South African Journal of Botany 92 (2014) 78–82

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Exogenous nitric oxide alleviates oxidative damage in turfgrasses under drought stress

A. Rahimian Boogar [a], H. Salehi [b], A. Jowkar [b,*]

[a] Department of Horticulture Science, University of Zabol, Zabol, Iran
[b] Department of Horticulture Science, College of Agriculture, Shiraz University, Shiraz, Iran

ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form 22 January 2014
Accepted 3 February 2014
Available online 13 March 2014

Edited by BS Ripley

Keywords:
Nitric oxide donors
Oxidative stress
Drought
Antioxidant enzymes
Turfgrasses

ABSTRACT

Oxidative stress caused by drought stress is a major limiting factor for grass cultivation in arid and semi-arid regions. In order to investigate the effect of nitric oxide (NO) donors on drought tolerance and recovery of *Poa pratensis* L. 'Balin', *Lolium perenne* L. 'Numan' and *Cynodon dactylon* [L.] Pers., sodium nitroprusside (SNP) and potassium nitrite (PN) were applied at 150, 200 and 250 μM concentrations with irrigation intervals of 3, 5, 7 and 9 days. The NO donors significantly increased the antioxidant enzymes' activity of the three grass species during drought stress. This enhancing effect was not dependent on the source of NO (SNP or NP), but rather reliant on their concentrations. The maximum activity of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and ascorbate peroxidase (APX) was obtained with the application of 200 μM SNP or PN. Nitric oxide donors caused the greatest increase in activities of antioxidant enzymes in plants subjected to 7 or 9 days of drought stress. Among different turfgrasses tested under water stress, warm season turfgrass *C. dactylon* had the highest activity for antioxidant enzymes.

1. Introduction

A great variety of abiotic stresses such as drought, salinity, UV light, air pollutants and heavy metals induce molecular damage in plants through the production of reactive oxygen species (ROS) (Mahajan and Tuteja, 2005). Drought stress promotes the production of superoxide (O_2^-), singlet oxygen ($^1O_2$), hydroxyl (OH^-), and hydrogen peroxide (H_2O_2), which can be detrimental to proteins, lipids, carbohydrates, and nucleic acids (Smirnoff, 1993). The plant cells are normally protected against this oxidative damage by a broad range of radical scavengers such as antioxidant enzymes which detoxify reactive oxygen species (Foyer et al., 1994; Møller et al., 2007; Bian and Jiang, 2009; Ashraf and Harris, 2013).

Although antioxidant enzymes' activity under drought stress depends on plant species, stress intensity and duration (DaCosta and Huang, 2007), maintaining a high level of antioxidant activity increases the capacity of protection mechanisms for drought tolerance (Sharma and Dubey, 2005; Türkan et al., 2005). In a study using three bentgrass species, *Agrostis canina* L. maintained antioxidant enzyme activities for a greater duration of drought treatment compared to both *Agrostis capillaris* L. and *Agrostis stolonifera* L. Higher capacity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) was linked to decrease lipid peroxidation of leaves and higher turf quality, leaf relative water content, and photosynthesis efficiency for a longer duration of drought stress compared with *A. capillaris* L. and *A. stolonifera* L. (DaCosta and Huang, 2007). Fu and Huang (2001) showed that in leaves of *Poa pratensis* and *Festuca arundinacea* when surface soil drying was prolonged, SOD activity increased while CAT and peroxidase (POD) activities remained unchanged. Further increase in the duration of drought stress resulted in a decline of SOD, POD and CAT activities. The drought tolerance of perennial grasses is attributed to the modification of the antioxidant metabolisms (Jiang and Huang, 2001; DaCosta and Huang, 2007; Bian and Jiang, 2009, Efeoğlu et al., 2009).

Nitric oxide is considered a small extremely diffusible gas and a versatile bioactive molecule which is implicated in the signal transduction pathway responsible for stress responses in plants (Crawford and Guo, 2005; Delledonne, 2005; Velikova et al., 2008). Furthermore, NO is involved in many other important physiological processes such as germination, mitochondrial function and floral regulation (Beligni and Lamattina, 2000; Lamattina et al., 2003; He et al., 2004; Hu et al., 2005). In recent years there has been increasing evidence that application of exogenous nitric oxide (NO) is useful to allay oxidative stresses caused by drought, high temperature and salinity (Lu et al., 2009; Bavita et al., 2012; Nalousi et al., 2013; Freschi, 2013).

The main objective of this study was to elucidate the effect of nitric oxide donors on relieving the oxidative stress induced by drought stress in cold and warm season turfgrasses. To our knowledge, this is the first report on using sodium nitroprusside on *P. pratensis*, *Lolium perenne* and *Cynodon dactylon* to improve their drought stress tolerance.

* Corresponding author. Tel.: +98 711 613 8158; fax: +98 711 228 6133.
E-mail address: ajowkar@shirazu.ac.ir (A. Jowkar).

http://dx.doi.org/10.1016/j.sajb.2014.02.005
0254-6299/

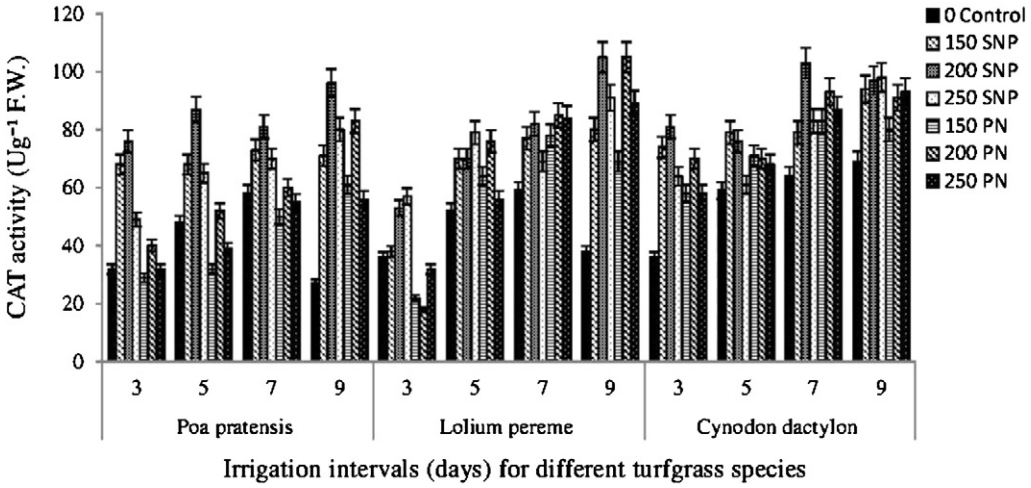

Fig. 1. The effect of NO donors on CAT activity at different water stresses of three turfgrass species.

2. Materials and methods

2.1. Plants and experiment condition

Turf seeds of three species of *P. pratensis* L. 'Balin', *L. perenne* L. 'Numan' and *C. dactylon* [L.] Pers. were cultivated in 5 L pots in April 2010 and April 2011 at a greenhouse located in the Department of Horticulture Science in Shiraz University, Shiraz, Iran. The plants were grown for 2 months after which shoots were clipped twice at 10 day intervals to a height of 5 cm above the soil surface in order to reach a uniform establishment and get ready for the treatments. The air temperature was set at 32–35 °C during daytime and at 25–27 °C during nighttime with a relative humidity of 60%.

2.2. Water stress treatments

Water stress was implemented from June until the end of September by withholding irrigation for 3, 5, 7 or 9 days. These irrigation frequencies ranged from 50% of field capacity at day 3, to the least retention of water near the permanent wilting point at day 9. Regular daily watering was resumed after the water stress period, to allow the plants to recover.

2.3. Nitric oxide (NO) donors

Prior to the drought stress treatments, in each pot 20 ml of freshly prepared sodium nitroprusside (SNP) or potassium nitrite (PN) solutions was applied as foliar spray at 150, 200 and 250 μmol L^{-1} concentrations. Deionized water was used as solvent and control for the treatments.

2.4. Antioxidant enzymes' activity

2.4.1. Catalase assay

Activity of CAT was measured in a cuvette using the method of Dhindsa et al. (1981) with slight modifications. The CAT reaction solution (1 mL) of 50 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2 was mixed rapidly with 50 μL enzyme extract. Reaction was initiated by adding the enzyme extract. Changes in absorption at 240 nm were read after 1 min with a WPA spectrophotometer (Biochrom, UK). One unit CAT activity was defined as an absorbance decrease of 0.01 unit min^{-1}.

2.4.2. Superoxide dismutase assay

The SOD activity was determined by measuring its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) (McCord and

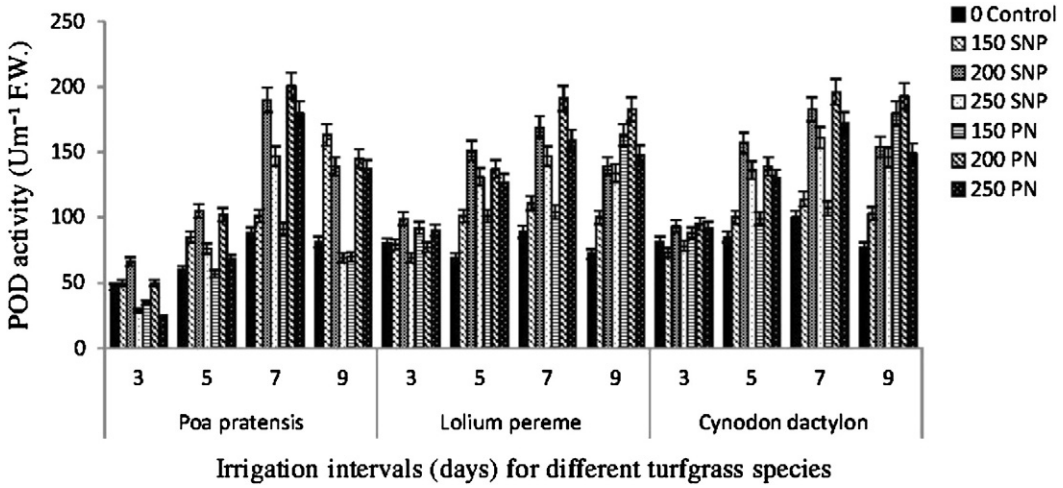

Fig. 2. The effect of NO donors on POD activity at different water stresses of three turfgrass species.

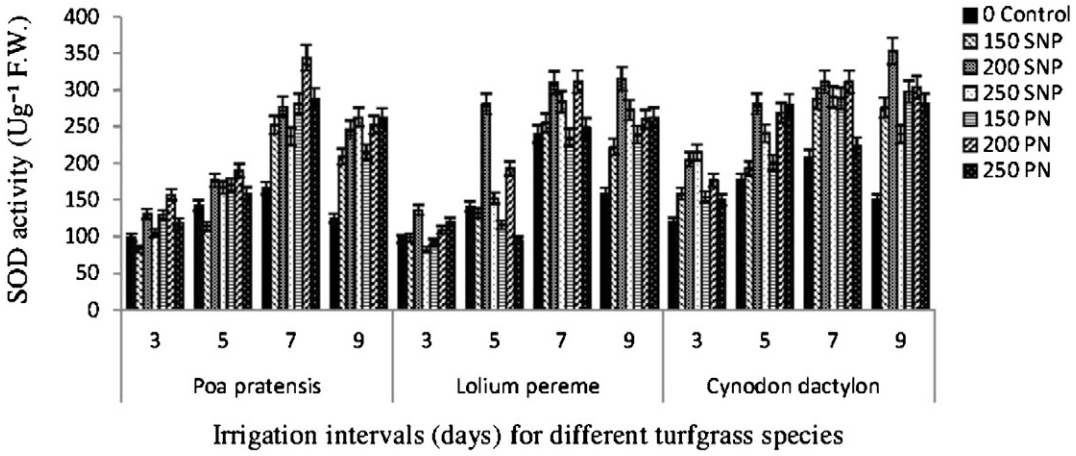

Fig. 3. The effect of NO donors on SOD activity at different water stresses of three turfgrass species.

Fridovich, 1969). Each 1 mL reaction solution contained: 75 μM NBT, 13 mM methionine, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), and 50 μL enzyme extract. Riboflavin (2 μM) was added at the last step to the reaction. Microtubes containing the reaction mixture were irradiated under fluorescent lamps at 78 μmol m^{-2} s^{-1} for 15 min. The light absorbance at 560 nm was determined with a WPA spectrophotometer (Biochrom, UK). One unit of SOD activity was defined as the amount of enzyme that would inhibit 50% of NBT photoreduction.

2.4.3. Peroxidase assay

Activity of peroxidase was determined using the method of Chance and Maehly (1995), with minor changes. The reaction solution (1 mL) contained 13 mM guaiacol, 5 mM H_2O_2 and 50 mM phosphate buffer (pH 7.0) which was mixed with 33 μL enzyme extract. The absorbance of the reaction mixture at 470 nm was read every 10 s for 1 min.

2.4.4. Ascorbate peroxidase assay

The ascorbate peroxidase (APX) activity was determined according to the method of Nakano and Asada (1981). The reaction solution (1 mL) contained 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM sodium ascorbate, 0.1 mM H_2O_2 and 50 μL enzyme extract. The light absorbance of the reaction solution was read after 1 min at 290 nm.

2.5. Experiment design and data analysis

Experiments were conducted in a randomized complete block design with four replications. Data were analyzed by factorial ANOVA at a significance level of $P < 0.05$ using SPSS v.16 and means were compared using LSD test.

3. Results

3.1. Antioxidant enzymes' activity during drought stress

3.1.1. CAT activity

Water stress significantly increased CAT activity in all treatments ($F_{83, 249} = 63.35$, $P < 0.01$) and species ($F_{2, 249} = 293.99$, $P < 0.01$); however, this increase was not always consistent over time and peaked at either day 7 or 9 (Fig. 1). The magnitude of this increase in CAT activity was different between species and treatments ($F_{12, 249} = 10.37$, $P < 0.01$). The 200 μmol SNP treatment resulted in the greatest increase in CAT activity in *P. pratensis* and *C. dactylon*, while the same concentration of either SNP or PN resulted in the greatest CAT activity in *L. perenne*. The highest increase in CAT activity was observed in *C. dactylon*.

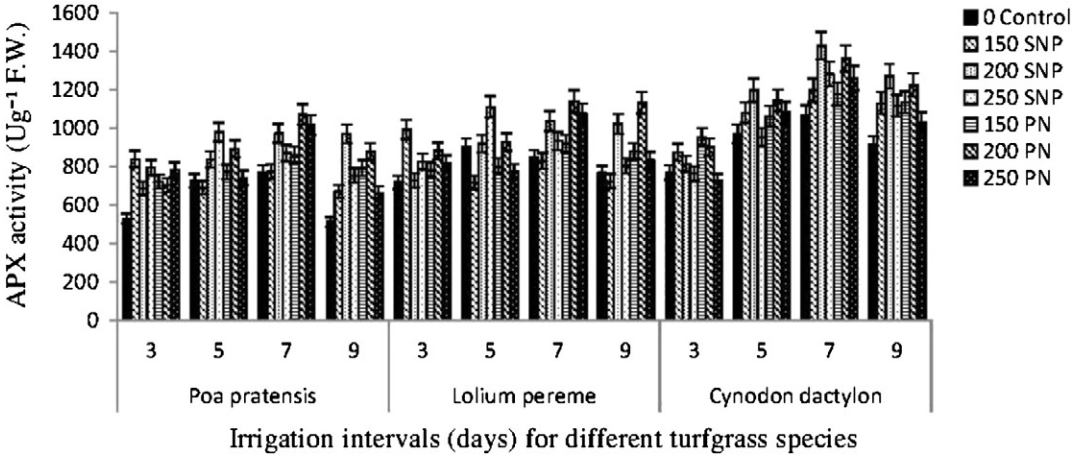

Fig. 4. The effect of NO donors on APX activity at different water stresses of three turfgrass species.

3.1.2. POD activity

Drought stress significantly raised POD activity in all treatments ($F_{83,\ 249} = 145.47$, $P < 0.01$) and species ($F_{2,\ 249} = 524.49$, $P < 0.01$), though this rise was not at all times constant over duration and hit the highest pointed at either day 7 or 9 (Fig. 2). The degree of this increase in POD activity was different between species and treatments ($F_{12,\ 249} = 18.89$, $P < 0.01$). The 200 μmol PN treatment resulted in the biggest increase in POD activity in *C. dactylon* and *L. perenne*, while the identical concentration of either SNP or PN resulted in the greatest POD activity in *P. pratensis*. The highest increase in POD activity was found in *C. dactylon*.

3.1.3. SOD activity

Water deficiency significantly enhanced SOD activity in all treatments ($F_{83,\ 249} = 222.17$, $P < 0.01$) and species ($F_{2,\ 249} = 763.89$, $P < 0.01$); nevertheless, this increase was not consistent across the different drought treatments over time and peaked at either day 7 or 9 (Fig. 3). The extent of this increase in SOD activity was different between species and treatments ($F_{12,\ 249} = 21.78$, $P < 0.01$). The 200 μmol SNP and PN treatments resulted in the greatest increase in SOD activity in *C. dactylon* and *P. pratensis*, respectively, whereas, the same concentration of either SNP or PN resulted in the greatest CAT activity in *L. perenne*. The greatest increase in SOD activity was seen in *C. dactylon*.

3.1.4. APX activity

Scarcity of water during drought stress significantly added to APX activity in all treatments ($F_{83,\ 249} = 541.02$, $P < 0.01$) and species ($F_{2,\ 249} = 8246.34$, $P < 0.01$), but this increase was not consistent over time and reached the maximum at either day 7 or 9 (Fig. 4). The amount of this increase in SOD activity was different between species and treatments ($F_{12,\ 249} = 83.19$, $P < 0.01$). The 200 μmol SNP treatment brought about the greatest increase in APX activity in *C. dactylon*, while the same concentration of PN resulted in the greatest APX activity in *P. pratensis* and *L. perenne*. The greatest increase in APX activity was detected in *C. dactylon*.

4. Discussion

Environmental stresses lead to intensive production of reactive oxygen species (ROS) setting off progressive oxidative damage and finally cell death (Asada, 1999; Reddy et al., 2004; Sharma et al., 2012; Ashraf and Harris, 2013). Drought is a major source of oxidative stress increasing ROS production and further lipid peroxidation, thus cellular homeostasis is disrupted and antioxidant enzymes' production and activity is diminished (Shi et al., 2007). Antioxidant enzymes' activity has a beneficial function on plant tolerance in drought stress (Møller et al., 2007; Bian and Jiang, 2009). SOD scavenges O_2^- to H_2O_2 (Bowler et al., 1992), and POD, CAT and APX detoxify H_2O_2 to H_2O at different cellular locations (Mittler, 2002). Our results showed that application of SNP and PN as NO donors comparably increased the production and activity of SOD, CAT, POD and APX in three turfgrasses. At longer drought stress, withholding water for 7 days, turfgrasses showed higher antioxidant enzymes' activity. Warm season turfgrass *C. dactylon* had the highest activity for antioxidant enzymes. Similar results have been seen in rice at greater drought intensities (Shehab et al., 2010). Nitric oxide donors have a positive role in raising the antioxidant enzymes' activities in different stressed plants (Shi et al., 2007; Tan et al., 2008; Tanou et al., 2009; Corpas et al., 2011; Nalousi et al., 2013; Egbichi et al., 2014). There are a few reports showing that drought stress has caused oxidative injury to Kentucky bluegrass and perennial ryegrass (Liu et al., 2008; Farfan-Vignolo and Asard, 2012). DaCosta and Huang (2007) showed that in three bentgrass species antioxidant metabolism is one essential process that improves drought tolerance. It is concluded that SNP and PN are useful NO donors which increase antioxidant enzymes' activity in plants under drought stress and may reduce the oxidative stress damage and adapt plants to deteriorating conditions.

Acknowledgments

The authors wish to thank Mr. A. Nikbakht and Mr. A. Lotfi from the Department of Horticulture Science at Shiraz University.

References

Asada, K., 1999. The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annual Review of Plant Physiology and Plant Molecular Biology 50, 601–639.
Ashraf, M., Harris, P.J.C., 2013. Photosynthesis under stressful environments: an overview. Photosynthetica 51, 163–190.
Bavita, A., Shashi, B., Navtij, S.B., 2012. Nitric oxide alleviates oxidative damage induced by high temperature stress in wheat. Indian Journal of Experimental Biology 50, 372–378.
Beligni, M.V., Lamattina, L., 2000. Nitric oxide induces seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. Planta 210, 215–221.
Bian, S., Jiang, Y., 2009. Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. Scientia Horticulturae 120, 264–270.
Bowler, C., Van Montagu, M., Inzé, D., 1992. Superoxide dismutase and stress tolerance. Annual Review of Plant Physiology and Plant Molecular Biology 43, 83–116.
Chance, B., Maehly, A., 1995. Assay of catalase and peroxidase. In: Colowick, S.P., Kaplan, N.O. (Eds.), Methods in Enzymology. Academic Press, New York, pp. 764–775.
Corpas, F.J., Leterrier, M., Valderrama, R., Airaki, M., Chaki, M., Palma, J.M., Barroso, J.B., 2011. Nitric oxide imbalance provokes a nitrosative response in plants under abiotic stress. Plant Science 181, 604–611.
Crawford, N.M., Guo, F.Q., 2005. New insights into nitric oxide metabolism and regulatory functions. Trends in Plant Science 10, 195–200.
DaCosta, M., Huang, B., 2007. Changes in antioxidant enzyme activities and lipid peroxidation for bentgrass species in responses to drought stress. Journal of American Society for Horticultural Science 132, 319–326.
Delledonne, M., 2005. NO news is good news for plants. Current Opinion in Plant Biology 8, 1–7.
Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A., 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. Journal of Experimental Botany 32, 93–101.
Efeoğlu, B., Ekmekçi, Y., Çiçek, N., 2009. Physiological responses of three maize cultivars to drought stress and recovery. South African Journal of Botany 75, 34–42.
Egbichi, I., Keyster, M., Ludidi, N., 2014. Effect of exogenous application of nitric oxide on salt stress responses of soybean. South African Journal of Botany 90, 131–136.
Farfan-Vignolo, E.R., Asard, H., 2012. Effect of elevated CO_2 and temperature on the oxidative stress response to drought in *Lolium perenne* L. and *Medicago sativa* L. Plant Physiology and Biochemistry 59, 55–62.
Foyer, C.H., Deascouveries, P., Kunert, K.J., 1994. Protection against oxygen radicals: important defense mechanism studied in transgenic plants. Plant, Cell and Environment 17, 507–523.
Freschi, L., 2013. Nitric oxide and phytohormone interactions: current status and perspectives. Frontiers in Plant Science 4, 1–22.
Fu, J., Huang, B., 2001. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. Environmental and Experimental Botany 45, 105–114.
He, Y., Tang, R., Hao, Y., Stevens, R.D., Cook, C.W., Ahn, S.M., Jing, L., Yang, Z., Chen, L., Guo, F., Fiorani, F., Jackson, R.B., Crawford, N.M., Pei, Z.M., 2004. Nitric oxide represses the *Arabidopsis* floral transition. Science 305, 1968–1971.
Hu, X., Neill, S.J., Tang, Z., Cai, W., 2005. Nitric oxide mediates gravitropic bending in soybean roots. Plant Physiology 137, 663–670.
Jiang, Y., Huang, B., 2001. Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolisms and lipid peroxidation. Crop Science 41, 436–442.
Lamattina, L., Garcı'a-Mata, C., Graziano, M., Pagnussat, G., 2003. Nitric oxide: the versatility of an extensive signal molecule. Annual Review of Plant Biology 54, 109–136.
Liu, J., Xie, X., Du, J., Sun, J., Bai, X., 2008. Effects of simultaneous drought and heat stress on Kentucky bluegrass. Scientia Horticulturae 115, 190–195.
Lu, S., Su, W., Li, H., Guo, Z., 2009. Abscisic acid improves drought tolerance of triploid bermudagrass and involves H_2O_2- and NO-induced antioxidant enzyme activities. Plant Physiology and Biochemistry 47, 132–138.
Mahajan, S., Tuteja, N., 2005. Cold, salinity and drought stresses: an overview. Archives of Biochemistry and Biophysics 444, 139–158.
McCord, J.M., Fridovich, I., 1969. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). Journal of Biological Chemistry 244, 6049–6055.
Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7, 405–410.
Møller, I.M., Jensen, P.E., Hansson, A., 2007. Oxidative modifications to cellular components in plants. Annual Review of Plant Biology 58, 459–481.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22, 867–880.
Nalousi, A.M., Ahmadiyan, S., Hatamzadeh, A., Ghasemnezhad, M., 2013. Protective role of exogenous nitric oxide against oxidative stress induced by salt stress in bell-pepper (*Capsicum annum* L.). American-Eurasian Journal of Agricultural and Environmental Sciences 8, 1085–1090.

Reddy, A.R., Ramachandra, R.K., Chaitanya, V., Vivekanandan, M., 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of Plant Physiology 161, 1189–1202.

Sharma, P., Dubey, R.S., 2005. Drought induces oxidative stress and enhances the activities of antioxidant enzyme in growing rice seedling. Plant Growth Regulation 46, 209–221.

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany. http://dx.doi.org/10.1155/2012/217037.

Shehab, G.G., Ahmed, O.K., El-Beltagi, H.S., 2010. Effects of various chemical agents for alleviation of drought stress in rice plants (*Oryza sativa* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca 38, 139–148.

Shi, Q., Ding, F., Wang, X., Wei, M., 2007. Exogenous nitric oxide protect cucumber roots against oxidative stress induced by salt stress. Plant Physiology and Biochemistry 45, 542–550.

Smirnoff, N., 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist 125, 27–58.

Tan, J., Zhao, H., Hong, J., Han, Y., Li, H., Zhao, W., 2008. Effects of exogenous nitric oxide on photosynthesis, antioxidant capacity and proline accumulation in wheat seedlings subjected to osmotic stress. World Journal of Agricultural Sciences 4, 307–313.

Tanou, G., Molassiotis, A., Diamantidis, G., 2009. Hydrogen peroxide- and nitric oxide-induced systemic antioxidant prime-like activity under NaCl-stress and stress-free conditions in citrus plants. Journal of Plant Physiology 166, 1904–1913.

Türkan, I., Bor, M., Özdemir, F., Koca, H., 2005. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Science 168, 223–231.

Velikova, V., Fares, S., Loreto, F., 2008. Isoprene and nitric oxide reduce damages in leaves exposed to oxidative stress. Plant Cell and Environment 31, 1882–1894.