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Review on Biotechnological Perspective of Reactive Oxygen Species (ROS) Mediated Stress Tolerance in Plants

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Abstract

This review covers recent advances in biotechnological perspective of reactive oxygen species. Reactive oxygen species (ROS) have been considered for a long time as undesirable by-product of the cellular metabolism, but recently the role of ROS in molecular signaling processes has been reported. Consequently, the cell must keep a fragile equilibrium between ROS production and the antioxidant defenses that protect cells in vivo against potential damages (oxidative stress) and, alternatively, allow the inter- and intra-cell communications. This equilibrium may become disturbed under different array of adverse conditions by an excessive generation of ROS or by an impaired antioxidant defenses. All environmental cues lead to develop secondary stress conditions like osmotic and oxidative stress conditions that reduce average crop yields by more than 50% every year. The univalent reduction of molecular oxygen (O_2) in metabolic reactions consequently produces superoxide anions (O_2□) and other reactive oxygen species (ROS) ubiquitously in all compartments of the cell that disturbs redox potential and causes threat to cellular organelles. Over the last decade our understanding of the role of ROS has progressed from the classical view of adverse toxic metabolic byproducts inadvertently associated with aerobic life to include the newly emerging role of biotechnological approach to regulate and coordinating responses to abiotic and biotic stress. A recent series of discoveries have given scientists new insights into ROS-dependent gene activation and the molecular mechanisms involved. The majority of information of the regulatory role of ROS on gene expression derived from experiments using: i) transgenic plants over expressing or suppressing antioxidant genes in order to reduce or increase the intracellular ROS levels, respectively; ii) mutants impaired in ROS generation or scavenging; iii) direct application of ROS; iv) application of ROS generating compounds. Results of these experiments provided significant information on ROS-dependent signaling pathways and ROS-responsive genes. A number of genes involved in defense, signal transduction, transcription, metabolism as well as cell structure have been identified revealing a highly dynamic and redundant network of ROS-producing and ROS-scavenging genes. The present review describes different biotechnological perspective of reactive oxygen species (ROS)-mediated stress tolerance in plants and their consequences under a biotic stress conditions and also described the approaches to overcome oxidative stress through genomics and genetic engineering. Finally, how all this wealth of information is being used with biotechnological purposes is revised.

Key words *Antioxidant, biotechnological, Genomics, ROS, and Stress*

Abbreviations

AOS	Reactive Oxygen Intermediates
ATP	Adenosine Tri Phosphote
cDNA	Complementary DNA
CESR	Common Environmental Stress Response
CESR	Common Environmental Stress Response
COTF	Co transcription cofactor
CYs	Cysteine
DHAR	Dehydroascorbate reductase
GST	Glutathione-S-transferase
GWAS	Genome-wide association
MAPK	Mitogen-activated protein kinase
LePHGPx	Lycopersicon esculentum phospholipid
PAL	Phenylalanine Ammonia Lyase
qRT-PCR	quantitative real time reverse transcriptase PCR
RNS	Reactive Nitrogen-Oxygen Species
ROI	Active Oxygen Species
ROS	Reactive Oxygen Species
RRTF1	Redox responsive transcription fator1
SNP	Single-Nucleotide Polymorphism
TF	Transcription factors

1. Introduction

1.1 Biotechnological Perspective of Reactive Oxygen Species

Plants produce excessive reactive oxygen radicals in response to stress caused as a result of environmental changes (Dabrowska *et al.*, 2007; Khan , 2014; Khan *et al*., 2014, 2015, 2016a, b). Reactive oxygen species (ROS) accumulate as a result of various abiotic stress factors such as salinity, UV radiations, heavy metals, extreme temperature changes, drought, air pollution, herbicides, nutrient deficiency, etc. (Wang *et al*. 2014; Feigl *et al*. 2015; Silveira *et al*. 2015; Thao *et al*. 2015; Farnese *et al*. 2016).

In daily life, plants encounter different abiotic stresses such as water deficit, extreme temperatures, high salinity, high light intensity, heavy metals and more often combination of these stresses under field conditions. However, plants cannot escape from these harsh environmental stresses due to their sessile life. Although all plants are equipped with adaptive mechanisms, to encounter such environmental cues, difference in their allelic constituency has left few crop plants vulnerable. It is estimated that the average yields are reduced to 50%, due to such abiotic stress factors (Vij and Tyagi , 2007).

The proteomic studies in relation to environmental stress caused oxidative stress, like identification of carbonyl groups in proteins; mainly depend on classical biochemical approaches. The immunological detection of modified protein residues under oxidative stress has also been a widely used method for the identification of the stress-induced modifications on proteins (Levine *et al*., 1994). However, all of these assays have great disadvantages in terms of large number of artifacts during sample preparation and can't differentiate between different pro oxidant mediated damages (Buss *et al*., 1997; Cumming RC *et al*. 2008). Moreover, focusing on a single oxidative modification does not render the complexity of the cellular response to oxidative stress, or regulation of redox protein. The cellular response to oxidative stress is extremely dynamic that merely results in the simple accumulation of modified proteins. Therefore, a classical approach to the event based on limited information will not reflect the complexity of redox response, which involves a variety of changes in protein levels (both reversible and irreversible), controlled post translational modifications, and oxidative damage to

proteins (Rabilloud *et al*., 2005). In this perspective, it has been found that biotechnological approach particularly proteomics is the best suited approach to this problem.

2. Review Literature

2.1 ROS Generation Sites in a Plant Cell

ROS production is an inevitable part of aerobic metabolism of a living organism due to the partial reduction nature of molecular oxygen. ROS are produced continuously at low concentration (below threshold levels) in normal plant cells, at sites that are actively engaged in electron transportation reactions (Choudhury *et al.*; 2013). According to estimation, about 1% of total O_2 consumed by plants is being utilized to generate ROS in various cellular organelles like chloroplasts, mitochondria and peroxisomes (Bhattacharjee, 2005). ROS is also known as reactive oxygen intermediates (ROI) or active oxygen species (AOS). ROS with potent damaging effect includes O_2□, singlet oxygen ($1O_2$), OH, perhydroxyl radical (HO_2), H_2O_2, alkoxy radical (RO), peroxy radical (ROO) and organic hydroperoxide (ROOH) (Konig *et al*. 2012; Mignolet-Spruyt *et al*., 2016).

The ROS are free radical and non-radical molecules (Sharma *et al*., 2012) and are the key components of the signaling pathways' network, which act as primary regulators of cellular responses and cell physiology of plant in response to environmental factors (Das and Roychoudhury, 2014). However, sudden rise in intracellular levels of ROS is caused due to the imbalance between production and scavenging of ROS under stress conditions (Mittler *et al*., 2004, Miller *et al*., 2010; Srivastava and Dubey, 2011). In plant tissues, a variety of reactions which consume 1–2% oxygen can lead to excess ROS production which results in cell structure damage (Bhattacharjee, 2005). The ROS are by-products of various metabolic activities which take place in the mitochondria, chloroplast and peroxisomes of the plant cell (Navrot *et al*., 2007; Luis, 2015).

In plants ROS are continuously produced as byproducts of various metabolic pathways localized in different cellular compartments (Foyer CH and Harbinson JC., 1994). Under physiological steady state conditions these molecules are scavenged by different antioxidative defense components that are often confined to particular compartments (Alscher RG *et al*., 1997). The equilibrium between production and scavenging of ROS may be perturbed by a number of adverse environmental factors. As a result of these disturbances, intracellular levels of ROS may rapidly rise (Elstner EF., 1991; Malan C *et al*., 1990; Prasad TK *et al* ., 1994 ; Tsugane K *et al* ., 1999). Plants also generate ROS by activating various oxidases and peroxidases that produce ROS in response to certain environmental changes (Allan AC *et al*., 1997 ; Bolwell GP *et al* ., 2002 ; Bolwell GP *et al*., 1998 ; Doke N. , 1985, Schopfer P ., 2001).

2.2 Proteomic and Genomic Approaches: Exploring Oxidative Stress in Plants

Environmental stresses cause significant decreases in the crop growth and productivity mainly via oxidative stress, which occurs due to redox imbalance. In fact, ROS are unwelcome by-product of aerobic metabolism and constantly produced during redox metabolisms in the cell (Foyer and Noctor , 2005). However, the redox homeostasis is tightly controlled by redundant antioxidative protective mechanism (Gill and Tuteja , 2010). ROS also found to have important regulatory and signaling properties in cellular physiology (Foyer and Noctor , 2005; Gill and Tuteja, 2010). Moreover, the origin of oxidative stress triggered by hyper accumulation of ROS [like, hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), superoxide radicals ($O_2.^-$), peroxy and alkoxy radicals (RO , RCO)] reactive nitrogen-oxygen species (RNS) [like, nitric oxide (NO•), peroxy nitrite ($ONOO^-$), etc.] escapes antioxidant defense machinery and may evoke metabolic dysfunction.

Among bio molecules, proteins are the major target of ROS that constitute about one third of the oxidized species. Since ROS are highly toxic, reactive, and extremely short-lived, it is very difficult to quantify them directly. This can be done by following indirect approach by measuring different components oxidized by these species that requires the use of sensitive, robust, and sophisticated techniques. The ROS-lead oxidative stress at genomic level is another area of investigation to understand plant responses to unfavorable environmental cues. The proteomic studies in relation to environmental stress-caused oxidative stress, like identification of carbonyl groups in proteins, mainly depend on classical biochemical approaches. The immunological detection of modified protein residues under oxidative stress has also been a widely used method for the identification of the stress-induced modifications on proteins (Levine *et al*., 1994). However, all of these assays have great disadvantages in terms of large number of artifacts during sample preparation and can't differentiate between different prooxidant-mediated damages (Buss *et al*., 1997; Caddihy *et al*., 2008). Moreover, focusing on a single oxidative modification does not render the complexity of the cellular response to oxidative stress, or regulation of redox protein. The cellular response to oxidative stress is extremely dynamic that merely results in the simple accumulation of modified proteins. Therefore, a classical approach to the event based on limited information will not reflect the complexity of redox response, which involves a variety of changes in protein levels (both reversible and irreversible), controlled posttranslational modifications, and oxidative damage to proteins (Rabilloud *et al*., 2005).

In this perspective, it has been found that proteomics is the best suited approach to this problem. The improvement in the sophisticated analytical techniques like mass spectrometry has by now provided more precise and more quantitative ways to measure oxidative modifications in the cell. A new branch of proteomics is called "redox proteomics," in which posttranslational modifications of proteins oxidative stress can be studied. This can be used in studying the proteins showing alterations under varied magnitude of oxidative stress (Moller *et al.;* 2007). Considering the significance of detection of genes for plant oxidative stress tolerance, it has been considered indispensable to get insights into the genetics of stress acclimation which will enable us ultimately to successfully develop transgenic plants to be grown under unfavorable environmental cues. With the help of identification of the stress tolerance gene and their

expression patterns, it has become possible to bring them under hybridization program or transgenesis for making oxidative stress-tolerant plants (Gill and Tuteja , 2010).

2.2.1 Genomic Approach for Understanding Oxidative Stress Responses in Plants

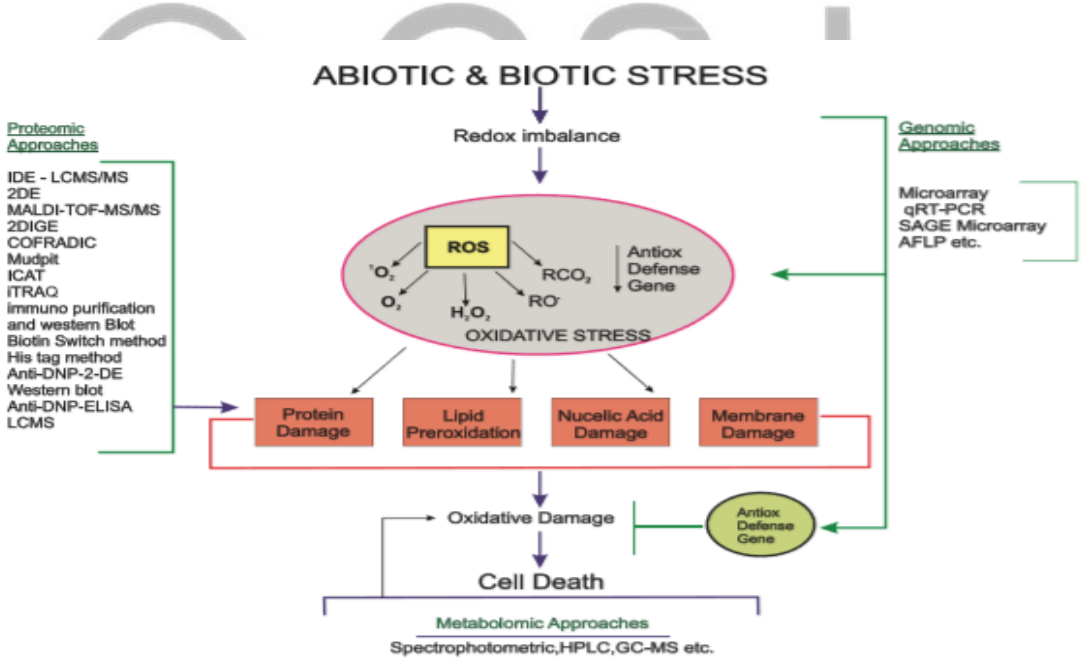
Large scale characterization of redox regulatory genes that help plants to tolerate oxidative stress is essential to understand genetics of plant stress adaptation and to successfully develop transgenic plants to be grown under adverse conditions. In fact, the initial strategy for improving crop production involves identification of redox regulatory genes. Nevertheless, association of the functions of different genes with certain biological processes can also be achieved by identifying the expression patterns of these genes, especially under stress conditions (Huang *et al.* 2014). Moreover, oxidative stress in plants also leaves traces of biomarkers at genomic level, which could be identified with technological breakthrough involving the approaches of genomics and transcriptomics followed by incorporating the data in bioinformatics for validating the same.

Plant responses to environmental stress are mainly polygenic in nature. Identification of genes responsible for intraspecific variation in plant abiotic stress response is therefore a prerequisite (Verslues *et al.* , 2014). Genome-wide association (GWAS) and quantitative real-time reverse-transcriptase PCR (qRT-PCR) are the prime approaches to identify the genes responsible for stress tolerance. Through GWAS, it is now possible to identify critical inducible genes and their alleles apart from successfully identifying loci for phenotypic variation (Ma *et al*. 2012). For unfolding the potential association between genetic variation and important agricultural traits, this method was widely exploited (Brachi *et al*. 2011; Ma *et al*. 2012). GWAS have been found to map gene with high resolution. GWAS with the single-nucleotide polymorphism (SNP) typing can identify small haplotype blocks. That ascorbate peroxidases are member of a multigenic family has been confirmed by GWAS (Najami *et al*. 2008). GWAS also helped to identify genomic regions that were correlated with Al tolerance (Famoso *et al.* 2011).

2.2.2 Proteomic Approaches Used for the Identification of ROS Modified Proteins

Several proteomic tools are in vogue for the detection of oxidatively modified proteins or identification of stress-responsive and/or stress-induced proteins and the analysis of their differential expression under stress. The list of different proteomic approaches or tools used for the analysis of redox modification of proteins has been summarized in Fig. 1.1, and the same have been discussed. In fact, under normal metabolism in plants, redox reactions take place in chloroplast, mitochondria, peroxisome, and plasma membrane which produces ROS by leakage of electrons onto molecular oxygen or by oxidoreductase enzymes which subsequently being scavenged by antioxidant defense mechanisms. However, under environmental stress, both abiotic and biotic, the redox homeostasis is disturbed, which is governed by elevated rate of ROS production and lesser elimination by antioxidant system. The rise in ROS in cells is sensed by the plant cells and transduced in the entire system by different signaling pathways for stress acclimation. The study of redox proteome helps us to reveal the route for signaling pathway during oxidative stress. ROS reversibly or irreversibly modify amino acid residues in oxidation-susceptible proteins. Cysteine, methionine, and selenocysteine undergo reversible oxidation, while tryptophan, tyrosine, arginine, histidine, phenylalanine, cysteine, valine, leucine, isoleucine, lysine, etc. undergo irreversible oxidation. These oxidative modifications alter the structure of proteins which is sensed by the plant and thus initiate signaling cascade. During oxidative stress, the amino acid residues of ROS vulnerable proteins are oxidized either directly by ROS or indirectly by reaction with secondary by products of oxidative stress (MLPO, PO products). The best studied proteins that have undergone oxidative posttranslational modifications are those enzymes involved in the Calvin cycle (Schurmann and Buchanan, 2008), sulfur metabolism (Kopriva et al. 2012), and starch metabolism (Glaring *et al*., 2012).The thiol of Cys is the most extensively characterized component of the redox proteome as Cys side chain contains sulfur atom at the core of the thiol which is electron-rich and its d-orbitals allow for multiple oxidation states. However, not all Cys residues in a protein are prone to ROS-mediated modifications, and the reactivity of different thiol-proteins toward ROS varies according to their physiological function and local redox environment. In individual Cys residues, the reactivity is strongly correlated with their pKa, i.e., the ability to form the anionic form of the sulfur, called

thiolate (R-S–), which is much more reactive than the thiol. If the pKa of the sulfur atom is higher than the pH of the solution, the protonated thiol will be the dominant species. However, if the pKa is lower than the pH, the majority of the thiols will be present as a thiolate (Cys prone to oxidation). The pKa of Cys residues is largely determined by the local electrostatic environment, i.e., the presence of proximal charged residues or dipoles and the hydrogen bonding between thiols/thiolates and neighboring residues (Harris and Turner, 2002). Thus cysteine oxidation permits various posttranslational modifications resulting in diverse regulatory effects. When cellular oxidative strength is low, reversible oxidation of cysteine residue to sulfenic acid takes place; this modification is highly unstable and leads to further modifications (Claiborne *et al*. 1993). An excess concentration of oxidant can lead to further oxidation to sulfenic acid (R-SO2H) and subsequently to irreversible sulfonic acid ($R\text{-}SO_3H$; Roos and Messens, 2011). Alternatively, sulfenic acid can react with free protein thiols to form intra- or intermolecular disulfide bonds (R-S-S-R/R-S-S-R′) or is modified by low molecular weight thiols (like GSH in plants), leading to Cys S-glutathionylation (Fig. 1.1).



Source: (Gill and Tuteja, 2010)

Fig. 1.1 Representing a list of different proteomic, genomic and metabolomic approaches used to detect oxidative stress-responsive changes in plants

2.3 ROS as signals for gene expression

Transcriptome analysis with full genome chips has revolutionized our knowledge regarding gene expression.Oxidative stress affects approximately 10%of the yeast transcriptome (Causton HC *et al* . , 2001, Chen D *et al* ., 2003 , Gasch A. *et al* , 200). Exposure of yeast cells to various stresses including H_2O_2 defines a large set of genes denoted as common environmental stress response (CESR).

CESR-induced genes play a role in carbohydrate metabolism, ROS detoxification, protein folding and degradation, organellar function, and metabolite transport. CESR-repressed genes are involved in energy consumption and growth, RNA processing, transcription, translation, and ribosome and nucleotide biosynthesis (Gasch A. *et al* , 200, Chen D *et al* ., 2003 , Gasch A *et al* ., 2000). In plants, ROS-induced genes have been identified for receptor kinase (Desikan R *et al* ., 2000), annexin (Moon H *et al* ., 2003) and peroxisome biogenesis (Desikan R *et al* ., 2000).

Recent approaches using cDNA profiling and DNA microarrays have analyzed large-scale gene expression in response to ROS. Following exposure of Arabidopsis cells to H_2O_2, a total of 175 genes (i.e., 1–2% of the 11,000 genes on the microarray) showed changes in expression levels Desikan R *et al*., 2001). Of the 113 induced genes, several encoded for proteins with antioxidant functions or were associated with defense responses or other stresses. Still others coded for proteins with signaling functions. Exposing a plant to sub lethal doses of one stress that results in protection from lethal doses of the same stress at a later time is termed stress acclimation. Global changes in gene expression were analyzed in tobacco plants that were treated with superoxide-generating methyl viologen after pretreatment with sub lethal doses (Vranova E *et al*., 2002). Approximately 2% of the tobacco genes were altered in their expression in acclimated leaves.

Genes with predicted protective or detoxifying functions and signal transduction were upregulated in acclimated leaves, implying a variety of cellular responses during acclimation tolerance. The effects of oxidative stress on the Arabidopsis mitochondrial proteome have been analyzed (Sweetlove LJ *et al*., 2002). Whereas two classes of antioxidant defense proteins, peroxiredoxins, and protein disulphide isomerase accumulated in response to oxidative stress, proteins associated with the TCA cycle were less abundant. By inhibiting H_2O_2 production, or facilitating its removal with scavengers such as CAT, genes encoding APX, pathogenesis-related (PR) proteins, glutathione Stransferase (GST), and phenylalanine ammonia-lyase (PAL) were identified (Desikan R *et al* ., 1998 ; Karpinski S *et al* ., 1999 , Levine A *et al* ., 1994). An alternative approach to study the effects of oxidative stress on the transcriptome is to induce oxidative stress by reducing antioxidant activity. CAT and ascorbate peroxidase antisense lines show elevated expression of SOD and GR (Rizhsky L *et al* ., 2002) Fig 1.2 .

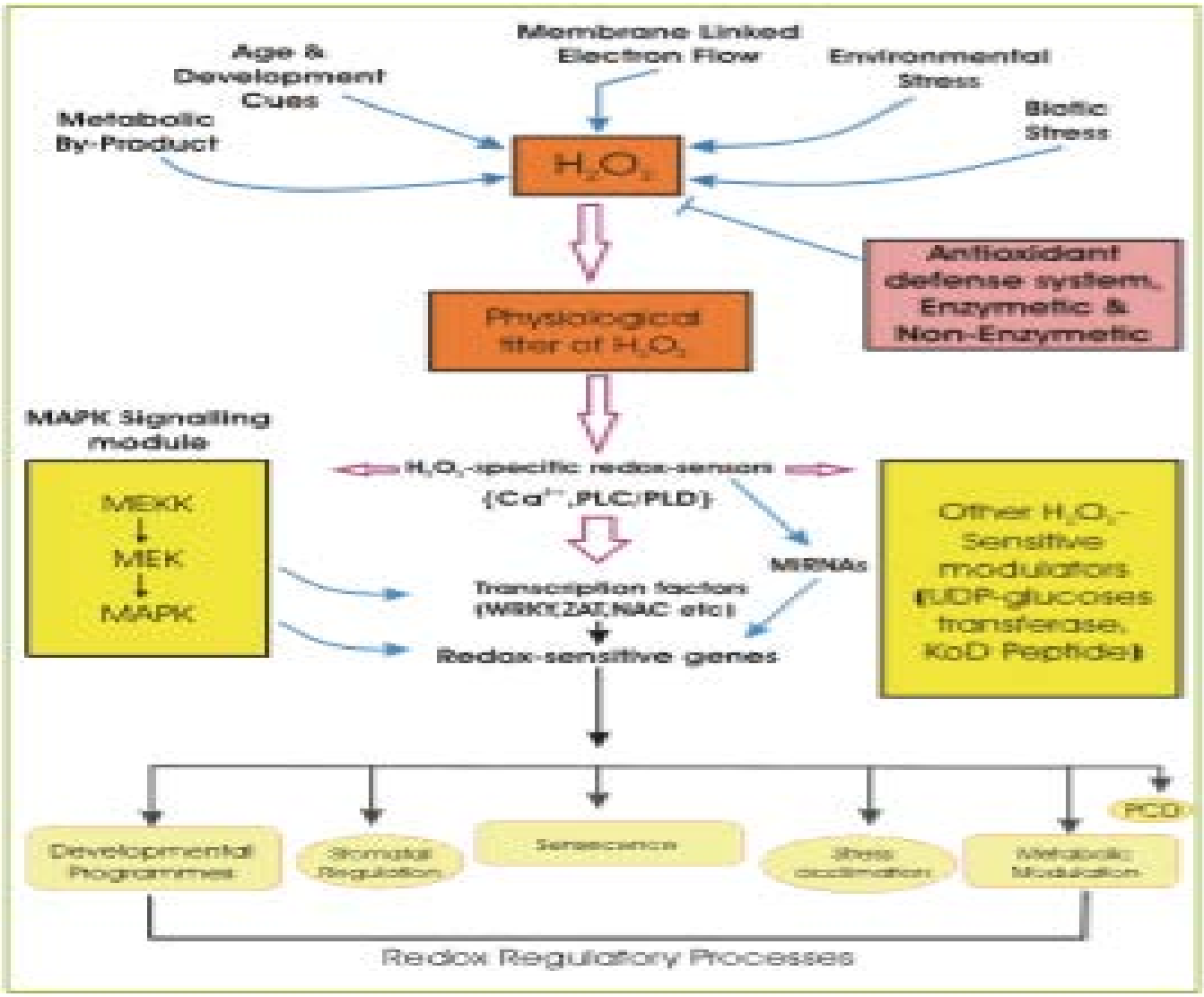


Fig. 1. 2. Schematic pathways showing the role of ROS in gene expression (ROS reactive oxygen species, MAPK mitogen-activated protein kinase, MAPKK MAPK kinase, MAPKKK MAPKK kinase, TF transcription factor, COTF transcription cofactor

In contrast, MDAR, a key enzyme for the regeneration of ascorbate, was upregulated in plants with experimentally reduced CAT and ascorbate peroxidase levels. An increase in expression of ROS detoxifying enzymes is compatible with compensatory mechanisms induced by oxidative stress. When tobacco plants deficient in CAT were grown in high-intensity light, they increased ROS production and PR protein levels, and showed enhanced disease resistance (Chamnongpol S *et al*., 1998).

2.4 ROS Biotechnology for Crop Improvement against Salt Stress

The last couple of decades has provided the crop physiologists with insightful understanding of different roles played by ROS in context of their damaging effects as well as their pivotal role in cellular signaling in plants (Mylona and Polidoros , 2010). Excess production of ROS leads to the toxic effects on cellular metabolism which in turn compromises the plant sustenance and eventually the yield, whereas low concentration of ROS acts as redox signals to maintain the many signal transduction pathways (Foyer and Noctor , 2005). Biotechnological advancements in the field of gene discovery and functional genomics have discovered many tools and lead to the identification of many possible gene targets, which could confer adaptation and improved productivity in hostile environments (Kumar *et al*. 2012).

Genes encoding either ROS-scavenging enzymatic antioxidants or enzymes which control the cellular antioxidant ability from several sources have been proven effective (Zhang *et al*. 2013). The primary goal of these approaches is betterment of crop species leading to minimization of massive loss in productivity. Such transgenic experimentation also helps in understanding the role of each and every scavenger imparting the tolerance to various abiotic factors including salinity. It also helps in understanding the coordinated mechanism between enzymatic and nonenzymatic antioxidant interactions in the complex signaling network. Hence as the total information about such coordinated network is available, it has been reported that rather than producing a transgenic line with a single transgene, it is beneficial to generate a transgenic line with co-expression of several antioxidant defense genes (Diaz-Vivancos *et al*., 2013; Xu *et al*. , 2014). Hence the following are some reports discussed about transgenic lines generated with improved salt tolerance. Overexpression of Cu/ZnSOD gene from Kandelia candel in tobacco

has showed reduction in ROS formation specifically in plastids and improved salinity tolerance (Jing *et al.* 2015). Shafi *et al*. (2015) have demonstrated the co-expression of SOD gene from Potentilla atrosanguinea (PaSOD) and APX gene from Rheum austral (RaAPX) in transgenic Arabidopsis line. This resulted into enhanced lignin deposition and biomass production (yield) under salinity stress.

Over expression of yeast and pea mitochondrial Mn SOD in plastids of rice conferred salt and oxidative stress tolerance (Wang et al. 2005). Also, manganese superoxide dismutase gene from halophilic archaeon, Natrinema altunense (NaMnSOD), is introduced into Nipponbare rice via agrobacterium-mediated transformation. The resultant transgenic line showed increment in total SOD and CAT activity and enhanced elimination of the ROS under salt stress (Chen *et al*. 2013). Transgenic lines of plum (Prunus domestica cv. Claudia Verde) have risen via Agrobacterium-mediated transformation under the control of CaMV35S promoter with cytosolic Cu/ZnSOD from Spinacia oleracea and cytosolic APX cDNA from Pisum sativum as transgenes. Transgenic plants showed higher accumulation of the AsA and GSH and lower H_2O_2 accumulation with enhanced enzymatic and nonenzymatic antioxidative activities to confer the salinity tolerance (Diaz-Vivancos *et al*. 2013).

Over expression of OsAPXa or OsAPXb in Arabidopsis enhanced salinity tolerance to dissimilar levels. Overexpression of OsAPXb improved and sustained APX expression to greater extent than OsAPXa (Lu *et al*. 2007). APX gene from Puccinellia tenuiflora (PutAPX) was overexpressed in transgenic Arabidopsis by Guan *et al.* (2015). This showed the reduction in lipid peroxidation levels and higher chlorophyll content in transgenic seedlings with significantly enhanced salinity tolerance. Peroxisomal APX from Salicornia brachiate was overexpressed in which it conferred not only the salinity tolerance Arachis hypogaea but also the improved vegetative growth and germination rate in transgenic tobacco lines (Singh *et al*. 2014a, b).

Wheat GPXs when expressed in Arabidopsis plastids showed greater growth rate and survival rate during salt stress. Transgenic lines also showed augmented peroxide scavenging capacity and enhanced tolerance to H_2O_2 (Zhai *et al*. 2013). Transient overexpression of the Lycopersicon esculentum phospholipid like GPX (LePHGPx) in tobacco leaves showed suppressed apoptotic feature during severe salt and high-temperature stresses to impart the better tolerance (Chen *et al*.

2004). Homoplastic chloroplast transformants of tobacco were generated by Le Martret *et al*. (2011), with either glutathione-S-transferase (GST) or dehydroascorbate reductase (DHAR) or combination of GST-GR/DHAR-GR. Transgenic progeny showed the better maintained redox state with respect to AsA and GSH content and improved tolerance to various abiotic stresses including salt, cold, and heavy metals. Overexpression of Zea mays CAT and SOD in plastids of Brassica campestris not only improved tolerance to salinity and sulfur dioxide with enhanced endogenous K+, Mg2+, and Ca2+, which help to maintain ionic balance (Tseng *et al*. 2007). Tobacco plants that concurrently express Cu/ZnSOD, APX, and DHAR in their chloroplasts showed improved tolerance to salinity and oxidative damage as compared to the transgenic lines with single- or double-gene transfer (Lee *et al*. 2007). All the above mentioned cases clearly specify that over expression of diverse iso forms of different genes amended the detoxification of ROS and imparted superior salinity tolerance in transgenic host

system.

Table 1 Summarizes the successful attempts made to enhance the salt tolerance of several transgenic plants via over expressing the ROS-detoxifying antioxidant genes.

Gene	Source organism	Transgenic plant	Improved characters	References
Cu/ ZnSOD	*Kandelia candel*	*Nicotiana tabacum*	Reduced ROS formation in plastids, improved salt tolerance	Jing et al. (2015)
PaSOD, RaAPX	*Potentilla atrosanguinea, Rheum austral*	*Arabidopsis thaliana*	Enhanced lignin deposition and yield under salinity stress	Shafi et al. (2015)
MnSOD	*Saccharomyces cerevisiae, Pisum sativum*	*Oryza sativa*	Enhanced salt and oxidative stress	Wang et al. (2005)
NaMnSOD	*Natrinema altunense*	*Oryza sativa*	Enhanced total SOD and CAT activity and ROS elimination under salt stress	Chen et al. (2013)
Cu/ ZnSOD, APX cDNA	*Spinacia oleracea, Pisum sativum*	*Prunus domestica*	Higher accumulation of the AsA and GSH and lower H_2O_2 accumulation, enhanced salinity tolerance	Diaz-Vivancos et al. (2013)
OsAPXa or OsAPXb	*Oryza sativa*	*Arabidopsis thaliana*	Enhanced salinity tolerance	Lu et al. (2007)
PutAPX	*Puccinellia tenuiflora*	*Arabidopsis thaliana*	Reduced lipid peroxidation, increased chlorophyll content, and enhanced salinity tolerance	Guan et al. (2015)
APX	*Salicornia brachiata*	*Arachis hypogaea, Nicotiana tabacum*	Enhanced vegetative growth and germination rate, improved salinity tolerance	Singh et al. (2014a, b)
GPX	*Triticum aestivum*	*Arabidopsis thaliana*	Enhanced growth rate and survival rate, increased peroxide scavenging capacity, enhanced tolerance to H_2O_2	Zhai et al. (2013)
LePHGPx	*Lycopersicon esculentum*	*Nicotiana tabacum*	Suppressed apoptotic during severe salt and high-temperature stresses imparting tolerance	Chen et al. (2004)
GSF or DHAR or GSF-GR/ DHAR-GR	*Oryza sativa, E. coli*	*Nicotiana tabacum*	Better maintained redox status, improved tolerance to various abiotic stresses including salt, cold, heavy metals	Le Martret et al. (2011)
CAT, SOD	*Zea mays*	*Brassica campestris*	Enhanced salinity tolerance, improved endogenous K^+, Mg^{2+}, and Ca^{2+} levels to maintain ionic balance	Tseng et al. (2007)

Source : (Vinay Kumar *et al*., 2017)

2.5 Transcription factors associated with ROS

Transcription factors (TFs) have been found to regulate the expression of several stress-inducible genes involved in stress acclimation and tolerance by interacting with cis-elements of genes involved in ROS-induced transcriptional changes. Large-scale expression analysis has confirmed the participation of various genes in abiotic stress and there are huge similarities in the transcriptional response upon treatment with ROS-forming compounds. In a microarray study, 32 TFs common to various oxidative stress treatments were found that were at least five-fold up regulated. Seven of them belonged to NAM/NAC family, eight to AP2/ERF family and six to WRKY families (Gajdev *et al.,* 2006). Additionally, few ER-bound TFs have also been shown to regulate abiotic stress responses (Jaspers Kangasjärvi , 2010).

Salt stress and heat stress inducible genes have been found to be up regulated by the activation of AtbZIP17 and AtbZIP28 respectively (Liu *et al*., 2007). Moreover, AtERF6 and redox responsive transcription fator1 (RRTF1) is also involved in ROS activated response and it binds with GCC Box to bring transcriptional changes (Wang *et al*., 2013; Matsuo *et al.,* 2015). Some genes are also down regulated during oxidative damage. An AP2/ERF TF, cytokinin response factor 6, also binds to GCC box and regulate down-regulation of cytokinin-related genes during oxidative stress (Zwack *et al.,* 2016). ROS-mediated stress response are also governed by other regulators like zinc finger TFs, NAC or NAM (no apical meristem), WRKY (Chen *et al.,* 2010) Zat, RAV, GRAS and Myb families (Rizhsky *et al*., 2004; Epple *et al*., 2003). Although, Zat10 works as a repressor of ROS-responsive genes (Mittler 2006), Zat6 and Zat12 regulate Apx1 expression positively and induce plant protection during oxidative stress (Shi *et al.,* 2014).

2.6. ROS-Induced Signaling and Gene Expression in Crops under Salinity Stress

Salinity is one of the most severe threats for crop production. Hypersaline conditions not only limit the global crop productivity but also affect the quality of produce. Salinity exerts threefold effect as reduced plant growth and yield take place through osmotic stress, ion toxicities and imbalances, and oxidative stress (Khare *et al*. 2015). As a consequence, decreased stomatal conductivity under low water potential, over-reduction of electron transport in cellular organelles, and overall declined photosynthetic electron transport take place, leading to excessive generation of reactive oxygen species (ROS) such as singlet oxygen, superoxide radical, hydrogen peroxide, and hydroxyl radical. These ROS cause serious damages to lipid membranes and other essential macromolecules including proteins and nucleic acids and ultimately induce cell death (Qureshi *et al*. 2013; Khare *et al.* 2015). However, notably, they have the abilities to work as signaling molecule and are critical in regulating the responses of development as well as various aspects of stress (Ismail *et al*. 2014).

There are recent reports of ROS-induced aberrant expression of multiple genes and altered signal transduction pathways, which implies the plant cellular strategies to use ROS as stimuli and signals that trigger and regulate numerous stress-responsive genetic networks in stressed plants. However, plants have evolved crucial biochemical strategies to keep a check on excess ROS generation to counter the deleterious effects of abiotic stress including salinity, which principally includes the induction of enzymatic and non enzymatic antioxidant machinery for ROS detoxification or scavenging (Khan and Khan 2014; Khan *et al*. 2014, 2015, 2016a, b; Qureshi *et al.* 2013; Yildiztugay *et al.* 2014). Owing to their significance, the antioxidant entities have been altered to engineer the salt tolerance in various crops through overexpression of their pathway genes (Kumar *et al*. 2010; Kumar and Khare 2016; Wani *et al*. 2016).

Through this review we are presenting here in a comprehensive and critical assessment and discussion of salinity stress-induced generation of different types of ROS in plant cells and tissues, cellular damages by the ROS, role of ROS as signaling molecules, and recent reports on

differential expression of antioxidants under saline conditions, besides ROS biotechnology for improved crop salt tolerance

2.7. ROS, Oxidative Stress and Engineering Resistance in Higher Plants

Reactive oxygen species (ROS) play an important role in the ability of plants to adapt to environmental conditions and respond to various stresses. The complex network of ROS scavenging systems keep the ROS levels in control throughout the life cycle of the plant. However, plants respond differently to various stresses. Under biotic stress the ROS production serves as a defense component rather than the toxic metabolic product. On the other hand, during abiotic stress response plants scavenge the increased ROS levels to prevent the damage. This chapter discusses the role of ROS in defense response and describes the recent advances in genetic engineering of oxidative stress resistance in higher plants (Damla D. Bilgin, 2006)

Oxygen in the atmosphere and water supports aerobic life. Plants consume oxygen during respiration and generate it by photosynthesis. During these metabolic processes, the production of reactive oxygen species (ROS) cannot be avoided in plants especially in organelles such as chloroplasts, mitochondria and paroxysms. The reactive nature of ROS led to the evolution of ROS scavenging mechanisms for protection. It is widely accepted that ROS play an important role in the ability of plants to adapt to environmental conditions and respond to various stresses (Scheel , 2002, Apel and Hirt, 2004, Foyer and Noctor, 2005, Torres and Dangl , 2005, Gechev *et al*. 2006, Pitzschke *et al.* 2006, Gadjev *et al*. 2008, Shetty *et al.*, 2008) . ROS, which under normal conditions, are produced and scavenged in a controlled manner, become in excess under stress. Overproduction of ROS is a key part of the defense mechanism against pathogens and induces defense-related gene expression. The distinct role of ROS as a signaling molecule is important for both plant development and defense. The purpose of this chapter is to discuss ROS scavenging mechanisms, signaling and how these concepts are used to produce plants resistant to oxidative stress. The role of ROS and detoxifying mechanisms in defense response against biotic and abiotic stresses will be described in detail. The major findings to genetically engineer plants are not reviewed in detail with an emphasis on defense against oxidative stress.

2.8 DNA Damage

Endogenously produced ROS under stress conditions can readily attack plant DNA. Though plant DNA is stable in nature, it cannot protect itself from ROS (OH•,O 2 •¯, and NO•) attack (Tuteja *et al*. 2009; Valko *et al*. 2006). $1O_2$ was found to attack guanine (Wiseman and Halliwell 1996), whereas all apparatus of DNA like purines, pyrimidines, and deoxyribose backbone gets attacked by OH•, making it highly reactive against DNA (Halliwell and Gutteridge 1999). The damage caused by ROS to DNA is of multiple sorts like fragmentation of DNA, base modification by alkylation or oxidation, cross-linking, base removal, and dimer formation (Tuteja *et al*. 2001; Tuteja and Tuteja 2001; Kumar *et al.* 2013). As DNA is the primary coding center in cell, its damage triggers the cascade secondary responses. These responses include decreased protein synthesis, cell membrane damage, and destruction of photosynthetic proteins which, in turn, greatly affects growth and development (Britt 1999).

Summery

With the advent of novel genetic tools in molecular biology, noticeable efforts are made to develop transgenic plants which have shown tolerance against abiotic stress and pathogen defence. The development of transgenic plants with altered enzymatic mechanisms has shown considerable tolerance against abiotic stress, and improved yield is being noticed. Earlier ROS accumulation was perceived as toxic, but now it is a well established fact that they mediate crosstalk between various signalling pathways to influence various developmental processes and making them adaptable towards extreme abiotic combating ROS induced stress, but the future belongs to the development of a more novel transgenic plants with improved genetic mechanisms to achieve plethora of abiotic stresses. Recently, many transgenic plants with overexpressed genes have shown promising role in combating ROS-induced stress. A substantial amount of research has revealed that ROS generation in plant cells and tissues is an unfortunate consequence of all the biotic and abiotic stress conditions and these ROS have multiple negative implications on plant growth and development. Though there is a recent advocacy for their significance as biotechnological molecules, critical for cellular functions and stress responses, however, greater insights are yet to be revealed in these areas and need further efforts from the scientific community. Several attempts have been made in the recent past to develop salt-tolerant plants via altering the expression levels of major antioxidant pathway genes. Therefore, ROS biotechnology presents a solid platform for developing a biotic stress tolerant crop. Due to the regulatory issues associated with GMOs, transgenic approach may be considered as lost approach, when the gene of interest is not available within the germplasm of host plant. Marker-assisted breeding (molecular breeding) may be considered as the right choice to identify and transfer the QTLs associated with trait of interest. Due to complexity of the trait, and functional sharing with salt, drought and heat stress, oxidative stress was given less focus in QTL identification and subsequent marker development programmes. Not many specific QTLs were identified associated with oxidative stress, but many QTL identified and markers associated were developed for heat tolerance that shares common genes for detoxifying ROS generated through oxidative stress. Bita and Gerats (2013) have described various QTLs associated with heat tolerance in different crop.

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