ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

Plant Abiotic Stress Challenges from the Changing Environment: How to develop plants capable of mitigating climate change

[1] Amrina Shafi, [2] Ashaq Hussain Mir, [3] Insha Zahoor, [4] Umar Mushtaq, [5] Saife Niaz

[1] Research Associate, Department of Biotechnology, CSIR-Institute of Himalayan Bioresource Technology, Palampur – 176 061, (HP) India

[2] Research Scholar, Department of Botany, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, 190006, India.

[3] Lecturer, Bioinformatics Centre, University of Kashmir, Srinagar, Jammu and Kashmir, 190006, India.

[4,5] Research Scholar, Department of Biotechnology, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, 190006, 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. Biotic and abiotic stress is a result of climate change. 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. Plants evolve defense mechanisms to withstand these stresses, e.g. antioxidants and antioxidant enzymes. In the present study, two different antioxidant enzymes namely copper-zinc superoxide dismutase derived from Potentilla astrisanguinea (Cu-Zn/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 salt stress. It was found that the transgenic plants over-expressing both the genes were more tolerant to salt stress than either of the single gene expressing transgenic plants during growth and development. Further, transcriptomic analysis showed that most of the genes related to secondary metabolite production and phytohormones were overexpressed in transgenic lines under stress conditions. Thus, genetically engineered plants or biotech crops can contribute significantly both to sustainability and for the mitigation of the arduous challenges associated with possible climate change and global warming.**

***Keywords*:-- Arabidopsis thaliana, Salinity, RNA sequencing, Phytohormones, Secondary metabolites, Climate Change**

I. INTRODUCTION

Salt stress is one of the major abiotic stresses experienced by plants worldwide, affecting approximately 7% of the world's total land area (Shabala and Cuin, 2008; Tran and Mochida, 2010). Mild salt stress primarily affects plant development, agronomy traits and agricultural productivity, but extremely high salinity stress can lead to plant death. In addition, climate change and declining water quality are of great concern because they contribute to land degradation by causing high salinity levels in soil. Thus, salt stress has been considered an increasingly serious problem underscoring the importance of developing salt-tolerant plants, through the use of genetic engineering, which are capable of surviving under saline conditions (Sobhanian et al., 2011; Roy et al., 2011). Salt stress negatively impacts photosynthesis, energy production, lipid metabolism, nutrient acquisition, the integrity of cellular membranes and the activity of various enzymes, thereby leading to a number of destructive processes, such as water deficit, hyperosmotic stress, secondary oxidative stress, homeostasis disruption and ionic toxicity (Ashraf, 2009; Chen and Polle, 2010; Munns and Tester, 2008). This salinity stress results in the production of reactive oxygen species (ROS) and oxidative stress, arising from an imbalance in the generation and removal of reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), is a challenge faced by all aerobic organisms (Finkel and Holbrook, 2000). Although ROS were originally considered to be detrimental to cells, it is now widely recognized that redox regulation involving ROS is a key factor modulating cellular activities (Allen and Tresini, 2000; Dat et al., 2000). Increasing evidence indicates that H2O2 functions as a signaling molecule in

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

plants. H2O2 generation during the oxidative burst is one of the earliest cellular responses to salinity stress (Shafi et al., 2015a, b; 2017). There are several possible sources of H2O2 in plants, and a number of abiotic and biotic stress stimuli induce H2O2 generation and thereby oxidative stress. Superoxide dismutase (SOD) converts superoxide radical to H2O2, while ascorbate peroxidase (APX) catalyses conversion of H2O2 (Shafi et al., 2014; 2015a, b). Furthermore, the phenomenon of cross tolerance, in which exposure to one stress can induce tolerance to other stresses, is one in which H2O2 is likely to play a pivotal role (Bowler and Fluhr, 2000). It is already known that H2O2 can induce the expression of genes involved in antioxidant defense (Mullineaux et al., 2000). Identification of genes and proteins regulated by H2O2 is thus an important step toward treatments that might confer tolerance of multiple stresses. To cope with salinity stress, plants employs various mechanisms, at both the whole plant and cellular levels, which are controlled by a variety of genes and signaling pathways and are expressed and activated at different times during the life of a plant (Roy et al., 2011; Ashraf, 2009). With the availability of genomic sequences from various plant species and recent advances in sequencing technologies, genes associated with high salinity tolerance have been identified on a large scale at a genome-wide level (Tran and Mochida, 2010; Yao et al., 2011; Li et al., 2011). Together with other omic technologies, such as proteomics and metabolomics, transcriptomics has contributed significantly to the elucidation of stress responses (Manavalan et al., 2009; Jogaiah et al., 2012). Thus the present study was designed to study the effect of salinity stress on transcriptome of WT (wild type) and transgenic lines overexpressing Cu/Zn-SOD and APX. We have found that the transgenic lines were more tolerant to stress conditions and accumulated significantly higher biomass under stress conditions. Further, RNAseq analysis showed that phytohormones level was elevated in transgenic lines under salinity stress, which was validated with real time expression analysis.

III. RESULTS AND DISCUSSION

Transgenic plant growth and expression analyses of selected genes

Transgenic lines (S26, APX and 18O) and WT plants were grown in pots under lab conditions (Fig.1A). Total RNA was isolated from each line and cDNA synthesis was carried out for expression analysis, using 26S rRNA as internal control. Primers (Table1) specific for SOD and APX genes were used to check expression of these genes in transgenic lines (Fig. 1B). It was observed that SOD expression was seen in S26 and 18O line and APX in APX20 and 18O line, whereas no expression of SOD and APX was seen in WT, which indicates that genes are showing expression only in transgenic lines (Fig. 1B).

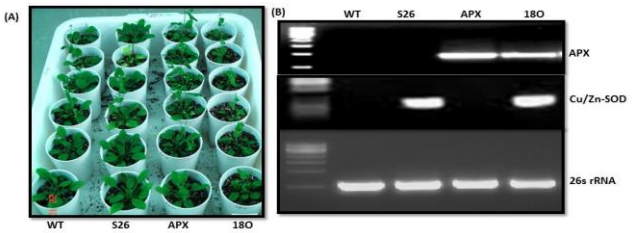

Fig.1. Arabidopsis plants growing on pots (A) and RT-PCR expression analysis of SOD and APX genes along with 26s rRNA as control (B).

Salt stress and its effect on SOD and APX enzyme activitiesEffect of salinity stress on plants can be seen and more noticeable effect was on WT under 100 and 150 mM stress (Fig.2A), as compared to transgenic lines (S26, APX and 18O) as they showed tolerance to stress. Total enzyme activities of SOD and APX were estimated in WT and transgenic samples collected at 1 and 24 h of salt stress (Fig.2B, C). Enzyme assays for total SOD and APX revealed that their activities increased with increase in magnitude of salt stress in WT and all the transgenic plants. Total enzyme activities increased gradually up to 100 mM NaCl and then decreased at 150 mM NaCl 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 (Fig. 2B, C). The increase in total SOD activity was 1.8 to 2 fold higher in PaSOD lines and 2.6 fold in dual transgenic lines as compared to WT (Fig. 2B) under 100 mM NaCl treatment (Fig. 2B). Nearly 2.5–4.3 fold increase in APX activity was observed in RaAPX lines and 1.7–1.8 fold in dual transgenic lines as compared to WT under 100 mM NaCl treatment. But the levels of APX activity were higher throughout the stress in transgenic plants, especially in APX where nearly 3 fold higher activity was recorded (Fig. 2C).

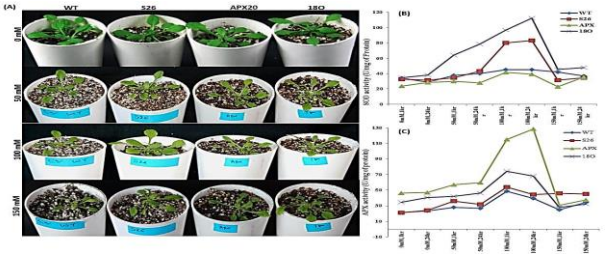

Fig.. 2. Arabidopsis WT and transgenic (S26, APX and 18O) plants growing under control (0 mM) and salinity stress (50, 100 and 150 mM) (A), .Biochemical analysis of

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

SOD (B) and APX (C) activity under control (0 mM) and salt stress (50, 100, 150 mM) after 1hr and 24hr of stress.

Effect of salt stress on H2O2 accumulation

H2O2 is now widely recognized as a key signalling molecule in all eukaryotes, including plants. Under control conditions, H2O2 content of WT and transgenic lines exhibited the same trend, while under salt stress conditions, enhanced H2O2 accumulation was observed in all transgenic lines with higher amounts detected in SOD (1.2–2.3 fold) and dual transgenic lines (1.1–2 fold) followed by APX (0.5–1.7 fold; Fig. 3). Under control conditions, WT and transgenic lines showed a very low H2O2 accumulation. While at 150 mM NaCl, H2O2 accumulation was found to be highest in WT followed by S26, APX20 and 18O (Fig. 3). The H2O2 accumulation at 50 and 100 mM NaCl, was found to be lower in transgenic lines than WT. Generation of H2O2 occurs under a diverse range of conditions, and it appears likely that H2O2 accumulation in specific tissues, and in the appropriate quantities, is of benefit to plants and can mediate cross tolerance toward other stresses (Bowler and Fluhr, 2000). H2O2 is intimately involved in plant defense responses, affecting both gene expression and the activation of proteins such as MAP kinases, which in turn function as regulators of transcription (Mittler et al., 1999; Kovtun et al., 2000).

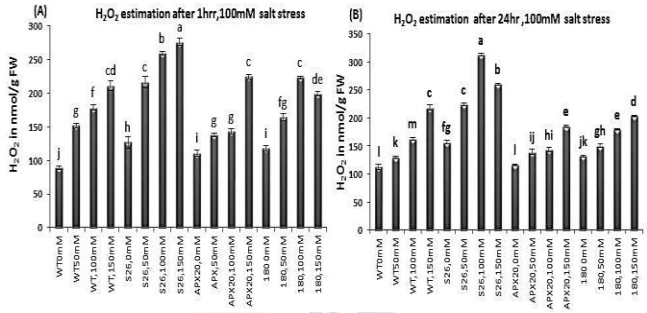

Fig. 3. Estimation of H2O2 in WT and transgenic lines (S26, APX20 and 18O) after 1h (A) and 24h (B) of salinity stress

Expression profiling of WT and transgenic plants using RNAseq under normal and salt stress conditions

Whole transcriptome profiling was done to determine which genes of phytohormone biosynthesis pathway are differentially expressed among three types of transgenics lines under different salt stress conditions. Based on co-expression analysis, phytohormone biosynthesis genes and certain candidate transcription factors were identified whose expression patterns were correlated with phytohormone production. Using high throughput sequencing on Illumina GAIIx (Fig. 4), a total of 495,692,298 reads were generated for all the 32 samples. The read quality score for all the samples was found to be >30 and after performing read filtering, a total of 387,748,946 reads were used for reference based assembly using TopHat and Cufflinks protocol. A total of 1,16,778 transcripts were obtained for whole transcriptome of 32 conditions. All the significant differentially expressed transcripts were identified in each comparative condition and GO enrichment analysis was performed using AgriGO (Fig.5). In condition I, among molecular processes, protein serine/threonine kinase activity (p value 3.96e−12) and in biological processes response to abiotic stimulus (6.48e−16), MAPKKK cascade (1.05e−26), regulation/ biosynthesis of H2O2 metabolic process (1.82e−15 to 0.00171) and positive regulation of flavonoid biosynthetic process (0.00267) were found to be highly enriched (Fig.5). In condition II, under molecular function, protein serine/threonine kinase activity (2.72e−10) and transcription factor activity (0.0171) were highly enriched (Fig.6). The most abundant TF families observed under stress condition were C3H (6–11 %), MADs (6–8 %), MYB-related (5–8 %), NAC (3–5 %), bHLH (4–6 %) and WRKY (2–4 %). However, bZIP (1–3 %), SNF2 (2−4 %) were also observed, but with relatively less abundance (Fig.6). It was found that in both the comparative conditions (condition I; 18O, 100 mM NaCl w.r.t. 0 mM at 24 h stress and condition II; 18O w.r.t. WT under 100 mM NaCl at 24 h stress), biological processes belonging to signalling, response to stimulus and the phytohormone pathways were highly enriched (Fig.7, 8).

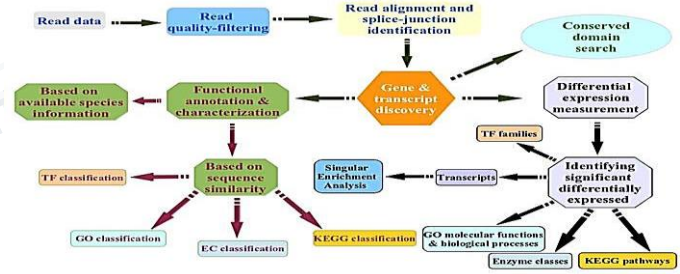

Fig.4. Workflow of developed pipe-line for transcriptome study.

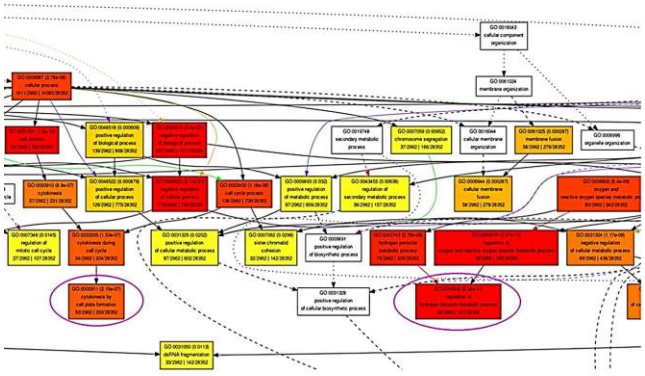

Fig.5. Enrichment analysis was performed using AgriGO.

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 12, December 2017

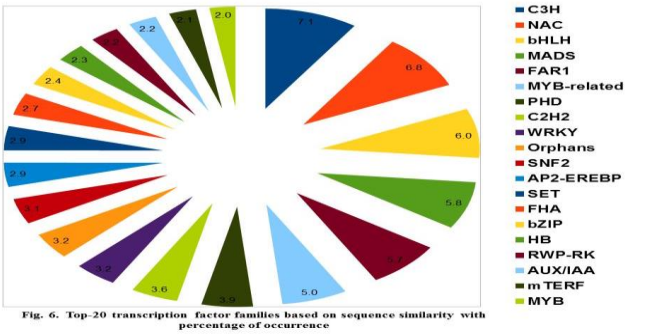

Fig. 6. Top-20 transcription factor families based on sequence similarity with percentage of occurrence

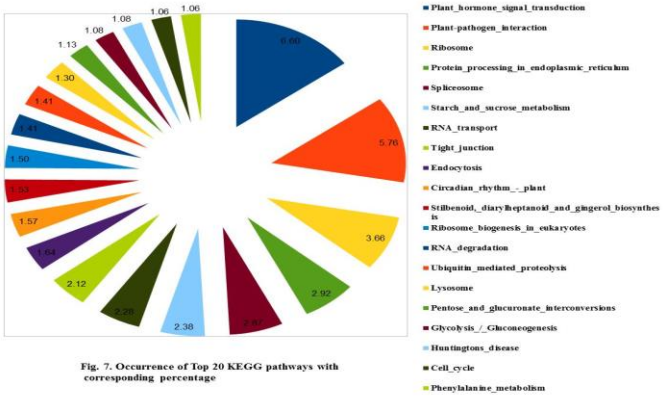

Fig. 7. Occurrence of Top 20 KEGG pathways with corresponding percentage

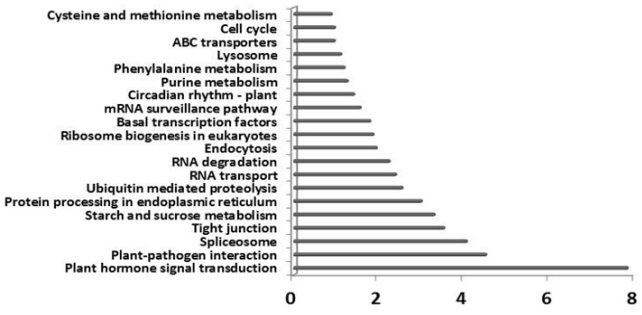

Fig.8. KEGG Pathway in Transgenic at 100mM stress

Effect of stress in phytohormone synthesis

To investigate the effect of overexpression of these antioxidant genes on phytohormones, it was intriguing to study the expression pattern of various genes associated with phytohormones biosynthesis. On the basis of gene IDs, 26 genes belonging to phytohormone biosynthesis pathway were identified and their FPKM based gene expression was validated using qPCR (Fig.). The data revealed that under control conditions (0 mM NaCl), only few genes showed upregulation in transgenic lines compared to WT. However, a major drift in expression pattern was observed after 24 h of 100 mM salt stress in 18O and S26 transgenic lines (Fig.) and less change was observed in APX20. Most of these genes involved in phytohormone biosynthesis exhibit upregulation in 18O line after 24 h of salt stress (Fig.). Phytohormones, including gibberellins (GA), cytokinins (CK), ethylene (ET) and jasmonic acids (JA), are involved in numerous developmental processes in plants. Various genes encoding transcription factors were induced by H2O2 suggesting that these transcription factors mediate further downstream H2O2 responses, and that several other genes are likely to be induced at later times. Some of the H2O2-sensitive genes could also be involved in plant hormone signaling. For example, a gene encoding a syntaxin was identified as H2O2 responsive by both microarray and RNA-blot analyses. Syntaxins are docking proteins involved in vesicle trafficking, and a role in the hormonal control of guard cell ion channels has been demonstrated for an ABA-inducible syntaxin in tobacco (Leyman et al., 1999). Because both elicitors and ABA induce H2O2 production in guard cells (Pei et al., 2000), it could be that induction of a syntaxin by H2O2 is involved in regulating guard cell functioning.

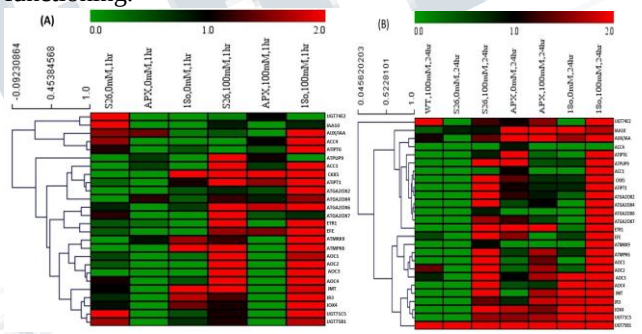

Fig. 5 Heat map showing differential expression of phytohorone biosynthesis genes under control and salt stress. Heat map represents relative expression ratio of each gene under control and salt stress treatment for 1 h (A) and 24 h (B) with respect to WT. Bar at the top indicates relative expression ratio whereby red, black and green colors represent upregulation, no change and downregulation, respectively

IV. CONCLUSION

Our data demonstrate that H2O2 can modulate the expression of a subset of genes belonging to phytohormones within the Arabidopsis genome. Furthermore, it is also clear from other studies that H2O2 can alter the activity of cellular proteins. The mechanisms by which these changes are effected remain to be elucidated. It is possible that in some cases H2O2 can interact directly with target proteins. In addition, it may be that plant cells contain redox sensors that detect and respond to signals such as H2O2.

V. ACKNOWELEDGEMETS

This work was supported by Grants from the Council of Scientific and Industrial Research (CSIR), New Delhi, India.

REFERENCE

[1] Ahuja PS, Kumar S. De novo sequencing and characterization of Picrorhiza kurrooa transcriptome at two temperatures showed major transcriptome adjustments. BMC Genom 13:126. (2012)

[2] Allen RG, Tresini M. Oxidative stress and gene regulation. Free Radic Biol Med 28: 463–499. (2000)

[3] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 215:403–410. (1990)

[4] Ashraf M. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnol Adv 27: 84–93. (2009)

[5] Bowler C, Fluhr R. The role of calcium and activated oxygens as signals for controlling cross-tolerance. Trends Plant Sci 5: 241–245. (2000)

[6] Bradford MM. 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)

[7] Chen S, Polle A. Salinity tolerance of Populus. Plant Biol (Stuttg) 12: 317–333. (2010)

[8] Czechowski T, Stitt M, Altman T, Udvardi MK, Scheible WR. Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. Plant Physiol 139:5–17. (2005)

[9] Dat J, Vandenbeele S, Vranova E, Van Montagu M, Inze D, Van Breusegm F. Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57: 779–795. (2000)

[10] Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 408: 239–247. (2000)

[11] Gahlan P, Singh HR, Shankar R, Sharma N, Kumari A, Chawla V, Ahuja PS, Kumar S (2012) De novo sequencing and characterization of Picrorhiza kurrooa transcriptome at two temperatures showed major transcriptome adjustments. BMC Genom 13:126.

[1] Ghawana S, Paul A, Kumar H, Kumar A, Singh H, Bhardwaj PK, Rani A, Singh RS, Raizada J, Singh K, Kumar S. An RNA isolation system for plant tissues rich in secondary metabolites. BMC Res Notes 4:85.

[2] Gill T, Kumar S, Ahuja PS, 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)

[3] Gill T, Sreenivasulu Y, Kumar S, Ahuja PS. Over-expression of superoxide dismutase exhibits lignification of vascular structures in Arabidopsis thaliana. J Plant Physiol 167:757–760. (2010b)

[4] Jogaiah S, Govind SR, Tran LS. System biology-based approaches towards understanding drought tolerance in food crops. Crit Rev Biotechnol;doi: 10.3109/07388551.2012.659174. (2012)

[5] Kovtun Y, Chiu W-L, Tena G, Sheen J. Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc Natl Acad Sci USA 97: 2940–2945. (2000)

[6] Leyman B, Geelen D, Qunitero FJ, Blatt MR. A tobacco syntaxin with a role in hormonal control of guard cell ion channels. Science 283: 537–540. (1999)

[7] Li D, Zhang Y, Hu X, Shen X, Ma L, et al. Transcriptional profiling of Medicago truncatula under salt stress identified a novel CBF transcription factor MtCBF4 that plays an important role in abiotic stress responses. BMC Plant Biol 11: 109. (2011)

[8] Manavalan LP, Guttikonda SK, Tran LS, Nguyen HT. Physiological and molecular approaches to improve drought resistance in soybean. Plant Cell Physiol 50: 1260–1276. (2009)

[9] Mittler R, Herr EH, Orvar BL, Van Camp W, Willekens H, Inze D, Ellis BE. Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hyperresponsive to pathogen infection. Proc Natl Acad Sci USA 96: 14165–14170. (1999)

[10] Mullineaux P, Ball L, Escobar C, Karpinska B, Creissen G, Karpinski S. Are diverse signalling pathways integrated in the regulation of Arabidopsis antioxidant defense gene expression in response to excess excitation energy? Philos Trans R Soc Lond 355: 1531–1540. (2000)

[11] Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol 59: 651–681. (2008)

[12] Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plantarum 15:473–497. (1962)

[13] Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate- specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880. (1981)

[14] Pei Z-M, Murata Y, Benning G, Thomine S, Klusener B, Allen GJ, Grill E, Schroeder JI. Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature 406: 731–734. (2000)

[15] Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 30:36. (2002)

[16] Roy SJ, Tucker EJ, Tester M Genetic analysis of abiotic stress tolerance in crops. Curr Opin Plant Biol 14: 232–239. (2011)

[17] Rozen S, Skaletsky H Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365–386. (2000)

[18] Schmid R, Blaxter ML annot8r: GO, EC and KEGG annotation of EST datasets. BMC Bioinformatics 9:180. (2008)

[19] Shabala S, Cuin TA Potassium transport and plant salt tolerance. Physiol Plant 133: 651–669. (2008)

[20] Shafi A, Chauhan R, Gill T, Swarnkar MK, Sreenivasulu Y, Kumar S, Kumar N, Shankar R, Ahuja PS and Singh AK. 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. (2015b)

[21] Shafi A, Dogra V, Gill T, Ahuja PS and Sreenivasulu Y. Simultaneous over-expression of PaSOD and RaAPX in transgenic Arabidopsis thaliana confers cold stress tolerance through increase in vascular lignifications. PLoS One, 9:e110302. (2014)

[22] Shafi A, Gill T, Sreenivasulu Y, Kumar S, Ahuja PS and Singh AK. Improved callus induction, shoot regeneration, and salt stress tolerance in Arabidopsis overexpressing superoxide dismutase from Potentilla atrosanguinea. Protoplasma, 252 41–51. (2015a)

[23] Shafi A, Pal AK, Sharma V, Kalia S, Kumar S, Ahuja PS and Singh AK. Transgenic Potato Plants Overexpressing SOD and APX Exhibit Enhanced Lignification and Starch Biosynthesis with Improved Salt Stress Tolerance. Plant Mol. Biol. Report. 35:504–518. (2017)

[24] Sobhanian H, Aghaei K, Komatsu S Changes in the plant proteome resulting from salt stress: Toward the creation of salt-tolerant crops? J Proteomics 74: 1323–1337. (2011)

[25] Tran LS, Mochida K Functional genomics of soybean for improvement of productivity in adverse conditions. Funct Integr Genomics 10: 447–462. (2010)

[26] Yao D, Zhang X, Zhao X, Liu C, Wang C, et al. Transcriptome analysis reveals salt-stress-regulated biological processes and key pathways in roots of cotton (Gossypium hirsutum L.). Genomics 98: 47–55. (2011)