http://dx.doi.org/10.1590/1678-4499.353

Nitric oxide reduces oxidative damage induced by water stress in sunflower plants

Inês Cechin ([1]*); Guilherme Sementili Cardoso ([1]); Terezinha de Fátima Fumis ([1]); Natália Corniani ([2])

([1]) Sao Paulo State University (UNESP), Faculty of Sciences, Department of Biological Sciences, 17033-360 Bauru (SP), Brazil.

([2]) UNESP, Faculty of Agricultural Sciences, Department of Plant Protection, 18610-307 Botucatu (SP), Brazil.

(*) Corresponding author: icechin@fc.unesp.br

Received: Oct. 8, 2014; *Accepted:* Jan. 26, 2015

Abstract

Drought is one of the main environmental constraints that can reduce plant yield. Nitric oxide (NO) is a signal molecule involved in plant responses to several environmental stresses. The objective of this study was to investigate the cytoprotective effect of a single foliar application of 0, 1, 10 or 100 µM of the NO donor sodium nitroprusside (SNP) in sunflower plants under water stress. Water stressed plants treated with 1µM SNP showed an increase in the relative water content compared with 0 µM SNP. Drought reduced the shoot dry weight but SNP applications did not result in alleviation of drought effects. Neither drought nor water stress plus SNP applications altered the content of photosynthetic pigments. Stomatal conductance was reduced by drought and this reduction was accompanied by a significant reduction in intercellular CO_2 concentration and photosynthesis. Treatment with SNP did not reverse the effect of drought on the gas exchange characteristics. Drought increased the level of malondialdehyde (MDA) and proline and reduced pirogalol peroxidase (PG-POD) activity, but did not affect the activity of superoxide dismutase (SOD). When the water stressed plants were treated with 10 µM SNP, the activity of PG-POD and the content of proline were increased and the level of MDA was decreased. The results show that the adverse effects of water stress on sunflower plants are dependent on the external NO concentration. The action of NO may be explained by its ability to increase the levels of antioxidant compounds and the activity of ROS-scavenging enzymes.

Key words: photosynthesis, reactive oxygen species, antioxidant enzymes, proline, malondialdehyde.

1. INTRODUCTION

Lower rainfall is observed in many regions worldwide, causing many negative plant responses such as lower dry weight accumulation and lower rates of carbon assimilation (Álvarez et al., 2011). Water stress also causes an overproduction of a variety of reactive oxygen species (ROS) such as hydrogen peroxide and singlet oxygen which are potentially harmful to all cellular components. Plant cell evolved an antioxidant system composed of nonenzymatic and enzymatic components that play a critical role in neutralizing the free radicals which can affect the cellular stability. The ability to maintain a high antioxidant system activity under water stress depends on plant species, stress intensity and duration (DaCosta & Huang, 2007), with the high antioxidant enzyme activity levels been positively related to drought resistance (Ghahfarokhi et al., 2015; Sairam & Srivastava, 2001).

Under a variety of stresses, active solute accumulation of compatible solutes such as proline is claimed to be an effective stress tolerance mechanism. Proline works as both an osmoprotectant and as a redox-buffering agent possessing antioxidant property under conditions of stress (KaviKishor & Sreenivasulu, 2014). Accumulation of proline under drought stress was found in several plants, particularly in young leaves (Cechin et al., 2006). Furthermore, foliar applied proline ameliorated the adverse effects of water stress on growth and photosynthetic capacity of two maize cultivars (Ali et al., 2007). The osmolyte accumulations in plant cells may therefore be important in maintaining various physiological processes in operation even under stressed conditions.

Nitric oxide (NO), a molecule that is highly diffusible through cellular membrane due to its lipophilic nature, is involved in several physiological, biochemical and developmental processes in plants (Krasylenko et al., 2010 and references therein). NO is itself a reactive nitrogen species (RNS) produced in a variety of cells and its effects on different types of cells have proved to be either protective or toxic, depending on its concentration (Beligni & Lamattina, 1999). Besides proline (Kahlaoui et al., 2014), other chemicals such as sodium nitroprusside (NO donor) are currently being applied to plants exposed to stressful conditions in order to improve growth and

yield (Farooq et al., 2009). In recent years, evidences have accumulated showing that exogenous NO can alleviate the harmful effects of environmental stresses in plants such as water stress (Boogar et al., 2014; Farooq et al., 2009; Liao et al., 2012). Therefore, the objective of the present study was to investigate whether sodium nitroprusside (SNP), a NO donor, plays an important role in protecting sunflower plants against water stress, as assessed by dry weight accumulation, gas exchange characteristics and antioxidant enzyme activities.

2. MATERIALS AND METHODS

Plant material and growth conditions

Seeds of sunflower (*Helianthus annuus* L. variety IAC-Iarama) were sown in 4 dm^3 pots filled with a 47:13:40% mixture of pinus bark: vermiculite: peat enriched with minerals. Seedlings were thinned to one per pot after emergence and were grown in a greenhouse under natural photoperiod. Maximum day and night temperatures were close to 32 and 18 °C, respectively.

Plants were supplied with tap water according to the requirements and supplemented with 250 mL of 70% full strength Long Ashton nutrient solution (Hewitt, 1966) 16 and 23 days after planting. After 27 days of sowing, the plants were separated into five distinct groups: (1) plants well hydrated and without SNP (control), (2) plants under water stress and without SNP, (3) plants under water stress with 1μM of SNP, (4) plants under water stress with 10 μM of SNP and (5) plants under water stress with 100 μM of SNP. The well watered plants were watered daily according to the previous conditions, whereas the stressed plants had the water supply discontinued. Applications of SNP coincided with the onset of drought stress. The plants received a single dose of SNP, at 1, 10 or 100 μM concentrations. The well watered plants were sprayed with distilled water.

Gas exchange measurements

On the 3rd day after the induction of stress, a portable infra-red gas analyzer (LCpro, ADC, Hoddesdon, UK) was used for measurements of photosynthesis (A), stomatal conductance to water vapor (g_s), transpiration (E) and intercellular CO_2 concentration (C_i) on the youngest fully expanded leaf. Measurements were made inside the greenhouse and a photosynthetic active radiation (PAR) of 1000 μmol m^{-2} s^{-1} was supplied by a light unit mounted on the top of leaf chamber. The leaf was kept under this PAR until a steady-state rate was achieved.

Chemical analysis

The photosynthetic pigments were measured on leaf discs of known area from a leaf next to the leaf used for gas exchange. Pigments were extracted in 80% aqueous acetone and the content was calculated according to the equations proposed by Lichtenthaler (1987). The proline content was determined according to the method described by Bates et al. (1973) and modified by Torello & Rice (1986) from oven dried and fine powder leaves and the concentration expressed on a leaf dry weight basis by using proline as standard.

The activity of pirogalol peroxidase (PG-POD; EC 1.11.1.7), superoxide dismutase (SOD; EC 1.15.1.1) and lipid peroxidation were determined on leaves next to those used for the above analysis. The extraction of PG-POD and SOD were determined according to Ekler et al. (1993). The activity of the enzymes was determined according to Teisseire & Guy (2000) and Beauchamp & Fridovich (1971) cited by Bor et al. (2003), respectively. The activity of PG-POD was expressed as μM min^{-1} mg^{-1} protein. SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). Protein content was measured using casein as standard according to the method of Bradford (1976). The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation, according to the method described by Heath & Packer (1968). The MDA content was calculated by its extinction coefficient of 155 mmol L^{-1} cm^{-1} and expressed as nmol MDA per g fresh weight.

Relative water content and dry mass determination

At the end of the experiment (4 days after stress induction), the leaf relative water content (RWC) was determined as:

$$(FW - DW)/(TW - DW) \times 100 \quad (1)$$

where FW is the fresh weight obtained immediately after the removal of leaf discs; TW is the turgid weight determined after rehydration of the discs for 3 h and DW is the dry weight obtained after drying the discs in an oven at 60 °C for 48 hours. Plants of each treatment were selected randomly for shoot dry weight determinations. The sunflower plants were divided into stem and leaves before been oven dried at 60 °C for 48 hours.

Statistical analysis

The statistical tests were conducted using the Software Statistical Package for the Social (SPSS/PC) version 9.0 at 5% significance level. Quantitative changes of different parameters were analyzed through analysis of variance (ANOVA), with Tukey's honestly significant difference multiple comparison test being used to determine significant differences among treatments.

3. RESULTS AND DISCUSSION

Similar to other environmental stresses, drought affects many physiological and metabolic processes within plants thus resulting in lower dry weight accumulation. Plant response to stress is first characterized by a rapid inhibition, followed by adaptation to the new condition (Skirycz & Inzé, 2010). After four days of stress imposition, leaf and shoot dry weight and RWC were reduced about 23%, 22% and 13%, respectively but stem dry weight accumulation was not affected (Table 1). The observed reduction in leaves dry weight is a result of high sensitivity of young leaves to changes in water supply (Cechin et al., 2006) since cell division and elongation are influenced by leaf water status or cell turgor (Heckenberger et al., 1998). Applications of SNP in wheat seedlings has been found to confer water deficit tolerance by maintaining more water than the well watered plants (García-Mata & Lamattina, 2001). In this study, althought short-term SNP applications of 1 μM under water stress resulted in an increase of 7% in RWC compared with 0 μM SNP this did not result in improvement of plant growth. Applications of NO have been used to improve crop growth under various abiotic stresses, with contradictory results. Foliar-applied NO has been reported to enhance growth only under non-stressed conditions (Kausar et al., 2013) or under both non-stressed and salt stressed conditions (Uchida et al., 2002). This conflicting results might be related to many factors such as species, SNP concentration and duration of applications. In this study, water stress did not occur abruptly, but developed slowly and increased with time in intensity. The lack of 10 μM SNP beneficial effect on sunflower plant dry weight accumulation under water stress as seen for proline, PG-POD and MDA (Figure 1) might be ascribed to the fact that the dry weight was measured after 4 days of the beginning of water stress. Under short period of water stress the physiological responses are easily seen whereas morphological changes are not.

David et al. (1998) found that dehydration increased the chlorophyll content in young leaves and that it was slightly decreased in the older ones, suggesting that the contribution of mesophyll limitations in the inhibition of photosynthesis increases with leaf age. The carotenoids that are intrinsic components of chloroplastic membranes have been reported to decrease (Yildiz-Aktas et al., 2009) or to increase significantly (Guha et al., 2012) under drought. No significant changes in the concentration of total chlorophyll or carotenoids were observed in sunflower plants under water stress without SNP when compared with non-stressed plants without SNP (Table 1). It has been found that NO applications increased leaf chlorophyll of plants under water stress, suggesting that NO treatment protected the photosynthetic apparatus (Fan & Liu, 2012). This phenomenon was not observed in the present study. The inconsistent findings observed in plant responses to water stress and to application of NO may be related to the different approaches used by the authors or to the variation in sensitivity among species.

The RWC of leaves and photosynthesis are under control of stomata and stomata closure is one of the first responses to drought. A small decline in stomatal conductance under mild water stress may have protective effects against stress by saving water and improving water-use efficiency by the plant. Sunflower plants showed a remarkable reduction in stomata conductance without significant reduction in transpiration rate after three days of water stress which were not altered by SNP concentration (Figures 2a,b). This results contrast to those reported by García-Mata & Lamattina (2001) who found that exogenous NO was able to induce stomatal closure under water stress. In addition, the authors showed that the stomata closure was correlated with a 10% increase in RWC. However, through elegant experiments, Ribeiro et al. (2009) reported that the effect of NO depends on the hydration conditions of the tissues. The authors demonstrated that the NO is not involved in the stomata closure under water stress conditions in *Arabidopsis thaliana* but in well-hydrated tissues. Althought in the present study the effect of NO in well-hydrated plants was not evaluated it seems that the observed effect of NO in water stressed plants is similar to that found by Ribeiro et al. (2009).

Stomatal closure without a change in mesophyll capacity results in lower concentration of intercellular CO_2. In this

Table 1. Relative water content (RWC; %), dry weight (g plant^{-1}) of leaves, stem and shoot, and total chlorophyll (Chl) and carotenoids (Car) concentrations (g m^{-2}) of well watered (WW) or water-stresed plus SNP (WS+SNP) sunflower plants. SNP, as NO donor, was added on the surface of the leaves. Values are means±SE of 5-6 plants. Values sharing the same letters within the same row are not significantly different at 5% significance level

	WW	WS + SNP (μM)			
		0	1	10	100
RWC	82.96±0.70a	72.33±0.84b	77.23±1.02c	74.69±1.18bc	75.94±1.05bc
Leaves	1.42±0.02a	1.09±0.08b	1.08±0.05b	1.19±0.05ab	1.18±0.05b
Stem	1.15±0.07a	0.92±0.08a	0.87±0.08a	0.86±0.01a	0.98±0.08a
Shoot	2.58±0.09a	2.02±0.17b	1.95±0.13b	2.05±0.06b	2.16±0.12ab
Chl	0.32±0.01a	0.37±0.01ab	0.35±0.01ab	0.38±0.01b	0.38±0.02b
Car	0.09±0.00a	0.10±0.00ab	0.10±0.00ab	0.11±0.00b	0.11±0.01b

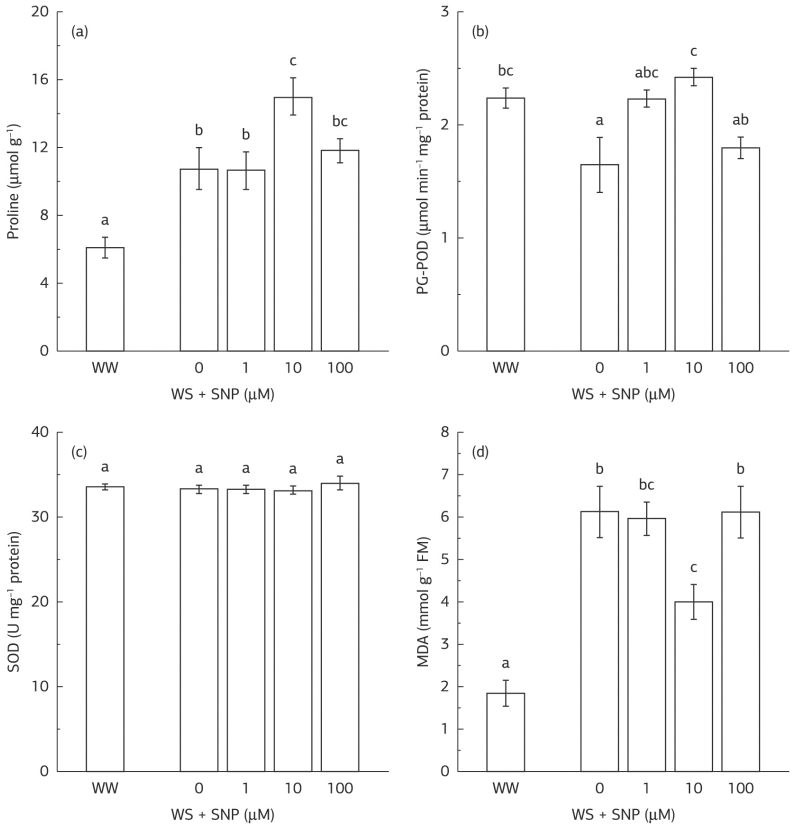

Figure 1. Proline content (a), pirogalol peroxidase (PG-POD) activity (b), superoxide dismutase (SOD) activity (c) and malondialdehyde (MDA) content (d) of well watered (WW) or water-stresed plus SNP (WS+SNP) sunflower plants. SNP, as NO donor, was added on the surface of the leaves. Values are means±SE of 4 plants. Values sharing the same letters are not significantly different at 5% significance level.

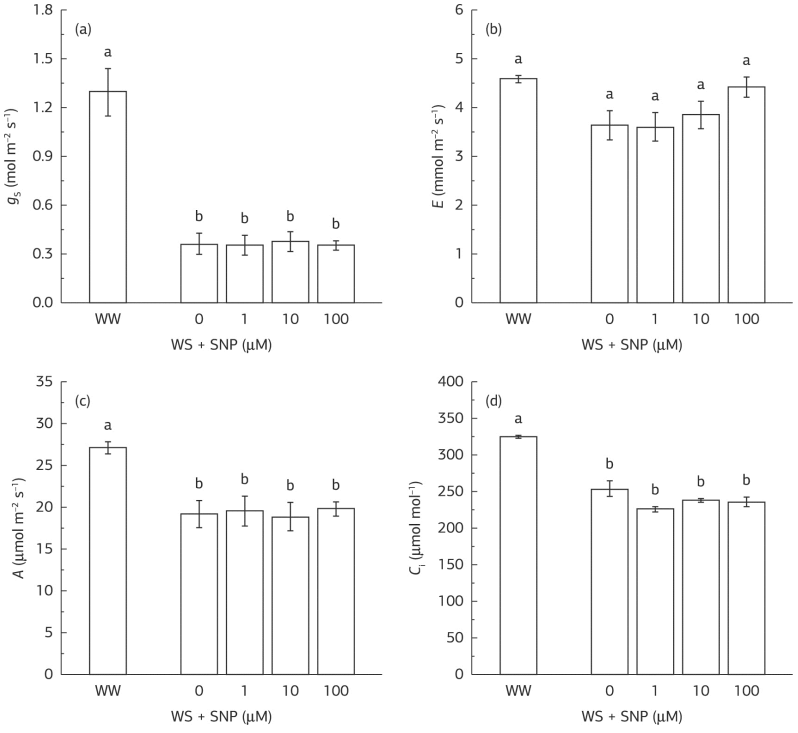

Figure 2. Stomatal conductance (g_s, a), transpiration (E, b), photosynthesis (A, c), and intercellular CO_2 concentration (C_i, d) of well watered (WW) or water-stresed plus SNP (WS+SNP) sunflower plants. SNP, as NO donor, was added on the surface of the leaves. Values are means±SE of 6 plants. Values sharing the same letters are not significantly different at 5% significance level.

study, the reduction in stomata conductance under water stress without or with application of SNP was accompanied by a significant reduction in the concentration of intercellular CO_2 and photosynthesis (Figures 2d,c). By removing the lower epidermis of sunflower leaves, Tang et al. (2002) were able to demonstrate that CO_2 depletion was responsible for reduction in photosynthesis only in the early phases of water stress. However, Tezara et al. (1999) stated that metabolic inhibition of photosynthesis also takes place at mild water stress and it becomes more important as the water stress intensifies. Although the decline in photosynthesis and stomata conductance are often taken to indicate that photosynthesis is affected via stomatal limitation it is interesting to note that in this study the reduction in stomata conductance was higher than in the concentration of intercellular CO_2. Substantial increase in intercellular CO_2 concentration was observed with prolonged water stress in sunflower plants, suggesting that the photosynthesis came gradually under control of mesophyll metabolism (Cechin et al., 2008). In present study, the decrease in photosynthesis observed in sunflower plants under three days of water stress with or whitought SNP was likely due to stomatal closure rather than reflecting a reduction in chloroplast activity, since intercellular CO_2 concentration decreased in response to water restriction.

NO is an important signaling molecule with diverse functions in plants and its production can be triggered by several abiotic stresses (Fan & Liu, 2012; Yang et al., 2011) or its endogenous level be increased by applications of NO donors under both normal and stress conditions (Boyarshinov & Asafova, 2011; Fan & Liu, 2012). Increase in endogenous NO via application of NO donors has been shown to improve the photosynthetic performance in water stressed plants (Fan & Liu, 2012), the improvement was associated with an increase in photosynthetic pigments. In this study, foliar spray of SNP failed to induce increase in photosynthetic pigments and photosynthetic performance which might be explained due to the that in the present study the plants were not pre-treated with SNP.

Different kinds of stress produce an increase in ROS, thus resulting in oxidative stress as a consequence of loss of oxidant/antioxidant balance. Increased MDA is a characteristic feature of oxidative membrane damage that has been reported as a common response to stress conditions. In this study, water stress withought SNP increased MDA of about 231% (up to 3 times) when compared with well watered plants (Figure 1d). Suppression (35%) of MDA accumulation in sunflower leaves was observed with foliar application of 10 μM SNP in comparison with water stressed and 0 μM SNP. As low MDA level has been found to be a characteristic feature of plants tolerant to drought (Sairam & Srivastava, 2001; Yildiz-Aktas et al., 2009), it seems that in the present study the cell membrane of sunflower plants was in some way protected against the oxidative stress induced by water stress under foliar application of 10 μM SNP. Plants respond to stress through changes in gene expression that might lead to the production of antioxidants and allowing recovery of growth (Xiong & Zhu, 2002). The elimination of ROS is achieved mainly by antioxidant compounds and by ROS-scavenging enzymes. Plants contain substantial amounts of carotenoids that serve as non-enzymatic scavengers of active oxygen species and the high carotenoid levels have been suggested to be a measure of drought tolerance (Chandrasekar et al., 2000). In this study, the level of carotenoids was not altered by neither water stress nor water stress plus SNP applications (Table 1).

Proline was found to be the major non-enzymatic antioxidant metabolite under water stress conditions, resulting in stress tolerance as a consequence of its ROS-scavenging ability (Guha et al., 2012). It has also been shown that water stress tolerance can be induced by exogenous NO which was attributed to high accumulation of proline in plants (Lei et al., 2007), the high accumulation of proline was attributed to exogenous NO on the activity of some key enzymes involved in the synthesis of proline (Zhang et al., 2008). Restriction of water supply for four days resulted in an increase in proline content of about 76% when compared with well watered plants withought SNP (Figure 1a). A further increase in proline accumulation of about 39% was observed in response to application of 10 μM SNP in comparison with water stressed plant plus 0 and 1 μM SNP. At 100 μM SNP the content of proline was decreased to values similar to water stress without or with 1 μM SNP. Although it was demonstrated that NO reduces hydrogen peroxide accumulation under water stress (Sang et al., 2008), it is well known that NO action can lead to opposite effects depending on its concentration (Tian & Lei, 2006). Plants control the level of ROS by several antioxidant enzymes such as pirogalol peroxidase (PG-POD) and superoxide dismutase (SOD) which are responsible for scavenging accumulated ROS. This ability to control the level of ROS is important in drought tolerance in arid and semiarid regions (Ghahfarokhi et al., 2015). Water stress withought SNP did not affect the activity of SOD nor did the application of SNP under water stress (Figure 1c). The figure 1b demonstrates that water stress withought SNP significantly decreased the activity of PG-POD, while the application of 10 μM SNP under water stress increased the activity of this enzyme. Thus, NO can effectively protect plants from damage probably by enhancing the activities of antioxidant enzymes (Boogar et al., 2014) or acting as a potent antioxidant in plants (Beligni & Lamattina, 2002). In addition, it is clear in the present study that NO also can protect the plants by enhancing the levels of proline.

4. CONCLUSION

This study show that the adverse effects of water stress on sunflower plants are dependent on the external NO concentration. A single application of 10 μM SNP as NO donor at the beginning of water stress proved to be beneficial in alleviating the negative effects of water stress on membrane integrity as seen by lower levels of MDA. The action of NO may be explained by its ability to increase the levels of antioxidant compounds and to increase the activity of ROS-scavenging enzymes.

ACKNOWLEDGEMENTS

The authors thank the Brazilian agencies FAPESP and FUNDUNESP for Financial support and Agronomic Institute of Campinas (IAC) that supplied the seeds.

REFERENCES

Ali, Q., Ashraf, M., & Athar, H. U. R. (2007). Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. Pakistan Journal of Botany, 39, 1133-1144.

Álvarez, S., Navarro, A., Nicolas, E., & Sanchez-Blanco, M. J. (2011). Transpiration, photosynthetic responses, tissue water relations and dry mass partitioning in *Callistemon* plants during drought conditions. Scientia Horticulturae, 129, 306-312. http://dx.doi.org/10.1016/j.scienta.2011.03.031.

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

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, 44, 276-287. http://dx.doi.org/10.1016/0003-2697(71)90370-8. PMid:4943714

Beligni, M., & Lamattina, L. (1999). Is nitric oxide toxic or protective? Trends in Plant Science, 4, 299-300. http://dx.doi.org/10.1016/S1360-1385(99)01451-X. PMid:10431217

Beligni, M. V., & Lamattina, L. (2002). Nitric oxide interferes with plant photo-oxidative stress by detoxifying reactive oxygen species. Plant, Cell & Environment, 25, 737-748. http://dx.doi.org/10.1046/j.1365-3040.2002.00857.x.

Boogar, A. R., Salehi, H., & Jowkar, A. (2014). Exogenous nitric oxide alleviates oxidative damage in turfgrasses under drought stress. South African Journal of Botany, 92, 78-82. http://dx.doi.org/10.1016/j.sajb.2014.02.005.

Bor, M., Özdemir, F., & Türkan, I. (2003). The effects of the salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritime* L. Plant Science, 164, 77-84. http://dx.doi.org/10.1016/S0168-9452(02)00338-2.

Boyarshinov, A. V., & Asafova, E. V. (2011). Stress responses of wheat leaves to dehydration: participation of endogenous NO and effect of sodium nitroprusside. Russian Journal of Plant Physiology: a Comprehensive Russian Journal on Modern Phytophysiology, 58, 1034-1039. http://dx.doi.org/10.1134/S1021443711060033.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3. PMid:942051

Cechin, I., Corniani, N., Fumis, T. F., & Cataneo, A. C. (2008). Ultraviolet-B and water stress effects on growth, gas exchange and oxidative stress in sunflower plants. Radiation and Environmental Biophysics, 47, 405-413. http://dx.doi.org/10.1007/s00411-008-0167-y. PMid:18404272

Cechin, I., Rossi, S. C., Oliveira, V. C., & Fumis, T. F. (2006). Photosynthetic responses and proline content of mature and young leaves of sunflower plants under water deficit. Photsynthetica, 44, 143-146. http://dx.doi.org/10.1007/s11099-005-0171-2.

Chandrasekar, V., Sairam, R. K., & Srivastava, G. C. (2000). Physiological and biochemical responses of hexaploid and tetraploid wheat to drought stress. Journal Agronomy & Crop Science, 185, 219-227. http://dx.doi.org/10.1046/j.1439-037x.2000.00430.x.

DaCosta, M., & Huang, B. (2007). Changes in antioxidant enzyme activities and lipid peroxidation for bentgrass species in response to drought stress. Journal of the American Society for Horticultural Science, 132, 319-326.

David, M. M., Coelho, D., Barrote, I., & Correia, M. J. (1998). Leaf age effects on photosynthetic activity and sugar accumulation in droughted and rewatered *Lupinus albus* plants. Australian Journal of Plant Physiology, 25, 299-306. http://dx.doi.org/10.1071/PP97142.

Ekler, Z., Dutka, F., & Stephenson, G. R. (1993). Safener effects on acetochlor toxicity, uptake, metabolism and glutathione S-transferase activity in maize. Weed Research, 33, 311-318. http://dx.doi.org/10.1111/j.1365-3180.1993.tb01946.x.

Fan, Q. J., & Liu, J. H. (2012). Nitric oxide is involved in dehydration/drought tolerance in *Poncirus trifoliata* seedlings through regulation of antioxidant systems and stomatal response. Plant Cell Reports, 31, 145-154. http://dx.doi.org/10.1007/s00299-011-1148-1. PMid:21938448

Farooq, M., Basra, S. M. A., Wahid, A., & Rehman, H. (2009). Exogenously nitric oxide enhances the drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). Journal Agronomy & Crop Science, 195, 254-261. http://dx.doi.org/10.1111/j.1439-037X.2009.00367.x.

García-Mata, C., & Lamattina, L. (2001). Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. Plant Physiology, 126, 1196-1204. http://dx.doi.org/10.1104/pp.126.3.1196. PMid:11457969

Ghahfarokhi, M. G., Mansurifar, S., Taghizadeh-Mehrjardi, R., Saeidi, M.; Jamshidi, A. M., & Ghasemi, E. (2015). Effects of drought stress and rewatering on antioxidant systems and relative water content in different growth stages of maize (*Zea mays* L.) hybrids. Archives of Agronomy and Soil Science, 61, 493-506. http://dx.doi.org/10.1080/03650340.2014.943198

Guha, A., Sengupta, D., Rasineni, G. K., & Reddy, A. R. (2012). Non-enzymatic antioxidant defence in drought-stressed mulberry (*Morus indica* L.) genotypes. Trees, 26, 903-918. http://dx.doi.org/10.1007/s00468-011-0665-4.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, 125, 189-198. http://dx.doi.org/10.1016/0003-9861(68)90654-1. PMid:5655425

Heckenberger, U., Roggatz, U., & Schurr, U. (1998). Effect of drought stress on the cytological status in *Ricinus communis*. Journal of Experimental Botany, 49, 181-189. http://dx.doi.org/10.1093/jxb/49.319.181.

Hewitt, E. J. (1966). Sand and water culture methods used in the study of plant nutrition. England: Commonwealth Agricultural Bureaux, Farnham Royal.

Kahlaoui, B., Hachicha, M., Rejeb, S., Rejeb, M. N., Hanchi, B., & Misle, E. (2014). Response of two tomato cultivars to field-applied proline under irrigation with saline water: Growth, chlorophyll fluorescence and nutritional aspects. Photosynthetica, 52, 421-429. http://dx.doi.org/10.1007/s11099-014-0053-6.

Kausar, F., Shahbaz, M., & Ashraf, M. (2013). Protective role of foliar-applied nitric oxide in *Triticum aestivum* under saline stress. Turkish Journal of Botany, 37, 1155-1165. http://dx.doi.org/10.3906/bot-1301-17.

KaviKishor, P. B., & Sreenivasulu, N. (2014). Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? Plant, Cell & Environment, 37, 300-311. http://dx.doi.org/10.1111/pce.12157. PMid:23790054

Krasylenko, Y. A., Yemets, A. I., & Blume, Y. B. (2010). Fuctional role of nitric oxide in plants. Russian Journal of Plant Physiology, 57, 451-461. http://dx.doi.org/10.1134/S1021443710040011.

Lei, Y., Yin, C., & Li, C. (2007). Adaptive responses of *Populus przewalskii* to drought stress and SNP application. Acta Physiologiae Plantarum, 29, 519-526. http://dx.doi.org/10.1007/s11738-007-0062-1.

Liao, W.-B., Huang, G.-B., Yu, J.-H., & Zhang, M.-L. (2012). Nitric oxide and hydrogen peroxide alleviate drought stress in marigold explants and promote its adventitious root development. Plant Physiology and Biochemistry, 58, 6-15. http://dx.doi.org/10.1016/j.plaphy.2012.06.012. PMid:22771430

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigment photosynthetic biomembranes. Methods in Enzymology, 148, 362-385.

Ribeiro, D. M., Desikan, R., Bright, J., Confraria, A., Harrison, J., Hancock, J. T., Barros, R. S., Neill, S. J., & Wilson, I. D. (2009). Differential requirement for NO during ABA-induced stomatal closure in turgid and wilted leaves. Plant, Cell & Environment, 32, 46-57. http://dx.doi.org/10.1111/j.1365-3040.2008.01906.x. PMid:19021879

Sairam, R. K., & Srivastava, G. C. (2001). Water stress tolerance of wheat (*Triticum aestivum* L.): variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. Journal Agronomy & Crop Science, 186, 63-70. http://dx.doi.org/10.1046/j.1439-037x.2001.00461.x.

Sang, J., Jiang, M., Lin, F., Xu, S., Zhang, A., & Tan, M. (2008). Nitric oxide reduces hydrogen peroxide accumulation involved in water stress-induced subcellular anti-oxidant defense in maize plants. Journal of Integrative Plant Biology, 50, 231-243. http://dx.doi.org/10.1111/j.1744-7909.2007.00594.x. PMid:18713446

Skirycz, A., & Inzé, D. (2010). More from less: plant growth under limited water. Current Opinion in Biotechnology, 21, 197-203. http://dx.doi.org/10.1016/j.copbio.2010.03.002. PMid:20363612

Tang, A. C., Kawamitsu, Y., Kanechi, M., & Boyer, J. S. (2002). Photosynthetic oxygen evolution at low water potential in leaf discs lacking an epidermis. Annals of Botany, 89, 861-870. http://dx.doi.org/10.1093/aob/mcf081. PMid:12102512

Teisseire, H., & Guy, V. (2000). Cooper-induced changes in antioxidant enzymes activities in frond of duckweed (*Lemma minor*). Plant Science, 153, 65-72. http://dx.doi.org/10.1016/S0168-9452(99)00257-5.

Tezara, W., Mitchell, V. J., Driscoll, S. D., & Lawlor, D. W. (1999). Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature, 401, 914-917. http://dx.doi.org/10.1038/44842.

Tian, X., & Lei, Y. (2006). Nitric oxide treatment alleviates drought stress in wheat seedlings. Biologia Plantarum, 50, 775-778. http://dx.doi.org/10.1007/s10535-006-0129-7.

Torello, W. A., & Rice, L. A. (1986). Effects of NaCl stress on proline and cation accumulation in salt sensitive tolerance turfgrass. Plant and Soil, 93, 241-247. http://dx.doi.org/10.1007/BF02374226.

Uchida, A., Jagendorf, A. T., Hibino, T., Takabe, T., & Takabe, T. (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress toerance in rice. Plant Science, 163, 515-523. http://dx.doi.org/10.1016/S0168-9452(02)00159-0.

Xiong, L., & Zhu, J. K. (2002). Molecular and genetic aspects of plant responses to osmotic stress. Plant, Cell & Environment, 25, 131-139. http://dx.doi.org/10.1046/j.1365-3040.2002.00782.x. PMid:11841658

Yang, W., Sun, Y., Chen, S., Chen, F., Fang, W., & Liu, Z. (2011). The effect of exogenously apllied nitric oxide on photosynthesis and antioxidant activity in heat stressed chrysanthemum. Biologia Plantarum, 55, 737-740. http://dx.doi.org/10.1007/s10535-011-0178-4.

Yildiz-Aktas, L., Dagnon, S., Gurel, A., Gesheva, E., & Edreva, A. (2009). Drought tolerance in cotton: involvement of non-enzymatic ROS-scavenging compounds. Journal Agronomy & Crop Science, 195, 247-253. http://dx.doi.org/10.1111/j.1439-037X.2009.00366.x.

Zhang, L. P., Mehta, S. K., Liu, Z. P., & Yang, Z. M. (2008). Copper-induced proline synthesis is associated with nitric oxide generation in *Chlamydomonas reinhardtii*. Plant & Cell Physiology, 49, 411-419. http://dx.doi.org/10.1093/pcp/pcn017. PMid:18252734