Available online at www.sciencedirect.com
ScienceDirect
Journal of Integrative Agriculture
2012, 11(11): 1828-1835
November 2012
RESEARCH ARTICLE

Influence of Diazotrophic Bacteria on Antioxidant Enzymes and Some Biochemical Characteristics of Soybean Subjected to Water Stress

Hamed Zakikhani[1], Mohammad Reza Ardakani[1], Farhad Rejali[2], Majid Gholamhoseini[3], Aydin Khodaei Joghan[3] and Aria Dolatabadian[3]

[1] *Division of Sustainable Agriculture, Agricultural Research Center, Islamic Azad University Karaj Branch, Karaj 31485-313, Iran*
[2] *Soil and Water Research Institute, Karaj 31785-311, Iran*
[3] *Agronomy Department, Faculty of Agriculture, Tarbiat Modares University, Tehran 14115-111, Iran*

Abstract

Drought stress is an abiotic stress that imposes serious constraints on plants. The present investigation was carried out to determine the inter-relationship between some physiological attributes of soybeans affected by drought stress and pure isolates of *Azotobacter* and *Azospirillum*. Drought stress and bacterial application increased catalase and glutathione peroxidase activity, whereas drought stress increased superoxide dismutase activity during the pod-filling stage. Abscisic acid and proline levels increased due to drought stress and bacterial application during the flowering stage, whereas total plant nitrogen was enhanced under well-watered conditions when plants were inoculated with bacteria. The close relationship between enzyme activity and drought stress with bacteria indicated that antioxidant enzymes play an important role in alleviating the detrimental effects of water stress. In addition, the enhancement of abscisic acid and proline could be positively linked with drought stress, and drought-induced abscisic acid could induce proline accumulation and the expression of antioxidant enzyme genes.

Key words: abscisic acid, *Azospirillum*, *Azotobacter*, proline, soybean, water stress

INTRODUCTION

Soybeans (*Glycine max* L. Merr.) are a substantial and globally widespread crop and are sensitive to water stress, which is a major limitation to the productivity of many crops (Araus *et al*. 2002). Therefore, it is essential to study the biochemical and physiological responses of soybeans to water-limited conditions to understand the plant's resistance mechanisms (Shao *et al*. 2005). Drought stress promotes the expression of some genes, the transduction of stress signal molecules and affects biochemical mechanisms in plants (Suprunova *et al*. 2004).

Many studies suggest that drought induces oxidative stress through the production of reactive oxygen species (ROS) during periods of stress (Zhang *et al*. 1995; Perdomo *et al*. 1996). Reactive oxygen species consist of free radicals, such as superoxide ($O_2^{\cdot-}$), hydroxyl radicals (OH^-), hydrogen peroxides (H_2O_2), and single oxygen ($^1O_2^-$). These radicals attack lipids, proteins and nucleic acids, resulting in lipid peroxidation, protein denaturation and DNA mutation, respectively (Yu and Rengel 1999). They also serve as signaling

Received 26 October, 2011 Accepted 23 December, 2011
Correspondence Aria Dolatabadian, Tel: +98-21-44196522-23, +98-21-44194911-4, Fax: +98-21-44196524, E-mail: aria_dolat2000@yahoo.com

molecules regulating important biological processes in both animal and plant cells (Halliwell 2006). Plants normally alleviate the detrimental effects of ROS using antioxidant systems that scavenge oxygen radicals by two mechanisms: enzymatic and non-enzymatic detoxification (Breusegem *et al*. 1998). Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) are designed to minimize the concentration of hydrogen peroxide and superoxide. Superoxide dismutase catalyzes the dismutation of O_2 into oxygen and H_2O_2, catalase dismutase catalyzes the dismutation of H_2O_2 into water and GPX acts as a H_2O_2 detoxifier (Chen *et al*. 2004). Glutathione peroxidase has been demonstrated to function as a reducing-oxidizing transducer in abscisic acid (ABA) and drought stress signaling (Miao *et al*. 2006).

Proline accumulation is a unique plant response to environmental stresses. Proline acts as an osmoprotectant and a hydroxyl radical scavenger, and it is capable of preventing membrane distortion (Matysik *et al*. 2002). Proline accumulation in response to drought stress has been reported in wheat (Kathju *et al*. 1998). Furthermore, ABA mediates some aspects of physiological responses to environmental stresses such as drought or osmotically induced stomata closure (Leung and Giraudat 1998). It has been reported that ABA can induce the activation of antioxidant enzymes and stimulate ROS generation (Murata *et al*. 2001).

Soil microorganisms are very important in the biogeochemical cycles of inorganic and organic nutrients in the soil and in the maintenance of soil health and quality (Jeffries *et al*. 2003). Soil-plant-microbe interactions are complex and can influence plant health and productivity in many ways (Kennedy 1998). Rhizobacteria are classified into two major groups: those that form symbiotic relationships with plants and those that do not (termed free-living rhizobacteria) (Saharan 2011). Beneficial bacteria such as plant growth-promoting rhizobacteria (PGPR) and N_2-fixing bacterial species belonging to the genera *Azospirillum*, *Azotobacter*, and *Bradyrhizobium* have been proposed as promising inocula for agriculture. *Azospirillum* is a N_2-fixing genus of bacteria associated with the roots of many economically important crops that can promote plant growth when inoculated onto the seed (Okon and Labandera-Gonzalez 1994). *Azotobacter* is another genus of N_2-fixing bacteria that inhabit soil. Enhancing inoculation and nitrogen concentration could be profitable when using inoculated cereal (Caba *et al*. 1994). Therefore, the present field experiment was carried out to evaluate the effects of pure *Azospirillum-Azotobacter* isolates and various irrigation regimes on antioxidant enzyme activity, lipid peroxidation, ABA and proline accumulation, and proline content in soybean plants. The objective of this study was to compare various methods of introducing bacteria to water-stressed soybean plants and to understand the effects of these bacteria on the activity of antioxidant enzymes and on some biochemical characteristics.

RESULTS AND DISCUSSION

Analysis of variance demonstrated that effect of water stress was significant on antioxidant enzyme activity, MDA (malondiadehyde) activity, ABA accumulation, proline content, and total plant N (Table 1). Bacterial application had a significant effect on all assayed attributes except SOD activity (Table 1). The interactions between water stress and bacterial application were significant for the mentioned attributes with the exceptions of SOD and MDA activities.

Catalase and glutathione peroxidase activities and malondialdehyde content

The data showed that CAT and GPX activities were higher under conditions of 80 and 60% water depletion during pod-filling (S_5 and S_4) than under drought stress induced during flowering (S_2 and S_3) (Figs. 1 and 2). According to Upadhyaya *et al*. (2008) drought stress leads to cell lipid peroxidation and an increase in antioxidant activity. The results demonstrated that total GPX activity was higher than total CAT activity while the plants were drought stressed; this might be attributable to the stronger affinity GPX for H_2O_2 than CAT (Bringelius-Flohe and Flohe 2003). In accord with the analysis of variation, significant differences were observed between bacterial applications regarding scavenging enzyme activities such as those of CAT and GPX. In the present study, we found that drought stress during pod-filling could result in increased membrane lipid

Table 1 Analysis of variance for the effects of irrigation regimes and bacteria application on the measured traits

SOV[1)]	*df*	CAT	GPX	MDA	SOD	Plant N	ABA	Proline
Replication	2	4.71 ns	993.35**	3 922.21 ns	141 186.81 ns	0.020**	6.06 ns	3.06 ns
Irrigation regimes (S)	4	34 849.44**	68 324.47**	93 845.95**	6 000 084.95**	2.281**	13 709.43**	3 794.54**
Error a	8	17.34	186.85	1 302.69	175 530.51	0.005	25.61	37.23
Bacteria application (b)	3	4 703.35**	4 218.06**	7 299.60*	313 744.96 ns	0.228**	3 011.86**	980.77**
S×b	12	285.08**	551.96**	3 961.44 ns	288 343.71 ns	0.013**	280.44**	105.25**
Error b	20	38.23	126.28	2 129.31	17 942.86	0.002	20.39	8.58
CV (%)		3.98	4.37	25.73	17.26	2.00	3.24	5.73

[1)] SOV, source of variations.

ns, not significant. * and **, significant at the 0.05 and 0.01 probability levels, respectively.

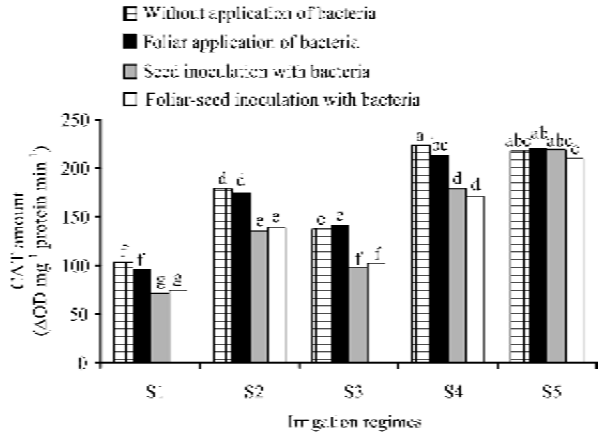

Fig. 1 Effects of different irrigation regimes and bacterial applications on CAT. Differences between means were compared by Fisher's least significance test. Different letters indicate significant differences P=0.05. The same as below.

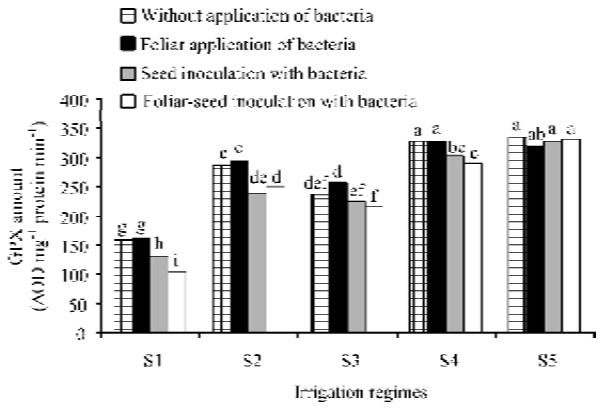

Fig. 2 Effects of different irrigation regimes and bacterial applications on GPX.

peroxidation (based on the enhancement of MDA activity, which is often used as an indication of lipid peroxidation resulting from oxidative stress). The resulting membrane fluidity leads to increased electrolytic leakage, and consequently, CAT activity will increase. Enhancement of CAT activity under drought stress has been reported in wheat and tomato (Shao *et al*. 2005).

Malondialdehyde activities were higher during pod-filling than flowering stages; nonetheless, all levels of stress treatments resulted in significantly higher activities than the control treatment (Table 2). Based on analysis of variation, the inoculated treatments were significant but the differences between the treatment levels were not significant (Table 2). Malondialdehyde enhancement indicated that drought stress significantly increased ROS levels. Antioxidant activities and the hormonal activity of the ABA hormone could play roles in modulating impairments due to ROS. Moreover, diazotrophic bacteria could also increase antioxidant enzyme activities by improving plant resistance.

Superoxide dismutase activity

Superoxide dismutase activity reached its highest level in the 60% water depletion treatment during pod-filling (S_4) and decreased in the S_3 treatment. Nevertheless, the total levels of SOD activity were significantly higher than those that occurred under the control conditions (Table 2), which could be attributed to the enhancement of MDA activity during the 80% water depletion experiments (S_3 and S_5). The higher activity of SOD during pod-filling stress treatments compared to flowering stress treatments could be attributed to the overproduction of SOD in chloroplasts to increase stress tolerance (Asiri *et al*. 1998), as observed in studies on the regulation of photosynthesis in transformed plants. This overproduction of SOD is related to enhanced H_2O_2 production (Prasad *et al*. 1994). Higher SOD activity has also been observed in maize (Jiang and Zhang 2002), safflower (Hojati *et al*. 2010), lupine (Yu and Rengel 1999), pea and tobacco plants (Yu and Rengel 1999)

Table 2 Effect of irrigation regimes and bacteria application on measured traits

Traits treatment[1)]	MDA (nmol mg^{-1} protein)	SOD (unite mg^{-1} protein)	CAT (unite mg^{-1} protein)	GPX (unite mg^{-1} protein)	ABA (ppm)	Proline (nmol g^{-1} FW)	Plant nitrogen (%)
Main effect							
Irrigation regimes							
S_1	62.50 e	1 344.3 d	85.83 e	139.41 e	574.33 a	26.35 e	3.38 a
S_2	176.67 c	2 632.8 b	156.75 c	268.16 c	462.33 b	55 b	2.71 b
S_3	133.92 d	2 220.8 c	119.75 d	233.91 d	351.58 d	75.88 a	2.43 c
S_4	231.75 b	3 098 a	196.75 b	312.75 b	406.50 c	46.37 d	2.41 c
S_5	292.33 a	2 971.1 ab	217 a	329 a	402.41 c	51.83 c	2.31 d
Main effect							
Bacterial application							
b_1	190 a	2 656.6 a	172.20 a	269.53 a	124.30 d	42.91 d	2.53 b
b_2	209.73 a	2 453.7 a	168.80 a	272.4 a	130.13 c	45.65 c	2.55 b
b_3	175 ab	2 370.7 a	140.467 b	245.46 b	149.05 b	56 b	2.74 a
b_4	150.6 b	2 332.5 a	139.40 b	239.20 b	153.5 a	59.78 a	277 a

[1)] S_1, 40% depletion of soil water during the whole plant growth period; S_2, 60% depletion water during flowering; S_3, 80% depletion water during flowering; S_4, 60% depletion water during pod filling; S_5, 80% depletion water during pod filling; b_1, without application; b_2, foliar application; b_3, seed inoculation; b_4, foliar-seed inoculation.

The given means within each column of each section followed by the same letter are not significantly differences (P=0.05).

under conditions of drought stress. The by-product of SOD activity is hydrogen peroxide, which is toxic and must be eliminated by conversion to H_2O through reactions catalyzed by peroxidase and CAT. The enhancement of hydrogen peroxide levels, which acts as a lipid peroxidant, may account for the increased CAT and GPX activities observed during the drought stress treatments, particularly during pod-filling. In contrast with the results of Dhanda *et al*. (2004), concomitant increases of SOD, CAT, GPX, and MDA resulted in a lower antioxidative ability, reflecting a lower drought resistance during severe drought stress (Table 2). Our results are in agreement with those of Masoumi and co-workers (2011).

Total plant N content

Examination of the interactions between drought stress and the inoculation treatments showed that normal irrigation accompanied by bacterial inoculation (b_3 and b_4) demonstrated significantly higher amounts of total plant N compared with the enhanced drought stress severity of other treatments (Fig. 3). This confirms the idea that the N_2 fixation resulting from pure isolates of *Azospirillum* and *Azotobacter* could improve total plant nitrogen levels (Okon and Itzigsohn 1995) during well-watered conditions and that severe drought stress decreases the effectiveness of inoculants leading to a decrease in nitrogen fixation (Sall and Sinclair 1991). ROS accumulation has been observed in alfalfa during the interaction between *Rhizobia* and legumes (Santos *et al*. 2001), and transient ROS changes have been observed in root hair cells moments after treatment with Nod factors in common beans (Cardenas *et al*. 2008). The inhibition of ROS production leads to a halt in the curling of root hairs and the formation of infection threads (Peleg-Grossman 2007). Hence, the production of ROS may not be a defense response to microbes, but could be a process that is needed for the development of interactions. Moreover, inoculation with *Azotobacter chrococcum* has been reported to alleviate oxidative stress by improving the defense ability of sugar beet leaves because the inoculated plants demonstrate enhanced activities of SOD, CAT, and peroxidases (Stajner *et al*. 1997). However, it is not clear whether this enhancement in oxidative stress tolerance is a direct or an indirect effect of inoculation with bacteria such as *Azotobacter*.

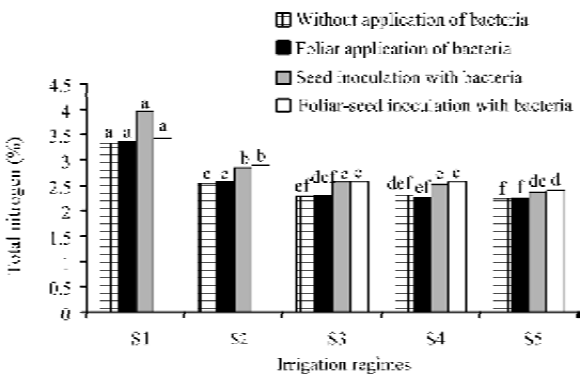

Fig. 3 Effects of different irrigation regimes and bacterial applications on total plant nitrogen.

Abscisic acid content

The interaction between drought stress and bacterial inoculation was significant (Table 1), as demonstrated by the observation that the highest amount of ABA accumulated in the plants that experienced the most severe level of drought stress during flowering coincident with bacterial application (Fig. 4). It appears that water stress can induce the accumulation of ABA. Additionally, oxidative stress in plant cells and drought stress-induced ABA accumulation can cause, at least in part, increased generation of O_2 and H_2O_2, induction of the expression of antioxidant genes (i.e., SOD and CAT) and, consequently, an increase in the activity of these enzymes. Our results are in agreement with those of Guan *et al*. (2000) and Williamsen and Scandalios (1992). Based on Figs. 1, 4 and Table 2, which show the preemptive defense signaling role of ROS and the enhancement of CAT activity, ABA accumulation, and MDA activity, it appears that H_2O_2 might be involved in ABA-induced CAT1 expression, and CAT1 is probably involved in its feedback regulation of H_2O_2 signaling apart from its ROS scavenging function (Xing *et al*. 2008).

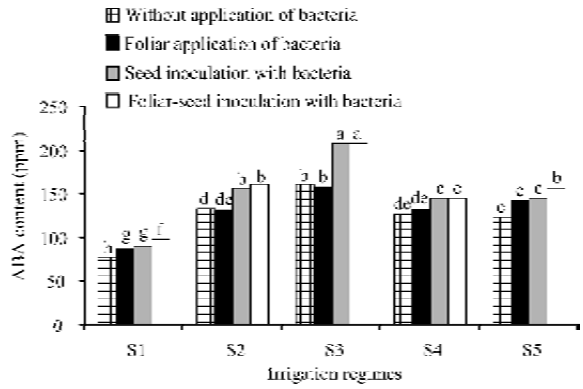

Fig. 4 Effects of different irrigation regimes and bacterial applications on ABA.

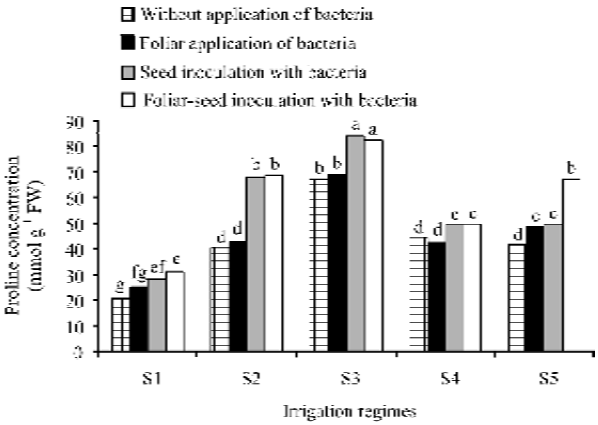

Fig. 5 Effects of different irrigation regimes and bacterial applications on proline.

Proline content

Our results demonstrated that the accumulation of proline increased considerably during water stress and during the inoculation treatments compared with control conditions (Fig. 5). The highest amount of proline was observed for the 80% water depletion treatments (S_3 and S_5) accompanied by seed and foliar-seed inoculations (b_3 and b_4). In general, the accumulation of proline was higher during the flowering than the pod-filling stages (Fig. 5). Under well-watered conditions, the total accumulation of proline was higher under inoculated than under non-inoculated treatments, but no significant differences between inoculated treatments were found (Fig. 5). The results of this study demonstrated that proline and ABA levels concomitantly accumulate to the highest point during the S_3 treatment, suggesting that ABA and proline accumulation are directly linked (Hare *et al*. 1999). Therefore, added ABA induces proline accumulation when ABA levels are maintained above a threshold level for few hours during stress. Rehydration after induced stress results in declines in ABA and proline accumulation (Stewart and Voetberg 1985). Based on Table 2, it appears that the accumulations of ABA and proline are positively correlated with ROS accumulation and drought induction. Drought stress also induces an increase in the activity of enzymes related to proline metabolism in bacteria (Kohl *et al*. 1991), and the accumulated proline might support nitrogenase activity (Pederson *et al*. 1996), resulting in better nitrogen fixation and increased plant nitrogen availability.

MATERIALS AND METHODS

Site description and experimental design

Field experiments were conducted in the spring season of

2009 (from 20 May to 21 September) at the Islamic Azad University of Karaj (IAUK) research station. Soybean (*Glycine max* L. *cv*. L17 (which is classified as having an indeterminate growth habit)) seeds were sown in plots of 6 m length and 3 m width. The plants were grown at a density of 350 000 plants ha^{-1}. The experimental design used a randomized complete block in a split-plot arrangement with three replications. The main plots included five irrigation regimes, and the subplots included four bacterial applications. In this study, five irrigation regimes were used as follows: 40% depletion of soil water during the entire plant growth period (S_1), 60% depletion of water during flowering (S_2), 80% depletion of water during flowering (S_3), 60% depletion of water during pod-filling (S_4), and 80% depletion of water during pod-filling (S_5) and four bacterial application methods were used as follows: no application (b_1), foliar application (b_2), seed inoculation (b_3), and foliar-seed inoculation (b_4). Mixed samples were collected separately during the flowering and pod-filling stages. All samples were frozen immediately in liquid nitrogen.

Bacterial growth, seed inoculation, and foliar application

Azospirillum brasilense and *Azotobacter chrococcum* were provided by the Soil Biology Department of the Soil and Water Research Institute of Iran. Seeds and leaves were inoculated with a combination of *Azospirillum* and *Azotobacter*. The bacterial populations were adjusted to 8×10^6 and 5×10^9 CFU mL^{-1}, respectively (Somasegaran and Hoben 1994). Seeds were treated with 20-mL inoculations at each application. To ensure full inoculation, the seeds were smeared with gum and shaken well in a polyethylene bag for 30 s. After exposure to a flow of air, the seeds were planted and watered immediately. The plants were sprayed with 1 L of cell culture at each treatment during flowering stages according to the methods of Fehr and Caviness (1977).

Irrigation

Irrigation was scheduled based on the daily changes of soil water content (DSW) at root development depth. This procedure used a deficit approach (the soil water content at field capacity (FC) represents no deficit) to estimate irrigation requirements. The plots were irrigated when the current daily deficit values reached 40% (for S_1 irrigation as the control treatment), 60 and 80% (for the S_2 and S_3 irrigation treatments during flowering), and 60 and 80% (for the S_3 and S_4 irrigation treatments during pod-filling) of available water remaining at the root development depth (60 cm). The growth stages for irrigation application were determined based on the methods of Fehr and Caviness (1977). Tube access probes (TRIME-FM, England) were used to measure soil-water content (θ_v) based on time domain reflectometry (TDR) at 0-60 cm soil depth in the plots. Soil volumetric water content data were collected daily during the growing season.

Physiological measurements

MDA concentration The MDA concentration was calculated as a measure of lipid peroxidation. Samples were homogenized in an aqueous solution of trichloroacetic acid (10% w/v), and aliquots of the filtrates were heated in 0.25% thiobarbituric acid. The specific absorbance of extracts was recorded at 532 nm, and the values were corrected for non-specific absorbance at 600 nm (Puckette *et al*. 2007).

Catalase activity CAT activity was estimated using the method of Cakmak and Horst (1991). The reaction mixture contained 100 μL crude extract, 500 μL 10 mmol L^{-1} H_2O_2 and 1.4 mL 25 mmol L^{-1} sodium phosphate buffer. The decrease in the absorbance was recorded at 240 nm for 1 min using a spectrophotometer (Cintra, GBC, Australia). The catalase activity of the extract was expressed as ΔOD mg^{-1} protein min^{-1}.

Superoxide dismutase activity SOD activity was determined by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Giannopolitis and Ries (1977). The reaction mixture contained 100 μL 1 μmol L^{-1} riboflavin, 100 μL 12 mmol L^{-1} L-methionine, 100 μL 0.1 mmol L^{-1} EDTA (pH 7.8), 100 μL 50 mmol L^{-1} Na_2CO_3 (pH 10.2), 100 μL 75 μmol L^{-1} NBT in 2 300 μL 25 mmol L^{-1} sodium phosphate buffer (pH 6.8), and 200 μL crude enzyme extract in a final volume of 3 mL. Glass test tubes that contained the reaction mixture were illuminated with a fluorescent lamp (120 W), and identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of the photochemical reduction of NBT. The superoxide dismutase activity of the extract was expressed as ΔOD mg^{-1} protein min^{-1}.

Total plant nitrogen content Plant samples were ground in a mill and then passed through a 2-mm sieve. Sub-samples (2 g) of plant materials were digested using the Kjeldhal method before the analysis of total nitrogen. The digestion mixture included 0.5% selenium as a catalyst and salicylic acid. Nitrogen uptake by grain and straw was calculated by multiplying their yield by their nitrogen content.

Abscisic acid content The extraction, purification and analysis procedures of ABA were carried out as previously described by Zhou *et al*. (2003). Leaf samples (0.3 g) were homogenized in 750 μL of a solution of acetone, distilled water and acetic acid (80:19:1 (v:v:v), respectively). The extract was centrifuged at 10 000×g for 2 min, after which the supernatant was removed, and the mixture was extracted again. The supernatant from the 2nd extraction

was removed and dried at room temperature; then, 200 μL of acetonitrile and distilled water (15:85, respectively) and acetic acid (12 mmol L^{-1} at pH 3.3) were added. Finally, 10-15 μL of the resulting sample was injected into an HPLC column (50 mm×2.1 mm, 3.5 μm) with isocratic elution at a flow rate of 0.6 mL min^{-1} using acetonitrile and water (90:10) as the mobile phase.

Proline content The proline concentration in soybean leaves was measured spectrophotometrically using the method of Bates *et al*. (1973). Leaf samples (0.5 g) were homogenized with 5 mL of sulfosalicylic acid (3%) using a mortar and pestle, and filtered. The filtrate was made up to 10 mL with sulfosalicylic acid, and 2.0 mL of filtrate was incubated with 2.0 mL of glacial acetic acid and 2.0 mL ninhydrin, and boiled in a water bath at 100°C for 30 min. Then, the mixture was cooled and 6.0 mL of toluene was added. The absorbance was read at 570 nm.

Glutathione peroxidase activity GPX activity was measured according to Urbanek *et al*. (1991) in a reaction mixture (2 mL) that contained 100 mmol L^{-1} phosphate buffer (pH 7), 0.5 μL enzyme extract, 5 mmol L^{-1} guaiacol, 15 mmol L^{-1} hydrogen peroxide, and 0.1 μmol L^{-1} EDTA. After the enzyme extract was added to the mixture, the increase in absorbance recorded at 470 nm was determined during 1 min based on the amount of tetraguaiacol present using the molar extinction coefficient (26.6 mmol L^{-1} cm^{-1}). Glutathione peroxidase content was expressed as units mg^{-1} protein.

References

Araus J L, Slafer G A, Reynolds M P, Royo C. 2002. Plant breeding and drought in C3 cereals: what should we breed for? *Annals of Botany*, **89**, 925-940.

Asiri A C M, Cornic G, Jouanin L, Foyer C H. 1998. Over expression of Fe-SOD IN transformed poplar modifies the regulation of photosynthesis at low CO_2 partial pressure or following exposure to the pro-oxidant herbicide methyl viologen. *Plant Physiology*, **117**, 565-574.

Bates L S, Waldern R P, Teare I K. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, **39**, 205-207.

Breusegem F, van Montagu M, van Inze D, van Breusegem F, van Montagu M. 1998. Engineering stress tolerance in maize. *Outlook on Agriculture*, **27**, 115-124.

Brigelius-Flohe R, Flohe L. 2003. Is there a role of glutathione peroxidases in signaling and differentiation? *Biofactors*, **17**, 93-102.

Caba J M, Lluch C, Ligero F. 1994. Genotypic variability of nitrogen metabolism enzymes in nodulated roots of vicia faba. *Soil Biology and Biochemistry*, **27**, 785-789.

Cakmak I, Horst W J. 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum*, **83**, 463-468.

Cardenas L, Martinez A, Sanchez F, Quinto C. 2008. Fast transient and specific intracellular ROS changes in living root hair cells responding to Nod factors (NFs). *The Plant Journal*, **56**, 802-813. (in Press)

Chen S, Vaghchhipawala Z, Li W, Asard H, Dickman M B. 2004. Tomato phospholipids hydro peroxide glutathione peroxidase plants. *Plant Physiology*, **135**, 1630-1641.

Dhanda S, Sethi G S, Behl R K. 2004. Indices of drought tolerance in wheat genotypes at early stages of plant growth. *Journal of Agronomy and Crop Science*, **190**, 6-12.

Fehr W R, Caviness C E. 1977. *Stages of Soybean Development*. Special Report 80, Agriculture and Home Economics Experiment Station, Lowa State University, IA, USA.

Giannopolitis C N, Ries S K. 1977. Superoxide dismutase in higher plants. *Plant Physiology*, **59**, 309-314.

Guan L, Zhao J, Scandalios J G. 2000. *Cis*-elements and transfactors that regulate expression of the maize Cat1 antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signalling molecule for the response. *The Plant Journal*, **22**, 87-95.

Halliwell B. Reactive species and antioxidants. redox biology is a fundamental theme of aerobic life. *Plant Physiology*, **141**, 312-322.

Hare P D, Cress V A, Staden J V. 1999. Proline synthesis and degredation: a model system for elucidating stress related signal transduction. *Journal of Experimental Botany*, **50**, 413-434.

Hojati M, Modarres-sanavy S A M, Karimi M, Ghanati F. 2010. Responses of growth and antioxidant systems in *Carthamus tinctorius* L. under water deficit stress. *Acta Physiologia Plantarum*, **33**, 105-112.

Jiang M Y, Zhang J H. 2002. Water stress induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *Journal of Experimental Botany*, **53**, 2401-2410.

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea J M. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils*, **37**, 1-16.

Kathju S, Vyas S P, Garg B K, Lahiri A N. 1988. Fertility induced improvements in performance and metabolism of wheat under different intensities of water stress. In: *Proceedings of the International Congress of Plant Physiology*. Society for Plant Physiology and Biochemistry, Watertechnorny Cern, Indian Agricultural Research Insltvl'e, New Delhi, India. pp. 854-858.

Kennedy A C. 1998. The rhizosphere and spermosphere. In: Sylvia D M, Fuhrmann J J, Hartel P G, Zuberer D A, eds., *Principles and Applications of Soil Microbiology*. Prentice-Hall, Englewood Cliff, NJ. pp. 389-407.

Kohl D H, Kennelly E J, Zhu Y, Schubert K R, Shearer G. 1991. Proline accumulation, nitrogenase (C_2H_2 reducing) activity and activities of enzymes related to proline

metabolism in drought-stressed soybean nodules. *Journal of Experimental Botany*, **42**, 831-837.

Leung J, Giraudat J. 1998. Abscisic acid signal transduction. *Annual Review of Plant Physiology and Plant Molecular Biology*, **49**, 199-222.

Masoumi H, Darvish F, Daneshian J, Normohammadi G, Habibi D. 2011. Effects of water deficit stress on seed yield and antioxidants content in soybean (*Glycine max* L.) cultivars. *African Journal of Agricultural Research*, **6**, 1209-1218.

Matysik J, Alia A, Bhalu B, Mohanty P. 2002. Molecular mechanism of quenching of reactive oxygen species by proline under water stress in plants. *Current Science*, **82**, 525-532.

Miao Y, Lv D, Wang P, Wang X C, Chen J, Miao C. 2006. An Arabidopsis glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. *The Plant Cell*, **18**, 2749-2766.

Murata Y, Pei Z M, Mori I C, Schroeder J I. 2001. Abscisic acid activation of plasma membrane Ca^{2+} channels in guard cells requires cytosolic NAD(P)H and is differentially disrupted upstream and downstream of reactive oxygen species production in abi1-1 and abi2-1 protein phosphatase 2C mutants. *The Plant Cell*, **13**, 2513-2523.

Okon Y, Itzigsohn R. 1995. The development of *Azospirillum* as a commercial inoculant for improving crop yields. *Biotechnology Advances*, **13**, 415-424.

Okon Y, Labandera-Gonzalez C A. 1994. Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. *Soil Biology and Biochemistry*, **26**, 1591-1601.

Pedersen A L, Felder H C, Rosendahl L. 1996. Effect of proline on nitrogenase activity in symbiosomes from root nodules of soybean (*Glycine max* L.) subjected to drought stress. *Journal of Experimental Botany*, **47**, 1533-1539.

Peleg-Grossman S, Volpin H, Levine A. 2007. Root hair curling and rhizobium infection in *Medicago* trancatula are mediated by phosphatidylinostide-regulated endocytosis and reactive oxygen species. *Journal of Experimental Botany*, **58**, 1637-1649.

Perdomo P, Murphy J A, Berkowitz G A. 1996. Physiological changes associated with performance of Kentucky bluegrass cultivars during summer stress. *Horticulture Science*, **31**, 1182-1186.

Prasad T K, Anderson M D, Martin B A, Stewart C R. 1994. Evidence for chilling induced oxidative stress in maize seedliongs and a regulatory role for hydrogen peroxide. *The Plant Cell*, **6**, 65-74.

Puckette M C, Weng H, Mahalingam R. 2007. Physiological and biochemical responses to acute ozone-induced oxidative stress in *Medicago truncatula*. *Plant Physiology and Biochemistry*, **45**, 70-79.

Saharan B S, Nehra V. 2011. *Plant Growth Promoting Rhizobacteria: A Critical Review*. Life Sciences and Medicine Research, LSMR-21, JK. pp. 1-30.

Sall K, Sinclair T R. 1991. Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration. *Plant Soil*, **133**, 31-37.

Santos R, Herouart D, Siguad S, Touati D, Puppo A. 2001. Oxidative burst in alfalfa-sinorhizobium meliloti symbiotic interaction. *Molecular and Plant Microbe Interaction*, **14**, 86-89.

Shao H B, Liang Z S, Shao M A, Sun A. 2005. Dynamic changes of antioxidative enzymes of ten wheat genotypes at soil water deficits. *Colloids and Surfaces* (B), **42**, 187-195.

Somasegaran P, Hoben H J. 1994. *Handbook for Rhizobia: Methods in Legumerhizobiu Technology*. Springer-Verlag, New York, USA. p. 450.

Stajner D, Kevreaan S, Gāsaíc O, Mimica-Dudíc N, Zongli H. 1997. Nitrogen and *Azotobacter chroococcum* enhance oxidative stress tolerance in sugar beet. *Biologia Plantarum*, **39**, 441-445.

Stewart C R, Voetberg G. 1985. Relationship between stress-induced ABA and proline accumulations and ABA-induced proline accumulation in excised barley leaves. *Plant Physiology*, **79**, 24-27.

Suprunova T, Krugman T, Fahima T, Cheng G, Shams I, Korol A, Nevo E. 2004. Differential expression of dehydrin genes in wild barley, Hordeum spontaneum, associated with resistance water deficit. *Plant Cell Environment*, **27**, 1297-1308.

Upadhyaya H, Panda S K, Dutta B K. 2008. Variation of physiological and oxidative responses in tea cultivars subjected to elevated water stress followed by rehydration recovery. *Acta Physiologia Plantarum*, **30**, 457-468.

Urbanek H, Kuzniak-Gebarowska E, Herka K. 1991. Elicitation of defense responses in bean leaves by Botrytis cinerea polygalacturonase. *Acta Physiologiae Plantarum*, **13**, 43-50.

Williamson J D, Scandalios J G. 1992. Differential response of maize catalase to abscisic acid: Vpl transcriptional activator is not required for abscisic acid-regulated Cat1 expression. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 8842-8846.

Xing Y, Jia W, Zhang J. 2008. AtMKK1 mediates ABA-induced CAT1 expression and H_2O_2 production via AtMPK6-coupled signaling in Arabidopsis. *The Plant Journal*, **54**, 440-451.

Yu Q, Rengel Z. 1999. Drought and salinity differentially influence activities of superoxide dismutases in narrow-leafed lupins. *Plant Science*, **144**, 1-11.

Zhang J, Cui S, Li J, Kirkham M B. 1995. Protoplasmic factors, antioxidant responses, and chilling resistance in maize. *Plant Physiology and Biochemistry*, **33**, 567-575.

(Managing editor WANG Ning)