

Role of nitric acid or H₂O₂ in antioxidant defense system of *Pisum sativum* L. under drought stress¹Helal Ragab Moussa and ²Mohamed Abd El-Fattah Hassan Mohamed¹Radioisotope Department, Atomic Energy Authority, Malaeb El-Gamaa St., P.O. 12311, Dokki, Giza, Egypt.Corresponding author: *E-mail: helal_moussa@hotmail.com*²Agriculture Research Center, Soils, Water & Environment Research Institute, Giza

SUMMARY. Water shortage is likely to be one of the major global environmental stresses of the 21st century. Drought is an important environmental constraint limiting the productivity of many crops worldwide. Experiments were conducted to investigate the effects of seed pretreatment by hydrogen peroxide at 70 mM or sodium nitroprusside (SNP; nitric oxide donor) at 10 μM on drought tolerance in pea seedlings. Osmotic stress was provoked by addition polyethylene glycol to the nutrient solution at the flowering stage. H₂O₂ or SNP are active molecules involved in mediation of various biotic and abiotic stress induced physiological responses in plants. H₂O₂ or SNP pretreatment alleviate oxidative damage, accelerate proline accumulation and enhance total chlorophyll, carotenoid, photosynthetic activity (¹⁴CO₂-assimilation), and total yield/plant in leaves of pea seedlings subjected to osmotic stress. The results showed that osmotic stress induced decrease in the enzyme activities of ascorbate peroxidase, glutathione peroxidase, catalase and overproduction of O₂^{•-} in pea leaves, which in turn caused exacerbation of lipid peroxidation and depression of photosynthesis. Application of H₂O₂ or SNP significantly increased the enzyme activities and decrease O₂^{•-} production and hence inhibited lipid peroxidation. Level of H₂O₂, proline and Evan blue uptake in seedlings pretreated with H₂O₂ or SNP were markedly lower than under drought stress, indicating the operation of antioxidant system in them. Moreover, seedlings arising from H₂O₂ or SNP pretreatment enhanced the membrane stability, as revealed from greatly reduced malondialdehyde content. The present data suggest that seed pretreatment in pea with H₂O₂ or SNP, a stress signal, could trigger the activation of antioxidants in seeds, which persists in the seedlings to alleviate the oxidative damage, leading to improvements in physiological attributes for the seedling growth under drought.

[Helal Ragab Moussa and Mohamed Abd El-Fattah Hassan Mohamed. **Role of nitric acid or H₂O₂ in antioxidant defense system of *Pisum sativum* L. under drought stress.** World Rural Observations 2011;3(2):76-81]; ISSN: 1944-6543 (Print); ISSN: 1944-6551 (Online). <http://www.sciencepub.net/rural>.

Key words: antioxidative enzymes, drought stress, photosynthesis, proline, pea

INTRODUCTION

Drought stress is one of the main causes for crop yield reduction in the majority of agricultural regions of the world (Moussa, 2011). Reactive oxygen species (ROS) are enhanced during drought stress through the disruption of electron transport system and oxidizing metabolic activities occurring in chloroplast, mitochondria and microbodies (Sofa et al., 2005). Plants eliminate ROS produced in non-stressful conditions through production of non-enzymatic and enzymatic antioxidants (Inze and Montagu, 2000), whereas during severe drought conditions the production of ROS exceeds the capacity of the antioxidative systems to remove them, causing oxidative stress (Sofa et al., 2005). In these conditions cells could be protected either by the endogenous molecular systems or exogenously applied compounds that mitigate the stress (Ingram and Bartels, 1996). 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). Among various technique strategies, pre-sowing treatment and priming of plant seeds are easy, low cost,

low risk and effective approaches to enhance plant tolerance to the stressful environments (Ashraf and Foolad, 2007). Hydrogen peroxide is a major kind of ROS in plant tissues. It acts as an important signal molecule involved in acclamatory signaling, triggering higher tolerance against various biotic and abiotic stresses at low concentrations, whereas at high concentrations, it is toxic to plant tissues and can trigger programmed cell death (Quan et al., 2008). H₂O₂ pretreatment could improve salt stress (Fedina et al., 2009), oxidative stress (Chen et al., 2009), and multiple stresses (Uchida et al., 2002). Many previous studies have reported presence of NO in the plant kingdom as an important endogenous plant signalling molecule and its involvement in growth, development and defense responses (Lamotte et al., 2005). As a relatively stable free-radical molecule, and due to its highly lipophilic nature, NO diffuses through membranes and may act as a synchronizing chemical messenger that is involved in many physiological processes in plants (Franciele et al., 2010). NO is an important signal molecule involved in plant response to biotic and abiotic stresses (Jinfang et al., 2008). However, there is very little information about the

effect of exogenously applied H₂O₂ or SNP and drought stress in pea seedlings.

The aim was to provide experimental basis for understanding the mechanism of drought tolerance in pea induced by H₂O₂ or SNP pretreatment.

MATERIALS and METHODS

Plant material and growth conditions

A homogenous lot of pea seeds (*Pisum sativum* L.), cv. Master; was obtained from the Crop Institute, Agricultural Research Center, Giza, Egypt. The caryopsis was kept at 4°C. They were surface sterilized in 0.1 % (w/v) sodium dodecyl sulphate solution and then thoroughly rinsed with sterile deionized water. Clean-healthy dry seeds were soaked for 20 h in solutions of H₂O₂ (70 mM) or sodium nitroprusside (10 µM) and with deionized water as the control. The H₂O₂ and SNP-treated and water-soaked seeds were sown in black polyethylene pots (40 cm diameter, 45 cm height) filled with silicon sands. Ten seeds were sown per pot. After the seedlings reached the first true leaf stage, they were thinned to four plants per pot. The sand-filled pots irrigated with half strength Hoagland's solution (Rafi and Epstein, 1999) as well-watered conditions. Pots were kept in a controlled-growth chamber at photo flux density of 240 µmole M⁻²S⁻¹ (12/12 h day/night period) at relative humidity of 55-60%, and 25±2 °C temperature. Cultural practices, such as weed control and irrigation, were performed as needed. Drought stress was achieved by adding polyethylene glycol (PEG-6000, -0.5 MPa) solution at the flowering stage (40 day after planting). The experiments conducted four times in complete randomization replicated design. Samples were collected 10 days after the water treatment was applied, between 9:30 and 10:30 a.m., and kept in liquid nitrogen until analyzed. At harvest, the effects of treatments on the total seed yield/plant were recorded.

Biochemical assays.

Free proline was determined according to the method described by Bates et al. (1973). The amount of total chlorophyll (*a+b*) and carotenoids were determined according to the method of Lichtenthaler and Wellburn (1983).

Determination of O₂^{•-} and H₂O₂ concentrations.

The superoxide radical (O₂^{•-}) was determined by 2, 3-bis (2-methoxy-4-nitro-5-sulphenyl) (2H) tetrazolium-5-carboxanilide (XTT) assay (Frahry and Schopfer, 2001). To determine H₂O₂ concentration, the root extract was mixed with 0.1% titanium chloride in 20% (v/v) H₂SO₄. The mixture was then centrifuged at 6 000 g for 15 min. The absorbance was measured at 410 nm (Hsu and Kao, 2007).

Determination of lipid peroxidation and Evans blue uptake.

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content using the thiobarbituric acid reaction as described by Madhava Rao and Sresty (2000). The loss of plasma membrane integrity was detected by the non-permeable dye (Evans blue) uptake in the root cells, which has also been used as an indicator of cell death (Baker and Mock, 1994).

Determination of antioxidant enzyme activities.

The catalase (CAT, EC 1.11.1.6) activity was assayed from the rate of H₂O₂ decomposition following the method of Aebi (1983). The ascorbate peroxidase enzyme activity (APX, EC 1.11.1.1) was determined as the decrease in absorbance at 290 nm due to ascorbate oxidation (Nakano and Asada, 1981). The glutathione peroxidase (GPX, EC 1.11.1.9) activity was determined as the decrease in absorbance at 340 nm due to the oxidation of NADPH (Navrot et al., 2007).

Photosynthetic activity (¹⁴CO₂-fixation).

Photosynthetic activity was measured in the atomic energy authority, radioisotope department, Cairo, Egypt, with the method of Moussa (2011). The seedlings from each treatment were placed under a Bell jar, which was used as a photosynthetic chamber. Radioactive ¹⁴CO₂ was generated inside the chamber by a reaction between 10% HCl and 50 µCi (1.87×10⁶ Bq) NaH¹⁴CO₃ + 100 mg Na₂CO₃ as a carrier. Then the samples were illuminated with a tungsten lamp. After 30 min exposure time, the leaves were quickly detached from the stem, weighed and frozen for 5 min to stop the biochemical reactions, then subjected to extraction by 80% hot ethanol. The ¹⁴C was assayed from the ethanolic extracts in soluble compounds using a Bray Cocktail (Bray, 1960) and a liquid scintillation counter (LSC2-Scaler Ratemeter SR7, Nuclear Enterprises, Edinburgh, UK).

Statistical analysis

All data were subjected to ANOVA and the means were compared using Duncan's multiple range tests (*P*<0.05).

RESULTS

Water deficit decreased significantly the total chlorophyll (*a+b*), carotenoid content, photosynthetic efficiency (¹⁴CO₂-assimilation) and total yield/plant (Table 1). However, pretreatment with H₂O₂ or SNP increased significantly the above parameters as compared to water deficit stressed plants (Table 1).

Table (1). Effects of H₂O₂ or SNP pretreatment on total chlorophyll *a+b* (mg/gFW), carotenoids (mg/gFW), photosynthetic efficiency (*KBq/mgFW) and total yield/plant (g) in pea seedlings under drought stress. Data presented are the means of four separate experiments.

Treatments	Total chlorophyll (<i>a+b</i>)	Carotenoids	Photosynthetic activity	Total yield/plant
Control	3.98 ^c	6.3 ^b	19829 ^a	58 ^a
Drought stress	2.57 ^e	3.9 ^c	15422 ^d	40 ^e
SNP	4.18 ^d	7.4 ^d	21498 ^e	63 ^d
Drought stress+ SNP	3.74 ^b	5.8 ^a	19032 ^b	55 ^c
H ₂ O ₂	4.05 ^d	6.8 ^e	20602 ^c	60 ^a
Drought stress+ H ₂ O ₂	3.51 ^a	5.5 ^a	18837 ^f	53 ^b

Values followed by the same letter within columns are not significantly different according to Duncan's multiple range tests ($P < 0.05$). * KBq: kilobequerel

H₂O₂ content increased significantly under drought stress as compared with the control (Table 2). Exogenous application of H₂O₂ or SNP in drought stressed plants decreased significantly H₂O₂ content under both normal and stress conditions (Table 2). Exposure to water stress increased malondialdehyde, proline concentration, Evans blue uptake and O^{•-} concentrations more significantly as compared with controls. Pretreatment with H₂O₂ or SNP alleviated Evans blue uptake, reduced the overproduction of O^{•-}, proline and MDA accumulation as compared with water deficit plants (Table 2). Water deficit markedly increased proline content and nearly tripled. Drought stress decreased significantly the activity of CAT, APX and GPX as compared with the control (Table 3). However pretreatment H₂O₂ or SNP application to drought stressed plants enhanced the activity of these enzymes (Table 3).

Table (2). Effects of H₂O₂ or SNP pretreatment on H₂O₂ (μM/gFW), MDA content (μM/gFW), O^{•-} concentration (μM/gFW), Evan blue uptake (OD_{600nm}/gFW), and free proline level (μM/gFW), in pea seedlings under drought stress. Data presented are the means of four separate experiments.

Treatments	H ₂ O ₂	MDA	O ^{•-}	Evans blue uptake	Free proline
Control	1.8 ^a	2.2 ^b	1.1 ^a	0.128 ^a	8.7 ^a
Drought stress	7.3 ^e	5.8 ^e	3.7 ^d	0.346 ^e	23.9 ^e
SNP	1.1 ^b	1.7 ^a	0.8 ^c	0.080 ^b	18.8 ^b
Drought stress+ SNP	2.6 ^c	3.0 ^d	1.7 ^b	0.204 ^d	14.3 ^c
H ₂ O ₂	1.3 ^b	1.9 ^a	0.7 ^c	0.075 ^b	17.1 ^b
Drought stress+ H ₂ O ₂	3.0 ^d	2.8 ^c	1.6 ^b	0.251 ^c	19.3 ^d

Values followed by the same letter within columns are not significantly different according to Duncan's multiple range tests ($P < 0.05$).

Table (3). Effects of H₂O₂ or SNP pretreatment on enzyme activities of APX (μM ascorbate/min.gFW), CAT (μMH₂O₂/min.gFW) and GPX (μM NADPH/min.gFW) in pea seedlings under drought stress. Data presented are the means of four separate experiments.

Treatments	APX	CAT	GPX
Control	2.6 ^b	1.8 ^a	5.1 ^b
Drought stress	1.7 ^a	0.8 ^c	3.5 ^c
SNP	2.4 ^b	2.3 ^b	8.4 ^e
Drought stress+ SNP	2.1 ^c	1.9 ^a	4.6 ^a
H ₂ O ₂	2.8 ^b	2.5 ^b	7.3 ^d
Drought stress+ H ₂ O ₂	2.2 ^d	1.7 ^a	4.6 ^a

Values followed by the same letter within columns are not significantly different according to Duncan's multiple range tests ($P < 0.05$).

DISCUSSION

Various environmental stresses such as drought can inhibit plant growth and development, leading to crop reduction. For food security in the

world and agricultural production, more efforts are needed to develop multiple effective strategies to improve crop stress tolerance (Moussa, 2011). In this study, we investigated the effects of seed treatment

with H₂O₂ or SNP on pea growth characteristics and physiological- biochemical response to drought stress. Overproduction of ROS, especially H₂O₂, is believed to be primarily responsible for drought triggered oxidative damage and cell death (Moussa, 2011). H₂O₂ acts as signal molecules in plant response to stress in low concentrations (Desikan et al., 2004; Liheng et al., 2009). H₂O₂ can effectively modulate the related gene expression and subsequently lead to the enhancement of plant tolerance to the stress (Hung et al., 2005 and Liheng et al., 2009). Proline is such an antioxidant which accumulates in response to biotic and abiotic stresses, including water stress (Zhang et al., 1995). The change in stability of biological membranes is a key indicator of cellular damage. Drought and other stresses always results in cellular membrane injures including the increase of membrane permeability and MDA content due to membrane lipid peroxidation (Agrawal and Rathore, 2007; Farooq and Azam, 2006). Like other physiological/biochemical processes affected by drought, the photosynthetic activity (¹⁴CO₂-assimilation), total chlorophyll and carotenoid content is also adversely affected by drought stress (Moussa, 2011 and Akram et al., 2007). H₂O₂ treatment enhanced photosynthetic efficiency in wheat under water stress condition (Liheng et al., 2009). Paralleling with the improvement of photosynthesis upon drought, the total yield/plant production largely increased. SNP promotes a significant increase in chlorophyll content and chloroplast membrane density in maize plants (Graziano et al., 2002). Also, SNP application stimulating the photosynthetic efficiency and inhibiting the degradation of chlorophyll in wheat (Tue et al., 2003). Opposite to this result, exposure to NO reduced photosynthesis in leaves of oats and alfalfa (Hill and Bennett, 1970). Franciele et al., 2010, reported that SNP treatment in soybean seedlings decreased H₂O₂ and Evans blue uptake concentration. Also, Tian and Lai (2006) investigated that pretreatment with SNP in wheat under drought stress increase the enzyme activities of CAT and GPX and decrease MDA content. Liheng et al. (2009) reported that H₂O₂ pretreatment enhanced membrane stability by greatly reduced MDA content and increasing the antioxidant enzyme activity of CAT and APX in drought stressed wheat seedlings (Liheng et al., 2009). The increased stress tolerance is attributed partly to an enhanced level of antioxidant defense system induced by H₂O₂ or SNP pretreatment, which alleviates ROS accumulation and oxidative damage during the subsequent stress conditions (Hu et al. 2005). 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 O₂^{•-}, 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[•]) and peroxy (LOO[•]) 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 MDA content. Thus NO may help plants to survive stressful conditions through its action as signaling molecule to activate antioxidative enzymes and reaction with active oxygen and lipid radicals directly. Tolerance to drought, is enhanced in wheat treated with SNP (García-Mata & Lamattina 2001, 2002). Incubation of soybean roots in solutions of SNP at very low concentrations (1–10 µM) promotes growth, whereas greater concentrations inhibit it (Hu et al., 2005). Proline as a cytosolic osmoticum and a scavenger of OH[•] radical can interact with cellular macromolecules such as DNA, protein and membranes and stabilize the structure and function of such macromolecules (Kavir Kishor et al., 2005). The data are of considerable value in understanding the mechanisms of plant stress tolerances and in developing effective methods for crop protection against environmental stresses. Thus, it may be a useful management tool in afforestation projects in arid and semiarid areas.

In conclusion, applying H₂O₂ or SNP prior to water deficit stress could partially alleviate the detrimental effect of water stress on growth of pea through increasing assimilation of ¹⁴CO₂, and improving antioxidant system and reduce the oxidative damage of cellular membranes. It was concluded that H₂O₂ or SNP minimized the pea yield loss caused by water deficits.

REFERENCES

1. Aebi H (1983). Catalase. In: Bergmeyer HU, ed. *Methods of Enzymatic Analysis*. 3. Weinheim-Verlag, Chemie, Germany. pp. 273–277.
2. Agrawal SB, Rathore D (2007). Changes in oxidative stress defense system in wheat (*Triticum aestivum* L.) and mung bean (*Vigna radiate* L.) cultivars grown with and without mineral nutrients and irradiated by supplemental ultraviolet-B. *Environ. Exp. Bot.* 59: 21-33.
3. Akram NA, Shahbaz M, M. Ashraf. M (2007). Relationship of photosynthetic capacity and proline accumulation with the growth of differently adapted populations of two potential grasses (*Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L.) to drought stress. *Pak. J. Bot.*, 39(3): 777-786.

4. Ashraf M and Foolad MR (2007). Roles of glycinebetaine and proline in improving plant abiotic resistance. *Environ. Exp. Bot.* 59: 206-16.
5. Baker CJ, Mock NM (1994) An improved method for monitoring cell death in cell suspension and leaf disc assays using Evans blue. *Plant. Cell. Tissue. Organ.* 39, 7-12.
6. Bates, L. S., Waldron, R. P., Teare, I. D. (1973): Rapid determination of free proline in water stress studies. *Plant Soil*, 29, 205-207.
7. Beligni M.V. & Lamattina L. (1999) Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plant tissues. *Planta* 208, 337-334
8. Bray, G. A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.*, 1, 276-285.
9. Chen XY, Ding X, Xu S, Wang R, Xuan W, Cao ZY, Chen J, Wu HH, e MB, Shen WB (2009). Endogenous hydrogen peroxide plays a ositive role in the upregulation of heme oxygenase and acclimationto oxidative stress in wheat seedlings leaves. *J. Integr. Plant Biol.* 51(10), 951-960.
10. Conner EM, Grisham MB (1996). Inflammation, free radicals and antioxidants. - *Nutrition* 12: 274-277, 1996.
11. Desikan R, Cheung MK, Clarke A, Golding S, Sagi M, Fluhr R, et al. (2004). Hydrogen peroxide is a common signal for darkness- and ABA-induced stomatal closure in *Pisum sativum*. *Funct. Plant Biol.* 31:
12. 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 (3): 307-313, 2008
13. Farooq S, Azam F (2006). The use of cell membrane stability (CMS) technique to screen for salt tolerant wheat varieties. *J. Plant Physiol.* 163: 629-637.
14. Fedina IS, Nedeva D, C, ic,ek N (2009) Pre-treatment with H₂O₂ induces salt tolerance in barley seedlings. *Biol. Plant.* 53, 321- 324.
15. Frahy G, Schopfer P (2001) NADH-stimulated, cyanide-resistant superoxide production in maize coleoptiles analyzed with a tetrazolium-based assay. *Planta*, 212, 175-183.
16. Franciele Mara Lucca Zanardo Bo'hm, Maria de Lourdes Lucio Ferrarese, Daniele Iara Lucca Zanardo, Jose Ronaldo Magalhaes, Osvaldo Ferrarese-Filho (2010).Nitric oxide affecting root growth, lignification and related enzymes in soybean seedlings. *Acta Physiol Plant*, 32:1039-1046
17. Frank S, Kämpfer H, Podda M(2000). 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.
18. Garcia-Mata C, Lamattina L (2001). Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. *Plant Physiology*, 126, 1196-1204.
19. Garcia-Mata C, Lamattina L (2002). Nitric oxide and abscisic acid cross talk in guard cells. *Plant Physiology*, 128, 790-792.
20. Graziano M, Beligni MV, Lamattina L (2002). Nitric oxide improves internal iron availability. *Plant Physiol.*, 130, 1852 - 1859.
21. Hill A., Bennett JH (1970). Inhibition of apparent photosynthesis by nitrogen oxides. *Atmospheric Environment* 4, 341-348.
22. Hsu YT, Kao CH (2007). Cadmium-induced oxidative damage in rice leaves in reduced by polyamines. *Plant Soil.*, 291, 27-37.
23. Hu X, Neill SJ, Tang Z, Cai W (2005). Nitric oxide mediates gravitropic bending in soybean roots. *Plant Physiol* 137:663-670
24. Hung SH, Yu CW, Lin CH (2005). Hydrogen peroxide functions as a stress signal in plants. *Bot. Bull. Acad. Sin.* 46: 1-10.
25. Ingram J, Bartels D (1996). The molecular basis of dehydration tolerance in plants. *Plant Physiol.*, 47, 377-403.
26. Inze J, Montagu MV (2000). Oxidative stress in plants. *TJ International Ltd, Padstow, Carnawell. Great Britain*, p.321.
27. Jinfang Tan, Huijie Zhao, Jianping Hong, Yanlai , Yanlai Han, Hui Li, Wencai Zhao (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 (3): 307-313.
28. Kavir Kishor PB, et al. (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Current science*, 88(3), 424-438.
29. Lamotte L, Courtois C, Barnavon L, Pugin A, Wendehenne D. (2005). Nitric oxide in plants: the biosynthesis and cell signaling properties of a fascinating molecule. *Planta* 221, 1-4.
30. Lamotte, O, Gould, K, Lecourieux D, Sequeira-Legrand, A, Lebun-Garcia A, Durner J., Pugin A, Wendehenne D (2004). Analysis of nitric oxide signaling functions in tobacco cells challenged by the elicitor cryptogein. - *Plant Physiol.* 135: 516-529.

31. Lichtenthaler H, Wellburn A (1983). Determination of total carotenoids and chlorophyll *a* and *b* of leaf extracts in different solvents. *Biochem. Soc. Trans.* 603:591–592.
32. Liheng He, Zhiqiang Gao, Runzhi Li (2009). Pretreatment of seed with H₂O₂ enhances drought tolerance of wheat (*Triticum aestivum* L.) seedlings. *African Journal of Biotechnology*, 8 (22), pp. 6151-6157.
33. Madhava Rao KV, Sresty T V (2000). Antioxidative parameters in the seedlings of pigeon pea (*Cajanus cajan* L. Millspaugh) in response to Zn and Ni stresses. *Plant Sci.*, 157, 113–128.
34. Moussa HR (2011). Low dose of gamma irradiation enhanced drought tolerance in soybean. *Acta Agronomica Hungarica*, 59(1), pp. 1–12 (2011)
35. Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.*, 22, 867–880.
36. Navrot N, Rouhier N, Gelhaye E, Jacquot JP (2007). Reactive oxygen species generation and antioxidant systems in plant mitochondria. *Physiol. Plant.*, 129(1), 185–195.
37. Quan LJ, Zhang B, Shi WW, Li HY (2008). Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *J. Integr. Plant Biol.* 50, 2–18.
38. Rafi MM, Epstein E (1999). Silicon absorption by wheat (*Triticum aestivum* L.). *Plant Soil.* 211:223–230.
39. Sofu A, Tuzio AC, Dichio B, Xiloyannis C (2005). Influence of water deficit and rewatering on the components of the ascorbate-glutathione cycle in four interspecific *Prunus* hybrids. *Plant Sci.*, 69, 403–412.
40. TIAN X. and Y. LEI (2006). Nitric oxide treatment alleviates drought stress in wheat seedlings. *BIOLOGIA PLANTARUM*, 50 (4): 775-778.
41. Tu J, Shen W., Xu LL (2003). Regulation of nitric acid on the aging process of wheat leaves. - *Acta bot. sin.*, 45: 1055- 1062.
42. Uchida A, Jagendorf AT, Hibino T, Takabe T, Takabe T (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Sci.*, 163, 515–523.
43. Zhang CS, Lu Q, Verma DPS (1995). Removal of Feedback Inhibition of P5CS, a Bifunctional Enzyme Catalyzing the First Two Steps of Proline Biosynthesis in Plants, *J. Biol. Chem.*, 270: 20491-20496.

4/5/2011