DOI: 10.1007/s10535-012-0277-x BIOLOGIA PLANTARUM 57 (2): 359-364, 2013

BRIEF COMMUNICATION

Over-expression of *PaSOD* in transgenic potato enhances photosynthetic performance under drought

A.K. PAL[1], K. ACHARYA[1], S.K. VATS[2], S. KUMAR[1]*, and P.S. AHUJA[1]

Division of Biotechnology[1] and Division of Biodiversity[2], CSIR-Institute of Himalayan Bioresource Technology, Council of Scientific and Industrial Research, Palampur-176061, H.P., India

Abstract

Drought stress enhances the production of superoxide radical ($O_2^{\cdot-}$) and superoxide dismutase catalyses dismutation of it to H_2O_2 and O_2, and hence provides a first line of defense against oxidative stress. Over-expression of a cytosolic *copper-zinc superoxide dismutase*, cloned from *Potentilla atrosanguinea* (*PaSOD*), in potato (*Solanum tuberosum* ssp. *tuberosum* L. cv. Kufri Sutlej) resulted in enhanced net photosynthetic rates (P_N) and stomatal conductance (g_s) compared to that in the wild type (WT) plants under control (irrigated) as well as drought stress conditions. Drought stress declined leaf water potential, P_N, g_s, photosystem II activity, and chlorophyll content, but increased proline and $O_2^{\cdot-}$ content more in WT than transgenic potato plants (SS5). The significantly higher SOD activity in SS5 coincided well with lower $O_2^{\cdot-}$ content suggesting its role in maintaining higher g_s and P_N in transgenic potato plants.

Additional key words: net photosynthetic rate, ROS, *Solanum tuberosum*, stomatal conductance, superoxide dismutase.

Drought is one of the major environmental factors limiting crop yield. Onset of drought generally prompts stomata to close which restricts the net photosynthetic rate (P_N) (Ort *et al*. 1994). Reduction in P_N may enhance production of reactive oxygen species (ROS). Apart from causing oxidative damages to cell, role of ROS as signaling molecules has also been reported (Jabs *et al*. 1996, Overmyer *et al*. 2000, Breusegem *et al*. 2001).

Plants are known to scavenge ROS for protection against the oxidative stress through various enzymatic and non-enzymatic mechanisms. Studies have shown that oxidative stress tolerance correlates with elevated levels of superoxide dismutase (SOD, EC 1.15.1.1) which catalyzes dismutation of superoxide radical ($O_2^{\cdot-}$) into H_2O_2 and O_2 (Asada 1999). Over-production of SOD in transgenic plants has been shown to impart significant protection under high oxidative stress (Sengupta *et al*. 1993, Breusegem *et al*. 2001, Attia *et al*. 2011).

One of the consequences of higher production of SOD would be lowered level of $O_2^{\cdot-}$ and so possible oxidative stress. Since $O_2^{\cdot-}$ is also a signaling molecule (Jabs *et al*. 1996, Overmyer *et al*. 2000, Breusegem *et al*. 2001), it is likely that SOD indirectly modulates other plant processes as well and thus could be an important determinant of plant functioning under stress. Potato plants are very sensitive to various stresses, therefore, transgenic potatoes have been produced to combat environmental stresses (Perl *et al*. 1993, Tang *et al*. 2006, Stiller *et al*. 2008, Movahedi *et al*. 2012). Over-expression of a cytosolic *copper-zinc superoxide dismutase* cloned from *Potentilla atrosanguinea* (*PaSOD*) (Gene Bank sequence acc. No. FB316647.1)

Received 28 October 2011, *accepted* 18 June 2012.

Abbreviations: g_s - stomatal conductance; NBT - nitroblue tetrazolium chloride; *PaSOD* - *Potentilla atrosanguinea copper-zinc superoxide dismutase* gene; P_N - net photosynthetic rate; PS II - photosystem II; ROS - reactive oxygen species; RM - regeneration media; SS5 - *PaSOD* transgenic potato line 5; WT - wild type.

Acknowledgments: The authors A.K. Pal and K. Acharya gratefully acknowledge Council of Scientific and Industrial Research (CSIR), New Delhi, India for providing research fellowships and financially support the research work under the network project entitled "Exploratory studies on climate change and adaptation of species complexes (NWP-020). Authors thank Dr. Y. Sreenivasulu and Dr. T. Gill for providing the construct (pCAMBIA1302 harbouring *PaSOD* under the control of CaMV35S promoter). This research was also financed partly by the Department of Biotechnology, New Delhi, India. Authors also thank Director, Central Potato Research Institute, Shimla, India for providing germplasm of potato cv. Kufri Sutlej.
IHBT Publication No. 2164.

*Author for correspondence; fax: (+91) 1894-230433, e-mail: sanjayplp@rediffmail.com

has shown enhanced lignifications of vascular structures and improved tolerance under salt stress (Gill *et al*. 2010a,b, 2012) in *Arabidopsis*. The present work was an attempt to understand the impact of over-expression of *PaSOD* in potato.

Potato (*Solanum tuberosum* ssp. *tuberosum* L. cv. Kufri Sutlej) shoots were grown *in vitro* from single node cutting on Murashige and Skoog (1962; MS) basal medium (pH 5.65) supplemented with 2 % sucrose and 5 µM silver thiosulfate (STS) under a 16-h photoperiod, irradiance of 60 - 70 µmol m^{-2} s^{-1} (cool white fluorescent tubes), temperature of 25 ± 2 ºC, and relative humidity of 50 - 60 %. The construct pCAMBIA1302 harbouring *PaSOD* under the control of CaMV35S promoter, mobilized in *Agrobacterium tumefaciens* strain GV3101 through tri-parental mating, was used in the present work (Gill *et al*. 2010a,b, 2012). The internodal segments (10 - 15 mm) were dipped in *Agrobacterium* suspension (absorbance, A_{600} 0.6 - 08) for 10 min, blotted dry on sterile *Whatman* filter paper, transferred onto potato regeneration media (RM) for 48 h, and cultured under similar conditions as mentioned earlier. The potato RM contained MS basal medium supplemented with 2.5 mg dm^{-3} triiodobenzoic acid (TIBA) (*Sigma-Aldrich*, Bangalore, India), 0.25 mg dm^{-3} zeatin (*Hi-Media*, Mumbai, India), and 5 mg dm^{-3} silver nitrate (*Sigma-Aldrich*, Bangalore, India). The co-cultivation continued for two days after which the explants were washed in sterile distilled water and kept on plant selection RM media supplemented with hygromycin (20 mg dm^{-3}; *Sigma-Aldrich*, Bangalore, India), cefotaxime (250 mg dm^{-3}; *HiMedia*, Mumbai, India), and carbenicillin (250 mg dm^{-3}; *Hi-Media*, Mumbai, India). Eight putative transgenic lines were obtained and rooting was induced on MS basal medium containing indole-3-acetic acid (IAA; 0.1 mg dm^{-3}; *Sigma-Aldrich*, Bangalore, India).

DNA from wild type (WT) and putative transgenic lines was isolated following the method of Doyle and Doyle (1990) to confirm the presence of *PaSOD* by polymerase chain reaction (PCR). PCR was performed using sense (5'-GCAAAGGGCGTTGCTGTACTTAG-3') and antisense (5'- GCCACCAGCATTTCCAGTGG-3') primers on a thermal cycler (*S 1000*^{TM}; *Bio-Rad,* Hercules, CA, USA) programmed for the following conditions: initial denaturation at 94 ºC for 3 min followed by 35 cycles of denaturation at 94 ºC for 1 min, annealing at 55 ºC for 1 min, elongation at 72 ºC for 2 min and final extension for 7 min at 72 ºC. Amplicons were checked on 1.2 % (m/v) agarose gel (Fig. 1*A*). RNA was isolated using the method of Ghawana *et al*. (2011) and cDNA was synthesized as described by Singh *et al*. (2004). For evaluating gene expression, PCR was performed essentially as described above except that cDNA was used in place of genomic DNA and the number of cycles used was 30 to have amplification in exponential phase. The expression of native *SOD* of potato (Gene Bank sequence acc. No. AF355460.1) was analyzed with sense (5'-GGTGCTCCTGAAGAT GAGGTGC-3') and antisense (5'-CAGGAGCAATTA ACCCTGGAGAC-3') primers under the above mentioned PCR conditions. Amplicons were checked on a 1.5 % agarose gel (Fig. 1*A,B,D*) and scanned on a gel documentation system (*Alpha DigiDoc*, *Alpha Innotech*, San Leandro, CA, USA). PCR and RT-PCR showed amplification with 8 different lines (Fig. 1*A*,*B*). Primers based on *26S rRNA* were used as an internal control. Integrated density value (IDV) of the amplicons was recorded with *AD-1000* software (Fig. 1*D*). Since *PaSOD* encodes for the autoclavable SOD (Yogavel *et al*. 2008), the crude enzyme was subjected to autoclaving and activity staining for SOD was performed on a native gel as described by Vyas and Kumar (2005). A prominent band of PaSOD was observed for *PaSOD* transgenic line 5 (SS5) in the autoclaved extract. Though lines SS1 and SS2 also exhibited faint bands of PaSOD in the autoclaved samples, a significant increase in SOD activity was obtained only for line SS5 (Fig. 1*C*) and hence it was considered for further analysis.

The transgenic plants along with the WT were hardened in the containment facility of our institute in pots and tubers were generated on large scale from each of them. Uniform sized tubers of WT and SS5 were sown at a depth of 7 cm, one in each pot (30 and 20 cm top and bottom diameter, respectively, and 30 cm height; filled with soil, organic manure and fine sand in the ratio of 2:1:1, respectively). The pots were maintained in the transgenic containment facility of the institute (11-h photoperiod, maximum irradiance of 540 - 610 µmol m^{-2} s^{-1}, day/night temperature of 21/6 °C, and air humidity 55/85 %) as approved by the competent authority. Tubers were allowed to sprout and irrigation was carried out on 7 d interval. Three weeks after sprouting, the pots were fully saturated with water and thereafter drought stress was imposed by withholding irrigation. Data indicating percent change with respect to irrigated control was collected the next day, termed as 0 day (d), and then at 15 d and 30 d of drought stress duration. Nine uniform plants of almost equal height and health were selected, each of wild type and *PaSOD* transgenic potato plants. Three plants each for 0, 15, and 30 d drought stress duration were considered for this study. A parallel experiment of well-irrigated control for both WT and SS5 was also laid for all the drought stress durations. Percentage increase/decrease in all parameters was compared to the respective well-irrigated control unless mentioned otherwise.

Leaf water potential (Ψ_w) was measured using a *Wescor HR-33T* dew point thermocouple psychrometer with a *C-52* sample chamber (model *2740*, *Wescor, Logan*, UT, USA) as described by Kumar *et al*. (2006). A portable photosynthetic system *LI-6400* (*Li-COR, Lincoln*, NE, USA) was used to estimate g_s and P_N for the second terminal leaflet of fully grown second leaf at an irradiance of 1 000 µmol m^{-2} s^{-1} and CO_2 concentration of 400 cm^3 m^{-3} as described by Kumar *et al*. (2006). The relative amount of chlorophyll was measured using a portable chlorophyll meter (*CCM200, Opti Sciences*, Hudson, NH, USA). Chlorophyll fluorescence emission

kinetics (F_v/F_m), which approximates the activity of PS II, was measured with a portable pulse modulated chlorophyll fluorimeter (model *FMS2*, *Hansatech Instruments*, King's Lynn, Norfolk, UK) as described by Kumar *et al.* (2007). $O_2^{\cdot-}$ was estimated using nitroblue tetrazolium (NBT) method of Vyas and Kumar (2005). Total SOD activity was estimated as a function of NBT reduction into formazan following the methods of Vyas and Kumar (2005), except that the reaction was carried out in 0.2 cm^3 reaction volume (195 mm^3 reaction mixture and 5 mm^3 enzyme extract) in a 96 well microplate format on a *Multimode Microplate Reader* (*Synergy^{TM} HT*, Winooski, VT, USA). The protein was estimated according to Bradford (1976) using bovine serum albumin as a standard. One unit of enzyme activity was defined as the amount of protein required for 50 % inhibition of NBT reduction at 25 ºC in 10 min. Proline was extracted from fresh leaf with 3 % (m/v) sulphosalycylic acid and estimated using the ninhydrin reagent (Bates *et al*. 1973).

The experiment was performed using a three factorial arrangement in completely randomized block design with number of observation on three plants for each treatment (Table 1). The statistical analysis was performed using *Statistica* software (*v.7*). The statistical significance between the mean values was assessed by analysis of variance (*ANOVA*) applying Duncan's Multiple Range Test (*DMRT*). A probability level of $P \leq 0.05$ was considered significant.

With the increasing duration of drought stress, leaf Ψ_w showed significantly higher decrease (50.0 %) in WT as compared to SS5 (38.0 %) at 30 d (Table 1). There is evidence that g_s might be regulated by changing leaf Ψ_w (Jarvis 1976, Tardieu *et al*. 1996, Gomes *et al.* 2004).

Table 1. Effect of withholding irrigation on physiological parameters in wild type (WT) and *PaSOD* transgenic potato line 5 (SS5). Values are the means ± SE, $n = 3$ (40 for g_s and P_N, 10 for chlorophyll content), values marked with different letters within each parameter show significant difference among plant type, water stress, and days of withholding irrigation at $P \leq 0.05$. Data in the parentheses indicate the percent change with respect to the irrigated control.

Parameter	Plant type	Water status	Time of withholding irrigation 0 d	15 d	30 d
Water potential [-MPa] (3)	WT	control	0.57±0.05d	0.65±0.05cd	0.67±0.07cd
		stress	0.57±0.04d	0.83±0.09c (+26.5)	1.00±0.00a (+50.0)
	SS5	control	0.57±0.05d	0.64±0.02d	0.67±0.01cd
		stress	0.57±0.06d	0.80±0.02bc (+25.0)	0.92±0.04ab (+38.0)
Stomatal conductance [mol(H_2O) m^{-2} s^{-1}] (40)	WT	control	0.27±0.03abc	0.26±0.03abc	0.26±0.03abc
		stress	0.25±0.00bc	0.20±0.01cd (−22.1)	0.10±0.00e (−61.0)
	SS5	control	0.34±0.02a	0.30±0.06ab	0.30±0.02ab
		stress	0.31±0.02ab	0.26±0.00abc (−13.6)	0.16±0.00de (−47.9)
Net photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}] (40)	WT	control	11.71±0.42b	10.10±0.29bc	8.87±0.23cd
		stress	11.45±0.64b	8.85±0.53cd (−12.4)	5.10±0.05e (−41.8)
	SS5	control	15.42±0.83a	13.91±1.04a	10.47±0.63bc
		stress	15.16±0.24a	11.54±0.01b (−17.1)	8.01±0.70d (−23.5)
Chlorophyll content [CCI] (10)	WT	control	15.23±0.50a	13.55±0.15abc	12.60±0.20bc
		stress	15.24±1.03a	7.78±0.87de (−42.6)	6.12±0.05e (−51.4)
	SS5	control	15.31±0.30a	14.18±0.21ab	12.83±0.16bc
		stress	15.06±1.49a	11.75±0.51c (−17.2)	8.39±0.38d (−34.6)
Fv/Fm (3)	WT	control	0.82±0.01abc	0.82±0.00abc	0.81±0.01bc
		stress	0.82±0.01abc	0.80±0.00cd (−2.3)	0.78±0.01d (−3.1)
	SS5	control	0.83±0.01ab	0.83±0.01ab	0.82±0.00abc
		stress	0.83±0.01a	0.81±0.01abc (−2.1)	0.81±0.01bc (−1.0)
SOD activity [U mg^{-1}(protein)] (3)	WT	control	97.58±8.87d	103.68±13.89d	104.63±5.95d
		stress	95.94±8.65d	119.68±9.38cd (+15.4)	141.16±3.80bc (+34.9)
	SS5	control	94.22±8.41d	99.77±4.15d	115.76±9.06cd
		stress	114.27±10.48cd	156.46±5.75b (+56.8)	202.22±14.97a (+74.7)
$O_2^{\cdot-}$ content [A_{580nm}] (3)	WT	control	0.28±0.03d	0.30±0.05d	0.31±0.05cd
		stress	0.27±0.04d	0.42±0.01b (+39.6)	0.52±0.02a (+67.7)
	SS5	control	0.26±0.01d	0.27±0.02d	0.27±0.19d
		stress	0.28±0.01d	0.39±0.01bc (+42.2)	0.44±0.01b (+59.8)
Proline content [mg g^{-1}(f.m.)] (3)	WT	control	4.70±0.20d	5.96±0.34d	6.52±0.54d
		stress	4.90±0.16d	17.34±0.46b (+191.0)	25.57±2.19a (+292.2)
	SS5	Control	4.12±0.18d	5.53±0.36d	6.44±0.17d
		Stress	3.92±0.07d	11.23±0.49c (+103.1)	14.86±2.49b (+130.9)

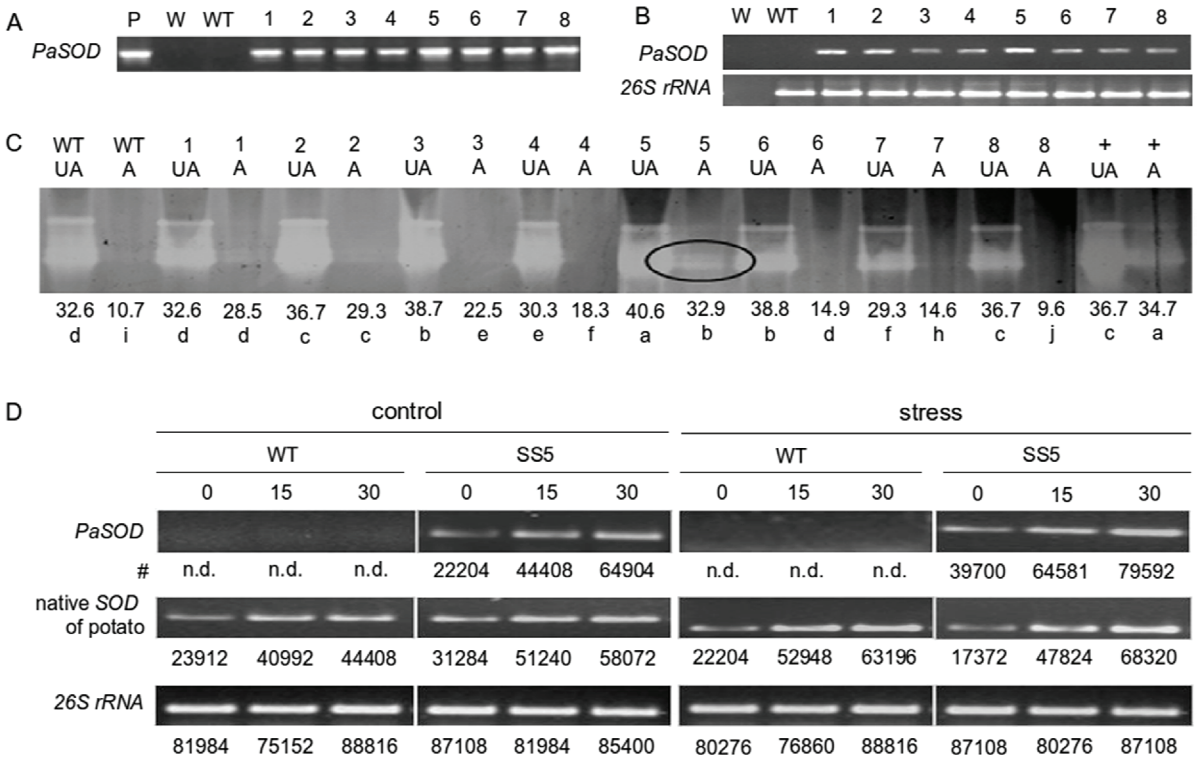

Fig. 1. Confirmation of *PaSOD* over-expressing transgenic potato lines and evaluation of gene expression through RT-PCR under different duration of drought stress. *A* - PCR confirmation of *PaSOD* in transgenic potato lines; *B* - RT-PCR analysis of transgenic *PaSOD* plants as compared to *26S rRNA*; *C* - native activity gel staining for PaSOD in un-autoclaved (UA) and autoclaved (A) enzyme samples of WT, *PaSOD* transgenic lines and *Potentilla atrosanguinea*. Value below each lane denotes SOD activity and different letters indicate significant differences at $P \leq 0.05$; *D* - RT-PCR analysis of *PaSOD* and native *SOD* of potato as compared to internal control *26S rRNA* (Singh *et al*. 2004) in WT and *PaSOD* over-expressing transgenic potato line (SS5) under drought stress. #Value below each lane indicates the integrated density value (IDV) of the amplicons as recorded with *AD-1000* software. Background values of IDV were deducted from each lane. n.d. - not detected, control - irrigated regularly, stress - irrigation was withheld. P - plasmid with *PaSOD* (positive control), W - water (negative control), WT - wild type, Lanes 1 to 8 - *PaSOD* over-expressing transgenic lines SS1 to SS8, (+) - positive control (enzyme sample from *Potentilla atrosanguinea*).

Stomata can also sense soil drought due to root-to-leaf signaling (Hebbar *et al*. 1994). Stomatal closure thus could be the indicator of the developing water stress (Medrano *et al*. 2002). In both WT and SS5 plants, a significant decrease in g_s at each stage of water stress indicated stomatal sensitivity to changing soil water status (Table 1). Limitations imposed by continuous drought stress on CO_2 assimilation have long been debated with regard to stomatal or non-stomatal constraints (Chaves and Oliveira 2004). In addition to reduction in g_s, water stress may lead to reduction of chlorophyll content, electron transport, ribulose bisphosphate carboxylase/oxygenase (Rubisco) activity, impaired ATP synthesis and ATP limited regeneration of ribulose bisphosphate, *etc*. (Flexas and Medrano 2002). A stomatal regulation of photosynthesis was observed in our study, where reduction in g_s by 61.0 % in WT and 47.9 % in SS5 also lead to decline in P_N by 41.8 and 23.5 % in WT and SS5, respectively, at 30 d of drought stress (Table 1). SS5 under drought stress showed higher $\Psi_{W \text{ and }} g_s$ at all stages suggesting ability of SS5 plants to react on drought with delay. It is likely that SS5 had smaller leaf area and despite its higher g_s exhausted less soil water. These possibilities, however, need to be supported by further studies.

Drought induced decrease in chlorophyll content is well known. The study showed reduction in chlorophyll content in WT (51.4 %) and SS5 (34.6 %) plants at 30 d. PSII activity was also less affected in SS5 line (3.1 and 1.0 % in WT and SS5 plants, respectively).

The stress related down regulation of photosynthesis is associated with enhanced production rates of ROS (Foyer and Shigeoka 2011). Evidence from a wide variety of plants has shown that ROS accumulation provides a measure of the degree of stress experienced by plants (Moran *et al*. 1994, Loggini *et al*. 1999). Non-stressed WT and SS5 did not show any significant difference in $O_2^{\cdot -}$ content but its production increased significantly with increasing drought stress duration in both plant types. After 30 d, the increase was 67.7 % in WT and 59.8 % in SS5 (Table 1).

SOD is a central enzyme which regulates the cellular concentration of $O_2^{\cdot -}$ and thereby protects the photo-synthetic machinery against oxidative damage

(Breusegem *et al*. 2001). The significantly higher SOD activity in SS5 coincided well with lower $O_2^{\cdot-}$ content. The progressive increase in expression of native *SOD* was higher in drought condition as compared to the irrigated control in both WT and SS5 plants (Fig. 1*D*). Though the *PaSOD* was under the control of constitutive promoter *i.e*. CaMV35S, its expression was higher at 30 d in SS5 plants under both well-irrigated and drought stress conditions as compared to that of the 0 d and 15 d. As expected, *PaSOD* could not be detected in the WT. Stress-mediated inducibility of *PaSOD*, in spite of being under the control of CaMV35S promoter, has been reported earlier by Gill *et al*. (2010a,b, 2012). Similar results have also been reported by Shi *et al*. (2003) and Singer *et al*. (2012). Transgene mis-expression, silencing and plant-to-plant variability is often observed in case of transgenic plants (Butaye *et al*. 2005). This could be a possible reason that out of eight PCR-positive lines, only SS5 exhibited significantly higher SOD activity with prominent expression of *PaSOD* (Fig. 1*C*). The total SOD activity increased with the duration of water stress in both WT and SS5. On 30 d, SOD activity was significantly higher in SS5 [202.22 U mg^{-1}(protein) compared to that in the WT [141.16 U mg^{-1}(protein)]. SOD activity increased by 34.9 % in WT and 74.7 % in SS5 on 30 d of drought stress (Table 1).

Drought stress down regulates several metabolic processes in plants with concomitant activation of internal defense mechanisms including enhanced production of stress proteins, up-regulation of anti-oxidants, and accumulation of compatible solutes (Wang *et al*. 2003). Proline is an osmolyte which could accumulate at a high concentration in the cytoplasm under drought stress (Szabados and Savouré 2010). Proline provides protection to cell membranes and stabilizes proteins and protein complexes in the chloroplast and cytosol. It also provides protection to the photosynthetic apparatus and enzymes involved in detoxification of ROS (Szabados and Savouré 2010). In maize plants, proline content increased up to three times under drought stress (Mohammadkhani and Heidari 2008). In the present study, proline content increased by 292.2 % in WT plants at 30 d of drought stress compared to 130.9 % in SS5 (values with respect to the respective irrigated control).

In conclusion, the present study showed significantly lower proline and $O_2^{\cdot-}$ content but enhanced SOD activity in SS5 compared to WT under drought stress which probably generated lower stress signal for stomata to close. This was indicated by significantly higher g_s in SS5 as compared to the WT. Since g_s also regulate sub-stomatal concentration of CO_2, SS5 plants exhibited significantly higher P_N. The present work suggests that manipulation of $O_2^{\cdot-}$ content could be a new way to modulate drought stress tolerance in plants.

References

Asada, K.: The water-water cycle in chloroplasts: scavenging of active oxygen species and dissipation of excess photons. - Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 601-639, 1999.

Attia, H., Karray, N., Masilini, N., Lachaal, M.: Effect of salt stress on gene expression of superoxide dismutases and copper chaperone in *Arabidopsis thaliana* - Biol. Plant. **55**: 159-163, 2011.

Bates, L.S., Waldren, B.P., Tease, J.D.: Rapid determination of free proline for water stress studies. - Plant Soil **39**: 205-207, 1973.

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

Breusegem, F.V., Vranova, E., Dat, J.F., Inze, D.: The role of active oxygen species in plant signal transduction. - Plant Sci. **161**: 405-414, 2001.

Butaye, K.M.J., Cammue, B.P.A., Delaure, De Bolle, M.F.C.: Approaches to minimize variation of transgene expression in plants. - Mol. Breed. **16**: 79-91, 2005.

Chaves, M.M, Oliveira, M.M.: Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. - J. exp. Bot. **55**: 2365-2384, 2004.

Doyle, J.J., Doyle, J.L.: A rapid total DNA preparation procedure for fresh plant tissue. - Focus **12**: 13-15, 1990.

Flexas, J., Medrano H.: Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. - Ann. Bot. **89**: 183-189, 2002.

Foyer, C.H., Shigeoka, S.: Understanding oxidative stress and antioxidant functions to enhance photosynthesis. - Plant Physiol. **155**: 93-100, 2011.

Ghawana, S., Paul, A., Kumar, H., Kumar, A., Singh, H., Bhardwaj, P.K., Rani, A., Singh, R.S., Raizada, J., Singh, K., Kumar, S.: An RNA isolation system for plant tissues rich in secondary metabolites. - BMC Res. Notes **4**: 85-89, 2011.

Gill, T., Dogra, V., Kumar, S., Ahuja, P.S., Sreenivasulu, Y.: Protein dynamics during seed germination under copper stress in *Arabidopsis* over-expressing *Potentilla* super-oxide dismutase. - J. Plant Res. **125**: 165-172, 2012.

Gill, T., Kumar, S., Ahuja, P.S., Sreenivasulu, Y.: Over-expression of *Potentilla superoxide dismutase* improves salt stress tolerance during germination and growth in *Arabidopsis thaliana*. - J. Plant Genet. Transgenics **1**: 1-10, 2010a.

Gill, T., Sreenivasulu, Y., Kumar, S., Ahuja, P.S.: Over-expression of superoxide dismutase exhibits lignification of vascular structures in *Arabidopsis thaliana*. - J. Plant Physiol. **167**: 757-760, 2010b.

Gomes, M.M.A., Lagôa, A.M.M.A., Medina, C.L., Machado, E.C., Machado, M.A.: Interactions between leaf water potential, stomatal conductance and abscisic acid content of orange trees submitted to drought stress. - Braz. J. Plant Physiol., **16**: 155-161, 2004.

Hebbar., K.B., Kumar, S., Sinha, S.K.: Action potential – a possible signal in root to shoot communication caused by water deficits around roots of sunflower seedlings. - Curr. Sci. **66**: 936-938, 1994.

Jabs, T., Dietrich, R.A., Dangl, J.L.: Initiation of runaway

cell death in an *Arabidopsis* mutant by extracellular superoxide. - Science **273**: 1853-1856, 1996.

Jarvis, P.G.: The interpretation of the variations in leaf water potential and stomatal conductance found in canopies in the field. - Phil. Trans. roy. Soc. Lond. Ser. B. **273**: 593-610, 1976.

Kumar, N., Kumar, S., Vats, S.K., Ahuja, P.S.: Effect of altitude on the primary products of photosynthesis and the associated enzymes in barley and wheat. - Photosynth. Res. **88**: 63-71, 2006.

Kumar, N., Vyas, D., Kumar, S.: Plants at high altitude exhibit higher component of alternative respiration. - J. Plant Physiol. **164**: 31-38, 2007.

Loggini, B., Scartazza, A., Brugnoli, E., Navari-Izzo, F.: Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. - Plant Physiol. **119**: 1091-1099, 1999.

Medrano, H., Escalona, J.M., Boto, J., Gulias, J., Flexas, J.: Regulation of photosynthesis of C_3 plants in response to progressive drought: Stomatal conductance as a reference parameter. - Ann. Bot. **89**: 895-905, 2002.

Mohammadkhani N, Heidari R.: Drought-induced accumulation of soluble sugars and proline in two maize varieties. - World Appl. Sci. J. **3**: 448-453, 2008.

Moran, J.F., Becana, M., Iturbe-Ormaetxe, I., Frechilla, S., Klucas, R.V., Aparicio-Tejo, P.: Drought induces oxidative stress in pea plants. - Planta **194**: 346-52, 1994.

Movahedi, S., Tabatabaei, B.E.S., Alizade, H., Ghobadi, C., Yamchi, A., Khaksar, G.: Constitutive expression of *Arabidopsis DREB1B* in transgenic potato enhances drought and freezing tolerance. - Biol. Plant. **56**: 37-42, 2012.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-497, 1962.

Ort, D.R., Oxborough, K., Wise, R.R.: Depressions of photosynthesis in crops with water deficits. - In: Baker, N.R., Bowyer, J.R. (ed): Photoinhibition of Photosynthesis from Molecular Mechanisms to the Field. Pp. 315-329. BIOS Scientific Publ., Oxford 1994.

Overmyer, K., Tuominen, H., Kettunen, R., Betz, C., Langebartels, C., Sandermann, H., Jr., Kangasjärvi, J.: Ozone-sensitive *Arabidopsis rcd*1 mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. - Plant Cell **12**: 1849-1862, 2000.

Perl, A., Perl-Treves, R., Galili, S., Aviv, D., Shalgi, E., Malkin, S., Galun, E.: Enhanced oxidative-stress defense in transgenic potato expressing tomato Cu, Zn superoxide dismutases. - Theor. appl. Genet. **85**: 568-576, 1993.

Sengupta, A., Webb, R.P., Holiday, A.S., Allen, R.D.: Overexpression of superoxide dismutase protects plants from oxidative stress; induction of ascorbate peroxidase in SOD over-expressing plants. - Plant Physiol. **103**: 1067-1073, 1993.

Shi, H., Lee, B.H., Wu, S.J., Zhu, J.K.: Overexpression of a plasma membrane Na^+/H^+ antiporter gene improves salt tolerance in *Arabidopsis thaliana*. - Natur. Biotechnol. **21**: 81-5, 2003.

Singer, S.D., Liu, Z., Cox, K.D.: Minimizing the unpredictability of transgene expression in plants: the role of genetic insulators. - Plant Cell Rep. **31**: 13-25, 2012.

Singh, K., Raizada, J., Bhardwaj, P., Ghawana, S., Rani, A., Singh, H., Kaul, K., Kumar, S.: 26S rRNA based internal control gene primer pair for reverse transcription - polymerase chain reaction - based quantitative expression studies in diverse plant species. - Anal. Biochem. **335**: 330-333, 2004.

Stiller, I., Dulai, S., Kondrak, M., Tarnai, R., Szabo, L., Toldi, O., Banfalvi, Z.: Effects of drought on water content and photosynthetic parameters in potato plants expressing the *trehalose-6-phosphate synthase* gene of *Saccharomyces cerevisiae*. - Planta **227**: 299-308, 2008.

Szabados, L., Savouré, A.: Proline: a multifunctional amino acid. - Trends Plant Sci. **15**: 89-97, 2010.

Tang, L., Kwon, S.-K., Kim, S.-H., Kim, J.-S., Choi, J.S., Kwang, K.Y., Sung, C.K., Kwak, S.-S., Lee, H.-S.: Enhanced tolerance of transgenic potato plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against oxidative stress and high temperature. - Plant Cell Rep. **25**: 1380-1386, 2006.

Tardieu, F., Lafarge, T., Simonneau, T.: Stomatal control by fed or endogenous xylem ABA in sunflower: interpretation of correlations between leaf water potential and stomatal conductance in anisohydric species. - Plant Cell Environ. **19**: 75-84, 1996.

Vyas, D., Kumar, S.: Tea (*Camellia sinensis* (L.) O. Kuntze) clone with lower period of winter dormancy exhibits lesser cellular damage in response to low temperature. - Plant Physiol. Biochem. **43**: 383-388, 2005.

Wang, W., Vinocur, B., Altman, A.: Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. - Planta **218**: 1-14, 2003.

Yogavel, M., Mishra, P.C., Gill, J., Kumar, P., Bhardwaj, P.K., Dutt, S., Kumar, S., Ahuja, P.S., Sharma, A.: Structure of a superoxide dismutase and implications for copper-ion chelation. - Acta Crystallogr. D **64**: 892-901, 2008.