Transgenic Plants and Antioxidative Defense: Present and Future?

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Abstract Environmental stresses considerably limit plant growth, distribution, and productivity. Biological systems generate a range of different reactive oxygen species (ROS) like superoxide ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2), during the course of normal metabolic reactions. If it is not effectively and rapidly removed, ROS damages a wide range of macromolecules, ultimately leading to cell death. ROS are also generated in response to various biotic and abiotic stresses. Cells have evolved both enzymatic and nonenzymatic defense mechanisms to protect cells from lethal effects of free radicals. ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) that play a crucial role in regulating ROS accumulation in cells. Transgenic plants expressing specific gene candidates have been proven to

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increase the tolerance to environmental stresses significantly. In recent years, several efforts have been made to improve the oxidative stress tolerance in plants by over-expressing plant or bacterial genes coding either for ROS-scavenging enzymes or for enzymes modulating the cellular antioxidant capacity. In this chapter, we have discussed some of the significant reports on transgenic plants with altered antioxidant capacity mainly focusing on the new insight into the antioxidant defense mechanisms. Finally, future focus of transgenic research to combat oxidative stress has been briefed.

Keywords Reactive oxygen species • Antioxidants • Superoxide dismutase • Ascorbate peroxidase • Glutathione peroxidase • Glutathione reductase • Catalase • Alternative oxidase • Uncoupling protein

1 Introduction

The rise in global population along with reduction in agricultural practices and diminishing availability of resources poses serious challenges to present community for continuous supply of agricultural products. To feed 9 billion people that is expected by 2050, a significant increase in grain yield of approximately 44 million metric tons per year is required (Tester and Langridge 2010). Global warming and other stress factors negatively influence the plant growth and yield, that is making it even more challenging (Reguera et al. 2012). Thus, the environmental stress is one of the major limiting factors that determine the crop yield. Stress injuries on plants cause oxidative damages both at the cellular and molecular levels.The critical role of reactive oxygen intermediates (ROI) in cellular damage has been well studied (Rennenberg and Polle 1994).

Over the last few decades, deep understanding of the role played by of ROS lead to discovery of signaling molecules regulating various physiological processes in plants (Mylona and Polidoros 2010) (Fig. 1). Recently, several reports proved that low concentration of ROS is acting as redox signal molecules playing a crucial role in various signal transduction pathways in plant cells (Foyer and Noctor 2005a, b). Environmental stresses like salinity, temperature, or drought result in a marked increase in ROS level leading to oxidative damage in plant cells. During the course of evolution, plant system also developed potential antioxidant defense system to protect itself from oxidative damage (Asada 1999; Mittler et al. 2004). Till date, different ROS-scavenging enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), and catalase (CAT) have been discovered, which are localized in different cellular compartments (Table 1). In general, there are more than one type of enzyme in a cell for scavenging different kind of ROS (Mittler et al. 2004).

ROS are continuously generated as by-products of various cellular processes like photosynthesis, photorespiration, fatty acid oxidation, electron transport chain, etc.

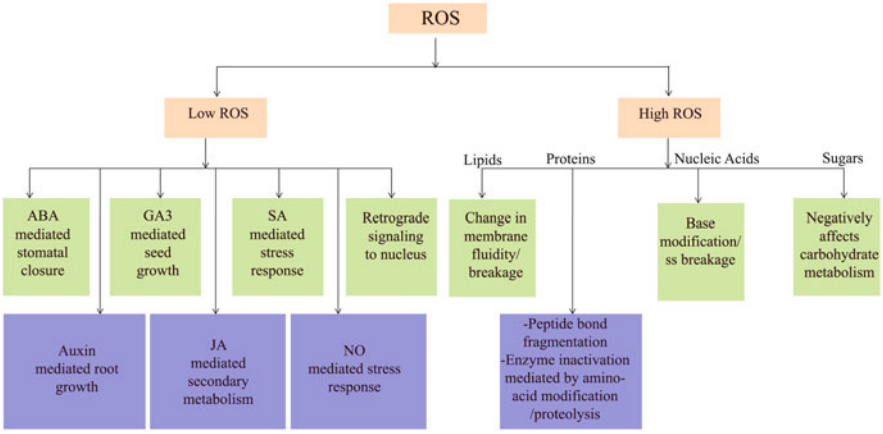

Fig. 1 Reactive oxygen species (ROS) at low levels act as secondary messengers in several physiological processes, whereas at high levels they negatively act on biomolecules

Table 1 Major ROS-scavenging enzymes, reaction mechanism, and cellular localization

Sl. No	ROS-scavenging enzymes	Mechanism	Localization	References
1	SOD	$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow 2H_2O_2 + O_2$	Cytosol, golgi, plastids, and mitochondria	Mittler et al. (2004)
2	APX	$AA + H_2O_2 \rightarrow DHA + 2H_2O$	Cytosol, plastids, peroxisomes, glyoxysomes, and mitochondria	Foyer and Noctor (2005a, b)
3	GPX	$H_2O_2 + 2GSH \rightarrow H_2O + GSSG$	Cytosol, plastids, nucleus, peroxisomes, and mitochondria	Ursini et al. (1995), Rodriguez et al. (2003)
4	GR	$GSSG + NAD(P)H \rightarrow 2GSH + NAD(P)^+$	Cytosol, plastids, peroxisomes, and mitochondria	Edwards et al. (1990), Creissen et al. (1995)
5	CAT	$2H_2O_2 \rightarrow O_2 + 2H_2O$	Peroxisomes, glyoxysomes	Mhamdi et al. (2010)

Up to 1 % of the molecular oxygen consumed by plant is metabolized to form ROS in different cellular compartments (Bartoli et al. 2000). ROS molecules include free radicals like superoxide ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), etc., and non-radical molecules like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), etc. (Shafi et al. 2015). Under normal conditions, ROS generated is efficiently metabolized by the multicomponent antioxidant system and protects them from potentially deleterious effects of ROS (Fig. 2). However, during stress (both biotic and abiotic),

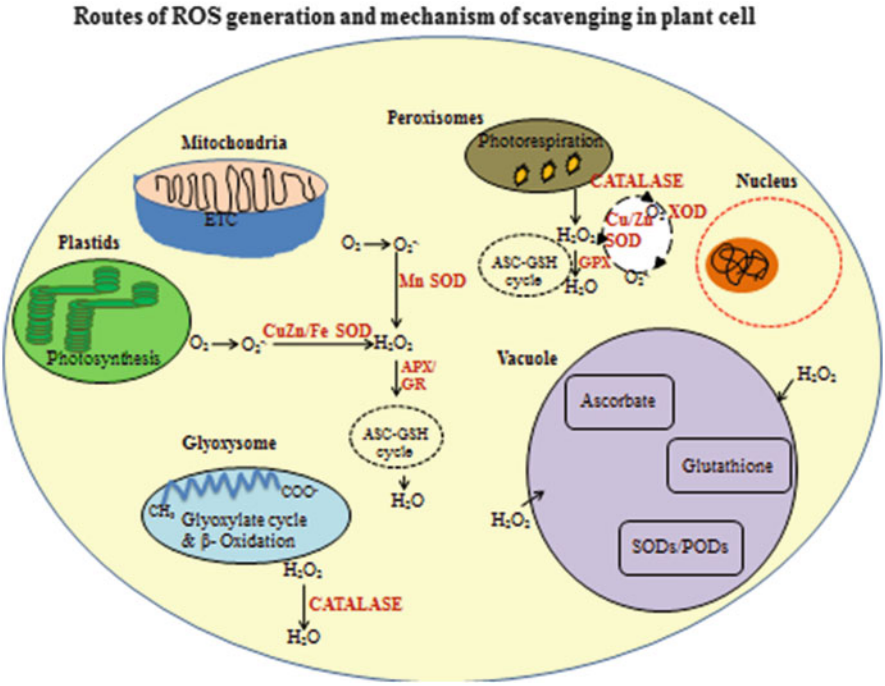

Fig. 2 Overview of cellular process generates ROS in different compartments and their scavenging in cells [Modified from Kuzniak (2002)]

ROS levels are elevated and thus the cellular antioxidant capacity can be overwhelmed leading to oxidative stress (Robinson and Bunce 2000). It is well known that much of plant injury as a result of environmental stresses are related to ROS-initiated oxidative damage. Hence, enhancing tolerance to several environmental stresses could be achieved through modulation of gene expression to different ROS-scavenging enzymes ultimately reducing ROS population in cells (Fig. 3).

Transgenic approaches are widely used for crop improvement programs in recent years. Gene discovery and functional genomics have revealed infinite mechanisms and led to the identification of many potential gene families, which could confer adaptation and improved productivity during adverse environmental conditions (Kumar et al. 2012). To improve oxidative stress tolerance in plants, overexpression of genes encoding either ROS-scavenging enzymes or enzymes modulating the cellular antioxidant capacity from various sources has been proved effective (Liu et al. 2013, 2014; Zhai et al. 2013; Zhang et al. 2013). The rationale behind these approaches is to minimize massive loss in productivity of crop plants by various environmental constraints. The study of transgenic lines expressing antioxidant genes revealed a complete understanding of the roles of individual enzymes in scavenging free radicals and imparting tolerance to various stress factors.

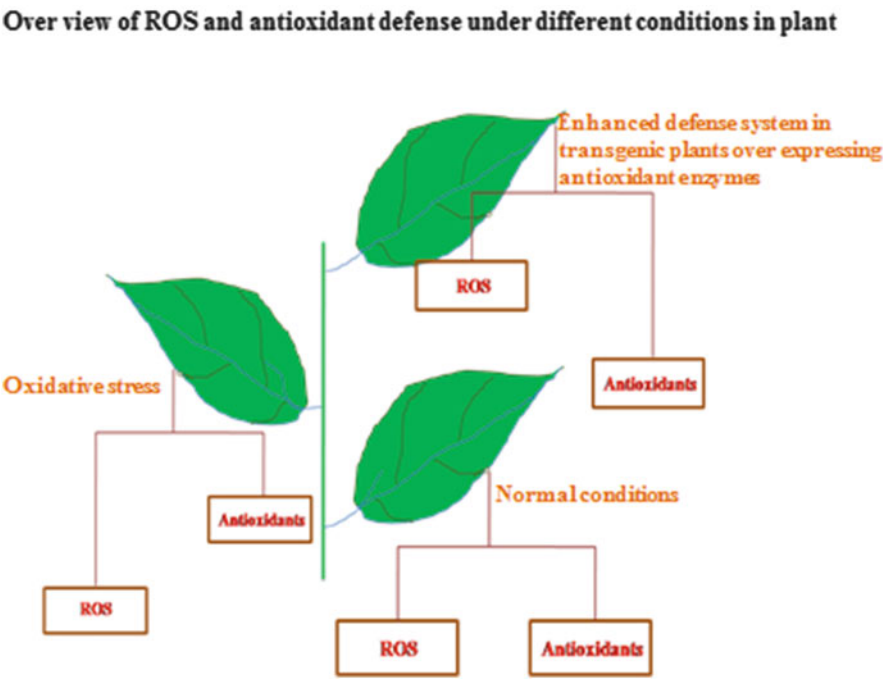

Fig. 3 Modulating ROS scavenging in transgenic plants by overexpression of antioxidant enzymes

Besides, transgenic lines with altered antioxidant capacity also provided new insights into the interrelations between various enzymatic and nonenzymatic antioxidant components and their signaling network. It has been already reported that the transgenic lines generated through gene pyramiding or co-expression of several antioxidant defense genes imparted more stress tolerance than the plants overexpressing either of genes alone (Lee et al. 2007; Wei et al. 2011; Diaz-Vivancos et al. 2013; Xu et al. 2014). In this chapter, we focus mainly on recent studies on transgenic plants with altered ROS-scavenging capacity by overexpression of antioxidant genes; readers are requested to refer to Gill and Tuteja (2010) and reference therein for transgenic plants for ROS scavenging by non-enzymatic antioxidants. Future focus is on the new insight in antioxidant defense mechanism by expressing genes like alternative oxidase, uncoupling protein has also been discussed briefly. In the end, future of transgenic research under field conditions has been emphasized.

2 Superoxide Dismutase (SOD)-Expressing Transgenic Plants

SOD and APX constitute the first line of defense response against ROS (Alscher et al. 2002), thus playing an important role in protecting cells against superoxide radicals. SODs are metallo-enzymes with different isoforms localized in various cellular compartments like cytosol, plastids, and mitochondria. Irrespective of their compartmentalization, they all catalyze dismutation of superoxide ions ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2) (Gill and Tuteja 2010). The endogenous plant genes encoding different SOD isoforms have been used for plant transformation studies. Initial attempts to express SOD in heterologous systems were not successful. *Petunia hybrida* plastidial Cu/ZnSOD when over-expressed in tobacco did not increase the photosynthetic efficiency after methyl viologen (MV) treatment (Tepperman and Dunsmuir 1990). Also, over-expression of *Petunia* SOD in transgenic tobacco lines did not alter the oxidative stress tolerance (Pitcher et al. 1991). Later, several reports revealed reasons for failure of initial attempts using plastidial Cu/Zn/Fe SOD are due to inactivation of SOD by peroxides, whereas MnSOD is not susceptible to peroxide inactivation (Allen et al. 1997). A chimeric MnSOD (native mitochondrial transit peptide was replaced by plastidal peptide) from *Nicotiana plumbaginifolia* when over-expressed in tobacco lead to reduced levels of membrane damage following exposure to MV, ozone, and photooxidative stress (Van Camp et al. 1994). Transgenic alfalfa lines expressing same chimeric MnSOD enhanced freezing tolerance and water deficit (McKersie et al. 1993, 1996).

Sen Gupta et al. (1993a, b) reported some interesting results with respect to expression of different isoforms of SOD in tobacco. Transgenic tobacco lines over-expressing plastidial Cu/ZnSOD or a chimeric chloroplastic MnSOD showed different tolerance level to MV. MnSOD-expressing lines showed superior protection after MV exposure, whereas Cu/ZnSOD-expressing plants revealed low level of tolerance. Since expression levels of SOD in both lines were similar, it was reported that peroxide sensitivity of Cu/ZnSOD could account for low level of tolerance relative to the chloroplastic MnSOD-expressing plants. It was also shown that tobacco lines expressing plastidial Cu/ZnSOD were least susceptible to photo-oxidative damage induced by excess light and also chilling temperatures; however, tobacco lines expressing chloroplastic MnSOD were susceptible. Adding to this, chloroplastic MnSOD-expressing lines did not protect MV-induced damage to photosystem II (PSII). Since plastidial Cu/ZnSOD is located on the thylakoid membrane, close association with photosystem I (PSI) enzyme rapidly scavenges $O_2^{\bullet -}$ before significant damage occurs. Since MnSOD is not native to the chloroplast, it is possible that it may not form a productive association with PSI. Hence, expression of peroxide insensitive MnSOD in plastids alleviate the damage to cellular membranes possibly by reducing the leakage of ROI from plastids to cytosol, but MnSOD does not appear to provide considerable protection to photosynthetic components.

Over-expression of Cu/ZnSOD from *Kandelia candel* in tobacco resulted in reduced ROS generation in plastids and enhanced tolerance to salinity stress (Jing et al. 2015). Transgenic *Arabidopsis* co-expressing *Potentilla atrosanguinea SOD* and *Rheum austral APX* enhanced lignin deposition along with higher biomass production and yield under salinity stress (Shafi et al. 2015). The involvement of SOD and peroxides in cell wall lignification has been also previously reported (Karpinska et al. 2001; Kim and Barbara 2008). It was also reported that H_2O_2 signaling enhanced genes involved in lignin biosynthesis indirectly through other secondary messengers like MAP kinases and transcription factors like NAC, MYB, and WRKY (Fujita et al. 2006). The same combination of genes (*PaSOD* and *RaAPX*) also conferred tolerance to chilling stress in *Arabidopsis* and had higher levels of total antioxidant enzymes and chlorophyll content and lower levels of ROS (Shafi et al. 2014). Transgenic rice either over-expressing or knocked down with *OsMnSOD* was tolerant and susceptible to heat stress, respectively. Plants over-expressing MnSOD upregulated ROS-scavenging enzymes, chaperone, and quality control systems in rice grain under heat stress (Shiraya et al. 2014). It was also shown that over-expressing lines had better quality rice grains compared to knock down lines revealing that constitutive expression of golgi/plastid targeted MnSOD is effective in regulating formation of perfect grains under unfavorable conditions like heat stress. Over-expression of both yeast MnSOD and pea mitochondrial MnSOD in plastids of rice conferred tolerance to salt and oxidative stress (Tanaka et al. 1999; Wang et al. 2005). Tolerance to oxidative and chilling stresses in transgenic Cassava plants co-expressing Cu/ZnSOD and APX was mediated through improved ROS scavenging and resulted in reduced peroxide accumulation and improved chilling stress resistance (Xu et al. 2014).

Transgenic potato lines over-expressing tomato plastidal Cu/ZnSOD had reduced levels of damage after MV treatment (Perl et al. 1993). Transgenic rice transformed with mangrove cytosolic Cu/ZnSOD showed superior tolerance to drought stress (Prashanth et al. 2008). *E. coli* MnSOD gene with a soybean-derived leader sequence over-expressed in tobacco chloroplasts showed superior tolerance to photooxidative stress (Van Assche et al. 1989). A FeSOD over-expressed in transgenic tobacco chloroplasts conferred protection from MV damage as well as PSII inactivation (Van Camp et al. 1996). However, expression of FeSOD did not enhance tolerance to chilling-induced photoinhibition in Poplar (Arisi et al. 1998) and salinity stress in tobacco (Van Camp et al. 1996). This could be due to that response of different stress factors is mediated through different routes, and superoxide anion scavenging capacity of the chloroplasts may not be rate limiting for each of them. Further physiological, biochemical, and molecular analyses of transgenic lines along with transgenic lines with silenced SOD activities will provide deeper insight into the functions of SOD in plant cells.

3 Ascorbate Peroxidase (APX) Expressing Transgenic Plants

Ascorbate peroxidase (APX) is the primary H_2O_2-scavenging enzyme in both cytosol and plastids (Asada 1992). APXs have also been reported from glyoxysome, where it is presumed to act with catalase to remove peroxides (Yamaguchi et al. 1995; Bunkelmann and Trelease 1996). Transgenic tobacco lines expressing either pea cytosolic APX or pea cytosolic APX fused with pea chloroplastic transit peptide of Cu/ZnSOD to target plastids showed 3 and 16-fold increase in SOD activity, respectively, with enhanced membrane stability. Also, both transgenic lines were tolerant to photooxidative stress, which is attributed to increase in scavenging of peroxides (Pitcher et al. 1994; Webb and Allen 1995, 1996). *Arabidopsis* over-expressing rice *OsAPXa* or *OsAPXb* enhanced salinity tolerance to different levels. Over-expression of *OsAPXb* enhanced and maintained APX expression to a much higher extent than *OsAPXa* (Lu et al. 2007). Transgenic tobacco lines expressing *C. annuum APX*-like 1 gene enhanced tolerance to oxidative stress and resistance to fungal pathogen *Phytophthora nicotianae*. Interestingly, transgenic lines showed different levels of susceptibility to other pathogens like *Pseudomonas syringae* and *Ralstonia solanacearum*. Tolerance to oxidative stress and *Phytophthora* is associated with overproduction of APX, which could have further increased the POD activity further strengthening an ROS-scavenging system (Sarowar et al. 2005).

Transgenic *A. thaliana* over-expressing peroxisomal *APX* from *Puccinellia tenuiflora* enhanced salt tolerance by reducing H_2O_2 accumulation (Guan et al. 2015). Similarly, peroxisomal *APX* from *Salicornia brachiata* imparted salinity tolerance when over-expressed in *Arachis hypogaea* (Singh et al. 2014a, b). Peroxisomal-specific *Sb APX* not only conferred salinity and drought tolerance but also enhanced vegetative growth and germination rate in transgenic tobacco lines (Singh et al. 2014a). A cytosolic APX from *Lycium chinense* enhanced salinity tolerance and photosynthetic rate and reduced peroxide accumulation in transgenic tobacco lines during salt stress (Wu et al. 2014). Transgenic tobacco expressing thylakoid targeted APX from *Jatropha curcas* enhanced ROS-scavenging system by reducing malondialdehyde (MDA) and H_2O_2 accumulation during salt stress (Liu et al. 2014). *Anthurium andraeanum APX* conferred chilling tolerance in transgenic tobacco lines, which corroborated with enhanced APX activity, reduced MDA content, and enhanced membrane stability (Liu et al. 2013). Rice *OsAPX 2* mutant negatively affected growth and development of rice seedlings and was sensitive to abiotic stress like cold, salinity, and drought. ROS metabolites like H_2O_2 and MDA levels were high in *OsAPX2* mutants; however, they were low in transgenic lines over-expressing *OsAPX2* after stress treatments (Zhang et al. 2013). Similarly, *Arabidopsis* cytosolic *APX* knockout mutants were highly sensitivity to wounding and MeJA treatment. In control plants, peroxide accumulated only in the vicinity of wound, but in leaves of *APX* knockouts, it accumulated both in damaged and undamaged regions. Transgenic *Medicago sativa* over-expressing rice *APX2* had

longer roots; ~3-fold more APX activity, less MDA content as compared to control plants (Guan et al. 2012). All the above studies clearly indicate that over-expression of different isoforms of *APX* improved detoxification of ROS and imparted enhanced tolerance in transgenic host system.

4 Glutathione Peroxidase (GPX) Expressing Transgenic Plants

GPX in mammals reduces H_2O_2 and other organic hydroperoxides to water and corresponding alcohols using glutathione (GSH), thus protecting cells from oxidative damage. Plant GPX family shares the highest sequence homology to animal phospholipid hydroperoxide GPXs (PHGPXs) and possess a cysteine (Cys) residue in catalytic site rather than Se-Cys seen in PHGPXs (Zhai et al. 2013). In plants, GPXs are localized in subcellular organelles like cytosol, nucleus, chloroplast, mitochondria, peroxisome, etc., and use thioredoxin (Trx) as a reducing agent (Ursini et al. 1995; Rodriguez et al. 2003). Plant GPXs are expressed in response to different kinds of abiotic factors like salinity, mechanical injury, high-light stress or treatment with Paraquat, etc. (Holland et al. 1993; Avsian-Kretchmer et al. 2004; Herbette et al. 2011; Gaber et al. 2012). *Arabidopsis* knockout mutants of *AtGPX8* were highly sensitive to oxidative damage by Paraquat treatment in roots, and transgenic lines over-expressing *AtGPX8* were less sensitive to oxidative damage than the wild-type controls (Gaber et al. 2012). *Mus musculus* GPX5 over-expressing transgenic tomato lines were more tolerant to oxidative damage after chilling exposure (Herbette et al. 2005). *MmGPX5* transgenic tomato lines were resistant to mechanical injury; however, transgenic plants were sensitive to biotic agents like *Oidium neolycopersici* and *Botrytis cinerea* (Herbette et al. 2011). Two wheat *GPXs* when expressed in plastids of *Arabidopsis* enhanced growth and survival rate during salt stress. Transgenic lines also had increased peroxide-scavenging capacity and enhanced tolerance to H_2O_2 (Zhai et al. 2013). Over-expression of a tobacco glutathione S-transferase having both GST and GPX activity lead to enhanced activities of both enzymes (GST/GPX) in transgenic tobacco seedlings. Also transgenic plants had elevated levels of monodehydro-ascorbate reductase activity and higher levels of glutathione and ascorbate compared to control plants (Roxas et al. 2000). Transient over-expression of tomato phospholipid like GPX (PHGPx) in tobacco leaves suppressed apoptotic-like features during severe salt and high-temperature stresses, and stable tobacco lines expressing *LePHGPx* also conferred protection against the fungus *B. cinerea* (Chen et al. 2004).

5 Glutathione Reductase (GR) Expressing Transgenic Plants

Maintenance of ascorbate and glutathione levels in a reduced state is a crucial factor in ROS-scavenging system in which glutathione reductase (GR) plays a critical role. A bacterial GR over-expressed in tobacco enhanced GR activity to ~4-fold, further reduced visible damage after MV exposure (Aono et al. 1991). Targeting of the *E. coli* GR to plastids in transgenic tobacco also showed similar level of resistance to MV and sulfur dioxide (which was not observed in above study), but not to ozone in both cases (Aono et al. 1993). Transgenic poplar plants over-expressing plastid directed *E. coli* GR resulted in ~500-fold transgene activity with close to two fold higher levels of both glutathione and ascorbate pools compared to control and cytosolic GR expressing lines. Transgenic lines were only resistant to photoinhibition by high-light intensity and chilling temperature (which is attributed to increased level of both GSH and ascorbate) and did not increase MV tolerance (Foyer et al. 1991). A pea cDNA when over-expressed in tobacco elevated GR activity in different sites like cytosol, plastid, and mitochondria, which was attributed to leaky targeting of GR due to presence of N-terminal transit peptide (Creissen et al. 1995). Transgenic lines with pea GR showed different levels of tolerance to ozone; however, none of them significantly reduced MV-induced damage (Broadbent et al. 1995). Surprisingly, few of transgenic lines with pea GR either with the native transit peptide or without any signal sequence showed tolerance to MV; again there was no correlation with the level of protection with the levels of GR expression. Payton et al. (1996) reported that tobacco over-expressing *Arabidopsis* GR had higher levels of GSH and also enhanced MV tolerance; however, some lines showed up to 20% reduction in GR activity, which could be due to sense suppression. Antisense glutathione reductase tomato lines accumulated more peroxides, leaked more electrolyte, and showed lower net photosynthetic rate as compared to WT plants (Feng Shu et al. 2011). Aono et al. (1995) generated antisense tobacco with reduced GR activity, which increased their susceptibility to MV-induced damage. Co-expression of both GR and SOD in cytosol of tobacco plants enhanced protection from MV treatment than transgenic lines with either gene expressed individually (Aono et al. 1995). Hence, it is clear that expressing combinations of different antioxidant enzymes can scavenge different ROS with synergistic effects further enhancing stress tolerance in transgenic lines.

6 Catalase Expressing Transgenic Plants

Catalases (CAT) are tetrameric heme containing enzymes, which directly dismutate H_2O_2 into water and molecular O_2, and they are indispensable for ROS detoxification during stressed conditions (Garg and Manchanda 2009). CAT has high

turnover rates with one molecule of CAT converting approximately 6 million molecules of peroxide per minute (Gill and Tuteja 2010). CAT plays an important role in scavenging peroxides generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration, and purine catabolism. Transgenic rice over-expressing endogenous type 1 metallothionein *OsMT1a* showed enhanced CAT activity and tolerance to drought stress (Yang et al. 2009). *B. juncea CAT 3* (*BjCAT3*) enhanced cadmium tolerance with two fold higher activity when compared to control plants (Guan et al. 2009). Over-expression of maize CAT and SOD in plastids of *B. campestris* not only enhanced tolerance to sulfur dioxide and salinity stress but also enhanced endogenous K^+, Mg^{2+}, and Ca^{2+}, which could maintain ion homeostasis and secondary signaling molecule (Tseng et al. 2007). Co-expression of *E. coli CAT* and Mn*SOD* in *B. campestris* also enhanced tolerance to sulfur dioxide and showed increased activity of APX and GR, which could reduce ROS accumulation. Peroxide accumulation in rice as a result of dismutation of $O_2^{\bullet -}$ is accelerated during chilling stress as SOD activity is normally enhanced during low temperature (Saruyama and Tanida 1995). A rapid increase in generation and accumulation of peroxides during chilling exposure was reported in wheat (Okuda et al. 1991). Although peroxides themselves are not lethal, they become highly toxic when they react with $O_2^{\bullet -}$ to form the highly reactive $OH^{\bullet}$ radical, which negatively act on various cellular components like proteins, DNA, and membrane lipids (Bowler et al. 1992). Expression of wheat CAT in rice reduced peroxide accumulation during chilling stress, and its superior tolerance is attributed to effective detoxification of peroxides in transgenic lines (Matsumura et al. 2002). Transgenic Cassava expressing endogenous catalase and Cu/ZnSOD had higher catalase, SOD activities, and lower MDA content than control plants after cold stress (Xu et al. 2014). Also, after harvest, the storage roots of over-expressing cassava lines showed a delay in postharvest physiological deterioration (PPD) response for approximately 10 days, which was accompanied by less mitochondrial oxidation and H_2O_2 accumulation. This was attributed to the combined expression of catalase and Cu/ZnSOD leading to an improved synergistic ROS-scavenging capacity in roots of transgenic plants (Xu et al. 2013). The physiological responses of tobacco with catalase-deficient plants revealed higher SOD activity (less catalase activity) in response to cadmium treatment (Iannone et al. 2015). On the other hand, a type 3 catalase from *B. juncea* enhanced tolerance to cadmium stress in tobacco. At 100 μM Cd, control plants became chlorotic, while *BjCAT*3 transgenic tobacco lines remained green and phenotypically normal. Transgenic lines had lower level of Cd-induced peroxide accumulation (Guan et al. 2009). In catalase-deficient *A. thaliana*, reduced peroxisomal catalase expression leads to increased sensitivity to ozone and photorespiratory peroxide-induced cell death (Vandenabeele et al. 2004). Transgenic tobacco expressing maize catalase 2 gene exhibited ~1.3-fold higher catalase activity and was not negatively affected by MV damage at moderate and high MV concentrations (Polidoros et al. 2001).

7 Current Limitations and Future Directions for the Usage of Transgenic Plants to Combat Oxidative Stress

It is clear from the above studies that modulating the expression of genes coding for ROS-scavenging enzymes should provide a better way to study their specific role, in stress tolerance, and interactions with other enzymes or nonenzymatic antioxidants or osmolytes. Although, modification of ROS-scavenging systems can cause considerable changes in oxidative stress tolerance, there are also several reports on the variance between levels of enzymes and plant tolerance to oxidative stress, which indicate that other cellular factors might be also involved in achieving such increased oxidative stress tolerance in transgenic plants. Also, reports of different transgenic plants expressing single enzymes has achieved only a slight increase in stress tolerance and failed to tolerate different kinds of stress factors. One of the major reason is the need for a balanced interaction of different ROS enzymes at similar levels. Besides, some of initial attempts did not increase stress tolerance (Tepperman and Dunsmuir 1990; Pitcher et al. 1991) due to enzyme sensitivity to products or lack of proper knowledge in targeting. Hence, from the enormous set of data on expression of ROS-scavenging enzymes known till date, it is obvious that an appropriate physiological balance of all the components of the antioxidative defense is crucial to achieve stress tolerance without affecting plants' normal physiological function. Moreover, studies with SOD and glutathione system have clearly indicated that in the future, enzyme should be engineered to overcome feedback inhibition or inhibition by-product.

Plants with the ability to scavenge and/or control the level of cellular ROS may be useful in the future to withstand harsh environmental conditions. Hence, alternate approaches like engineering of plants to reduce ROS levels using proteins like mitochondrial alternative oxidase (AOX), uncoupling protein (UCP), plastid targeted DnaJ protein, etc., are few of the promising genes, which have been proven already to reduce ROS in plants. AOX plays a role in lowering ROS formation in plant mitochondria. Besides function of thermogenesis in plants, the possible explanation for reduced ROS by AOX is explained below. AOX lowers ROS levels by acting on a second oxidase downstream of the ubiquinone pool maintains upstream components of electron-transport chain in a more oxidized state and hence lowering ROS generation by over reduced electron carriers (Maxwell et al. 1999; Umbach et al. 2005; Sugie et al. 2006; Zidenga et al. 2012; Vanlerberghe 2013).

Mitochondrial UCP is a specialized protein that uncouples electron transport from ATP synthesis (Nicholls and Rial 1999). Although thermogenesis was initially attributed to UCPs also, their ubiquitous presence suggests that this protein may have other functions including reducing ROS generation or maintaining its level. It has been already reported that UCP expression in transgenics positively regulated important physiological process like reduced ROS, stomatal conductance, transpiration rates, net photosynthesis, enhance seed viability, improved water balance, higher biomass, etc. (Begcy et al. 2011).

DnaJ proteins act as molecular chaperones (along with Hsp 70) and play a crucial role in maintaining protein conformation and cellular protein homeostasis under harsh environments like heat, high light, MV, chilling stresses, etc. (Piippo et al. 2006; Scarpeci et al. 2008; Rajan and D'Silva 2009). *Arabidopsis* knockout lines of DnaJ proteins induced global response to oxidative stress (Chen et al. 2010). Transgenic tomato lines over-expressing DnaJ accumulated lower ROS level during chilling stress (Kong et al. 2014).

8 Conclusion

New approaches using systems biology are opening doors to generate an all-inclusive transgenic line that are able to maintain its crop productivity even under stressed and changing environmental conditions. As already described above, numerous genes associated with plant response(s) to oxidative stress have been identified and used for generation of transgenic plants to overcome the stress. However, most of these studies were conducted only under controlled laboratory conditions applying artificial stress conditions using model plants like tobacco, *Arabidopsis*, *Medicago*, etc., with a main focus only on recovery from a particular type of stress. In fact plants in real time situations are exposed to different stresses under field conditions. Therefore, in the future, more emphasis should be on the development of transgenic crops (rather than model plants), and the testing should mimic the field condition (i.e., combination of environmental stresses) and focus should be on the crop yield.

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