Enzymatic Antioxidant

Subjects: Plant Sciences

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Plant enzymes are superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferases (GST), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR), which work as part of the antioxidant defence system. These enzymes together form a complex set of mechanisms to minimise, buffer, and scavenge the reactive oxygen species (ROS) efficiently.

Keywords: antioxidant enzymes ; reaction mechanism ; stressors ; reactive oxygen species ; secondary metabolites

1. Introduction

Plants are immobile; they cannot escape from biotic, (i.e., pathogens, parasites, grazing) and abiotic (such as drought, flooding, salinity, low-high temperatures, ultraviolet radiation, nutrient deficiency, heavy metal (HM) toxicity) stresses. Plant growth, development and productivity are influenced by a variety of environmental stresses. These stresses often perturb the homeostasis and ion distribution in plant cells and induces osmotic stress, leading to an enhancement in the accumulation of reactive oxygen species (ROS) [1]. The production and accumulation of ROS in the plants result in severe destruction of cell organelles and functions cause membrane peroxidation, leading to damage in the cell membrane, degradation of biological macromolecules and ultimately cell death. The ability of plants to scavenge the toxic effects of ROS seems to be the most important determinant for their tolerance to different stresses. Antioxidants are the first line of defence against the damages caused by free radicals and are critical for the optimum health of plant cells [2][3][4][5]. Plant antioxidants play a significant role in assisting plant development through a wide variety of mechanisms and functions.

There are several antioxidant enzymes associated with ROS scavenging in plants, and the synthesis of these enzymes is known to be enhanced during the exposure to oxidative stresses [6]. The ROS comprise free radicals, such as superoxide radicals ($\bullet O_2^-$), hydroxyl radicals (•OH), perhydroxyl radicals (HO_2^-) and alkoxy radicals, and non-radical forms, i.e., hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), present in the intra- and extra-cellular locations of the plant. Superoxide radicals ($\bullet O_2^-$) can be generated by a single electron transfer (e^-) to dioxygen (O_2).

Chloroplasts and mitochondria are the two main sites for the generation of ROS. The photosynthetic electron transport system (ETS) is one of the important sites for the generation of ROS, and this site has the potential to generate singlet oxygen ^1O and superoxide ($\bullet O_2^-$). Plant mitochondria differ from animal as it possesses O_2 and carbohydrate-rich environment [7], and also being associated with photorespiration. The mitochondrial ETC (mtETC) is also a source of generation of ROS as it houses sufficiently energised electrons to reduce the O_2. The major parts of the mtETC responsible for producing ROS are Complex I and Complex III [8]. Other sources of ROS production in the mitochondria are from the different enzymes present in the matrix. There are other sites as well for the generation of ROS, such as the endoplasmic reticulum, cell membrane, cell wall and apoplast.

Evolution has equipped plants with a wide range of defence measures, which include various enzymatic strategies to scavenge free ROS in plant cells [9][10]. The tolerance mechanisms in stressed plant include a number of physio-biochemical strategies, which includes many enzymatic components, such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POX), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferases (GST), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR), and non-enzymatic components, such as ascorbic acid (AA), glutathione (GSH), phenolic compounds, alkaloids, flavonoids, carotenoids, free amino acids and α-tocopherols [11][12][13]. However, in the present review, we have exclusively focussed on the role and mechanisms of enzymatic components in the plant to scavenge the ROS and to cope with the stress conditions. These enzymes are selected on the basis of majority of the research reports available and with their proven utility in transgenic plants to cope with the stress conditions (Table 1). During stresses, SOD catalyses the removal of $\bullet O_2^-$ by dismutating it into O_2 and H_2O_2, CAT converts the H_2O_2 into water and molecular oxygen (O_2) and POX works in the extra-cellular space for scavenging H_2O_2. Plant GPX catalyses the reduction of H_2O_2 and HO_2 to water and lipid

alcohols, respectively, using thioredoxin as an electron donor. Glutathione reductase catalyses the reduction of oxidised glutathione (GSSG; dimeric) to reduced glutathione (GSH; monomeric) and APX utilises ascorbate as specific electron donor to scavenge H_2O_2 to water.

These enzymes not only protect various components of the cells from damages, but also play an important role in plant growth and development by modulating cellular–sub-cellular processes such as mitosis [14], cell elongation [15], senescence [16] and cell death [17], and are also involved in a wide range of processes, such as cell differentiation [18], cell growth/division [19], regulation of senescence and sulphate transport [20][21], detoxification of xenobiotics [22], conjugation of metabolites [23], regulation of enzymatic activities [24], synthesis of proteins and nucleotides [25][26], phytochelatins [27] and expression of stress responsive genes [28]. The antioxidant defence system protects the unsaturated membrane lipids, nucleic acids, enzymes and other cellular structures from the negative impacts of free radicals [29]. Therefore, the antioxidant defence system of plants has been attracting considerable interest of the scientific community [29][30].

2. Enzymatic Antioxidant Defence Systems in Plants

The antioxidant defence system in the plant comprises several different enzymes. They are mainly involved in either preventing the Haber-Weiss reaction (Figure 1) or the Foyer–Halliwell–Asada pathway, which reduces the H_2O_2 and utilises the reducing potential of NADPH.

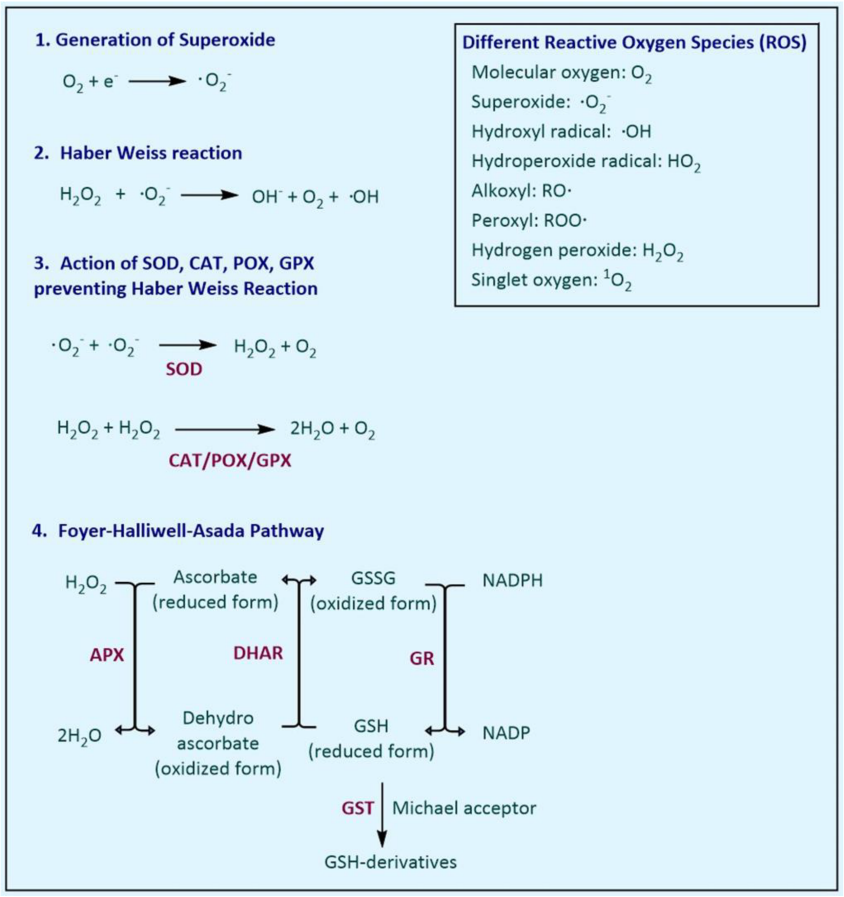

Figure 1. Basic reactions catalysed by the antioxidant enzyme system.

3. Applications of Antioxidant Enzymes in Developing Stress-Tolerant Transgenic Plants

Sustainable agriculture production is a key factor in ensuring global food security. However, there are multiple stress conditions that influences the crop growth and yield. In order to overcome these stress conditions, developing stress-tolerant plants is an important step. Understanding the role of individual gene under the influence of different stress condition can be useful in developing stress-tolerant plants. The overexpression of different genes of different antioxidant enzymes has resulted in the increase in tolerance in transgenic plants to various environmental stress conditions. Several stress-tolerant genetically engineered plants have been developed in the recent past, and the significant findings of these research reports are briefly described in Table 1. The majority of these studies focussed on abiotic stress caused due to salinity, heat, chilling, drought, flood and HM, but very few reports are available on understanding the role of these enzymes to cope with biotic stress. Furthermore, in all of these studies, the gene of the antioxidant enzyme is

overexpressed under a strong promoter in transgenic lines, thereby increasing the tolerance potential of the plant to stress condition. Thus, these findings are crucial for developing stress-resistant plants, and the knowledge gained will be helpful for sustained growth and productivity of various crops in variable environmental conditions.

Table 1. Recent studies of transgenic overexpression of different genes encoding antioxidant enzymes in enhancing stress tolerance in transgenic plants along with significant findings.

S.No.	Transgenic Plant(s)	Gene(s)/Source	Stress Condition	Significant Finding(s)	Reference
1.	Transgenic *S. lycopersicum*	FeSOD gene from *Arabidopsis*	Salt stress	Overexpression of antioxidant enzymes significantly mitigates the harmful effects of salt stress on cytoskeleton structural organisation in roots of the transgenic line cells.	[172]
2.	Transgenic *S. tuberosum*	Cu-ZnSOD (*StSOD1* gene overexpressed under CaMV 35S promoter)	Low temperature	Activity of SOD is 1.38-fold higher compared to non-transgenic lines. Furthermore, the activity of POX and CAT were also enhanced in transgenic line, signifying the fact that increasing the activity of one antioxidant enzyme can influence the activity of other defence enzymes via cross-talk.	[173]
3.	Transgenic *Citrus sps*	CsPIF8 influencing SOD gene expression	Low temperature	Phytochrome-interacting transcription factor CsPIF8 positively regulate CsSOD expression in citrus, highlighting the cross-talk between phytochrome genes and antioxidant enzymes. In this study, it is found that CsPIF8 directly bound to the E-box (CANNTG) of CsSOD promoter and activated the promoter of CsSOD.	[50]
4.	Transgenic *Arabidopsis*	*CmSOD* gene (from winter squash; *Cucurbita moschata*) and *AtSOD* gene (from *Arabidopsis*) under a ubiquitin promoter	Low temperature	Increased resistance to chilling and less oxidative injury in transgenic lines than wild type, indicating that the overexpression of *AtSOD* and *CmSOD* led to higher SOD activity in *Arabidopsis*-enhanced chilling tolerance by eliminating $\bullet O_2^-$. Furthermore, the activity of SOD in transgenic lines is influenced by ABA, indicating the role of plant hormone in the cross-talk with enzymes of the antioxidant defence system.	[174]
5.	Transgenic *Arabidopsis*	Cu-Zn SOD gene (*SaCu/Zn SOD*), from *Sedum alfredii*	Oxidative stress due to Cadmium	Cadmium stress induces the production of ROS, leading to oxidative stress. Cd-hyperaccumulator plant *S. alfredii* is used as a source of SOD gene, resulting in enhanced antioxidative defence capacity in transgenic *Arabidopsis* plants. The *SaCu/Zn SOD* is implicated as being responsible for conferring Cd tolerance.	[175]

S.No.	Transgenic Plant(s)	Gene(s)/Source	Stress Condition	Significant Finding(s)	Reference
6.	Transgenic tobacco	Cu/Zn-SOD gene, *SiCSD* from *Saussurea involucrata*	Drought, cold and oxidative stress	Higher activities of SODs, CAT and APX are reported in transgenic lines, and SOD is found as a positive regulator in drought and cold stress by reducing oxidant injury.	[176]
7.	Transgenic *C. grandis*	The basic helix-loop-helix (bHLH) family of transcription factors (*PtrbHLH*) from *Poncirus trifoliata*	Low temperature	Transgenic plant was found to exhibit lower electrolyte leakage and malondialdehyde content after chilling stress, lower ROS levels and elevated activity of antioxidant enzymes, including CAT, POX and SOD. Interestingly, PtrbHLH was found to bind to the promoter and activate the PtrCAT gene, thereby implicated as regulating the CAT gene activity.	[177]
8.	*Manihot esculenta*	SOD (*MeCu/ZnSOD*) and catalase (*MeCAT1*)	Biotic stress (Mite *Tetranychus cinnabarinus*)	The transgenic approach led to mite-resistant traits, as survival, reproduction and development of *T. cinnabarinus* feeding on transgenic cassava is significantly inhibited. Furthermore, the activities of SOD and CAT in transgenic cassava plants damaged by *T. cinnabarinus* significantly increased. This study highlights the role of antioxidant enzymes in developing pest resistant crops.	[178]
9.	Transgenic *Ipomoea batatas*	Peroxidase gene *swpa4* in *I. batatas*	Salt stress	Overexpressing the *swpa4* gene under CaMV 35S promoter led to 3- to 13-fold higher expression in transgenic sweet potato. Transgenic plants also showed increased tolerance to salinity conditions, with 13–26% less damage than control plants. Furthermore, photosynthetic capacity and total chlorophyll contents were less severely impacted in transgenic plants.	[179]
10.	Transgenic *Arabidopsis*	Glutathione peroxidase-like 5 gene (*AtGPXL5*) from *Arabidopsis*	Salt stress	Constitutive overexpression of *AtGPXL5* led to an increase in gene expression by 17–24 times in 6-week-old plants. It results in an increase in GSH pool and more negative redox potential than wild type and increased salt tolerance.	[91]
11.	Transgenic *Arabidopsis*	*AtGR1* encoding glutathione reductase (GR) from *Arabidopsis*	Aluminium toxicity	The overexpression of *AtGR1* led to a higher GSH pool and improved ratio of GSH/GSSG, and increased aluminium tolerance, with better root growth in comparison to the wild type under aluminium stress. Increased GSH levels were found to increase the capacity of RCS detoxification, which indicates that GR overexpression contributes to the mitigating of not only ROS, but also RCS.	[180]

S.No.	Transgenic Plant(s)	Gene(s)/Source	Stress Condition	Significant Finding(s)	Reference
12.	Transgenic *O. sativa*	*OsGSTU5* (a tau class GST in *O. sativa*)	Biotic stress	Overexpression of *OsGSTU5* provided tolerance against sheath blight disease, caused by *Rhizoctonia solani*.	[181]
13.	Transgenic *Arabidopsis*	Glutathione S-transferase from *Thermosynechococcus elongatus BP-1* (*TeGST*)	Thiocyanate (SCN^-) stress	Overexpression of *TeGST* in transgenic plant increased the tolerance to thiocyanate (SCN-) up to 5 mmol L^{-1}. This approach was found to be potentially effective to enhance the phytoremediation of environmental thiocyanates.	[182]
14.	Transgenic *Arabidopsis*	Ascorbate peroxidase (*AgAPX1*) from *Apium graveolens*	Drought tolerance	Overexpression of the *AgAPX1* gene enhanced ascorbate content, antioxidant capacity and drought resistance. Furthermore, increased antioxidant capacity does not affect the growth parameters of the plant much, as a comparatively smaller decrease in the net photosynthetic rate is observed, and a high survival rate of transgenic *Arabidopsis* lines after drought is reported.	[43]
15.	Transgenic *Arabidopsis*	Ascorbate peroxidase gene (*DaAPX*) from *Dioscorea alata*	Flood/Chilling stress	This study reports the effect of different types of stress on the expression of *DaAPX*. Yam variety Minghuai 1 (MH1), when exposed to a flood situation, showed an increase in the expression of *DaAPX*; however, chilling stress did not influence the expression profile of *DaAPX*, thereby making this variety sensitive to chilling stress. However, overexpression of *DaAPX* in *Arabidopsis* led to increased tolerance towards several abiotic stress, including flooding and chilling.	[152]
16.	Transgenic *Brassica juncea*	Ascorbate peroxidase gene (*Apx1*) from *Arabidopsis*	Salt stress	Overexpression of cytosolic *AtApx1* gene increased salinity stress tolerance in *B. juncea*. APX, along with higher activity of other enzymes such as GPX, CAT and POX, maintains the ROS homeostasis and provides tolerance to the cell, greater proline accumulation, increased chlorophyll stability index and lower chlorophyll a/b ratio.	[150]

S.No.	Transgenic Plant(s)	Gene(s)/Source	Stress Condition	Significant Finding(s)	Reference
17.	Transgenic *Nicotiana tabacum*	Monodehydroascorbate reductase from *S. lycopersicum* (*SlMDHAR*)	Salt stress	Overexpression of *SlMDHAR* in transgenic tobacco is found to increase salt stress tolerance and NO accumulation and the S-nitrosyalted *SlMDHAR* levels were found to be higher in transgenic tobacco. Results suggested that *SlMDHAR* confers salt stress tolerance by probably involving the S-nitrosylation (post-translational modification of cysteine thiol by nitric oxide group) of MDHAR.	[183]
18.	Transgenic *Arabidopsis*	Monodehydroascorbate reductase (*BvM14*-MDHAR) from *B. vulgaris*	Salt stress	The MDHAR gene is constitutively expressed in *Arabidopsis*, resulting in an enhanced salt stress tolerance phenotype, with higher AsA/DHA levels than wild-type. In addition, the overexpression seedlings showed higher activities of MDHAR and DHAR and decreased cell membrane damage.	[184]
19.	Transgenic *Arabidopsis*	DHAR (*AcDHAR1* and *AcDHAR2*) from *Actinidia chinensis* (kiwi fruit)	Salt stress	Transgenic overexpression of these two genes (separately) in *Arabidopsis* plants was found to significantly enhance the ascorbic acid concentration and enhance the tolerance to salinity.	[185]