BIOLOGIA PLANTARUM 50 (4): 775-778, 2006

BRIEF COMMUNICATION

Nitric oxide treatment alleviates drought stress in wheat seedlings

X. TIAN* and Y. LEI**[1]

*School of Biological Resources and Environmental Sciences, Jishou University, Hunan, 416000, P.R. China**
*Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, Chengdu 610041, P.R. China***

Abstract

The effects of sodium nitroprusside (SNP; nitric oxide donor) treatment on drought stress induced by PEG for different periods of time in wheat seedlings were investigated. Our results suggested that treatment for 2, 4 and 6 d with 15 % PEG could be termed as mild, moderate and severe stress, respectively. Drought stress induced accumulation of hydrogen peroxide and resulted in lipid peroxidation. On the other hand, activities of SOD, CAT and PAL increased under mild stress to counteract the oxidative injury and then decreased when the stress became severe (6 d). As the effect of SNP treatment, 0.2 mM enhanced wheat seedlings growth and kept high relative water content and alleviated the oxidative damage. However, 2 mM SNP aggravated the stress as a result of uncontrolled generation of reactive oxygen species and ineffectiveness of antioxidant systems.

Additional key words: ascorbate peroxidase, catalase, guaiacol peroxidase, L-phenylalanine ammonia lyase, reactive oxygen species, superoxide dismutase, *Triticum aeslivum.*

Drought stress is one of the main causes for crop yield reduction in the majority of agricultural regions of the world (Bajaj *et al*. 1999). A common effect of drought stress is the disturbance between the generation and quenching of reactive oxygen species (ROS) (Smirnoff 1998). ROS are highly reactive and in the absence of effective protective mechanism, can seriously damage plants by lipid peroxidation, protein degradation, breakage of DNA and cell death (Beligni and Lamattina 1999). Plant cells can tolerate ROS by endogenous protective mechanisms involving nonenzymic as well as enzymatic systems (Asada 1994).

Many previous studies have reported presence of NO in the plant kingdom and its involvement in growth, development and defense responses (Beligni and Lamattina 1999). Tu *et al*. (2003) found that 0.1 mM SNP delayed the senescence of wheat leaves by inhibition of the degradation of chlorophyll and soluble proteins, especially Rubisco, while 0.5 mM would accelerate the process. However, there is very little information about the effect of exogenously applied NO and drought stress in wheat seedlings. In this study, we used SNP as NO donor to determine the effect of NO on drought stress induced by PEG treatment in wheat seedlings.

Selected wheat (*Triticum aestivum* L. cv. W7) seeds, provided by Dr. T. Wang, Chengdu Institute of Biology, CAS, were surface sterilized with 0.1 % $HgCl_2$ (m/v) for 3 min, washed thoroughly under tap water and finally with distilled water. Then they were sown in silicon sands and after germination the seedlings were cultivated in phytotron, with day/night temperature of 22/18 °C and a 14-h photoperiod with irradiance of 120 μmol m^{-2} s^{-1} (determined by *CI-301PS*, *CID Inc.*, Vancouver, Washington, USA) provided by fluorescence lamps at 400 - 700 nm and a relative humidity of 60 %. The seedlings were irrigated with half strength Hoagland's solution containing 0, 0.2, 2 mM SNP respectively. After complete extension of the second leaf, the seedlings were treated with 15 % PEG for 0, 2, 4, 6 d and the shoots were harvested, immediately frozen in liquid N_2, and then stored at -20 °C for further analysis.

The relative water content (RWC) of shoots was calculated according to the formula: 100 × [(fresh mass - dry mass)/(saturated mass - dry mass)]. Saturated mass was determined after immersion of the shoots in water for 24 h at room temperature. Dry mass was measured following over drying at 105 °C till constant mass.

The contents of H_2O_2 were measured following the

Received 30 March 2005, *accepted* 18 October 2005.
Abbreviations: AsA - ascorbic acid; APX - ascorbate peroxidase; CAT - catalase; EDTA - ethylene diamine tetraacetic acid; GPX - guaiacol peroxidase; H_2O_2 - hydrogen peroxide; PAL - L-phenylalanine ammonia lyase, ROS - reactive oxygen species; RWC - relative water content; SNP - sodium nitroprusside; SOD - superoxide dimutase; TBARS - thiobarburic acid reacting substance.
[1] Corresponding author; fax: (+86) 28 85222753, e-mail: leiyb@cib.ac.cn

method of Brennan and Frenkel (1977). The lipid peroxidation was determined according to the content of thiobarbituric acid reacting substances (TBARS) as described by Hodges *et al.* (1999). The concentration of TBARS was calculated based on the absorbance at 532 nm and 600 nm.

Frozen shoots were crushed into a fine power with mortar and pestle under liquid nitrogen. Soluble proteins were extracted by homogenizing the powder in 10 cm^3 of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1 % polyvinylpyrrolidone. The homogenate was centrifuged at 12 000 *g* for 20 min at 4 °C and then the supernatant was used for the following enzyme assays. Total SOD (EC1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme required to cause 50 % inhibition of the reduction of NBT as monitored at 560 nm. CAT (EC1.11.1.6) activity was determined by following the consumption of H_2O_2 (coefficient of absorbance 39.4 mM^{-1} cm^{-1}) at 240 nm for 3 min (Aebi 1983). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.2 cm^3 of enzyme extract in a 3 cm^3 volume. GPX (EC 1.11.1.7) activity was based on the determination of guaiacol oxidation (coefficient of absorbance 26.6 mM^{-1} cm^{-1}) at 470 nm by H_2O_2. The reaction mixture contained 100 mM potassium phosphate buffer (pH 6.5), 16 mM guaiacol and 0.1 cm^3 of 10 % H_2O_2 in a 3 cm^3 volume. The reaction was initiated by adding 0.1 cm^3 enzyme extract and was followed for 3 min (Lin and Wang 2002). PAL activity was assayed according to the method of D'Cunha *et al.* (1996). The reaction mixture contained 100 mM Tris-HCl buffer (pH 8.5), 1 mM 2-mercaptoethanol, and 50 mM L-phenylalanine and enzyme extract. The mixture was incubated at 30 °C for 15 min. The reaction was terminated by the addition of 6 M HCl and the clear solution was measured at 290 nm. One unit represents the conversion of 1 μmol substrate to product per min. Total soluble protein contents were determined according to the method of Bradford (1976), using bovine serum albumin as standard.

Statistical analyses were conducted using *SPSS for Windows* (version *11.0*). Difference (LSD) test was employed to determine differences among the treatments ($P < 0.05$ or $P < 0.01$).

Water stress is one of the most important environmental factors that regulate plant growth and development, and limit plant production. As our results show that the water content decreased continuously with the aggravation of drought stress (Table 1). Relative water contents of 2, 4 and 6 d after stress were decreased by 10.19, 22.4 and 37.35 % compared to the control (0 d) respectively (Table 1). According to Hsiao (1973), drought stress for 2, 4 and 6 d could be termed as mild (RWC lowered by 8 to 10 %), moderate (RWC lowered less than 20 %) and severe stress (RWC lowered more than 20 % but less than 50 %), respectively. At the first two days, fresh mass of wheat shoots increased a lot. But afterwards drought stress inhibited the growth completely and the fresh mass began to decrease. After 6 d of treatment, fresh mass decreased to 84.8 % compared to the control (Table 1).

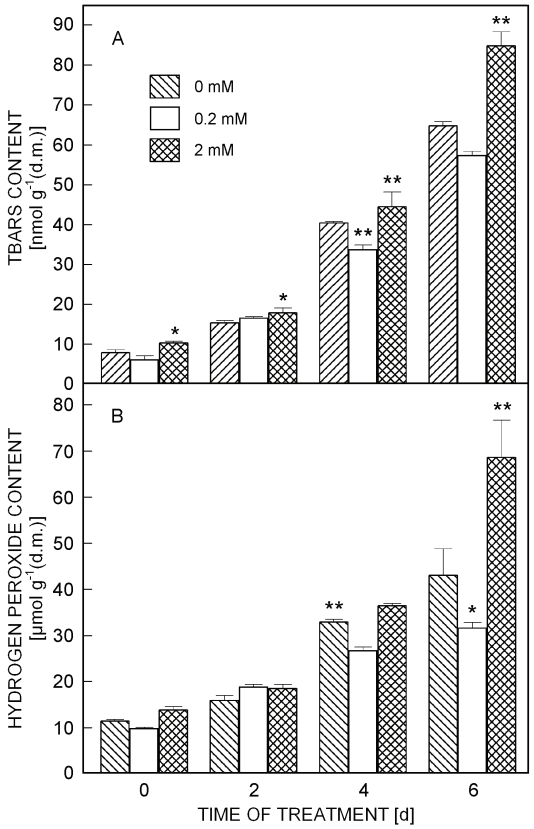

Fig. 1. Effects of drought stress and SNP treatment on contents of TBARS and H_2O_2 of wheat shoots. *A* - TBARS content, *B* - H_2O_2 content. Means ± SE of three separate measurements. The significance of differences between 0.2, 2 mM SNP and control (0 mM) was determined by one-way *ANOVA* (* - $P < 0.05$; ** - $P < 0.01$).

Drought stress significantly increased the content of H_2O_2 and the lipid peroxidation indicated by the content of TBARS. The contents of H_2O_2 and TBARS after 6 d treatment were 3.77 times and 8.12 times compared with the control (0 d), respectively ($P < 0.01$) (Fig. 1*A*,*B*). Moreover, there was significant correlation between H_2O_2 (x, μmol g^{-1} d.m.) and TBARS (y, nmol g^{-1} d.m.): $y = 0.6793\ x + 6.6114$ ($r^2 = 0.9189$).

SOD increased significantly under mild and even moderate stress; but under severe drought stress SOD activity decreased a lot, which were in well agreement with Chaparzaseh *et al.* (2004) who found that high salinity reduced leaf SOD activity in *Calendula officinalis* while low salinity led to an increase in the root. Drought caused a continuous increase in the activity of GPX: after 6 d of stress, it increased to 1.52 times compared to the control (0 d). In our results, CAT only increased a little under mild stress and then decreased

markedly with the commitment of the increase in H_2O_2 content. The down-regulation of CAT under stress may indicate that the plant is unable to maintain protection against active oxygen particularly at severe drought stress. The decrease in CAT activity measured under severe water stress has been reported previously (Baisak *et al*. 1994, Huang and Guo 2005). The change pattern of PAL activity was similar with SOD: firstly increased a lot until reached the highest level after 4 d treatment and then decreased a little under severe stress. PAL is considered the key enzyme in phenolic biosynthesis, which is involved in responses to many biotic and abiotic stresses and may also function as antioxidants because of their free-radical trapping properties (Haslam 1998). Studies with several different species of plants have shown PAL is activated by many environmental factors, which is consistent to the increase in PAL activity in wheat shoots in our experiment.

Table 1. Effects of drought stress and SNP [mM] treatment on the relative water content, RWC [%] and fresh mass [mg shoot^{-1}] of wheat shoots. Means of three replicates ± SD. The significance of differences between 0 (control), 0.2, 2.0 mM SNP was determined by one-way *ANOVA* (* - $P < 0.05$; ** - $P < 0.01$).

	SNP	0 d	2 d	4 d	6 d
RWC	0	81.34 ± 0.95	73.05 ± 1.54	62.99 ± 1.52	50.96 ± 2.66
	0.2	82.47 ± 0.88	76.39 ± 1.87*	67.04 ± 1.19**	56.55 ± 1.97**
	2.0	80.45 ± 0.71	72.14 ± 1.61	58.41 ± 2.01**	42.71 ± 2.07**
Fresh mass	0	127.60 ± 8.12	146.93 ± 3.93	127.10 ± 1.82	111.80 ± 3.21
	0.2	167.67 ± 1.74**	173.73 ± 5.31**	155.10 ± 1.93**	125.70 ± 3.11**
	2.0	135.07 ± 5.11	150.40 ± 4.51	101.80 ± 4.20**	80.47 ± 5.03**

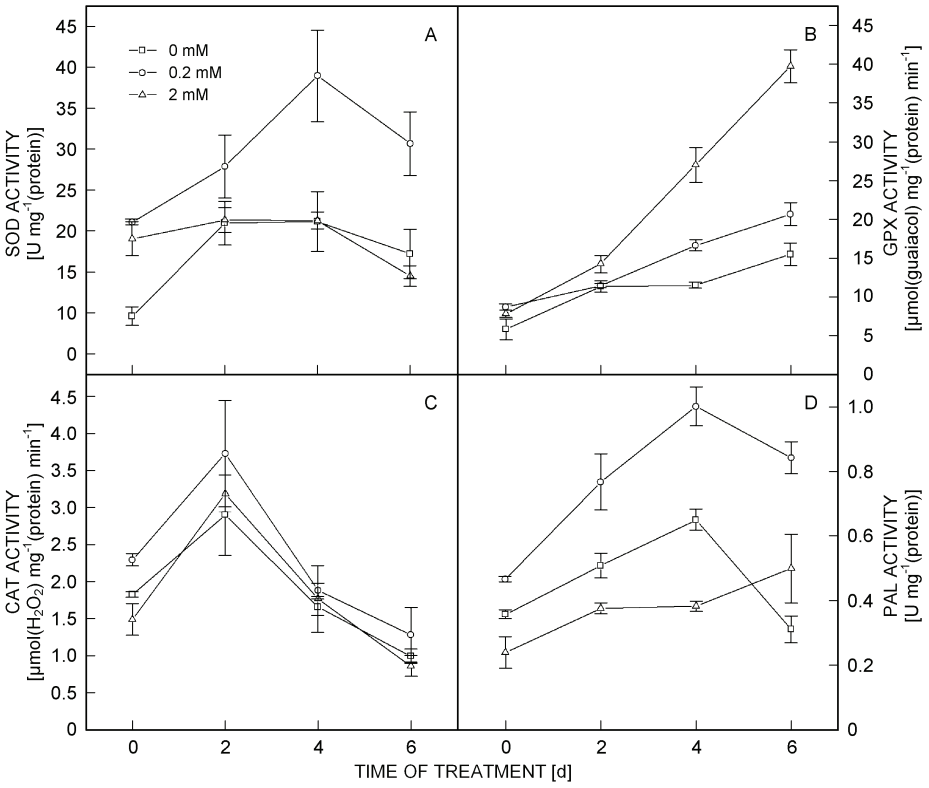

Fig. 2. Effect of drought stress and SNP treatment on enzyme activities of wheat shoots. *A* - SOD, *B* - GPX, *C* - CAT, *D* - PAL. Means ± SE of three separate measurements.

Whether NO is protective or toxic to plants is found to be quite concentration dependent. Low concentration (0.2 mM) could inhibit the loss of water content and strengthen the wheat seedling growth, while high concentration (2 mM) of SNP had the opposite effect (Table 1). It was most obvious at 4 and 6 d after stress

($P < 0.01$). Plants treated with 0.2 mM SNP had the lowest content of H_2O_2 and the highest antioxidative enzyme activities except GPX. As is now commonly accepted NO as a second messenger in plants, it is supposed that low concentration of NO might be a signal molecule to induce/stabilize the expression of many antioxidative enzymes including SOD, CAT (Frank *et al*. 2000). The protective effect of NO may also be related to its ability to react with some ROS, such as $O_2^{\cdot-}$, making NO act as a chain breaker and show its proposed antioxidant properties (Conner and Grisham 1996). Moreover it has been reported that NO can react with lipid alcoxyl ($LO^{\bullet}$) and peroxyl ($LOO^{\bullet}$) radicals, leading to the expectation that NO could stop the propagation of radical-mediated lipid oxidation in a direct fashion (Lamotte *et al*. 2004), which is in well agreement with our result in the decrease of TBARS content (Fig. 1*A*). Thus NO may help plants to survive stressful conditions through its action as signalling molecule to activate antioxidative enzymes and reaction with active oxygen and lipid radicals directly. However, it has also proved that the effect of NO is in concentration-dependent manner. High concentration of SNP (2 mM) inhibited wheat growth as suggested by Tu *et al.* (2003).

In conclusion, PEG induced drought stress could cause oxidative damage to wheat seedlings growth through excessive generation of ROS and proper concentrations of exogenous NO could improve the dehydration tolerance through enhancing the antioxidant systems. Our results provide some evidences to the important functions of NO in plant kingdom, which need further research.

References

Aebi, H.E.: Catalase. - In: Bergmeyer, H.U. (ed.): Methods of Enzymatic Analysis. Vol. 3. Pp. 273-282. Verlag-Chemie, Weinheim 1983.

Asada, K.: Production and action of active oxygen species in photosynthetic tissues. - In: Foyer, C.H., Mullineaux, P.M. (ed.): Causes of Photooxidative Stress and Amelioration of Defense System in Plants. Pp. 77-103, CRC Press, Boca Raton 1994.

Baisak, R., Rana, D., Acharya, P.B.B., Kar, M.: Alterations in activities of oxygen active scavenging enzymes of wheat leaves subjected to water stress. - Plant Cell Physiol. **35**: 489-495, 1994.

Bajaj, S., Jayaprakash, T., Li, L., Ho, T.H.D., Wu, R.: Transgenic approaches to increase dehydration-stress tolerance in plants. - Mol. Breed. **5**: 493-503, 1999.

Beligni, M.V., Lamattina, L.: Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plant tissues. - Planta **208**: 337-344, 1999.

Bradford, M.M.: A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Brennan, T., Frenkel, C.: Involvement of hydrogen peroxide in the regulation of senescence in pear. - Plant Physiol. **59**: 411-416, 1977.

Chaparzadeh, N., D'Amico, M.L., Khavari-Nejad, R.A., Izzo, R., Navari-Izzo, F.: Antioxidant responses of *Calendula officinalis* under salinity conditions. - Plant Physiol. Biochem. **42**: 695-701, 2004.

Conner, E.M., Grisham, M.B.: Inflammation, free radicals and antioxidants. - Nutrition **12**: 274-277, 1996.

D'Cunha, G.B., Satyanarayan, V., Nair, P.M.: Purification of phenylalanine ammonia-lyase from *Rhodotorula glutinis*. - Phytochemistry **42**: 17-20, 1996.

Frank, S., Kämpfer, H., Podda, M.: Identification of copper/zinc superoxide dismutase as a nitric oxide-regulated gene in human (HaCaT) keratinocytes: implications for keratinocyte proliferation. - Biochem. J. **346**: 719-728, 2000.

Giannopolitis, C.N., Ries, S.K.: Superoxide dismutase I: Occurrence in higher plants. - Plant Physiol. **77**: 309-314, 1977.

Haslam, E.: Practical Polyphenolics: From Structure to Molecular Recognition and Physiological Action. - Cambridge University Press, Cambridge 1998.

Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K.: Improving the thiobarbituric acid-reactive-substance assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. - Planta **207**: 604-611, 1999.

Hsiao, T.C.: Plant response to water stress. - Annu. Rev. Plant Physiol. **24**: 519-570, 1973.

Huang, M., Guo, N.: Responses of antioxidative system to chilling stress in two rice cultivars differing in sensitivity. - Biol. Plant. **49**: 81-84, 2005.

Lamotte, O., Gould, K., Lecourieux, D., Sequeira-Legrand, A., Lebun-Garcia, A., Durner, J., Pugin, A., Wendehenne, D.: Analysis of nitric oxide signaling functions in tobacco cells challenged by the elicitor cryptogein. - Plant Physiol. **135**: 516-529, 2004.

Lin, J.S., Wang, G.X.: Doubled CO_2 could improve the drought tolerance better in sensitive cultivars than in tolerant cultivars in spring wheat. - Plant Sci. **163**: 627-637, 2002.

Smirnoff, N.: Plant resistance to environmental stress. - Curr. Opin. Biotechnol. **9**: 214-219, 1998.

Tu, J., Shen, W.B., Xu, L.L.: Regulation of nitric acid on the aging process of wheat leaves. - Acta bot. sin. **45**: 1055-1062, 2003.