione and glutathione disulfide in biological samples. Meth. Enzymol., 113:548-555.

Apel, K., Hirt, H. (2004) Reactive oxygen species: metabolism, oxidative stress, and signaling transduction. *Annu. Rev. Plant Biol. 55*:373–399.

Arasimowicz, M., Floryszak-Wieczorek, J. 2007. Nitric oxide as a bioactive signalling molecule in plant stress responses. Plant Sci. 172 (5):876–887.

Arasimowicz-Jelonek, M., Floryszak-Wieczorek, J., Kubis, J. (2009) Interaction between polyamine and nitric oxide signaling in adaptive responses to drought in cucumber. J Plant Growth Regul., 28:177–186.

Astaneh, RK., Bolandnazar, S., Nahandi, FZ. 2022. Exogenous nitric oxide protect garlic plants against oxidative stress induced by salt stress. J. Plant Stress Physiol., 5:100101.

Barnawal D, Singh R. Role of plant growth promoting rhizobacteria in drought tolerance. In: Singh AK, Kumar A, Singh PK, editors. PGPR Amelioration in Sustainable Agriculture.WoodHead Publishing, An imprint Elsevier 2019:107–128.

Basu, S., Ramegowda, V., Kumar, A., Pereira, A. 2016. Plant adaptation to drought stress. F1000Research, 5:1554 doi:10.12688/f1000research.7678.1

Bates, LS., Waldren, RP., Teare, ID. 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39:205–207.

Blatt, MR. 2000. Ca21 signaling and control of guard-cell volume in stomatal movements. CurrOpin Plant Biol., 3:196–204.

Bohnert, HJ., Nelson, DE., Jensen, RG. 1995. Adaptations to Environmental Stresses. Plant Cell, 7(7):1099–111.

Boogar, AR., Salehi, H., Jowkar, A. 2014. Exogenous nitric oxide alleviates oxidative damage in turfgrasses under drought stress. South African Journal of Botany, 92: 78-82.

Bright, J., Desikan, R., Hancock, J.T., Weir, I.S., Neill, S.J. 2006. ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H_2O_2 synthesis. Plant J., 45:113–122.

Cakmak, I., Marschner, H.1992. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol., 98:1222–1227.

Corpas, FJ., Leterrier, M., Valderrama, R., Airaki, M., Chaki, M., Palma, JM., Barroso, JB., 2011. Nitric oxide imbalance provokes a nitrosative response in plants under abiotic stress. Plant Sci., 181 (5):604–611.

Cruz de Carvalho, MH. 2008. Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signal. Behav., 3(3):156-165.

da Silva Leite, R., do Nascimento, MN., Tanan, TT., Neto, LPG., da Silva Ramos, C A., da Silva, AL. 2019. Alleviation of water deficit in *Physalisangulata* plants by nitric oxide exogenous donor. Agric. Water Manag., 216:98-104.

Dey P, Datta D, Pattnaik D, Dash D, Saha D, Panda D, Bhatta BB, Parida S, Mishra UN, Chauhan J, Pandey H, Singhal RK. Physiological, biochemical, and molecular adaptation mechanisms of photosynthesis and respiration under challenging environments. In: Aftab T, Roychoudhury A, editors. Plant Perspectives to Global Climate Changes, 1st Ed. Elsevier Academic press, 2021.

Domingos, P., Prado, A.M., Wong, A., Gehring, C., Feijo, J.A. 2015. Nitric oxide: a multitasked signaling gas in plants. Mol Plant., 8: 506–520.

Eckardt, NA. 2009. A new chlorophyll degradation pathway. Plant Cell, 21(3):700.

Etesami, H., Maheshwari, D.K. 2018. Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. Ecotoxicol. Environ. Saf., 156:225–246.

FAO, 2020. The State of Food and Agriculture 2020: Overcoming Water Challenges in Agriculture. FAO, Rome. https://doi.org/10.4060/cb1447en.

Farooq M, Hussain M, Wahid A, Siddique KHM. Drought stress in plants: an overview. In:Aroca R, editor. Plant responses to drought stress. Springer, Berlin: Heidelberg, 2012:1-33.

Gan, L., Wu, X., Zhong, Y. 2015. Exogenously applied nitric oxide enhances the drought tolerance in hulless barley. Plant Prod. Sci., 18(1):52-56.

Georgiadou, E. C., Kowalska, E., Patla, K., Kulbat, K., Smolinska, B., Leszczynska, J., Fotopoulos, V. 2018. Influence of heavy metals (Ni, Cu and Zn) on nitro-oxidative stress responses, proteome regulation and allergen production in basil (*Ocimum basilicum* L.) plants. Front. Plant Sci., 9:862.

Gogoi, A., Tripathi, B., 2019. https://www.indiaspend.com/42-indias-land-area-underdrought-worsening-farm-distress-in-election-year/.

Hajihashemi, S., Skalicky, M., Brestic, M., Pavla, V. 2021. Effect of sodium nitroprusside on physiological and anatomical features of salt-stressed Raphanus sativus. Plant Physiol. Biochem., 169:160–170.

Hasanuzzaman, M., Bhuyan, M., Anee, TI., Parvin, K., Nahar, K., Mahmud, JA., Fujita, M. 2019. Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants,8:384.

Hasanuzzaman, M., Hossain, MA., Fujita, M. 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.

Hasanuzzaman, M., Nahar, K., Rahman, A., Inafuku, M., Oku, H., Fujita, M. 2018. Exogenous nitric oxide donor and arginine provide protection against short-term drought stress in wheat seedlings. PhysiolMolBiol Plants., 24(6):993-1004.

Hiscox, JT., Israelstam, G. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Canad. J.Bot., 57(12):1332-1334.

Javanmardi, J., Stushnoff, C., Locke, E., and Vivanco, JM. 2003. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chem., 83:547–550.

Kaur, N., Kaur, J., Grewal, SK., Singh, I. 2019. Effect of Heat Stress on Antioxidative defense system and its amelioration by heat acclimation and salicylic acid pre-treatments in three pigeonpea genotypes. Indian J. Agric. Biochem., 32:106–110.

Khan, MA., Shirazi, MU., Khan, MA., Mujtaba, SM., Islam, E., Mumtaz, S., Shereen, A., Ansari, RU., Ashraf, MY. 2009. Role of proline, K/NA ratio and chlorophyll content in salt tolerance of wheat (*Triticumaestivum*L.). Pak. J. Bot., 41(2):633-638.

Khurana, A., Khurana, JP., Babbar, SB. 2011. Nitric oxide induces flowering in the duckweed LemnaaequinoctialisWelw. (Syn. L. *paucicostataHegelm*.) Under non inductive conditions. J. Plant Growth Regul., 30 (3):378–385.

Labra, M., Miele, M., Ledda, B., Grassi, F., Mazzei, M., Sala, F. 2004. Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars. Plant Sci., 167:725-731.

Li, X., Gong, B., Xu, K. 2014. Interaction of nitric oxide and polyamines involves antioxidants and physiological strategies against chilling-induced oxidative damage in *Zingiber officinale* Roscoe. Sci. Hortic., 170:237–248.

Lipton, SA., Choi, YB., Pan, ZH., Lei, SZ., Chen, HSV., Sucher, NJ., Loscalzo, J., Singel, DJ., Stamler, JS. 1993. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature., 364:626–632.

Loughrin, J. H., Kasperbauer, M. J. 2001. Light reflected from colored mulches affects aroma and phenolic content of sweet basil (*Ocimumbasilicum* L.) leaves. J. Agric. Food Chem., 49:1331-1335.

Majeed, S., Nawaz, F., Naeem, M., Ashraf, MY., Ejaz, S., Ahmad, KS., Mehmood, K. 2020. Nitric oxide regulates water status and associated enzymatic pathways to inhibit nutrients imbalance in maize (*Zea mays* L.) under drought stress. Plant Physiol. Biochem., 155:147-160.

Mazid, M., Khan, TA., Mohammad, F. 2011. Role of Nitric oxide in regulation of H_2O_2 mediating tolerance of plants to abiotic stress: a synergistic signaling approach. J. Stress Physiol. Biochem.,7:2.

Mishra, S., Jha, A. B., Dubey, RS. 2011. Arsenite treatment induces oxidative stress, upregulates antioxidant system, and causes phytochelatin synthesis in rice seedlings. Protoplasma., 248(3):565–577.

Mittler, R. 2017. ROS are good. Trends Plant Sci., 22: 11–19.

Mukherjee, SP., Choudhuri, MA. 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and

hydrogen peroxide in Vigna seedlings. Physiol. Plant., 58: 166–170.

Nahar, K., Hasanuzzaman, M., Alam, MM., Fujita, M. 2015. Glutathione induced drought stress tolerance in mung bean: coordinated roles of the antioxidant defense and methylglyoxal detoxification systems. AoB Plants 7:1–18.

Narwal, SS., Bogatek, R., Zagdanska, BM., Sampietro, DA., Vattuone, MA. Plant Biochemistry. Studium Press LLC: Houston, TX, USA, 2009.

Okunlola, GO., Olatunji, OA., Akinwale, RO., Tariq, A., Adelusi, AA., 2017. Physiological response of the three most cultivated pepper species (*Capsicum spp.*) in Africa to drought stress imposed at three stages of growth and development. Sci. Hortic. 224:198–205.

Pirzad, D., Berger, MR., Zehtab-Salmasi, S., Mohammadi, SA., Darvishzadeh, R., Samadi, A., 2011. Effect of water stress on leaf relative water content, chlorophyll, proline and soluble carbohydrates in *Matricaria chamomilla* L. J. Med. Plant Res., 5(12):2483-2488.

Proch´azkov´a, D., Wilhelmov´a, NA. 2011. Nitric oxide, reactive nitrogen species and associated enzymes during plant senescence. Nitric Oxide., 24 (2):61–65.

Proch´azkov´a D, Sumaira J, Wilhelmov´a NA, Proch´azkov´a D, Sz´kov´ J. Reactive nitrogen species and the role of NO in abiotic stress. In: Ahmad P, Rasool S, editors. Emerging Technologies and Management of Crop Stress Tolerance.Academic Press,2014:249-66.

Rahman, I., Kode, A., Biswas, SK. 2006. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nat. Protoc., 1(6):3159-3165.

Rao, MV., Paliyath, G., Ormrod, DP. 1996. Ultraviolet-B- and ozoneinduced biochemical changes in antioxidant enzymes of Arabidopsis thaliana. Plant Physiol., 110:125–136.

Rastogi, S., Meena, S., Bhattacharya, A., Ghosh, S., Shukla, RK., Sangwan, NS., Lal, RK., Gupta, MM., Lavania, UC., Gupta, V., Nagegowda, DA., Shasany, AK. 2014. De novo sequencing and comparative analysis of holy and sweet basil transcriptomes. BMC Genomics., 15:588.

Sahay, S., Khan, E., Gupta, M. 2019. Nitric oxide and abscisic acid protects against PEG-induced drought stress differentially in Brassica genotypes by combining the role of stress modulators, markers and antioxidants. Nitric Oxide., 89:81-92.

Santos, C. 2004. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Sci. Hortic., 103(1):93-99.

Sharma, P., Jha, AB., Dubey, RS., Pessarakli, M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot., 2012(1): Article ID: 217037. https://doi.org/ 10.1155/2012/217037.

Shi, Q., Ding, F., Wang, X., Wei, M. 2007. Exogenous nitric oxide protect cucumber roots against oxidative stress induced by salt stress. Plant Physiol. Biochem., 45: 542–550. https://doi. org/10. 1016/j.plaphy.2007.05.005.

Siddiqui, M.H., Al-Whaibi, M.H., Basalah, M.O. 2011. Role of nitric oxide in tolerance of plants to abiotic stress. Protoplasma., 248: 447–455.

Sírov˘a, ´ J., Sedla´˘rov´a, M., Piterkova, ´ J., Luhova, ´ L., Pet˘rivalský, M. 2011. The role of nitric oxide in the germination of plant seeds and pollen. Plant Sci., 181 (5):560–572.

Somerville, C., Briscoe, J. 2001. Genetic engineering and water, Science.,292:2217.

Su, X., Howell, AB., D'Souza, DH. 2010. Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates–a time dependence study in vitro. Food Microbiol., 27: 985–991.

Walter, J., Nagy, L., Hein, R., Rascher, U., Beierkuhnlein, C., Willner, E., Jentsch, A., 2011. Do plants remember drought? Hints towards a drought-memory in grasses. Environ. Exp. Bot., 71:34–40.

Wang, X., Liu, H., Yu, F., Hu, B., Jia, Y., Sha, H., Zhao, H. 2019. Differential activity of the antioxidant defense system and alterations in the accumulation of osmolyte and reactive oxygen species under drought stress and recovery in rice (*Oryza sativa* L.) tillering. Sci. Rep., 9:1–11.

Yan, K., Chen, W., He, XY., Zhang, GY., Xu, S. 2010. Responses of photosynthesis, lipid peroxidation and antioxidant system in leaves of *Quercus mongolica* to elevated O_3. Environ. Exp. Bot., 69:198-204.

Zhang, J., Kirkham, MB. 1996. Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytol., 132:361–373.

Zottini, M., Formentin, E., Scattolin, M., Carimi, F., Lo Schiavo, F., Terzi, M. 2002. Nitric oxide affects plant mitochondrial functionality in vivo. FEBS Lett., 515: 75–78.

****