Plant Abiotic Stress Challenges from the Changing Environment: How to Develop Plants Capable of Mitigating Climate Change

Amrina Shafi[1], Insha Zahoor[2], Mudasir A Mir[3]
[1]Division of Biotechnology, CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, 170761, India.
[2]Department of Biotechnology, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, 190006, India.
[3]Centre for Plant Biotechnology, Division of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, 191121, India.

Abstract: Climate change is a multifaceted phenomenon with a wide range of impacts on the environment. Currently, with the competing uses of land and the growing world population, we are challenged to produce more in less area with diminishing resources, confronted with climate change and the unpredictable local microclimate adversely affecting crop productivity. Abiotic stresses will remain a challenge to the natural environment and agriculture. The challenges before us in plant biology and crop improvement are to integrate the systems level information on abiotic stress response pathways, identify stress protective networks, and engineer environmentally stable crops that yield more. In the present study, two different antioxidant enzymes namely copper-zinc superoxide dismutase derived from *Potentilla astrisanguinea* (SOD) and ascorbate peroxidase (APX) from *Rheum austral* both of which are high altitude cold niche area plants of Western Himalaya were cloned and simultaneously over-expressed in *Arabidopsis thaliana* to alleviate polyethylene glycol (PEG) stress. It was found that the transgenic plants over-expressing both the genes were more tolerant to PEG-induced stress during growth and development. In both transgenic plants higher levels of total antioxidant enzyme activities, total soluble sugars, proline content and lower levels of ROS, ion leakage were recorded when compared to the wild type (WT) during PEG stress. The transgenics in the present study showed 2-3 fold increase in SOD and APX activity resulting in the enhanced stress tolerance to PEG stress than WT. In terms of growth and development in number of leaves, rosette area and root length, which was observed to be significantly higher in transgenic lines compared to WT. Also, the transgenic lines showed higher germination percentage at various levels of PEG. Overall, SOD and APX transgenic lines were able to express greater drought tolerance and thus the present work would pave way for the judicious use of these genes effectively into the relevant crop plants leading to optimum growth and enhanced yield under environmentally stressed conditions.

Keywords: Climate Change, Abiotic stress, Antioxidant genes, Arabidopsis thaliana, PEG

I. INTRODUCTION

The challenges of abiotic stress on plant growth and development are evident among the emerging ecological impacts of climate change (Bellard et al., 2012), and the constraints to crop production exacerbated with the increasing human population competing for environmental resources (Wallace et al., 2003). Climate change is predicted to affect agricultural production the most, with adverse effects of increasing carbon dioxide and high temperature, challenging researchers toward devising adaptation strategies (Rosenzweig et al., 2014). These constraints to global food supply and a balanced environment encourage research and development of climate smart crops, resilient to climate change (Wheeler and Von Braun, 2013).

Plant abiotic stress encompasses all studies on abiotic factors or stressors from the environment that can impose stress on a variety of species (Sulmon et al., 2015). These stressors include extreme levels of light, radiation (UV), temperature, water (drought, flooding, and submergence), chemical factors (heavy metals and pH), salinity due to excessive Na^+ and other less frequently occurring stressors. Abiotic stresses adversely affect the growth and development of plants. Plants being non-motile cannot alter their environment under adverse conditions. Plant response to stresses comprises of a wide range of molecular, biochemical and physiological perturbations (Zhu, 2002). A common feature of most of the abiotic stresses, is the enhanced production of ROS (Boyer, 1982). During normal conditions, the cellular system is in homeostasis where the ROS produced are taken care of by the cellular antioxidant machinery by the mechanism known as the Asada–Halliwall Pathway, however, during stress the cellular homeostasis is perturbed and the increased ROS cause cellular damage to the cell and they react with lipids, proteins and nucleic acids (Allen, 1995). Thus, plant cell have highly regulated and controlled mechanisms to modulate their intracellular ROS concentrations to ensure minimum damage and optimal functioning. They have well-developed defense systems against ROS, involving the abatement of ROS formation and concomitantly instituting its removal. Enzymatic defense include superoxide dismutases, catalases, peroxidases and antioxidant molecules like ascorbate and glutathione. Superoxide dismutase which converts the superoxide radical to H_2O_2, and ascorbate peroxidise (APX) which triggers the conversion of H_2O_2 to water and oxygen (Asada, 1999). Plant stress tolerance can be increased by increasing the level of antioxidant enzymes (Allen, 1995) as they appear to be a critical component of defense against oxidative stress.

A fruitful strategy for the identification of stress tolerance genes has been by reverse genetics analyses of candidate genes identified through gene expression studies and other bioinformatics methods. The biological role of such candidate genes has been most often tested by the analysis of overexpression, knockout/knockdown genotypes in model, and crop plants (Todaka et al., 2015). Overexpression studies with transcription factors and other regulatory genes have been popular in transgenic crops, with the objective of improving their stress tolerance and productivity (Mickelbart et al., 2015), and often enabling applications across plant species. Over-expression of Potentilla atrosanguinea copper zinc superoxide dismutase (PaSOD) has improved copper and salt stress tolerance in Arabidopsis thaliana (Gill et al. 2010b; 2012; Shafi et al. 2014; 2015a,b). Apparently, overexpression of the same SOD in potato, enhanced photosynthetic performance under drought stress (Pal et al. 2013). In another study, over accumulation of lignin in vascular bundles was found to be the molecular mechanism which underlies the improved stress tolerance induced by the over-expression of PaSOD in Arabidopsis thaliana (Gill et al. 2010b). Genetic manipulation of antioxidant enzymes is one of the effective measures to impart stress tolerance in plants. Activities of superoxide dismutase and catalases were increased in plants subjected to stress (Scebba et al. 1999).

High altitude ecosystems are often inhabited by a very few plant species due to prevailing harsh environmental conditions. The genes/proteins isolated from high altitude plants are being used as molecular tools for engineering crop and other plants for better stress tolerance and adaptability against the present scenarios of climate change. In this pursuit, previously we identified and characterized a thermo-tolerant copper–zinc superoxide dismutase from a high altitude plant *Potentilla atrosanguinea* (*PaSOD*), which retains its activity even after autoclaving and also in the presence of NaCl. In the present study, we have

developed two types of transgenics in *Arabidopsis*, (a) overexpressing *PaSOD* gene, which converts superoxide anions into H_2O_2, (b) overexpressing ascorbate peroxidase gene, *RaAPX* from another high altitude plant *Rheum australe*, which converts H_2O_2 molecules into water. The detailed analysis revealed that these transgenics exhibit improved PEG tolerance as compared to wild type (WT). This study will elaborate and validate that higher altitude plants can serve as ample source of potential molecular tools which can be successfully utilized for engineering abiotic stress tolerance in plants and yet not disturbing native physiology of the plants.

II. RESEARCH METHODOLOGY

2.1 Plasmid construction and transgenic plant development

Full length cDNAs of Copper-Zinc Superoxide Dismutase (PaSOD) and Ascorbate Peroxidase (now onwards RaAPX) from high altitude plants Potentilla atrosanguinea (which grows at daytime air temperatures of 3–10uC in Lahaul and Spiti districts of Himachal Pradesh: altitude 4517 m; 32°24' 20" N; 077° 38' 400" E) and Rheum australe (Rohtang Pass in Himachal Pradesh: altitude 4000 m; 32° 22' 19" N; 077° 14' 46" E), respectively, from Western Himalaya, were cloned in Arabidopsis thaliana as described earlier by Gill et al. (2010). Briefly, coding nucleotide sequences of these genes were amplified using the gene specific primers with incorporated Nco1 and BglII restriction sites at 39 end. PCR products were cloned into a cloning vector pGEMT easy (Promega) and then sub-cloned into binary plant vector pCAMBIA1302 under the Cauliflower mosaic 35 S promoter. The prepared plasmid construct was mobilized into Arabidopsis plants via Agrobacterium mediated vacuum infiltration method (Bechtold et al. 1993). Seeds were collected and screened in Murashige and Skoog (Murashige and Skoog, 1962) medium supplemented with 20 mg ml 21 hygromycin.

2.2 SOD and APX enzyme activity assay

Total enzyme activity of SOD and APX was estimated at different time points during cold stress i.e. 0–192 h. Total SOD activity was estimated as described earlier (Gill et al. 2010b) while APX activity was determined according to Nakano and Asada (1981). Briefly, leaf samples (100 mg) were homogenized in a pre-cooled mortar in homogenizing buffer containing 2 mM EDTA, 1 mM DTT, 1 mM PMSF, 0.5% (v/v) Triton-X100 and 10% (w/v) PVPP in 50 mM phosphate buffer pH 7.8. For APX activity homogenizing buffer contained ascorbate in addition and the buffer pH was 7.0. The homogenate was transferred to 1.5 ml Eppendorf and centrifuged at 13,000 rpm for 20 min at 4uC. The supernatant was used to estimate total SOD and APX activities. The total SOD activity was measured by adding 5 ml enzyme extract to a reaction mixture (200 ml) containing 1.5 mm Riboflavin, 50 mm NBT, 10 mM DL-Methionine and 0.025% (v/v) Triton-X100 in 50 mM phosphate buffer. One unit of enzyme activity was defined as the amount of enzyme required for 50% inhibition of NBT reduction at 25°C. Total protein content was estimated according to the dye binding method of Bradford (1976) using BSA as standard.

2.3 Gene-specific semi-quantitative

Total RNA was isolated from control and PEG treated transgenic and the wild type Arabidopsis plants using Total RNA extraction kit (Real Genomics). One microgram of total RNA was used for oligo (dT) primed first-strand cDNA synthesis in 20 ml reaction using of Superscript III Reverse transcriptase (Invitrogen). Transcripts of SOD and APX were quantified with PCR using gene specific primers. Constitutively expressed 26s RNA was amplified simultaneously in 27 cycles to ensure equal amounts of cDNA used.

2.4 Evaluation of PEG stress tolerance

Arabidopsis (ecotype coloumbia) plants were grown on soil mixture of vermiculite: peat moss: perlite (1:1:1) in the greenhouse under a 16 h light and 8 h dark cycle at 20±1°C. For stress treatment, 21d old seedlings of wild type, and hygromycin selected transgenic seedlings were given PEG stress (0, 2.5, 5, 7.5 %). Samples were collected PEG treatment for the analyses of transcript levels, enzyme activity, proline and soluble sugars accumulation.

2.5 Estimation of electrolyte leakage, relative water content, total soluble sugars, proline content

Electrolyte leakage was measured using an electrical conductivity meter as described by Lutts et al. (1996). Relative water content (RWC) was measured according to Barrs and Weatherley (1962). Total soluble sugar (TSS) content was determined by anthrone method. Free proline content was estimated using the acid ninhydrin method described by Bates et al. (1973).

2.6 In-situ ROS staining

In situ ROS staining was done in accordance with Beyer and Fridovich (1987), on the basis of the principle of NBT (nitroblue tetrazolium) reduction to blue formazan by $O_2^{-\cdot}$. The intracellular concentration of ROS ($O_2^{-\cdot}$) was directly proportional to the development of intensity of blue color in the leaves. Briefly, leaf tissue was detached from the wild type and transgenic plants and vacuum infiltrated with 10 mM sodium azide (NaN3) in 10 mM potassium phosphate buffer for 1 min. The infiltrated leaf tissue was incubated in 0.1% NBT (nitroblue tetrazolium) in 10 mM potassium phosphate buffer; pH 7.8 for 30 min. The stained leaf tissue was boiled in acetic acid:glycerol:ethanol (1:1:3) solution to remove other pigments and the stain content was visually documented under Carl-Zeiss Stereo DiscoveryV12 with Axiovision software. This experiment was repeated three times from three biological replicates.

2.7 Statistical analysis

All experiments were conducted with at least three independent repetitions in triplicate. All values are shown as the mean±standard deviation. 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.

III. RESULTS AND DISCUSSION

3.1 Overexpression of *PaSOD* and *RaAPX* in *Arabidopsis*

RT-PCR analysis of the four single copy inserts was performed with WT as negative control. The order of expression of the four transgenic single copy insert lines (S7, S15, S26 and S31) in the case of *Cu/Zn-SOD* was S26>S7>S15>S31 (Fig. 1). The *APX* single copy insert lines (A2 and A18) have slightly higher levels of gene expression than the lines A10 and A20 when amplified with gene specific reverse primer (Fig. 2). Of the four single copy lines 3 lines (A2, A18 and A20) have been selected for *APX* enzyme activity under control and various concentrations of NaCl (50, 100 and 150 mM) with WT plants as control (Fig. 2). A2 line showed higher level of total *APX* activity and was taken for further analysis.

3.2 Transgenic Arabidopsis over-expressing SOD in combination with APX enhanced PEG tolerance

Arabidopsis transgenic plants over expressing SOD under the control of CaMV35 S promoter were generated as reported earlier (Gill et al. 2012). Similarly transgenic Arabidopsis plants over expressing ascorbate peroxidase gene from Rheum (APX) under the control of CaMV35 S promoter were also developed. Presence of these transgenes was confirmed by PCR by using genomic DNA from these plants as template and their expression by the presence of its transcripts from leaf tissues by semi-quantitative RT-PCR (Fig. 1, 2). On the basis of 3:1 segregation and with high enzyme activities, homozygous transgenic lines S26, S15 (for SOD) and A2, A10 (for APX) were selected for further analysis.

3.3 Effect of PEG on antioxidant enzyme activities

Total enzyme activities of SOD and APX were estimated in WT and transgenic samples collected after PEG stress. Enzyme assays for total SOD and APX revealed that their activities increased with increase in magnitude of PEG stress in WT and all the transgenic plants. Total enzyme activities increased gradually up to 5% PEG and then decreased at 7.5 % PEG in WT and all the transgenic lines, after which the minimal levels were maintained. However, total SOD and APX activities were significantly higher in transgenic plants as compared to WT under control as well as under salt stress, as the genes were overexpressed under constitutive CaMV35S promoter. The increase in total SOD activity was 1.8 to 2 fold higher in *PaSOD* lines and nearly 2.5–4.3 fold increase in APX activity was observed in APX lines (Fig.3A, B).

3.4 SOD and APX induced PEG stress tolerance alters Arabidopsis growth and biomass

After three weeks of plant growth on various concentration of PEG (0, 2.5, 5 and 7.5 %), number of leaves, rosette area and root length, were compared as general indicators of plant growth and development under stress (Fig. 4). Transgenic plants of S26 line had increased root length as compared to WT and P5 plants at all concentration of PEG stress (Fig. 4 A). On the same stress level, the number of leaves in transgenic line S26 was 2-4 leaves more than that of same in WT and P5 plants (Fig. 4 C). The rosette area of transgenic plants was significantly larger in S26 at all concentrations of PEG induced osmotic stress as compared to WT as well as P5 (Fig. 4 B). The rosette area markedly decreased with the increase in the concentration of PEG in the MS0 medium. The decrease in rosette area was relatively lower in S26 plants when compared with both the WT and P5 plants (Fig. 4 B).

After three weeks of plant growth on PEG stress, rosette area, number of leaves and root length was compared as general indicators of plant growth and development under PEG stress (Fig. 5). Average root length, particularly at 7.5% PEG stress was in 3.13 cm in WT as compared to 4.27 to 4.56 for the transgenic plants (A2, A10 and A18) (Fig. 5). On the same stress level, the number of leaves was significantly higher in transgenics plants at (8.20-8.91) when compared to the WT (7.33). At lower levels of PEG stress (2.5 and 5 %) the difference between the number of leaves between and WT was not significant (Fig. 5). The rosette area of transgenic plants was significantly larger at 5% PEG. The WT plants had the area of 0.95 cm^2 with transgenic lines showing a range of rosette area of 1.19 -2.36 cm^2 (Fig. 5).

Higher germination rates of transgenic Arabidopsis under PEG stress

Germination percentage of SOD transgenic lines (S15 and S26) was compared with WT at 0, 2.5, 5.0 and 7.5% of PEG in the MS0 medium. Germination percentage in WT and transgenic lines S15 and S26 was the same without PEG induced osmotic stress. On 2.5% PEG stress, WT seeds had 90% germination whereas seeds of S15 and S26 100% germination (Fig. 6). On 5 % PEG stress, S15 and S26 showed 90% germination (Fig. 6), whereas WT recorded 80% germination. On 7.5% PEG stress, the seeds of S15 and S26 evinced a germination of 90% each whereas WT germination was recorded at 60 % (Fig. 6). Overall, the transgenic lines exhibited increased germination percentages than the WT under PEG induced osmotic stress. Germination percentage of *APX* transgenic lines (A2, A10 and A18) was compared with WT at 0, 2.5, 5.0 and 7.5% of PEG in the MS0 medium. Germination percentage in WT and transgenic lines for *APX* was the same without PEG stress. On 2.5% PEG stress, WT seeds showed 80% germination whereas seeds of A2 and A18 recorded germination percentage of 95% and A10 showed germination percentage of 90% (Fig 7). On 5.0% PEG stress, A2 showed 85% germination, A10 and A18 both recorded a germination percentage of 80% as compared to that of WT seeds which recorded a percentage germination of 65%. On 7.5% PEG stress, the seeds of A2 showed 75% germination whereas both A10 and A18 evinced a germination of 70% each whereas WT germination was recorded at 50% (Fig. 7).

Reduced accumulation of ROS contents under PEG stress

In situ detection of ROS showed that the amount of $O_2^{\cdot -}$ (ROS) increased significantly with higher degree of PEG stress in WT and transgenic lines. Blue coloration intensity signifies the amount of ROS produced during stress. However, the $O_2^{\cdot -}$ content was found to be higher in WT as compared to transgenic lines under PEG stress. At higher levels of PEG stress (7.5%), the transgenic lines showed least $O_2^{\cdot -}$ content than WT plants (Fig. 8).

Response of transgenics under salt stress

Electrolyte leakage analysis revealed that, there was no significant difference in electrolyte leakage in WT and transgenic lines under control conditions. Under PEG stress, electrolyte leakage was found to be significantly lower in transgenic lines as compared to WT plants (Table1). Among transgenic lines, least

electrolyte leakage was found in SOD line followed by APX line under PEG stress. Relative water content (RWC) was observed to be similar in both, WT and transgenics under control conditions. However under PEG stress, WT suffered significant reduction in RWC as compared to transgenic lines. In all transgenic lines, the reduction in RWC was not significant at all tested PEG stress (Table1). The transgenic lines under PEG stress possess increased levels of proline and soluble sugars. The accumulation of proline was two fold higher in SOD lines, 1.4 fold in APX lines transgenic lines as compared to WT (Table 1). Soluble sugar content was also significantly higher (two fold) in all the transgenic lines as compared to WT, where no significant change in soluble sugar levels was observed at the same PEG stress conditions (Table1). Our results suggest that the higher tolerance of the transgenic lines to PEG stress is a result of the accumulation of more proline and total soluble sugars as compared to WT.

IV Discussion

As compared to other living organisms, plants need to protect themselves from different environmental, abiotic and biotic stresses. These stresses cause oxidative damage, ion toxicity and disruption of cellular homeostasis through the production of reactive oxygen species (ROS). ROS induces various complex biochemical, molecular, cellular, and physiological changes in plants including damage of DNA, proteins and membrane lipids (Gill et.al 2010b; Munns and Tester, 2008). The responses to abiotic stresses can generally be separated into two categories: those that regulate signal transduction and gene expression in response to the stress, and those that protect against environmental stress by redirection of metabolism. Resistance to abiotic stress requires a number of changes in gene expression (Fowler and Thomashow, 2002; Zhu, 2001; Xiong et al. 2002). Plants have developed a wide range of enzymatic and non-enzymatic mechanisms to scavenge ROS. Among the enzymatic methods, superoxide dismutase (SOD) is one of the crucial enzymes in the plant's defense mechanism that converts superoxide anion radicals ($O_2^{2\cdot}$) to hydrogen peroxide (H_2O_2), there by imparting protection against the harmful effects of highly reactive $O_2^{2\cdot}$ (Fridovich, 1975). This in turn with the help of other antioxidant enzymes such as APX and catalase converts them in to safer molecules like H_2O and O_2. In the present study transgenic line overexpressing SOD and APX lines, showed better tolerance and stability than WT under PEG stress. The alpine climate is very harsh with low temperatures, the length of the daily photoperiod, and the quality of the incident light influences the plant growth in these areas. Because of having morphological, physiological and genetical adaptations to these stresses, the alpine vegetation can grow even in such harsh condition. So it is expected that these plants are known to have better enzymatic system whose activities are enhanced or are better adapted than their counterparts from plains. High altitude plants with efficient and active protective systems such as autoclavable superoxide dismutase from Potentilla (Sahoo et al. 2001), make these plants sustain under stress conditions. Dissection of these adaptations in these plants has provided insights towards unraveling of the possible mechanisms of stress tolerance (Gill et al. 2010a; b). This work clearly demonstrates that the modulation of endogenous ROS scavenging capacity against abiotic stresses can be successfully engineered by the simultaneous over expression of PaSOD and RaAPX in Arabidopsis. In addition, the results outlined the importance of the cytosolic antioxidant machinery in the crossprotection from multiple stresses in agriculturally important plants.

Acknowledgment

A.S acknowledge Fellowship by the CSIR, New Delhi, India. This work was supported by Grants from the Council of Scientific and Industrial Research (CSIR), New Delhi, India under CSIR Network Projects: SIMPLE (BSC0109) and PlaGen (BSC0107) and Indo-German Science and Technology Centre (IGSTC).

V References

[1] Scebba, F. Sebastiani, L. Vitagliano, C. 1999. Protective enzymes against activated oxygen species in wheat (Triticum aestivum L.) seedlings: Responses to cold acclimation. Journal of Plant Physiology 155: 762–768.

[2] Gill, T. Kumar, S. Ahuja, PS. Sreenivasulu, Y. 2010b. Over-expression of Potentilla superoxide dismutase improves salt stress tolerance during germination and growth in Arabidopsis thaliana. Journal of Plant Genetics and Transgenic 1: 1–10.

[3] Gill, T. Dogra, V. Kumar, S. Ahuja, PS. Sreenivasulu, Y. 2012. Protein dynamics during seed germination under copper stress in Arabidopsis over-expressing Potentilla superoxide dismutase. Journal of Plant Research 125: 165–172.

[4] Pal, AK. Acharya, K. Vats, SK. Kumar, S. Ahuja, PS. 2013. Over-expression of PaSOD in transgenic potato enhances photosynthetic performance under drought. Biology Plantarum 57: 359–364.

[5] Gill, T. Sreenivasulu, Y. Kumar, S. Ahuja, PS. 2010a. Over-expression of superoxide dismutase exhibits lignifications of vascular structures in Arabidopsis thaliana. Journal of Plant Physiology 167: 757–760.

[6] Bechtold, N. Ellis, J. Pelletier, GC. 1993. In planta Agrobacterium mediated gene transfer by infiltration of adult Arabidopsis plants. C. R. Acad. Sci. Paris. 316: 1194–1199.

[7] Murashige, T. Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiology 15: 473–476.

[8] Nakano, Y. Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiology 22: 867–880.

[9] Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein dye binding. Analytical Biochemistry 72: 248–254.

[10] Lutts, S. Kinet, JM. Bouharmont, J. 1996. NaCl-induced senescence in leaves of rice (Oryza sativa L.) cultivars differing in salinity resistance. Annals of Botany 78: 389–398.

[11] Barrs, HD. Weatherley, PE. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Biol Sci 15: 413–428.

[12] Bates, L. Waldren, R. Teare, ID. 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39: 205–207.

[13] Beyer, WF. Fridovich, I. 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161: 559– 566.

[14] Munns, R. Tester, M. 2008. Mechanisms of salinity tolerance. Ann. Rev. Plant Biol. 59: 651–681.

[15] Fowler, S. Thomashow, MF. 2002. Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell 14: 1675–1690.

[16] Zhu, JK. 2001 Plant salt tolerance. Trends Plant Sci. 6: 666–671.

[17] Xiong. L, Shumaker. KS, Zhu. JK 2002. Cell signaling during cold, drought and salt stresses. Plant Cell 14: 165–183.

[18] Fridovich, I. 1975. Superoxide Dismutase. Ann. Rev. Biochem. 44: 147–159.

[19] Sahoo, R. Kumar, S. Ahuja, PS. 2001. Induction of a new isozyme superoxide dismutase at low temperature in Potentilla astrisanguinea Lodd. Variety argyrophylla (Wall.ex. Lehm) Griers. J. Plant Physiol.158: 1093–1097.

[20] Bellard, C. Bertelsmeier, C. Leadley, P. Thuiller, W. and Courchamp, F. 2012. Impacts of climate change on the future of biodiversity. Ecological Letters 15: 365–377.

[21] Wallace, JS. Acreman, MC. and Sullivan, CA. 2003. The sharing of water between society and ecosystems: from conflict to catchment–based co–management. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 358: 2011–2026.

[22] Rosenzweig, C. Elliott, J. Deryng, D. Ruane, AC. Müller, C. Arneth, A. et al. 2014. Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. Proc. Natl. Acad. Sci. U.S.A. 111: 3268–3273.

[23] Wheeler, T. and Von Braun, J. 2013. Climate change impacts on global food security. Science 341: 508–513.
[24] Sulmon, C. Van Baaren, J. Cabello-Hurtado, F. Gouesbet, G. Hennion, F. Mony, C. et al. 2015. Abiotic stressors and stress responses: what commonalities appear between species across biological organization levels? Environment Pollution 202: 66–77.
[25] Zhu, JK. 2002. Salt and drought stress signal transduction in plants. Annual Review on Plant Biology 53: 247–73.
[26] Boyer, JS. 1982. Plant productivity and environment. Science 218: 443–448.
[27] Allen, RD. 1995. Dissection of oxidative stress tolerance using transgenic plants. Plant Physiology 107: 1049–1054.
[28] Asada, K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. Annual Review in Plant Physiology and Plant Molecular Biology 50: 601–39.
[29] Todaka, D. Shinozaki, K. and Yamaguchi-Shinozaki, K. 2015. Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants. Frontier in Plant Science 6:84.
[30] Mickelbart, M.V. Hasegawa, PM. and Bailey-Serres, J. 2015. Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nature Reviews on Genetics 16: 237–251.
[31] Shafi, A. Dogra, V. Gill, T. Ahuja, PS. Sreenivasulu, Y. 2014. Simultaneous over-expression of PaSOD and RaAPX in transgenic Arabidopsis thaliana confers cold stress tolerance through increase in vascular lignifications. PLoS One 9:e110302.
[32] Shafi, A. Gill, T. Sreenivasulu, Y. Kumar, S. Ahuja, PS. Singh, AK. 2015a. Improved callus induction, shoot regeneration, and salt stress tolerance in Arabidopsis overexpressing superoxide dismutase from Potentilla atrosanguinea. Protoplasma 252:41–51.
[33] Shafi, A. Chauhan, R. Gill, T. Swarnkar, MK. Sreenivasulu, Y. Kumar, S. Kumar, N. Shankar, R. Ahuja, PS. Singh, AK. 2015b. Expression of SOD and APX genes positively regulates secondary cell wall biosynthesis and promotes plant growth and yield in Arabidopsis under salt stress. Plant Molecular Biology 87:615–631.

Figures and Tables

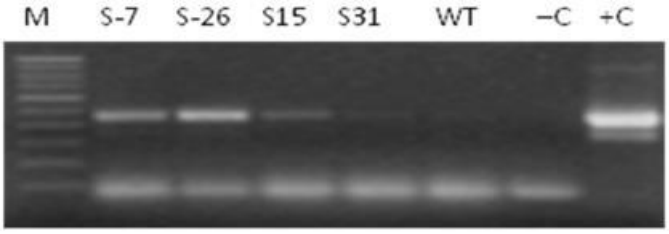

Fig. 1 PCR amplification of cDNA with *Cu/Zn-SOD* specific primer of single copy inserts lines. M- 100bp ladder, S7, S15, S26 and S31 (*Cu/Zn-SOD* single copy insert transgenic lines), WT- WT control, -C is the negative control and +C is a positive control

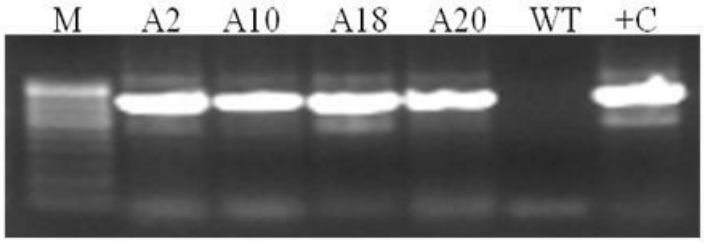

Fig. 2: PCR amplification of cDNA with *APX* reverse primer of single copy inserts lines. Lane 1 is 100 bp ladder, lanes 2-5 are *APX* single copy insert transgenic lines (A2, A10, A18 and A20), lane 6 is the WT control and lane 7 is the positive control

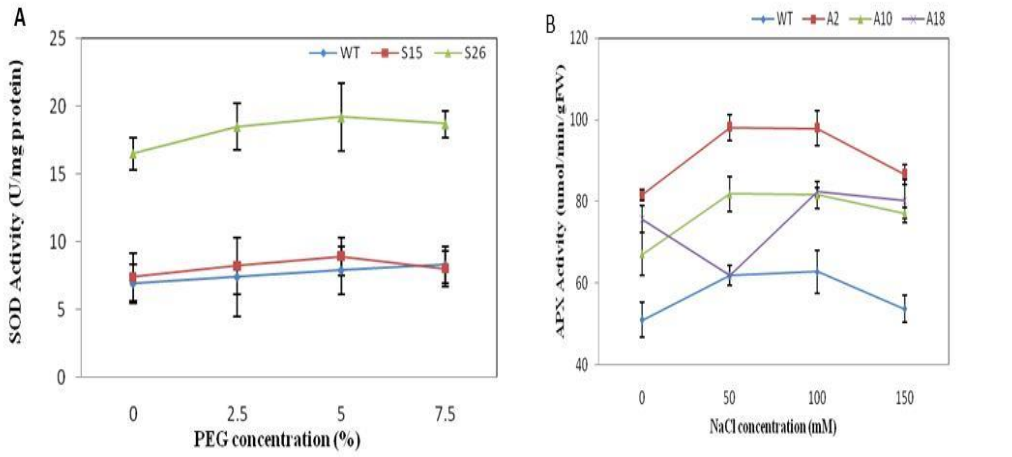

Fig. 3 Percentage SOD and APX activity in WT , SOD transgenic lines (S15 and S26) APX transgenic lines (A2, A18 and A20) under PEG stress.

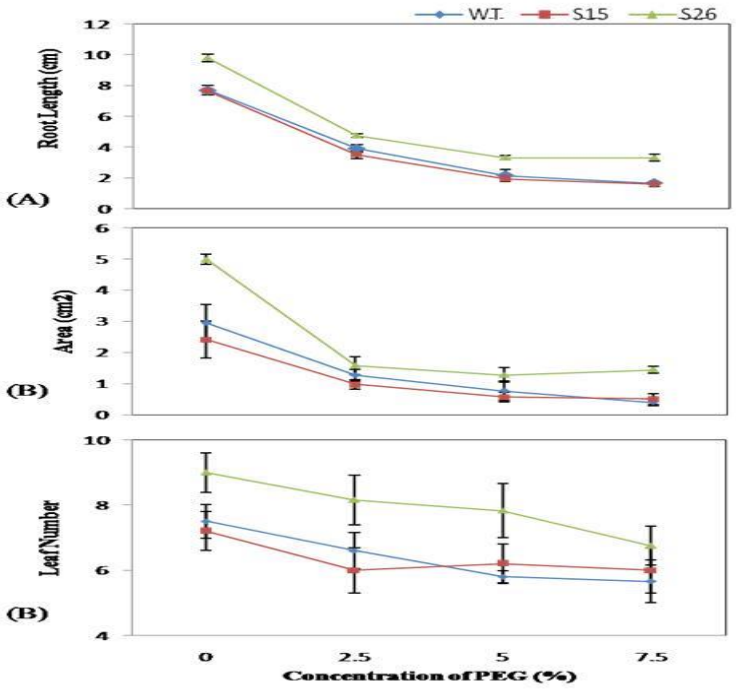

Fig.4 Overexpression of *Cu/Zn-SOD* gene in *Arabidopsis* improves (A) root length; (B) rosette area and (C) leaf number. WT and transgenic plant after three weeks growth on MS medium supplemented with different concentrations of PEG.

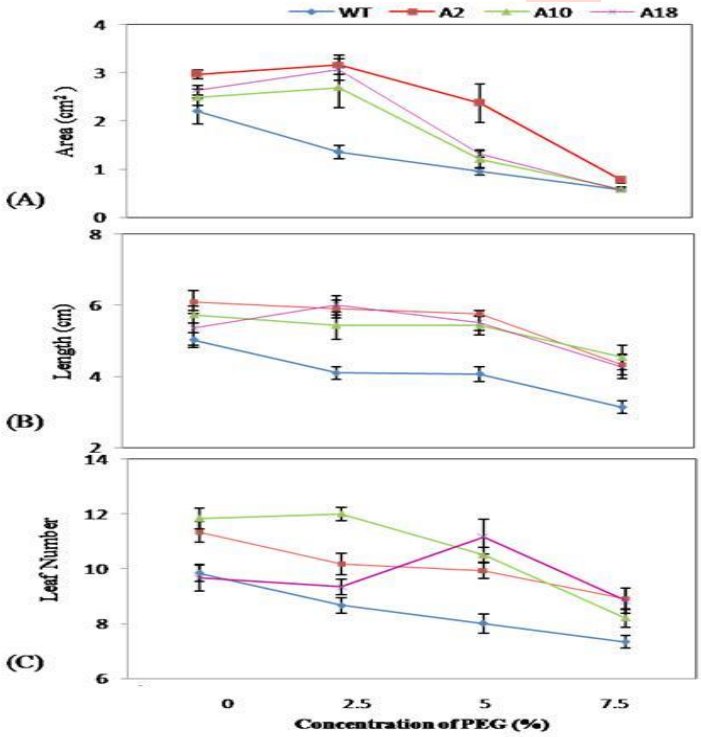

Fig.5 Overexpression of *APX* gene in *Arabidopsis* improves (A) root length; (B) rosette area and (C) leaf number. WT and transgenic plant after three weeks growth on MS medium supplemented with different concentrations of PEG.

ISSN: 2320-2882

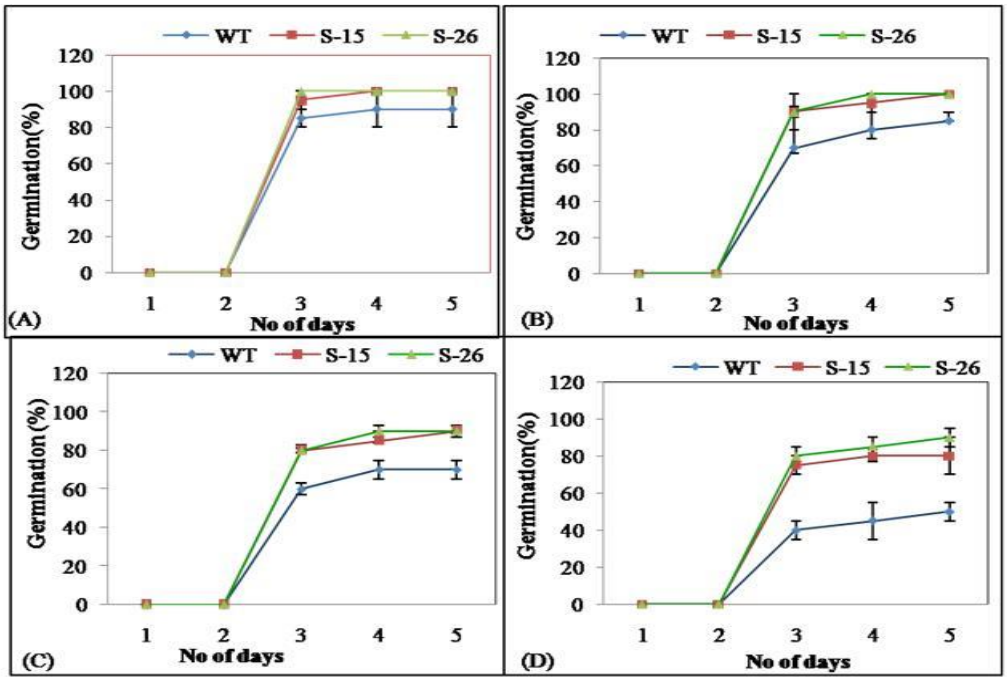

Fig. 6 Percentage germination of *Arabidopsis* (WT) and *Cu/Zn-SOD* transgenic lines (S15 and S26) under controlled conditions and PEG stress (A) control; (B)2.5% PEG; (C) 5.0% PEG and (D) 7.5% PEG.

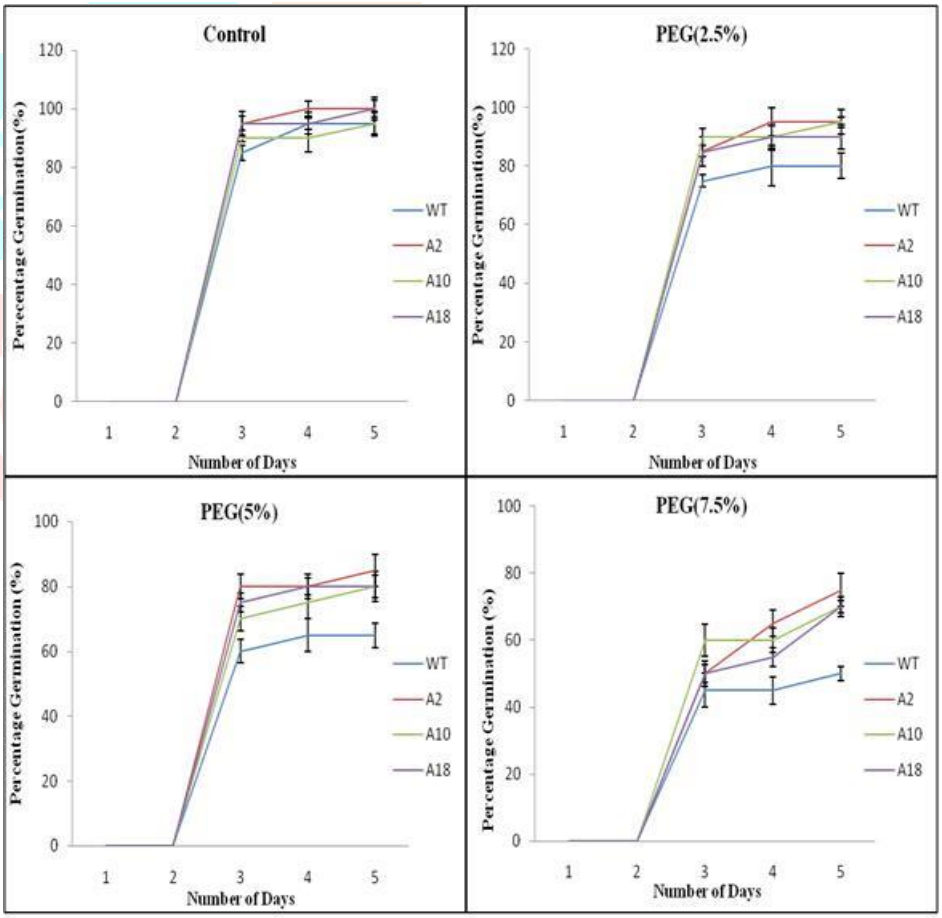

Fig.7 Germination percentage of WT and APX transgenic lines (A2, A18 and A20) under controlled conditions and Osmotic (PEG) stress (2.5, 5.0 and 7.5%) Error bars represent ±SE

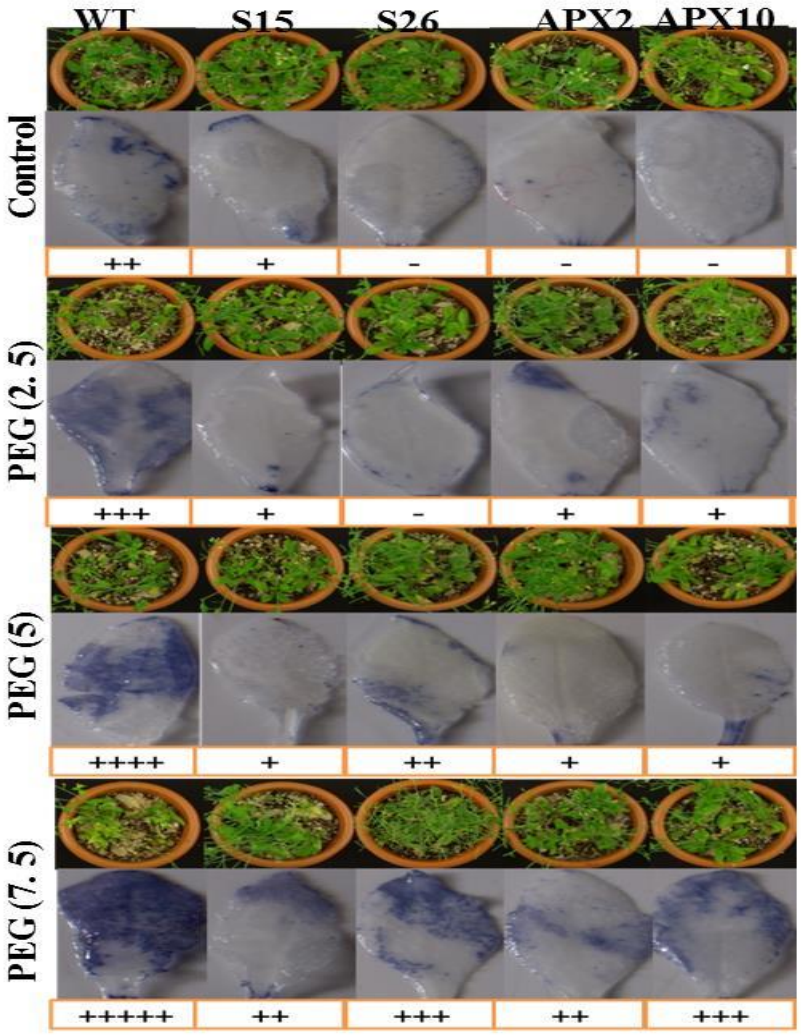

Fig.8 Insitu ROS detection in WT , SOD (S15, S26) and APX transgenic lines (A2, A18 and A20) under controlled conditions and Osmotic (PEG) stress (2.5, 5.0 and 7.5%).

Table 1. Biochemical analysis of WT and transgenic lines under PEG stress (2.5, 5, 7.5%) and control conditions.

	0 % PEG			2.5 % PEG			5 % PEG			7.5 % PEG		
Attributes	WT	S26	APX10	WT	S26	APX10	WT	S26	APX10	WT	S26	APX10
Relative water content(%)	90.1 ± 1.52^{a}	83.66 ± 2.27^{bc}	84.47 ± 1.12^{bc}	83.12 ± 1.46^{c}	78.53 ± 2.96^{cde}	77.03 ± 1.59^{de}	72.44 ± 1.88^{f}	76.81 ± 2.87^{de}	76.07 ± 1.97^{de}	62 ± 2.77^{g}	68.33 ± 0.61^{ef}	69.29 ± 2.07^{ef}
Electrolyte Leakage (%age)	24.26 ± 1.5^{de}	19.94 ± 2.36^{fg}	17.35 ± 2.94^{g}	29.89 ± 2.26^{d}	17.85 ± 2.36^{g}	20.96 ± 1.25^{ef}	38.43 ± 2.9^{ab}	21.64 ± 1.84^{ef}	27.62 ± 2.74^{de}	43.05 ± 1.76^{a}	30.78 ± 1.01^{c}	33.76 ± 3.19^{b}
TSS (mg/ml FW)	11.49 ± 0.03^{h}	14.60 ± 0.04^{i}	14.84 ± 0.04^{i}	11.54 ± 0.04^{h}	20.23 ± 0.15^{d}	15.55 ± 0.21^{f}	11.6 ± 0.04^{h}	22.31 ± 0.06^{cd}	23.5 ± 0.21^{c}	13.21 ± 0.11^{g}	23.29 ± 0.06^{c}	27.05 ± 0.09^{a}
Proline content (mg/gFW)	4.82 ± 0.20^{i}	3.68 ± 0.20^{i}	3.86 ± 0.60^{i}	5.87 ± 0.02^{h}	7.86 ± 0.04^{g}	9.50 ± 0.67^{f}	12.41 ± 0.60^{e}	27.70 ± 0.03^{a}	18.13 ± 0.04^{d}	24.33 ± 0.41^{b}	24.33 ± 0.60^{b}	15.35 ± 0.27