Review Article

Molecular Approach towards the Understanding of Defensive Systems against Oxidative Stress in Plant: A Critical Review

R. S. Pal*, P. K. Agrawal, J. C Bhatt
Division of Crop Improvement, Vivekananda Parvatiya Krishi Anusandhan Sansthan (Indian Council of Agricultural Research) Almora 263601, Uttarakhand, India.
***Corresponding author's E-mail:** ramesh_bio2006@rediffmail.com

Accepted on: 22-07-2013; **Finalized on:** 30-09-2013.

ABSTRACT

Scientific interest in oxidative stress has grown considerably over the last two decades. Studies have demonstrated that several reactive oxygen species (ROI) such as superoxide radical, hydrogen peroxide, and hydroxyl radicals, participate in the cause of oxidative stress under different environmental conditions. Plants have defense mechanisms comprising mainly enzymatic defense, which removes the intermediates of dioxygen reduction, has received much attention. Enzymes like ascorbate peroxidase, glutathione reductase, superoxide dismutase, catalase, are key enzymes and various studies on the activities/induction/expression of these enzymes during a range of stresses indicate the importance of ROI scavenging components in tolerance to the oxidative stress being imposed upon by $O_2^{\cdot-}$. The interpretation of these studies is often difficult due to the complex stress responses in plants. This review deals with the use of gene transfer technology for the ability to modify the levels of different components in single genetic strain(s) without using any chemical agents or stress treatments.

Keywords: Oxidative stress, reactive oxygen species, Genetic Engineering, SOD.

INTRODUCTION

Plants are continuously exposed to environmental cues such as varying temperature, solar radiations and unpredictable limitations of availability of water or nutrients. The cues would make plants to adopt adaptive strategies at different level of organization to survive and complete its life cycle. One of the consequences of the stressful environment is the activation of paramagnetic oxygen leading to the generation of superoxide radical and hydrogen peroxide, which react with each other to produce highly reactive hydroxyl radical. These reactive oxygen intermediates (ROI) react with proteins, nucleic acids, fatty acids and virtually with all other organic compounds causing colossal damage to the cell and consequently, to the plant. Oxygen is activated by many diverse cellular reactions; chloroplasts and mitochondria being the major site[1]. Plants have defense mechanisms comprising of ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and other peroxidases to scavenge ROI and hence protecting the plant. However, when the rate of production of ROI exceeds the rate of scavenging, damage occurs and the plant is said to be under oxidative stress. Under stressful environment the stability, activity, and sensitivity of these enzymes would determine the adaptation conferred to the plant[2,3]. SOD is the key enzyme catalyzing dismutation of super oxide anion ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) and oxygen (O_2) 10^4 times faster than the spontaneous dismutation reaction[4]. In plants, the role of SOD during environmental adversity has received much attention since ROI have been found to be produced during many stress situations. Several reports have shown that under stressful environments, such as in drought, low temperature, there is an increase in SOD activity with concomitant appearance of new isozymes of SOD [5]. It was shown that SOD orchestrating with APX and GR are critical to lower oxidative stress induced damage and improve plant growth and development[6-8]. Further, working with high altitude flora *Potentilla atrosanguinea* (potentilla) a novel Cu/Zn-superoxide dismutase (Cu/Zn-SOD) enzyme was discovered that can be autoclaved and can function at temperature ranging from <-10 ºC to >60 ºC. Its gene was cloned and a heterologous expression system was developed for production of potential SOD in *E. coli*[5]. Using this gene transgenic plants of arabidopsis and potato have been developed. Transgenic plants under stress conditions performed much better as compared to the non-transgenic control. Thus one of the approaches to improve drought tolerance would be to mitigate the production of ROI such as superoxide radical, hydrogen peroxide, and hydroxyl radicals. In fact, exposure of plants to abiotic stresses such as drought causes oxidative stress in which increased production of ROI is evident[9,7]. Studies have shown that over-expression of ROI scavenging enzymes had marked effect on yield enhancement[10 - 12].

Conditions Leading to Oxidative Stress

Transformation of life from O_2 free reducing atmosphere to O_2 rich oxidizing atmosphere, some of 2450 million years ago, necessitated modulation in the metabolism to account for the change[13]. While O_2 provided advantage to aerobic organisms in terms of high-energy production and better substrate utilization, generation of toxic species of O_2, known as ROI, was inevitable. ROI are produced at almost all the sites where O_2 is involved in metabolic processes[14]. The major sites of ROI production in plants

include chloroplasts, mitochondria, endoplasmic reticulum, microbodies, cell wall and plasma membranes. ROI are fatal to cells. Recently, these are believed to act as signaling molecules as well. Toxicity of ROI is conferred due to their capability to cause lipid peroxidation, protein denaturation, DNA mutation and virtually oxidizing any biomolecule present in the cell. Therefore, the fitness of aerobic organisms depends upon their ability to maintain the balance between the production and scavenging of ROI. This delicate balance is well maintained by the plants under the optimal growth conditions. However, non-optimal growth conditions shift the balance towards their increased accumulation forcing the system to undergo oxidative stress[15]. Recent literature indicates the involvement of ROI in several important reactions during different stages of growth and development in plants. ROI play important roles in the plant's defence system against pathogens[16,17], mark certain developmental stages such as tracheary element formation, lignification and other cross-linking processes in the cell wall[18,19] and act as intermediate signaling molecules to regulate the expression of genes[20]. On the other hand, ROI are extremely reactive and react virtually with all the organic molecules present in the cell. Although, ROI are produced even during the normal metabolic reactions, their production increases enormously under certain physiological conditions that are responsible for causing stress. Environmental conditions such as exposures to high light intensities, UV irradiation, extremes of temperature, drought, flooding, heavy metals, high salt concentration, air pollutants including ozone, herbicides, mechanical and physical stress and biotic factors like invasion by various pathogens are responsible for imposition of oxidative stress in plants[9]. Protective enzymes and antioxidants are constitutively present in leaves. However, the capacity of this system is not constant but responds to intrinsic as well as exogenous factors. Several types of environmental and physiological stresses have been found to reduce or enhance the activities of various ROI scavenging systems in both prokaryotes and eukaryotes. Much variation was however, observed in the expression of these in relation to the type/severity of stresses, the species type and the type/physiological status of tissues[21]. Detectable increase in total ascorbate, APX and GR activity was observed in pea plants grown in winter[22]. However, significantly higher amounts of ascorbate, APX, GR and dehydroascorbate reductase (DHAR) were found at high light intensities in the same. Jahnke *et al*.[23] observed low specific activities at 5ºC of SOD, GR, APX and DHAR in sensitive variety of maize. SOD and APX activity were double in tolerant variety. High light stress when combined with low temperatures enhanced the activities of ROI scavenging enzymes except CAT[24]. A large number of studies implicate the role of various components of ROI scavenging system during various stressful physiological conditions.

Defence Systems against Oxidative Stresses

The defence systems of plant cell are not restricted to the intracellular compartments but are also found in the apoplast to a limited extent. Antioxidative defence in plants consists of enzymatic as well as non-enzymatic constituents. Non-enzymatic components are generally small molecules including ascorbate, glutathione, carotenoids and lipid soluble α-tocopherol. The non-enzymatic machinery largely restrict ROI dependent chain reaction by capturing the ROI. The enzymatic defence includes SOD, APX and other peroxidases, CAT and the enzymes involved in the synthesis and regeneration of the reduced forms of the antioxidants[25]. In spite of having an array of antioxidants, the first and best defence for the plants is avoidance. This is achieved by cytochrome oxidase and similar enzymes, which by dint of several metal prosthetic groups; carry out the tetravalent reduction of the dioxygen to water without releasing intermediates. Cytochrome oxidase is responsible for most of the dioxygen reduction in respiring cells and thus markedly decreases the cell burden of O_2^- and H_2O_2.

Enzymatic Defence

Enzymatic defence, which removes the intermediates of dioxygen reduction, has received much attention. SOD dismutates O_2^- into H_2O_2 plus O_2; the catalases catalyses conversion of H_2O_2 into H_2O and O_2; and the peroxidases utilize a variety of electron donors to reduce H_2O_2 into H_2O. SOD is differentiated by the type of metal cofactor as, Cu/Zn, Mn and Fe-SOD. There are catalases based upon heme and others, which contain Mn (III) at their active sites. Peroxidases also occur in great variety[26], some utilizing aromatic amines and phenols as the electron donor, while the other utilizes NADPH, glutathione, and even halides.

Glutathione and Glutathione Reductase

Numerous studies have shown that foliar Glutathione (GSH) contents increase in winter[27,18]. Therefore, it has been suspected that GSH plays a role in protecting against freezing injury [29]. However, maximum content of GSH is found in young needles during emergence of the plant [30,31] when they are particularly freezing-sensitive[32]. In herbaceous species with artificially enhanced GSH contents, elevated GSH concentrations neither protected leaves from freezing injury nor provided enhanced cold tolerance[33]. These observations do not support a role of GSH as a cryoprotectant *per se*. Correlative evidence suggests, however, that GSH is involved in protection of plants from photooxidative stress in winter as a substrate of glutathione reductase[30]. Cold-hardening and enhanced cold tolerance were generally accompanied by significant increases in glutathione reductase activities in herbaceous and coniferous plants[34,35]. In peas, cold treatment resulted in about 2-fold increase in glutathione reductase activity, but not in mRNA levels, suggesting that the glutathione reductase present in cytoplasmic tissues get activated due to cold treatments[36]. These results

emphasize that glutathione reductase activity plays a central role in mediating resistance from cold-induced photooxidative stress.

Ascorbate and Ascorbate Peroxidase

Increases in ascorbate content and ascorbate peroxidase activity have been observed in evergreens in winter like in needles of eastern white pine[37]. In contrast, dehydroascorbate reductase activity was lower in spruce needles in winter than in summer, suggesting that this enzyme may not be involved in frost tolerance. In spruce needles in winter, monodehydroascorbate reductase activity was about two times higher than in light-protected dormant buds[38]. In herbaceous plants such as maize and spinach, and in foliar buds of beech, monodehydroascorbate radical reductase activity seemed to be more important for the maintenance of the ascorbate pool under cold conditions than dehydroascorbate reductase[39,23]. Taken together with the observation that glutathione reductase activity also increases in winter, it appears that leaves have an increased need for the regeneration of antioxidants at low temperatures.

Superoxide Dismutase

Accumulating data on correlative and experimental studies have indicated that SOD's play a significant role(s) in protecting organisms against oxidative stress. Its expression under various physiological conditions makes it a stress responsive enzyme in the true sense. The response of SOD in lower organism and yeast depend much on the growth conditions than on the environmental factors. Little is known about the regulation of Cu/Zn-SODs in prokaryotes; however, Cu/Zn-SODs in *E. coli* are induced upon entry into stationary phase[40]. Expression of Fe-SODs in *E. coli* is generally unaffected by a variety of conditions including oxidative stresses. However, Mn-SOD production in microorganism is modulated in a variety of environmental stimuli such as presence of oxygen, where it is induced. Campbell and Laudenbach[41] analyzed the response of SOD genes in cynobacterium *P. boryanum* to the environmental conditions and found that the cytosolic protein is produced only in stress conditions, including iron and nitrate starvation and oxidative stress. Other environmental conditions such as heat shock and factors such as metals, pH and DNA topology also influences SOD expression in microorganisms[42]. In yeast, *S. cerevisae*, induction of Mn-SOD was found by pretreatment with 8% ethanol or by low-level heat shock[43]. However, little induction of SOD by menadione, a dye that generates $O_2^{\cdot-}$, was observed by Jamieson *et al*.[44] in yeast. It was however, attributed to high constitutive level of expression of Cu/Zn-SOD. Induction of Mn-SOD to high oxygen status (Autor[45]) and paraquat (Pinkham[46]) has also been reported. The activity of Cu/Zn-SOD in yeast is always present at a fairly high level and is further induced by various conditions, including but not limited to aerobic growth, high oxygen, drug induced oxidative stress, copper ions, growth on respiratory carbon sources and entry into stationary phase[47]. A concept of cross tolerance has conceptualized in which tolerance to certain environmental stresses can clearly arise by several possible mechanisms, each likely to involve pleiotropic effects, and a biotype tolerant to one condition can also be tolerant to others[48]. This was first observed in *C. ellipsoidea* where sublethal concentration of paraquat (which induces Mn-SOD activity) can decrease the injury resulting from chilling mediated photoinhibition[49]. The diurnal expression of chloroplastic SOD in light and dark was not affected much, but when the plants were kept in three days dark prior to illumination, dramatic increase in the Fe-SOD mRNA level was observed[50]. When 3-(3,4-dichloro phenyl)-1, 1- dimethylurea (DCMU) was used to block the $O_2^{\cdot-}$ production in PS II, this induction could not be obtained[51], thereby suggesting that the induction in the levels of SOD is not the direct response to light but to the increased $O_2^{\cdot-}$ formation. The exposures to UV-B, which is common in light conditions, also enhanced the total SOD activities in *A. thaliana*[52].

Low temperature induces SOD activity in *A. thaliana* of which Cu/Zn-SOD formed the major chunk during early stages whereas; Mn-SOD was more during the senescent stages[53]. In another study, Abarca *et al*.[54]showed that mild stress treatments of low temperatures and high light increases mRNA levels of chloroplastic Cu/Zn-SOD, *Csd2*, up to the growth period of 48 h. The complementary increase in the activities of chloroplastic Cu/Zn-SOD was recorded up to 40%. Increase in the levels of SOD activity was observed in roots of wheat during exposures to low temperature followed by cold acclimation, whereas, leaves showed decrease in the SOD activity[55]. Studies on the differentially chilling sensitive genotypes in maize[56,57] showed that the tolerant cultivar had better management of SOD activity than the susceptible cultivar. Chilling stress enhances SOD activities in a number of other plants like cucumber[58], maize[59], potato[3] and rice[60]. These results thus indicate that the inhibition of photosynthesis and the occurrence of photooxidative damage due to chilling stress in light may largely depend on the oxy-radical scavenging capacities of the plant. A large number of other conditions also lead to induction in the activity of SOD. These include anoxia[61], water stress[62], paraquat[63], NaCl[64], ozone[65], fungal infection[66], ethylene[67], ABA and high osmoticum[68], salicylic acid[69], H_2O_2[70] and SO_2[71]. Sharma *et al*.[38] correlated different environmental conditions such as drought, chilling, anoxia and pathogenic injury with SOD activity levels.

Genetic Engineering of Oxidative Stress

The corroborative studies on the activities/induction/ expression of SOD during a range of stresses indicate the importance of ROI scavenging components in tolerance to the oxidative stress being imposed upon by $O_2^{\cdot-}$. However, these are only correlative studies and indicative to the importance of scavenging system. The interpretation of these studies is often difficult due to the

complex stress responses in plants. Therefore, the use of gene transfer technology provides the ability to modify the levels of different components in single genetic strains without using chemical agents or stress treatments.

Overexpression of Superoxide Dismutase

Of all the ROI scavenging components, SOD attracted maximum attention in development of transgenics. This is perhaps for its central role in the involvement of $O_2^{\cdot-}$ and H_2O_2. Allen[2] predicted yield enhancement due to the expression of antioxidant genes as it is speculated that frequent, mild oxidative stresses occur in the field situation and that these stresses inhibit photosynthesis and, therefore, yield meaning that SOD transgenic plants should improve the yield. Samis *et al*.[12] found that single transgene of either mit Mn-SOD or chl Mn-SOD resulted in greater plant and storage organ biomass. Southern analysis revealed that each of the parents had single insertion regions of the Mn-SOD cDNA and the inheritance followed the expected Mendelian ratios. Fe-SOD overexpression reduced secondary injury symptoms and thereby, enhanced recovery from the stresses experienced during winter stress[73]. Van Breusegem *et al*.[74] were able to get significant reduction in foliar damage in maize with overproduction of tobacco Mn-SOD when exposed to low temperature. Transgenic SOD activity contributed to 20% of the total SOD activity in the leaves. To examine the relationship between oxidative and freezing stress, Mckersie *et al*.[75] produced transgenic alfalfa with Mn-SOD cDNA from tobacco with mitochondria and chloroplast directing transit peptide. The analysis of transformants showed that they had enhanced SOD activity, increased tolerance to acifluorfen and increased regrowth after freezing stress. The plants with functional Mn-SOD transgene had more rapid regrowth following freezing stress than those lacking functional Mn-SOD transgene suggesting the protective role of Mn-SOD after freezing stress. These plants were also found to be tolerant to the injuries caused by the water deficit stress as determined by chlorophyll fluorescence, electrolyte leakage and regrowth from crowns. Three-year field trials of the transgenic plants supported the importance of SOD for adaptation to stress environment by virtue of increased yield and survival of transgenic plants[76]. Van Camp *et al*.[77] earlier found enhancement of oxidative stress in tobacco plants that overexpress Fe-SOD in choloplasts. The cDNA coding for Fe-SOD was taken from *Arabidopsis thaliana*. Fe-SOD overproduced plants do not confer tolerance to chilling induced photoinhibition or to salt stress at the whole plant level. The induction of water-soluble chloroplastic APX was promoted like in non-transgenic plants, whereas, induction of cytosolic and chloroplastic Cu/Zn-SOD was suppressed unlike in transgenic where it is shown to have 2-3 fold increase in activity. This difference was correlated to the difference in the membrane affinities of transgenic Fe-SOD and Mn-SOD. Earlier, two tomato cDNA's for cytosolic and chloroplast located Cu/Zn-SOD were cloned and potato tuber discs were infected using *Agrobacterium* strains[78]. Lines of both chloroplastic and cytosolic Cu/Zn-SOD harbouring transgenic plants showed significantly higher tolerance to oxidative stress than control. However, the response is tissue specific as chloroplastic Cu/Zn-SOD lines were more tolerant than cytosolic Cu/Zn-SOD and roots of cytosolic Cu/Zn-SOD were more tolerant than chloroplastic Cu/Zn-SOD. Transgenic tobacco plants, those expressed a chimeric gene encoding for chloroplast localized Cu/Zn-SOD from pea were prepared by Sen Gupta *et al*[79]. These plants were subjected to chilling temperatures with moderate and high light intensities. During moderate stress conditions, transformed plants showed greater resistance to degradation in rates of photosynthesis than control. In case of severe stress the rates of photosynthesis in control and transformed plants dropped to almost the same level. However, recovery of the transgenic plants was higher than untransformed ones. Increase in protein and mRNA levels of APX, in chloroplast located Cu/Zn-SOD overexpressing plants, were observed by Sen Gupta *et al*[80]. They could not find any increase in dehydroascorbate reductase and glutathione reductase specific activities of transgenic and control plants. It was thus inferred that enhancement in tolerance of oxidative stress as measured by the rates of photosynthesis in transgenic plants was not only from increased SOD levels, but from the combined increase in SOD and APX activity. Bowler *et al*.[9] for the first time demonstrated that transgenic plants with elevated levels of SOD could have significantly increased protection from oxidative stress. They developed transgenic tobacco plants that expressed a chimeric Mn-SOD directed towards chloroplast. The leaves of transgenic plants showed reduced levels of membrane damage following exposure to UV and light. Total Mn-SOD activity of these plants was estimated to be between 1.5 and 2 fold higher than in untransformed. Increased level of SOD also showed tolerance to other stresses including heavy metal toxicity[81] and salt stresses[82]. Apart from these successes, a large number of variations were also observed in the expression of the transgene of SOD in plants. Samis *et al*.[12] found that when two elite selected plants were hybridized, progenies containing both transgenes (mit Mn-SOD and chl Mn-SOD) had lower shoot and storage organ biomass compared to siblings having only one or the other transgene. McKersie *et al*.[10] had earlier found no significant difference in the winter survival of the transgenic alfalfa that was only 1°C more freezing tolerant than control. The primary freezing injury was similar in chloroplastic-targeted Mn-SOD overexpressing and the control plants. Transgenic tobacco plants that expressed high levels of petunia chloroplastic Cu/Zn-SOD were reported by Tepperman and Dunsmuir[83]. In addition transgenic tomato plants that expressed the same petunia Cu/Zn-SOD gene were found to be equally sensitive to photooxidative stress as untransformed control plants. This is perhaps for the reason that the alteration of SOD activity has a number of other possible

physiological effects since the product of the SOD reaction is H_2O_2. The ratio of $O_2^{\cdot-}$ and H_2O_2 were key in determining the cellular damage mediated by ROI. SOD overproduction shifts the balance towards H_2O_2 production, which is then eliminated using light dependent H_2O_2 scavenging systems in the chloroplasts. The sensitivity of Cu/Zn-SOD to the H_2O_2 produced, was also cited to be the reason for inactivation of the transgene. Also, the visibility of effects only on the high levels of Mn-SOD overproduction by Bowler *et al*.[9] is supported by shifting of reaction in dark almost completely over to H_2O_2, thus, ensuring little $O_2^{\cdot-}$ is available for $^{\cdot}OH$ production. However, low levels of the Mn-SOD overexpression merely serve to upset the normally optimized balance between $O_2^{\cdot-}$ and H_2O_2, thereby, leading to the formation of $^{\cdot}OH$ and consequently increase in the damage.

Since, different methodologies were adopted by various research groups in developing and analyzing transgenic plants, therefore, the detailed comparisons in transgenic plants is difficult to make. However, elevated levels of SOD in a variety of cellular compartments lead to detectable increase in cellular protection from oxidative stresses. It is clear that SOD can be manipulated to give resistance to oxidative stresses although there is clearly a fine line between benefit and injury. This depends upon the $O_2^{\cdot-}$: H_2O_2 ratio, which in fact is affected by the type of SOD used, site of overproduction, level of overproduction and the endogenous scavenging systems of the organisms.

Overexpression of Ascorbate Peroxidase

APX is a primary H_2O_2 scavenging enzyme in the cytosolic and chloroplastic compartments of the plant cells. Its presence in the glycosomal membranes indicates that it may also augment catalase activity. Overexpression of cytosolic and chloroplastic APX could provide increased protection compared with untransformed control plants. The increase in enzymatic activity was found to be three fold in cytosolic APX overexpressing plants whereas; it was 16 fold in chloroplastic APX overexpressing plants. The levels of protection in both lines were comparable to the plants that express chloroplastic Mn SOD. It was also reported that these plants have higher tolerance to the photooxidative stresses. Photosynthesis of 55% of the transformed plants with both cytosolic and chloroplastic APX returned to the pre-stress rates unlike only 35% of the control plants[84,85]. Earlier, the same group could not obtain any significant increase in protection from UV mediated membrane damage in chloroplastic overexpressing plants. The use of antisense technology in the development of the transgenic plants further elucidated the role of APX in oxidative stress tolerance [86].

Overexpression of Glutathione Reductase

Of the enzymes that maintain the ascorbate and glutathione pools in a reduced state, Glutathione Reductase (GR) has been most extensively studied. Initially, the effect of overexpression of bacterial GR was studied on the oxidative stress tolerance in tobacco. Aono *et al*.[87] found 3.5 fold increases in extractable GR activity and leaves of these plants were reported to have reduced visible damage after exposure to UV but no increase in ozone tolerance. These transgenic plants contained reduced levels of ascorbate after UV exposure than control plants. Visible foliar damage to the plants in which foreign GR was transmitted to chloroplast was lower to both, UV and sulfur dioxide, in the presence of light[88]. However no such results were obtained in case of ozone. A causal relationship between the magnitude of glutathione reductase activity and resistance to low temperature was demonstrated by Foyer *et al*.[89]. Photosynthesis in transgenic poplar plants overexpressing glutathione reductase in the chloroplasts was found to be protected from temperature stress as compared to the wild type. Transgenic poplar that expressed chloroplast targeted *E. Coli* GR was found to have GR activities 500 times higher than the untransformed plants. No difference was found in the inhibition of CO_2 assimilation induced by UV, however, they were found to be more resistant to photoinhibition caused by high light intensity and chilling temperature. Transgenic tobacco plants that expressed pea GR showed variable stress tolerance phenotypes. There were differences in these plants showing tolerance to damage caused by ozone and UV. Also, no correlation was found in the level of GR expression and the level of protection. Aono *et al*.[90] found decreased expression of GR activity in transgenic tobacco plants. Allen *et al*.[91] also identified several plants whose GR activity levels were reduced to 10-20%. Sense suppression was believed to be the reason for this decrease in activity.

CONCLUSION

Depending on the nature of the ROS species, some are highly toxic and rapidly detoxified by various cellular enzymatic and nonenzymatic mechanisms. Whereas plants are surfeited with mechanisms to combat increased ROS levels during abiotic stress conditions, in other circumstances plants appear to purposefully generate ROS as signaling molecules to control various processes including pathogen defense, programmed cell death, and stomatal behavior. The present article is an attempt where critical examination of various mechanisms of ROS described under biotic and abiotic stress conditions is being reviewed. Enzymes like ascorbate peroxidase, glutathione reductase, superoxide dismutase, catalase, are key enzymes and various studies on the activities/induction/expression of these enzymes during a range of stresses indicate the importance of ROI scavenging components. Since, different methodologies were adopted by various research groups in developing and analyzing transgenic plants. Studies have shown that majority of transgenic plants expressing proteins of unknown function and showing enhanced tolerance to oxidative stress; therefore, the detailed comparisons and inferences from transgenic plants is difficult to make. However, elevated levels of these enzymes in a variety of

cellular compartments leads to detectable increase in cellular protection from oxidative stress. Considering recent ROS-induced genome-wide expression analyses, the possible functions and mechanisms for ROS sensing and signaling in plants are compared with those in animals and yeast. Oxidative Stress is a serious problem of aerobic metabolism. However it cannot be avoided fully and is constantly attracting researchers to identify all possible approaches to find the most suitable cure, which will eventually help in understanding newer mechanisms and targets. This article is an attempt to review such targets available in the literature till date and to understand the developments for a meaningful application in crop improvement.

REFERENCES

1. Foyer CH, Descourvie'res P, Kunert KJ, Protection against oxygen radicals: an important defence mechanism studied in transgenic plants, Plant, Cell & Environment, 17, 1994507-523.
2. Allen .D, Dissection of oxidative stress tolerance using transgenic plants, Plant Physiology, 107, 1995, 1049-1054.
3. Seppänen MM, Fagerstedt K, The role of superoxide dismutase activity in response to cold acclimation in potato, Physiologia Plantarum, 108, 2000, 279-285.
4. Fridovich I, Quantitative aspects of the production of superoxide anion radical by milk xanthine oxidase, Journal of Biological Chemistry, 245, 1970, 4053-4057.
5. Sahoo R, Kumar S, Ahuja PS, Induction of new isozyme of superoxide dismutase at low temperature in *Potentilla astrisanguinea* Lodd. Var. argyrophylla (wall. Ex. Lehm) Griers, Journal of Plant Physiology, 158**,** 2001, 1093-1097.
6. Vyas D, Kumar S, Ahuja PS, Tea (*Camellia sinensis*) clones with shorter periods of winter dormancy exhibit lower accumulation of reactive oxygen species, Tree Physiology, 27, 2007, 1253-1259.
7. 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 Physiology and Biochemistry, 43, 2005a, 383-388.
8. Vyas D, Kumar S, Purification and partial characterization of a low temperature responsive Mn-SOD from tea (*Camellia sinensis* (L.) O. Kuntze). Biochemical and Biophysical Research Communications, 329, 2005b, 831-838.
9. Bowler, C., Slooten, L., Vandenbranden, S., Rycke, R.D., Botterman, J., Sybesma, C., Montagu, M.V. and Inze, D., Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants, EMBO Journal, 10, 1991, 1723-1732.
10. McKersie BD, Bowley SR, Murnaghan, J Jones KS, Winter survival of transgenic alfalfa overexpressing superoxide dismutase, Plant Physiology, 119, 1999, 839-847.
11. Pan SM, Hwang GB, Liu H C, Overexpression of rice Cu/Zn SOD in E.Coli. Botanical Bulletin of Academia Sinica, 40, 1999, 275-281.
12. Samis K, Bowley S, McKersie BD, Pyramiding Mn-superoxide dismutase transgenes to improve persistence and biomass production in alfalfa, Journal of Exerimental Botany, 53, 2002, 1343-135.
13. Pin C, Gonzalo D, Fernando G, Juan AO, Effect of Modified Atmosphere Composition on the Metabolism of Glucose by *Brochothrix thermosphacta,* Applied and Environmental Microbiology, 68, 2002, 4441-444.
14. Elstner EF, Mechanisms of oxygen activation in different compartments of plant cells In: *Active oxygen/oxidative stress and plant metabolism* (E.J. Pell and K.L. Steffen, Eds.). Amer. Soc. Plant Physiol. Rockville, M.D., 1991, pp. 13-25.
15. Gambarova NG, Gins MS, Characteristics of oxidative stress of plants with C3 and C4 photosynthesis during salinization, Russian Agricultural Sciences, 2008, 2, 7-10
16. Alvarez ME, Lamb C, Oxidative burst-mediated defense responses in plant disease resistance. In: *Oxidative stress and the molecular biology of antioxidant defenses* (J.G. Scandalios, Ed.). Cold Spring Harbour Laboratory Press, 1997, pp. 815-839.
17. Bolwell GP, Bindschedler LV, Blee KA, Butt VS, Davies DR, Gardner SL, Gerrish, C, Minibayeva F, The apoplastic oxidative burst in response to biotic stress in plants: A three-component system. Journal of Exerimental Botany, 53, 2002, 1367-1376.
18. Jacobson MD, Reactive oxygen species and programmed cell death. Trends in Biochemical Sciences, 21, 1996, 83-86.
19. Fath A, Bethke P, Belligni V, Jones R, Active oxygen and cell death in cereal aleurone cells, Journal of Exerimental Botany, 53, 2002, 1273-1282.
20. Vranova E, Inzé D, Van Breusegem F, Signal transduction during oxidative stress. Journal of Exerimental Botany, 53, 2002, 1227-1236.
21. Chaudhary B, Hovav R, Flagel L, Mittler R, Wendel JF, Parallel expression evolution of oxidative stress-related genes in fiber from wild and domesticated diploid and polyploidy cotton (*Gossypium*), BMC Genomics, 10, 2009, 278-390.
22. Gillham D, Dodge AD, Chloroplast superoxide and hydrogen peroxide scavenging systems from pea leaves: seasonal variations, Plant Science, 50, 1987, 105-109.
23. Jahnke LS, Hull MR, Long SP, Chilling stress and oxygen metabolizing enzymes in *Zea mays* and *Zea diploperennis*, Plant Cell Environment, 14, 1991, 97-104.
24. Mishra PN, Mishra KR, Singhal SG, Changes in the activities of anti-oxidant enzymes during exposure of intact wheat leaves to strong visible light at different temperature in the presence of protein synthesis inhibitors, Plant Physiology, 102, 1993, 903-910.
25. Inzé D, Van Montagu M, Oxidative stress in plants, Current Opinion in Biotechnology, 6, 1995, 153-158.
26. Ozdemir M T, Miller G, Song L, Kim J, Sodek A, Koussevitzky S, Misra A N, Mittler R, Shintani D, Plants subjected to oxidative stress was accompanied by enhanced expression of transcripts encoding thiamin biosynthetic enzymes, Plant Physiology, 151, 2009, 421-432.
27. Smith IK, Polle A, Rennenberg H, Glutathione. In: *Stress responses in plants: Adaptation and acclimation mechanisms* (R.G. Alsher and J.R. Cumming, Eds.). Wiley-Liss, New York, 1990, pp. 201-217.
28. Polle A, Rennenberg H, Photooxidative stress in trees. In: *Causes of photooxidative stress and amelioration of defence systems in plants* (C.H. Foyer and P.M. Mullineaux, Eds.). CRC Press, Boca Raton, Florida, 1994, pp. 199-218.
29. Levitt J, Response of plants to environmental stresses. Academic Press, 1980, New York.
30. Esterbauer H, Grill D, Seasonal variation of glutathione and glutathione reductase in needles of *Picea abies*, Plant Physiology, 61, 1978, 119-121.
31. Schupp, R. and Rennenberg, H. Changes in sulphur metabolism during needles development of Norway spruce. Botanica Acta, 105, 1992, 180-189.
32. Larcher W, Temperature resistance and survival. In: *Temperature and life* (H. Precht et al., Eds.). Springer-Verlag, Berlin, 1973, pp. 203-231.

33. Polle A, Schwanz P, Rudolf C, Developmental and seasonal changes of stress responsiveness in beech leaves (*Fagus sylvatica* L.), *Plant Cell Environ*ment, 24, 2001, 821-829.

34. Doulis AG, Hausladen A, Mondy B, Alscher RG, Chevone BI, Hess JL, Weiser RL, Antioxidant response and winter hardiness in red spruce (*Picea rubens* Sarg.), New Physiologist, 123, 1993, 365-374.

35. Hausladen A, Alscher RG, Cold-hardiness-specific glutathione reductase isozymes in red spruce: thermal dependence of kinetic parameters and possible regulatory mechanisms, Plant Physiology, 105, 1994, 215-223.

36. Edwards EA, Enard C, Creissen G, Mullineaux PM, Synthesis and properties of glutathione reductase in stressed peas, Planta, 192, 1994, 137-143.

37. Anderson J, Chevone BI, Hess JL, Seasonal variation in the antioxidant system of eastern white pine needles, Plant Physiology, 98, 1992, 501-508.

38. Sharma P, Jha AB, Dubey RS, Pessarakli M, Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. Journal of Botany, 2012.Article ID 217037, 26 pages.

39. Schöner S, Krause GH, Protective systems against active oxygen species in spinach: Response to cold acclimation in excess light, Planta, 180, 1990, 383–389.

40. Imlay KC, Imlay JA, Cloning and analysis of *SodC*, encoding the copper, zinc superoxide dismutase of *Escherichia coli,* Journal of Bacteriology, 178, 1996, 2564-2571.

41. Campbell WS, Laudenbach DE, Characterization of four superoxide dismutase genes from a filamentous cynobacterium, Journal of Bacteriology, 177, 1995, 964-972.

42. Touati D, Superoxide dismutases in bacteria and pathogen protists. In: *Oxidative stress and the molecular biology of antioxidant defenses* (J.G. Scandalios, Ed.). CSHL Press, USA, 1997, pp. 623-666.

43. Costa V, Reis E, Quintanilha A, Moradas-Ferreira P, Acquisition of ethanol tolerance in *Saccharomyces cerevisiae*: The key role of the mitochondrial superoxide dismutase, Archives of Biochemistry and Biophysics, 300, 1993, 608-614.

44. Jamieson DJ, Rivers SL, Stephen DW, Analysis of *Saccharomyces cerevisiae* proteins induced by peroxide and superoxide stress, Microbiology, 140, 1994, 3277-3283.

45. Autor AP, Biosynthesis of mitochondrial manganese superoxide dismutase in *Saccharomyces cerevisiae*. Precusor form of mitochondrial superoxide dismutase made in the cytoplasm, Journal of Biological Chemistry, 257, 1982, 2713-2718.

46. Pinkham JL, Transcriptional regulation of *SOD2*, the manganese superoxide dismutase gene in *Saccharomyces cerevisiae*. In: *The oxygen paradox* (K.J.A. Davies and F. Ursini, Eds.). CLEUP University Press, Padova, Italy, 1995, pp. 393-404.

47. Gralla EB, Kosman DJ, Molecular genetics of superoxide dismutases in yeasts and related fungi, Advances in Genetics, 30**,** 1992, 251-319.

48. Bowler C, Van Montagu M, Inzé D, Superoxide dismutase and stress tolerance, Annual Review of Plant Physiology and Plant Molecular Biology, 43, 1992, 83-116.

49. Clare DA, Rabinowitch HD, Fridovich I, Superoxide dismutase and chilling injury in *Chlorella ellipsoidea*, Archives of Biochemistry and Biophysics, 231, 1984, 158-163.

50. Tsang EWT, Bowler C, Hérouart D, Van Camp W, Villarroel R, Genetello C, Van Montagu M, Inzé D, Differential regulation of superoxide dismutase in plants exposed to environmental stress, Plant Cell, 3, 1991, 783-792.

51. Asada K, Kiso K, Yoshikawa K, Univalent reduction of molecular oxygen by spinach chloroplasts on illumination, Journal of Biological Chemistry, 249, 1974, 2175-2181.

52. Rao MV, Paliyath G, Ormrod DP, Ultraviolet-B and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana,* Plant Physiology, 110, 1996, 125-136.

53. Abarca D, Martin M, Sabater B, Differential leaf stress responses in young and senescent plants, Plant Physiology, 113, 2001a, 409-415.

54. Abarca D, Roldan M, Martin M, Sabater B, *Arabidopsis thaliana* ecotype Cvi shows an increased tolerance to photo-oxidative stress and contains a new chloroplastic copper/zinc superoxide dismutase isoenzyme, Journal of Experimental Botany, 52, 2001b, 1417-1425.

55. Scebba F, Sebastiani L, Vitagliano C, Changes in activity of antioxidative enzymes in wheat (*Triticum aestivum*) seedlings under cold acclimation, Physiologia Plantarum, 104, 1998, 747-752.

56. Hodges DM, Andrews CJ, Johnson DA, Hamilton RI, Antioxidant enzyme responses to chilling stress in differentially sensitive inbreeds maize lines. Journal of Experimental Botany, 48, 1997, 1105-1113.

57. Lannelli MA, Van Breusegem F, Van Montagu M, Inzé D, Massacci A, Tolerance to low temperature and paraquat-mediated oxidative stress in two maize genotypes. Journal of Experimental Botany, 50, 1999, 523-532.

58. Lee HD, Lee BC, Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: In gel enzyme activity assays, 159, Plant Science, 2000, 75-85.

59. Pinhero RG, Rao MV, Paliyath G, Murr DP, Fletcher RA, Changes in activities of antioxidant enzymes and their relationship to genetic and paclobutrazol-induced chilling tolerance of maize seedlings, Plant Physiology, 114, 1997, 695-704.

60. Oidaira H, Sano S, Koashiba T, Ushimaru T, Enhancement of antioxidative enzymes activities in chilled rice seedlings, Journal of Plant Physiology, 56, 2000, 811-813.

61. Biemelt S, Keetman U, Mock PH, Grimm B, Expression and activity of isoenzyme of superoxide dismutase in wheat roots in response to hypoxia and anoxia, Plant Cell Environment, 23, 2000, 135-144.

62. Borsani O, Diaz P, Agius FM, Valpuesta V, Monza J, Water stress generates an oxidative stress through the induction of a specific Cu/Zn superoxide dismutase in *Lotus corniculatus* leaves, Plant Science, 161, 2001, 757-763.

63. Donahue JL, Okpodu CM, Cramer CL, Grabau EA, Alscher RG, Responses of antioxidants to paraquat in pea leaves: Relationships to resistance, Plant Physiology, 113, 1997, 249-257.

64. Miszalski Z, Slesak I, Niewiadomska E, Baczer-Kwinta R, Luttge U, Ratajczak,R, Subcellular localization and stress responses of superoxide dismutase isoforms from leaves in the C_3-CAM intermediate halophyte *Mesembryanthemum crystallinum* L., Plant Cell Environment, 21, 1998, 169-179.

65. Lee E, Bennett J, Superoxide dismutase: A possible protective enzyme against ozone injury in snap beans (*Phaseolus vulgaris*), Plant Physiology, 69, 1982, 1444-1449.

66. Williamson JD, Scandalios JG, Plant antioxidant gene responses to fungal pathogens, Trends in Microbiology, 1, 1992, 239-245.

67. Megawati S, Harold MG, Evaluation of the Ethylene Effect on Well Defined Copper Enzymes, Jurnal Matematika dan Sains, 11, 1996, 25-31.

68. Zhu D, Scandalios JG, Differential accumulation of manganese superoxide dismutase transcripts in maize in response to abscisic acid and high osmoticum, Plant Physiology, 106, 1994, 173-178.

69. Scandalios JG, Molecular genetics of superoxide dismutases in plants. In: *Oxidative stress and the molecular biology of antioxidant defenses* (J.G. Scandalios, Ed.) CSHL Press, USA, 1997, pp. 527-568.

70. Kernodle SP, Scandalios JG, A comparison of the structure and function of the highly homologous maize antioxidant Cu/Zn superoxide dismutase genes, *Sod4* and *Sod4A*. Genetics, 143, 1996, 317-328.

71. Tanaka K, Sugahara K Role of superoxide dismutase in defence against SO_2 toxicity and an increase in superoxide dismutase activity with SO_2 fumigation, Plant Cell Physiology, 21, 1980, 601-611.

72. McKersie BD, Murnaghan J, Jones KS, Bowley SR, Iron-superoxide dismutase expression in transgenic alfalfa increase winter survival without a detectable increase in photosynthetic oxidative stress tolerance, Plant Physiology, 122, 2000, 1427-1437.

73. Van Breusegem F, Slooten L, Stassart JM, Botterman J, Moens T, Van Montagu M, Inzé D, Effects of overproduction of tobacco MnSOD in maize chloroplasts on foliar tolerance to cold and oxidative stress, Journal of Experimental Botany, 50, 1999, 71-78.

74. McKersie BD, Chen YR, deBeus M, Bowley SR, Bowler C, Inzé D, D'Halluin K, Botterman J, Superoxide dismutase enhances tolerance of freezing stress in transgenic alfalfa (*Medicago sativa* L.), Plant Physiology, 103, 1993, 1155-1163.

75. McKersie BD, Bowley SR, Harjanto E, Leprince O, Water deficient tolerance and field performance of transgenic alfalfa overexpressing superoxide dismutase, Plant Physiology, 111, 1996, 1177-1181.

76. Van Camp W, Van Montagu M, Inzé D, Superoxide dismutases: Roles in stress tolerance. In: *Causes of photooxidative stress and amelioration of defence systems in plants* (C.H. Foyer and P.M. Mullineaux, Eds.). CRC Press, Boca Raton, Florida, 1994, 317-341.

77. Perl A, Perl-Treves R, Galili S, Aviv D, Shalgi E, Malkin S, Galun E, Enhanced oxidative-stress defence in transgenic potato expressing tomato Cu, Zn superoxide dismutases, Theoretical and Applied Genetics, 85, 1993, 568-576.

78. Sen Gupta A, Heinen JL, Holaday AS, Burke JJ, Allen RD, Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase, Proceedings of the National Academy of Sciences USA, 90, 1993a, 1067-1073.

79. Sen Gupta A, Webb RP, Holaday AS, Allen RD, Overexpression of superoxide dismutase protects plants from oxidative stress: induction of ascorbate peroxidase in superoxide dismutase-overexpressing plants, Plant Physiology, 103, 1993b, 1067-1073.

80. Basu U, Good AG, Taylor GJ, Transgenic *Brassica napus* plants overexpressing aluminium-induced mitochondrial manganese superoxide dismutase cDNA are resistant to aluminium, Plant Cell Environment, 24, 2001, 1269–1278.

81. Tanaka Y, Hibino T, Hayashi Y, Tanaka A, Kishitani S, Takabe T, Yokota S, Takabe T, Salt tolerance of transgenic rice overexpressing yeast mitochondrial Mn-SOD in chloroplasts, Plant Science, 148, 1999, 131-138.

82. Tepperman JM, Dunsmuir P, Transformed plants with elevated level of chloroplastic SOD are not more resistant to superoxide toxicity, Plant Molecular Biology, 14, 1990, 501-511.

83. Christine H, Foyer, Shigeru S, Understanding Oxidative Stress and Antioxidant Functions to Enhance Photosynthesis1, Plant Physiology, 155, 2011, 1 93-100.

84. Webb RP, Allen RD, Overexpression of pea cytosolic ascorbate peroxidase confers protection against oxidative stress in transgenic *Nicotiana tabaccum*, Plant Physiology**,** 111, 1996, 48 abstr.

85. Örvar BL, Ellis BE, Transgenic tobacco plants expressing antisense RNA for cytosolic ascorbate peroxidase show increased susceptibility to ozone injury, Plant Journal, 11, 1997, 1297-1305.

86. Aono M, Kubo A, Saji H, Natori T, Tanaka K, Kondo N, Resistance to active oxygen toxicity of transgenic *Nicotiana tabaccum* that expresses the gene for glutathione reductase from *Escherichia coli*, Plant Cell Physiology, 32, 1991, 691-698.

87. Aono M, Kubo A, Saji H, Tanaka K, Kondo N, Enhanced tolerance to photooxidative stress of transgenic *Nicotiana tabaccum* with high chloroplastic glutathione reductase activity, Plant Cell Physiology, 34, 1993, 129-136.

88. Foyer CH, Souriau N, Perret S, Lelandais M, Kunert KJ, Pruvost C, Jouanin, L, Overexpression of glutathione reductase but not glutathione synthetase leads to increase in antioxidant capacity and resistance to photoinhibition in poplar trees, Plant Physiology, 109, 1995, 1047-1057.

89. Aono M, Saji H, Fujiyama K, Sugita M, Decrease in activity of glutathione reductase enhances paraquat sensitivity in transgenic *Nicotiana tabaccum*, Plant Physiology, 107, 1995, 645-648.

90. Allen RD, Webb RP, Schake SA, Use of transgenic plants to study antioxidant defences, Free Radical Biology and Medicine, 23, 1997, 473-479.

91. Song L, Sultan CY, Jeffery H, John C, Ron M, Enhanced Tolerance to Oxidative Stress in Transgenic Arabidopsis Plants Expressing Proteins of Unknown Function, Plant Physiology**,** 148, 2008, 280–292.

Source of Support: Nil, **Conflict of Interest:** None.