# Chapter 5
# Mechanism of Free Radical Scavenging and Role of Phytohormones in Plants Under Abiotic Stresses

**Parvaiz Ahmad, Shahid Umar, and Satyawati Sharma**

**Abstract** Environmental stresses result in the generation of reactive oxygen species (ROS) in plants. ROS accumulate in cells and lead to the oxidation of proteins, chlorophyll, lipids, nucleic acids, carbohydrates etc. Cells have evolved intricate defense systems including enzymatic (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductases (GR), monodehydroascorbate reductases (MSHAR), dehydroascorbate reductases (DHAR), glutathione peroxidase (GPX), guaicol peroxidase (GOPX) and glutathione-*S*- transferase (GST) and non-enzymatic systems such as ascorbic acid (ASH), glutathione (GSH), phenolic compounds, alkaloids, non-protein amino acids and α-tocopherol, which can scavenge the indigenously generated ROS. Plant stress tolerance mediated by antioxidants has been shown by many workers. Antioxidant resistance mechanisms may provide a strategy to enhance plant stress tolerance. Various enzymes involved in ROS-scavenging have been manipulated, over-expressed or down-regulated to add to the present knowledge and understanding of the role of antioxidant system. ROS induce the synthesis of several plant hormones, such as ethylene, salicylic acid (SA), jasmonic acid, brassinosteroids, abscisic acid (ABA) etc. These Phytohormones are required for growth and development of plants and defense responses during environmental stresses. The present review throws light on the enzymatic and non-enzymatic antioxidants in plants to enhance stress tolerance in plants and also in particular the role of brassinosteroids and ethylene during abiotic stress tolerance in plants.

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## Contents

## 1 Introduction

Environmental stresses like temperature, drought, alkalinity, salinity, UV radiation are dangerous to plant life (Van Breusegem et al. 2001). According to FAO (2004) approximately, 22% of the world agricultural land is saline and the land under drought stress is expanding at an alarming rate (Burke et al. 2006).

Abiotic stress environment can induce a wide number of responses in plants ranging from readjustments of transport and metabolic processes leading to growth inhibition (Jaleel et al. 2007b, 2008; Ahmad et al. 2008a). During the exposure of plants to stress, a number of genes and gene products are expressed including proteins and they may be responsible for tolerance to these stresses (Mathur et al. 2008).

The primary effect of abiotic stress is ion imbalance and hyper-osmotic stress. During stress molecular oxygen receives electrons from high energy level to produce reactive oxygen species (ROS) (Mittler 2002) that are harmful to plant cells at high concentrations. ROS such as hydrogen peroxide, superoxide ions, singlet oxygen, peroxides etc. are toxic molecules for plant metabolism (Apel and Hirt 2004). All cellular macromolecules including DNA are damaged due to the deleterious effects of ROS (Tuteja et al. 2009) (Table 5.1).

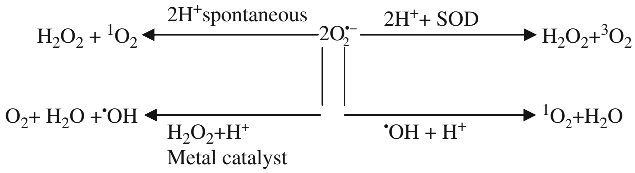
**Table 5.1** Reactive oxygen species and oxidative stress

| Name | Basic sources |
|---|---|
| Singlet oxygen (1st excited singlet state) $1O_2$ | Photoinhibition; UV irradiation; PS II e- transfer reactions (chloroplasts) |
| Superoxide anion $O_2^{\bullet -}$ | Formed in many photooxidation reactions (flavoprotein, redox cycling); Mehler reaction in chloroplasts; mitochondrial e- transfer reactions; glyoxysomal photorespiration; peroxisomal activity; nitrogen fixation; reactions of $O_3$ and $OH^{\bullet}$ in apoplastic space; defense against pathogens; oxidation of xenobiotics |
| Hydrogen peroxide $H_2O_2$ | Formed from $O_2^{\bullet -}$ by dismutation; photorespiration; ß-oxidation; proton-induced decomposition of $O_2^{\bullet -}$; defense against pathogens |
| Hydroxyl radical $OH^{\bullet}$ | Decomposition of O3 in apoplastic space; defense against pathogens; reactions of $H_2O_2$ with $O_2^{\bullet -}$ (Haber-Weiss); reactions of $H_2O_2$ with $Fe^{2+}$ (Fenton); highly reactive with all macromolecules |
| Perhydroxyl radical $O_2H^{\bullet}$ | Protonated form of $O_2^{\bullet -}$; reactions of $O_3$ and $OH^{\bullet}$ in apoplastic space |
| Ozone $O_3$ | UV radiation or electrical discharge in stratosphere; reactions involving combustion products of fossil fuels and UV radiation in troposphere |

## 2 ROS Production

The main site of ROS production in plants through photorespiration during light is chloroplast and peroxisomes (Foyer and Noctor 2003) and mitochondria during darkness (Moller 2001). Chloroplast is a major producer of superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$) in plants. Asada (2006) has demonstrated that the sites of ROS production in chloroplast thylakoids are PSI and PSII.

Superoxide ($O_2^{\bullet -}$) is produced as byproduct at complexes I and III of mitochondria. Superoxides ($O_2^{\bullet -}$) and hydrogen peroxide ($H_2O_2$) are produced during metabolism. The former is produced by NADPH oxidase in plasma membrane and has an important role in several metabolic processes (Torres and Dangl 2005). The most important reactive oxygen species are oxygen derivatives (Tuteja et al. 2001; 2009) that are produced through the complete reduction of $O_2$, as shown below:



Hydroxyl radicals are produced from hydrogen peroxide which is an oxidizing agent. It can affect biomolecules of the cell. Hydroxyl radicals are produced through Harber-Weiss reaction (1934).

$$O_2^{\bullet-} + H_2O_2 \xrightarrow{\text{Cu and/or Fe}} O_2^{\bullet-} + {}^{-}OH + {}^{\bullet}OH$$

Radiations are also known to generate hydroxyl radicals in plants. The high energy of radiations (X-rays or gamma-rays) in the cell sap splits the covalent bonds of water.

$$H_2O \xrightarrow{\text{Radiation}} \begin{array}{c}\text{Intermediate}\\ \text{steps}\end{array} \longrightarrow {}^{\bullet}H + {}^{\bullet}OH$$

The life span of hydroxyl radicals is very short (micro-seconds) but they are highly reactive among radicals studied so far.

Plant systems are equipped with enzymatic and non-enzymatic antioxidants such as: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione redutase (GR), ascorbic acid (AsA), glutathione etc. They minimize the deleterious effects of ROS. Every compartment of the cell contains one or more antioxidants that act on a particular ROS and detoxifies it (Nobuhiro and Mittler 2006). Introduction or over-expression of selected genes is the promising way to generate stress tolerant plants (Mathur et al. 2008).

## 3 Enzymatic Antioxidants

### *3.1 Superoxide Dismutase (SOD; EC 1.15.1.1)*

Superoxide dismutase is a metalloenzyme, which coverts $O_2^{-\bullet}$ to $H_2O_2$. It was first found in maize (Scandalios 1993). SOD is classified on the basis of metal ions attached to their active site, as Cu/Zn-SOD, Mn-SOD, Fe-SOD, and Ni-SOD. Cu/Zn-SOD is found in the cytosol and chloroplast of the plant cell, whereas Mn-SOD in the mitochondrial matrix and peroxisomes. SOD regulates the concentration of superoxide anionic radical, and it has received great attention because of its protective effect against oxygen toxicity (Nordberg and Arner 2001). Hence, SOD has gained considerable interest in the pharmaceutical and food industries (Meyer et al. 2005). The over-expression of SODs combats the negative effects of oxidative stress and has a significant role in tolerance and survival of plants. Experimental results showed that during salt stress, SOD activity increases in pea, maize, tea, mustard and mulberry (Ahmad et al. 2008b; Tuna et al. 2008; Upadhyaya et al. 2008; Ahmad 2010; Ahmad et al. 2010). Arbona et al. (2008) also showed the strong induction of SOD (up to 1.4 fold) in Carrizo citrange as compared to that in Cleoptra mandarin in response to flooding. In other studies, SOD activities were found to be low in salt sensitive cultivars and high in salt tolerant cultivars of tomato and *Plantago* under salt stress (Shalata et al. 2001; Sekmen et al. 2007). Total SOD activity showed marked enhancement under salinity in *Morus alba* (Harinasut et al. 2003). *Picea asperata* has been shown to have increased SOD activity during high light and drought stress (Yang et al. 2008). Qiu-Fang et al. (2005) demonstrated that under high NaCl concentration, chloroplast SOD, thylakoid bound SOD and stroma SOD were enhanced, and the increase being more in chloroplast SOD. Zhang et al. (2008) observed over-expression of 9-*cis*-epoxycarotenoid dioxygenase (NCED) gene *SgNCED1* in transgenic tobacco plants which induced the activity of SOD thereby resulting in improved growth in transgenic tobacco under drought and NaCl stresses. SOD activity increased under drought stress in *Euphorbia esula* (Davis and Swanson, 2001), maize (Pastori *et al*. 2000; Jiang and Zhang 2002), wheat (Singh and Usha 2003; Shao et al. 2005), rice (Wang et al. 2005), *Phaseolus acutifolius* (Turkan et al. 2005), and the SOD activity was higher under salinity stress in *Catharanthus roseus* (Jaleel et al. 2007a). Expression of Cu/Zn-SOD and APX genes in transgenic fescue plants showed tolerance to methyl viologen (MV), and heavy metal stress (Lee et al. 2007). Expression of Fe-SODs in *Lycopersicon esculentum* seedlings may help plants in the development of heat-shock tolerance (Camejo et al. 2007). Constitutive over-expression of Cu/Zn-SOD in the transgenic tobacco cytosol, reduced the ozone-induced necrosis (Pitcher and Zilinskas 1996).

## *3.2 Catalases (EC 1.11.1.6)*

Catalases, mainly localized in the peroxisomes, are responsible for the conversion of $2H_2O_2$ to $O_2 + 2H_2O$ (Srivalli et al. 2003; Ben-Amor et al. 2005). They are present in all aerobic eukaryotes and are important in the detoxification of $H_2O_2$ generated in peroxisomes (microbodies), involved in β-oxidation of fatty acids, the glyoxylate cycle (photorespiration) and purine catabolism. Multiple isozyme forms of catalase have been found in plants. Castor bean and *Arabidopsis* contain two and six isozyme forms, respectively (Frugoli et al. 1996). They can direct dismutation of $H_2O_2$. Plants have been shown to contain catalase in multiple forms, e.g., maize contains three isoforms, *CAT 1*, *CAT 2* and *CAT 3*, which are located on separate chromosomes and are differentially expressed and independently regulated (Scandalios 1990). Peroxisomes and cytosol contains *CAT 1* and *CAT 2,* and *CAT 3* is located in mitochondria. Plants contain multiple CAT isozymes, e.g., two in *Hordeum vulgare* (Azevedo et al. 1998), and as many as 12 isozymes in mustard (Frugoli et al. 1996). CAT isozymes have been shown to be regulated temporally and spatially and may respond differently to light (Skadsen et al. 1995). Catalases are the principal scavenging enzymes which directly dismutate $H_2O_2$ into $H_2O$ and $O_2$ during stress (Van Breusegem et al. 2001). There are reports which show that increasing catalase activity helps the plant to adapt the harsh conditions and maintains the metabolic processes by minimizing the toxic level of $H_2O_2$ (Sekmen et al. 2007; Vital et al. 2008). Abiotic stress leads to the up-regulation of the genes responsible for the expression of catalase in alfalfa nodule, tea, cotton and tobacco (Sekmen et al. 2007; Upadhyaya et al. 2008; Vital et al. 2008; Zhang et al. 2008). Sekmen et al. (2007) demonstrated that increase in catalase activity was more in salt tolerant *Plantago maritima* than that in salt-sensitive *Plantago media*. Continuous waterlogging in *Citrus melo CPB 4475* and *Carrizo citrange* showed that CAT activity increased 1.7 fold and 3.0 fold, respectively as compared to that in control plants (Arbona et al. 2008). Yang et al. (2008) observed that CAT activity significantly increased in dragon spruce (*Picea asperata* Mast.) seedlings subjected to the combined effect of drought and high light.

Catalase activity increased in maize (Pastori et al. 2000; Jiang and Zhang 2002); *Allium schoenoprasum* (Egert and Tevini 2002), and wheat (Dalmia and Sawhney 2004; Shao et al. 2005); *Phaseolus acutifolius* (Turkan et al. 2005) under drought stress. An increase in catalase activity was reported in many higher plants under drought stress (Reddy et al. 2004). Similar results were found in *Lotus corniculatus* (Borsani et al. 2001) and rice (Wang et al. 2005). However, Harinasut et al. (2003) showed that CAT activity did not respond to increasing salt concentration in salt tolerant mulberry cultivar, Pei. Decrease in CAT activity in leaves of *Bruguiera parviflora* under NaCl stress was also observed by Parida et al. (2004). The decreasing CAT activity in some plants reflects the importance of peroxidase as well as SOD/ascorbate-glutathione cycle as oxygen reactive scavenging systems (Harinasut et al. 2003).

## *3.3 Ascorbate Peroxidase (APX, EC 1.11.1.1)*

Ascorbate peroxidase is an essential antioxidant enzyme, which has a leading role in detoxification or scavenging of $H_2O_2$ in water-water and ascorbate-glutathione cycles. The excess of $H_2O_2$ is reduced to $H_2O$ and $O_2$ in the presence of APXs (Kangasjärvi et al. 2008). Five different isoforms of APX family have been found in different compartments of the cell (Noctor and Foyer 1998).

Ascorbate peroxide activity increased under drought stress in *Euphorbia esula* (Davis and Swanson 2001), *Zea mays* (Jiang and Zhang 2002), wheat (Dalmia and Sawhney 2004), *Phaseolus acutifolius* (Turkan et al. 2005) and soybean (Van Heerden and Kruger 2002). Increased APX activity was observed under drought stress in *Vigna* (Manivannan et al. 2007) and *Catharanthus* plants under salt stress (Jaleel et al. 2007a). Zhang et al. (2008) reported that transgenic tobacco overexpressing 9-cis-epoxycarotenoid dioxygenase (NCED) gene *SgNCED1* showed increased activity of APX and improved growth under mannitol-induced drought stress. The mRNA of cytosolic ascorbate peroxidase showed up-regulation during drought stress in alfalfa nodules (Naya et al. 2007). Different abiotic stress increases APX activity in different plants, e.g., waterlogging in citrus (Arbona et al. 2008), NaCl and paraquat stress in cotton calli (Vital et al. 2008), salt stress in *Arabidopsis* (Lu et al. 2007). Giacomelli et al. (2007) observed that *Arabidopsis thaliana* deficient in two chloroplast ascorbate peroxidases (stromal APX and thylakoid APX) showed accelerated necrosis induced by light at lower levels of AsA in the cell. Simultaneous over-expression of Cu/Zn-SOD and APX genes in chloroplasts of transgenic fescue plants showed resistance to abiotic stresses (Lee et al. 2007).

### *3.4 Glutathione Reductase (GR, EC 1.6.4.2)*

GR is a flavo-protein oxidoreductase, found in both prokaryotes and eukaryotes that catalyses the NADPH-dependent reduction of oxidized glutathione (GSSG) to its reduced form (GSH). In the cell, GR is located in the chloroplast stroma, mitochondria, cytosol and peroxisomes. Plants have multiple forms of this enzyme, eight in pea (Edwards et al. 1990) and two in wheat (Dalal and Khanna-Chopra 2001). There are reports which showed that different environmental stresses induce GR activity. For example, GR activity significantly increased with chilling stress in cucumber (*Cucumis sativus* L.), and wheat (*Triticum aestivum*) with high temperature (Keles and Oncel 2002), and in alfalfa nodules during water stress (Naya et al. 2007), and in cotton calli during NaCl and paraquat stress (Vital et al. 2008). Semane et al. (2007) also demonstrated that a significant increase in the messenger RNA level of genes involved in GSH synthesis (*gsh1 and gsh2*) during Cd stress in *Arabidopsis* (Semane et al. 2007).

Interestingly, higher glutathione levels were also observed in transgenic tobacco, which over-expressed human DHAR gene (dehydroascorbate gene) and the increased GR levels protected these plants from membrane damage when subjected to MV and NaCl (Lee et al. 2007). DHAR over-expressing plants also had improved tolerance for other abiotic stresses like low temperature and high salinity levels. However, with increasing NaCl concentration, a decrease in GR activity has been reported in roots of salt sensitive genotypes of wheat (BR5001) by Azevedo-Neto et al. (2006). More decrease in GR activity was observed in salt-sensitive *Plantago media* than that in salt tolerant *Plantago maritima* (Sekmen et al. 2007). Upadhyaya et al. (2008) observed that some clones of *Camellia sinensis* showed increased GR activity under water stress and rehydration treatments decreased the GR activities in all the tested clones. Ding et al. (2007) also reported increased GR activity in mango fruit after exogenous oxalic acid or salicylic acid treatment under chilling stress.

## 4 Non-enzymatic Antioxidants

### *4.1 Ascorbic Acid (Vitamin C)*

Among the non-enzymatic antioxidants AsA is the most extensively studied molecule and is found in various plant cell types (Horemans et al. 2000; Smirnoff 2000). Although the precursor of L-ascorbic acid is D-glucose, its biosynthetic pathway is still unclear (Foyer and Noctor 2005). Normally, ascorbate occurs in the reduced form (AsA). (90% of the ascorbate pool) and its intracellular concentration ranges from 20 m*M* in the cytosol to 300 mM in the chloroplast (Noctor and Foyer 1998). The synthesis of ascorbate takes place in mitochondria and is transported to other cell components through a proton-electrochemical gradient or through facilitated diffusion (Horemans et al. 2000). AsA has effects on different physiological processes including growth regulation, differentiation and metabolism of plants. The basic role of AsA is to protect plants from the deleterious effects of $H_2O_2$ and other toxic derivatives of oxygen. AsA acts essentially as a reductant and it scavenges many types of free radicals. In the ascorbate–glutathione cycle, APX utilizes ascorbic acid and reduces $H_2O_2$ to water and generates monodehydroascorbate (MDA). MDA can also be reduced directly to AsA. The electron donor is usually NADPH and catalyzed by monodehydroascorbate reductase (MDAR). AsA can directly scavenge $^1O_2$, $O_2^{\bullet-}$ and $^{\bullet}OH$ radicals produced in the cell. AsA helps to regenerate tocopherol from tocopheroxyl radical which in turn provides protection to the membranes against oxidative stress. The synergistic action of AsA with other antioxidants plays a significant role in reducing the damaging effect of oxidative stress and gives tolerance to plants against environmental stresses (Foyer and Noctor 2005). Over-expression of *A. thaliana* MDAR gene (*AtMDAR1*) in tobacco plants showed enhanced tolerance to ozone, salt and PEG (Eltayeb et al. 2007). This tolerance may be due to the increased levels of AsA which mainly resulted from the enhanced activity of MDAR (Eltayeb et al. 2007). AsA reacts non-enzymatically with superoxide, hydrogen peroxide, and singlet oxygen.

## 4.2 Vitamin E (α-Tocopherols)

Tocopherols, a lipid soluble antioxidant found in all plant parts and are potential scavengers of ROS and lipid radicals (Kruk et al. 2005). Kagan (1989) has reported that tocopherols are important part of membranes in biological systems, where they play both antioxidant and non-antioxidant functions. Out of four isomers of tocopherols (α-, β-, γ-, δ-) found in plants (Kamal-Eldin and Appelqvist 1996), α-tocopherol is extensively studied. The molecular stricture of α-tocopherol has three methyl groups that give the molecule highest antioxidant property. Tocopherols are shown to be scavengers of oxygen radicals, especially $^1O_2$ and during chain propagation step, lipid auto-oxidation is prevented by tocopherols and this makes them effective free radical traps (Serbinova and Packer 1994). Munne-Bosch (2005) demonstrated that one molecule of α-tocopherol can scavenge up to 120 $^1O_2$ molecules by resonance energy transfer. It is well established that oxidative stress up-regulates the genes for tocopherol synthesis in plants (Wu et al. 2007). Antioxidants including α-tocopherol and AsA contributes to chilling tolerance in tomato plants and plays a protective role in oxidative stress induced damages to membranes. Many workers have reported that water stress is accompanied by increasing levels of tocopherols (Wu et al. 2007; Shao et al. 2007). α-tocopherol is synthesized from $\gamma$-tocopherol in chloroplasts by $\gamma$-tocopherolmethyltransferase ($\gamma$-TMT; VTE4). Leaves of many plant species including *Arabidopsis* contain high levels of $\alpha$-tocopherol, but are low in $\gamma$-tocopherol. It has been suggested that $\gamma$-tocopherol or its respective derivative 5-nitro-$\gamma$-tocopherol (5-N$\gamma$T), may prolong early development by reducing the level of NOx. The germinating seeds of mustard, tobacco and *Arabidopsis* have been found to contain 5-N$\gamma$T (Desel et al. 2007). Bergmüller et al. (2003) reported that during oxidative stress (high light, high temperature, cold treatment) the amounts of $\alpha$-tocopherol and $\gamma$-tocopherol increased in wild type, and $\gamma$-tocopherol in *Arabidopsis* mutant line (*vte4-1*). However, chlorophyll content and photosynthetic quantum yield were very similar in wild type and *vte4-1*, suggesting that $\alpha$-tocopherol can be replaced by $\gamma$-tocopherol in *vte4-1* to protect the photosynthetic apparatus against oxidative stress. Giacomelli et al. (2007) found that the concentrations of α-tocopherol, ascorbate and glutathione showed increase in response to high light in different genotypes of *Arabidopsis,* and the four ascorbate deficient *vtc2* genotypes accumulated more glutathione under control light than the others. Tocopherol cyclase (VTE1) encoded by *VTE1* gene acts as a catalyst in the synthesis of tocopherol (Liu et al. 2008). Over-expressing *VTE1* from *Arabidopsis* in transgenic lines of tobacco showed decreased lipid per-oxidation, electrolyte leakage and $H_2O_2$ content in comparison with the wild type. Thus, they concluded that increase in vitamin E is due to expression of *VTE1* in plants and this also leads to enhanced tolerance to environmental stresses (Siefermann-Harms 1987).

## *4.3 Glutathione (GSH)*

GSH may be the most important intracellular defense against damage by ROS. The tripeptide (γ-GluCysGly) glutathione is one of the crucial metabolites in plants. Plant tissues contain GSH in reduced form which is abundantly found in all compartments of the cell (Jimenez et al. 1998). It plays a central role in several physiological processes, including regulation of sulfate transport, signal transduction, conjugation of metabolites, detoxification of xenobiotics (Xiang et al. 2001) and the expression of stress-responsive genes (Mullineaux and Rausch 2005). GSH has also been associated with several growth and development related events in plants (Rausch and Wachter 2005); its role is to maintain the reduced state of cells and is an important scavenger of $^1O_{2,}$ $H_2O_2$ and $OH^\bullet$ (Larson 1988; Smirnoff 1993; Noctor and Foyer, 1998). In an anti-oxidative defense system, GSH has an important role as it regenerates ascorbic acid (another antioxidant) *via* the Ascorbate–Glutathione cycle (Foyer and Halliwell 1976; Foyer et al. 1997). It also plays an indirect role in protecting membranes by maintaining α-tocopherol and zeaxanthin in the reduced state. Increase in stress levels showed a gradual decrease in glutathione concentrations and the redoxed forms were changed in to oxidized forms, leading to metabolic system failure (Tausz et al. 2004). GSH is a precursor of PCs (Phytochelatins), which are able to control heavy metal concentrations in the cell. The role of GSH in the antioxidant defense system provides a strong basis for its use as a stress marker. Freeman et al (2004) have demonstrated that increasing concentration of GSH is correlated with oxidative stress tolerance in plants during metal stress. *Arabidopsis* plants with low concentrations of glutathione were susceptible to even low concentrations of Cd (Xiang et al. 2001).

Manipulation of GSH biosynthesis increases resistance to oxidative stress (Sirko et al. 2004). It has been observed that upon Cd exposure, one of the main responses observed was the up-regulation of genes involved in sulfur assimilation–reduction and glutathione metabolism in the roots of *Arabidopsis* (Herbette et al. 2006). Feedback inhibition of $\gamma$-glutamylcysteine synthase ($\gamma$-ECS) by GSH is a basic central point for GSH synthesis (Noctor and Foyer 1998). Oxidation of GSH to GSSG decreases GSH levels and allows increased $\gamma$-ECS activity under stressed conditions (Noctor and Foyer 1998). Environmental stresses trigger an increase in ROS levels in plants and the response of glutathione can be crucial for adaptive responses. Antioxidant activity in leaves and chloroplast of *Phragmites australis* was associated with a large pool of GSH, protecting the activity of many photosynthetic enzymes against the thiophilic bursting of Cd exerting a direct important protective role in the presence of Cd (Pietrini et al. 2003). Increased concentration of GSH has been observed with increasing Cd concentration in *Brassica juncea* (Qadir et al. 2004), *Pisum sativum* (Metwally et al. 2005), and *Sedum alfredii* (Sun et al. 2007). However, decay in GSH content in *Pinus sylvestris* roots (Schutzendubel et al. 2001), *Populus* × *Canescens* roots (Schutzendubel and Polle 2002) and *Oryza sativa* leaves (Hsu and Kao 2004) has been reported under Cd stress. Cadmium-induced depletion of GSH has been mainly attributed to phytochelatin synthesis (Grill et al. 1985). Vacuoles of tobacco leaves and *Avena sativa* have been shown to accumulate PC-heavy metal complexes (Vogelli-Lange and Wagner 1990) and these complexes were reported to transport through the tonoplast (Vogelli-Lange and Wagner 1990). The decline in the levels of GSH might also be attributed to an increased utilization for ascorbate synthesis or for a direct interaction with Cd (Pietrini et al. 2003). The variety of responses to oxidative stress induced by heavy metals like Cd, is not only due to the Cd levels but it also depends on the plant parameters like species, age of the plant and duration of the treatment.

Srivastava et al. (2005) reported an appreciable decline in GR activity and GSH pool under copper stress, but a significant increase under NaCl stress. ROS scavenging enzymes and GSH concentration have been found to be in higher concentrations in the leaves of cultivar Pusa Bold than in CO 4 cultivar of *Vigna radiata*, and the higher concentrations of oxidized glutathione (GSSG) were detected in cultivar CO 4 as compared to that in Pusa Bold (Sumithra et al. 2006). Hence, it was concluded that Pusa Bold has an efficient antioxidative system that is responsible for its protection against oxidative damage than cultivar CO 4.

## 5 Phytohormones

### *5.1 Brassinosteroids (BRs)*

Brassinosteroids (BRs) are potent plant growth regulators of steroidal nature that are synthesized by plants affecting many aspects of plant growth. The most abundant one is brassinolide. It was first isolated from the pollen of *Brassica napus*. It plays an important role in growth and development of plants and is involved in different plant physiological responses (Sasse 2003). It is suggested that BRs have high biological activity and they regulate several morpho-physiological processes in plants, such as growth, germination, flowering, senescence, proton pump activation, stress tolerance, xylem differentiation and gene expression (Clouse 1996; Clouse and Sasse 1998; Li and Chory 1999). So far 42 BRs and four brassinosteroids conjugates have been characterized (Fujioka 1999). Li et al. (1998) observed that application of brassinolide to water stressed maize seedlings increased the activities of enzymatic and non-enzymatic antioxidants, whereas Vardhini and Rao (2003) showed that during osmotic stress BRs increase the activity of CAT and decrease the peroxidase and AsA oxidase activities in sorghum. Increase in anti-oxidative enzymes by BRs has also been reported in salt stressed rice seedlings (Núñez et al. 2003) and cadmium stressed chickpea (Hasan et al. 2008). Hayat et al. (2007) have also reported that BRs increase anti-oxidative activities and photosynthesis in mustard plants under cadmium stress. The foliar application of 24-epiBL or 28-homoBL improved growth and increased anti-oxidative enzymes in *Vigna radiata* under aluminum stress (Ali et al. 2008a) and in *Brassica juncea* under salt and nickel stresses (Alam et al. 2007; Ali et al. 2008b). Increases in photosynthesis and relative water content have also been observed in the above-mentioned plant species. Positive correlations have been seen between BR levels and tolerance to cold stress and photo-oxidation in cucumber plants (Xia et al. 2009). BR treatment induced the expression of genes *MAPK1, MAPK3* and *RBOH* and those related to anti-oxidative defense (Xia et al. 2009). Fariduddin et al. (2009) showed that treatment of *Brassica juncea* seedlings raised from the seeds treated with 28-homobrassinolide (HBL) improved growth, photosynthetic parameters and antioxidant enzymes under copper stress. The elevated antioxidant enzyme and proline might be responsible to overcome the toxic effects of copper in *B. juncea*.

## 5.2 Ethylene ($C_2H_4$)

Ethylene (Eth) is produced in most living plant cells and is considered as a plant hormone. Ethylene has many roles in various physiological processes, such as germination, growth, development, senescence and abscission as well as in defense and resistance (Wang et al. 2002). Environmental stress induces the production of ethylene in large amounts (Wang et al. 2006). Induction of ethylene biosynthesis has been shown in spring wheat during osmotic stress (Li et al. 2004) and in maize under UV-B radiation (Wang et al. 2006).

The biosynthesis of ethylene has two main steps: (i) Conversion of S-adenosyl L- methionine to ACC (1-aminocyclopropane-1-carboxylic acid) in the presence of catalyzing enzyme ACS (ACC synthase) and (ii) Cleavage of ACC to ethylene in the presence of ACO (ACC oxidase) (Fig. 5.1) (Zarembinski and Theologis 1994). Eth production in the tissues is very less as the activity of ACS enzyme is very low. During stress, the ACS activity is increased which in turn increases the production of ethylene. Tomato exposed to ozone stress induces ACS expression like *LE-ACS1A*, *LE-ACS2*, and *LE-ACS6*, and potato also shows the expression of *ST-ACS4* and

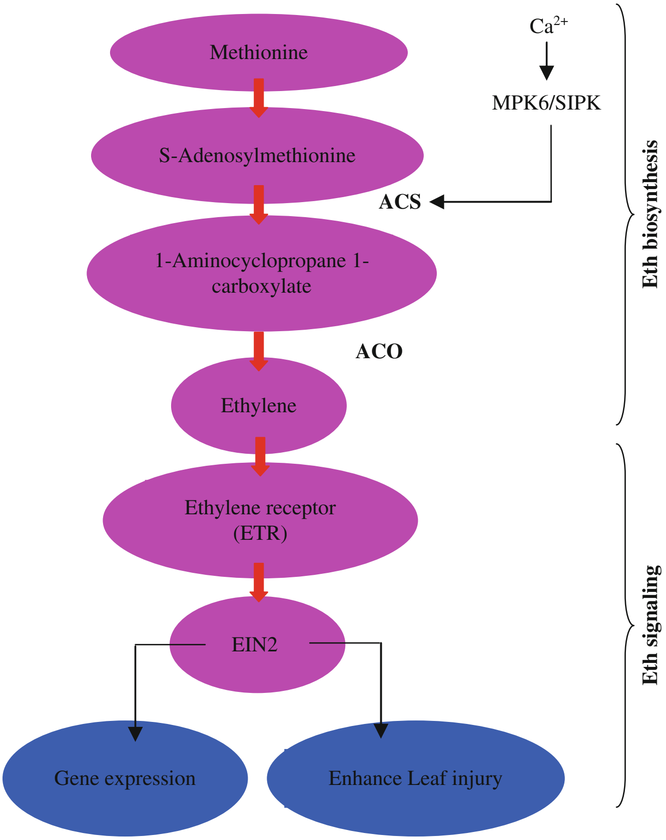


**Fig. 5.1** Ethylene Biosynthesis pathway and signaling in stressed plants. In MPK 6 in Arabidopsis and SIPK in tobacco regulates ACC synthase (ACS) whose activity is controlled with cytosolic free $Ca^{2+}$. Ethylene gets attached to ethylene receptots (ETR) and signaling is transmitted through EIN2

*ST-ACS5* (Tuomainen et al. 1997; Schlagnhaufer et al. 1997). Liu and Zhang (2004) observed that ACS accumulation is due to MPK6 induced phosphorylation in ACS2 and *ACS6* (Fig. 5.1) and thus leads to elevated levels of cellular ACS activity, indicating that ozone-induced ethylene evolution might be regulated not only by the transcription level of *ACS6*, but also post-transcriptionally through the MAPK signaling pathway. There is a strong correlation between ROS and ethylene levels in plant physiological responses. For example, it was found that this phytohormone and active oxygen species are responsible for the initiation of root nodules and it also acts as a transducer of downstream of the *Nod* factor response in the tropical, semi-aquatic legume *Sesbania rostrata* (D'Haeze et al. 2003). Tanaka et al. (2005) showed that ABA induced stomatal closure is inhibited by ethylene in *Arabidopsis*. $H_2O_2$

induced stomatal closure results in loss of function in *Arabidopsis* mutants, which suggests an important role of ethylene in guard cell ROS signaling and stomatal closure (Desikan et al. 2005). Pretreatment with ethylene increases ozone tolerance in pea (*Pisum sativum*) and mung bean (*Vigna radiata*) (Mehlhorn 1990). A dual role for ethylene in ozone tolerance has also been observed in different genotypes of silver birch (*Betula pendula* Roth). An ozone-tolerant silver birch clone produced little ethylene in response to ozone treatment, and ethylene production occurred temporally (Vahala et al. 2003).

## 6 Conclusion

Abiotic stress disturbs the balance between the production and removal of ROS which are in equilibrium at normal metabolic conditions. ROS induce oxidative damages to many biomolecules like membrane lipids, proteins, nucleic acids, chlorophyll etc. The $OH^\bullet$ is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone. The polyunsaturated fatty acids (PUFAs) linoleic acid and linolenic acid are particularly susceptible to attack to $^1O_2$ and $HO^\bullet$, giving rise to complex mixtures of lipid hydroperoxides. Extensive PUFA peroxidation decreases the fluidity of the membrane, increases leakiness and causes secondary damage to membrane proteins. ROS also leads to oxidations of proteins and are essentially irreversible, whereas, a few involving sulfur-containing amino acids are reversible. Protein oxidation is widespread and often used as a diagnostic marker for oxidative stress. Mounting evidence links oxidants and oxidative stress to senescence, impaired photosynthesis and necrosis in plants.

To control the level of ROS and to protect the cells under unfavourable environmental conditions, plants posses the ability to scavenge/detoxify ROS by producing different types of ROS Scavenging antioxidants. The components of antioxidant defense system are enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include SOD, CAT, APX, MDHAR, DHAR and GR and non-enzymatic antioxidants are GSH, AA (both water soluble), carotenoids and tocopherols (lipid soluble). Interestingly, higher plants also developed specific ROS-scavenging systems in different organelles to efficiently remove the ROS produced in these cellular parts; and, in particular under environmental stress such as salt stress, they coordinately work to provide plant cells with a highly efficient machinery for detoxifying ROS. A great deal of research has established that the induction of the cellular antioxidant machinery is important for protection against salt stress, metal stress, drought etc. various organelles have their own ROS scavenging system so that the organelles remove ROS more efficiently. They coordinately work to protect plant cells from ROS induced oxidative damage, e.g., Cytosolic APX1 can protect chloroplasts during light stress, which is a cross-compartment protection of thylakoid and stromal/mitochondrial APXs by cytosolic APX1.

## 7 Future Perspective

Plant biotechnologists are with the aim to increase the resistance of plants through genetic engineering. Up-regulation of certain anti-oxidative genes resulting in detoxification of ROS has been successful to some extent. This has added to the current knowledge in this area, but many reports are ambiguous at the same time. Improving the metabolic activities intricately involving superoxide scavenging, needs to be considerably taken care of, rather than enhancing the activity of antioxidant enzymes alone. Also the antioxidant mechanism of plants can be fortified by manipulating the antioxidant enzymes. Multiple genes which are affected under abiotic stresses indicate that there could not be a single marker for stress tolerance. Plant hormones are also responsible for the development of the plant and have a role in defense during environmental stresses. Much effort is still required to uncover in detail each product of genes induced by abiotic stress and signal transduction pathways. Researchers should look forward for defined set of markers to predict tolerance towards a particular type of stress.

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## References

Ahmad P (2010) Growth and antioxidant responses in mustard (*Brassica juncea* L.) plants subjected to combined effect of gibberellic acid and salinity. Arch Agro Soil Sci, (in Press)

Ahmad P, Sarwat M, Sharma S (2008a) Reactive oxygen species, antioxidants and signaling in plants. J Plant Biol 51:167–173

Ahmad P, Jhon R, Sarwat M, Umar S (2008b) Responses of proline, lipid peroxidation and antioxidative enzymes in two varieties of *Pisum sativum* L. under salt stress. Int J Plant Produc 2:353–366

Ahmad P, Jaleel CA, Sharma S (2010) Antioxidative defense system, lipid peroxidation, proline metabolizing enzymes and Biochemical activity in two genotypes of *Morus alba* L. subjected to NaCl stress. Russian J Plant Physiol (in press)

Alam MM, Hayat S, Ali B, Ahmad A (2007) Effect of 28-homobrassinolide on nickel induced changes in *Brassica juncea*. Photosynthetica 45:139–142

Ali B, Hasan SA, Hayat S, Hayat Q, Yadav S, Fariduddin Q, Ahmad A (2008a) A role for brassinosteroids in the amelioration of aluminum stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). Environ Exp Bot 62:153–159

Ali B, Hayat S, Fariduddin Q, Ahmad A (2008b) 24-Epibrassinolide protects against the stress generated by salinity and nickel in *Brassica juncea*. Chemosphere 72:1387–1392

Apel K, Hirt H (2004) Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399

Arbona V, Hossain Z, Lopez-Climent MF, Perez-Clemente RM, Gomez-Cadenas A (2008) Antioxidant enzymatic activity is linked to waterlogging stress tolerance in citrus. Physiol Plant 132:452–466

Asada, K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Azevedo RA, Alas RM, Smith RJ, Lea PA (1998) Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in leaves and roots of wild type and catalase deficient mutant of barley. Physiol Plant 104:280–292

Azevedo-Neto AD, Prisco JT, Enéas-Filho J, Braga-de-Abreu CE, Gomes-Filho E (2006) Effect of salt stress on Antioxidative enzymes and lipid peroxidation in leaves and roots of salt tolerant and salt sensitive maize genotypes. Environ Exp Bot 56: 87–94

Ben-Amor N, Hamed KB, Debez A, Grignon C, Abdelly C (2005) Physiological and antioxidant response of the perennial halophytes *Crithmum maritimum* to salinity. Plant Sci 168: 889–899

Bergmüller, E, Porfirova S, Dörmann P (2003) Characterization of an Arabidopsis mutant deficient in $\gamma$-tocopherol methyltransferase. Plant Mol Biol 52:1181–1190

Borsani O, Valupesta V, Botella M (2001) Evidence for role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. Plant Physiol 126:1024–1030

Burke EJ, Brown SJ, Christidis N (2006) Modeling the recent evolution of global drought and projections for the twenty-first century with the Hadley centre climate model. J Hydrometer 7:1113–1125

Camejo D, Martí MC, Nicolás E, Alarcón JJ, Jiménez A, Sevilla F (2007) Response of superoxide dismutase isoenzymes in tomato plants (*Lycopersicon esculentum*) during thermo-acclimation of the photosynthetic apparatus. Physiol Plant 131:367–377

Clouse SD (1996) Molecular genetic studies confirms the role of brassinosteroids in plant growth and development. Plant J 10:1–8

Clouse SD, Sasse JM (1998) Brassinosteroids: Essential regulators of plant growth and development. Anu Rev Plant Physiol Plant Mol Biol 49:421–451

Dalal M, Khanna-Chopra R (2001) Differential response of antioxidant enzymes in leaves of necrotic wheat hybrid and their parents. Physiol Plant 111: 297–304

Dalmia A, Sawhney V (2004) Antioxidant defense mechanism under drought stress in wheat seedlings. Physiol Mol Biol Plants 10:109–114

Davis DG, Swanson HR (2001) Activity of stress-related enzymes in the perennial weed leafy spurge (*Euphorbia esula* L.) Environ Exp Bot 46:95–108

Desel C, Hubbermann EM, Schwarz K, Krupinska K (2007) Nitration of γ-tocopherol in plant tissues. Planta 226:1311–1322

Desikan R, Hanckok JT, Bright J, Harrison J, Weir I, Hooley R, Neill SJ (2005) A role for ETR1 in hydrogen peroxide signalling in stomatal guard cells. Plant Physiol 137:831–834

D'Haeze W, De Rycke R, Mathis R, Goormachtig S, Pagnotta S, Verplancke C, Capoen W, Holsters M (2003) Reactive oxygen species and ethylene play a positive role in lateral root base nodulation of a semiaquatic legume. Proc Natl Acad Sci USA 100:11789–11794

Ding ZS, Tian SP, Zheng XL, Zhou ZW, Xu Y (2007) Responses of reactive oxygen metabolism and quality in mango fruit to exogenous oxalic acid or salicylic acid under chilling temperature stress. Physiol Plant 130: 112–121

Edwards EA, Rawsthorne S, Mullineaux PM (1990) Subcellular distribution of multiple forms of gluththione reductase in leaves of pea (*Pisum sativum* L.). Planta 180:278–284

Egert M, Tevini M (2002) Influence of drought on some physiological parameters sympatic for oxidative stress in the leaves of chives (*Allium schoenoprasum*) Environ Exp Bot 48:43–49

Eltayeb AE, Kawano N, Badawi GM, Kaminaka H, Sanekata T, Shibahara T, Inanaga S, Tanaka K (2007) Overexpression of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt and polyethylene glycol stresses. Planta 225:1255–1264

FAO (Food, Agriculture Organization of the United Nations) (2004) FAO production year book. FAO, Rome

Fariduddin Q, Yusuf M, Hayat S, Ahmad A (2009) Effect of 28-homobrassinolide on antioxidant capacity and photosynthesis in *Brassica juncea* plants exposed to different levels of copper. Environ Exp Bot 66:418–424

Foyer CH, Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant 119:355–364

Foyer CH, Noctor G (2005) Oxidant and antioxidant signaling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ 28:1056–1071

Foyer CH, Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133:21–25

Foyer CH, Lopez-Delgardo H, Dat JF, Scott IM (1997) Hydrogen peroxide and glutathione associated mechanisms of acclimatory stress tolerance and signaling. Physiol Plant 100:241–254

Freeman JL, Persan MW, Nieman K, Albrecht C, Peer W, Pickering IJ, Salt DE (2004) Increased glutathione biosynthesis plays a role in nickel tolerance in *Thlaspi* nickel hyperaccumulators. Plant Cell 16:2176–2191

Frugoli JA, Zhong HH, Nuccio ML, McCourt P, McPeek MA, Thomas TL, McClung CR (1996) Catalase is encoded by a multigene family in *Arabidopsis thaliana* (L.) Heynh. Plant Physiol 112:327–336

Fujioka S (1999) Natural occurrence of brassinosteroids in the plant kingdom. In: Sakurai A, Yokota T, Clouse SD (eds) Brassinosteroids: steroidal plant hormones. Springer, Tokyo, Japan, pp 21–45

Giacomelli L, Masi A, Ripoll DR, Lee MJ, Van Wijk KJ (2007) *Arabidopsis thaliana* deficient in two chloroplast ascorbate peroxidases shows accelerated light-induced necrosis when levels of cellular ascorbate are low. Plant Mol Biol 65:627–644

Grill E, Winnacker EL, Zenk MH (1985) Phytochelatins: The principal heavy metal complexing peptides of higher plants. Science 230:674–676

Harber F, Weiss J (1934) The catalytic decomposition of hydrogen peroxide by iron salt, Proc Royal Soc London, A Math Phys Sci 147:337–351

Harinasut P, Poonsopa D, Roengmongkoi K, Charoensataporn R (2003) Salt effects on antioxidant enzymes in mulberry cultivar. ScienceAsia 29:109–113

Hasan SA, Hayat S, Ali B, Ahmad A (2008) 28-homobrassinolide protects chickpea (*Cicer arietinum*) from cadmium toxicity by stimulating antioxidants. Environ Poll 151:60–66

Hayat S, Ali B, Hasan SA, Ahmad A (2007) Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. Environ Exp Bot 60:33–41

Herbette S, Taconnat L, Hugouvieux H, Piette L, Magniette MLM, Cuine S, Auroy P, Richaud P, Forestier C, Bourguignon J, Renou JP, Vavas-seur A, Leonhardt N (2006) Genome wide transcriptome profiling of the early cadmium response of *Arabidopsis* roots and shoots. Biochimie 88:1751–1765

Horemans N, Foyer CH, Asard H (2000) Transport and action of ascorbate at the plasma membrane. Trends Plant Sci 5: 263–267

Hsu YT, Kao CH (2004) Cadmium toxicity is reduced by nitric oxide in rice leaves. Plant Growth Regul 42:227–238

Jaleel CA, Gopi R, Manivannan P, Panneerselvam R (2007a) Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. Acta Physiol Plant 29:205–209

Jaleel CA, Manivannan P, Kishorekumar A, Sankar B, Gopi R, Somasundaram R, Panneerselvam R (2007b) Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit. Coll Surf B: Biointerf 59:150–157

Jaleel CA, Sankar B, Murali PV, Gomathinayagam M, Lakshmanan GMA, Panneerselvam R (2008) Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*; impacts on ajmalicine accumulation. Coll Surf B: Biointerf 62:105–111

Jiang M, Zhang J (2002) Water Stress induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up regulates the activities of antioxidant enzymes in maize leaves. J Exp Bot 53:2401–2410

Jimenez A, Hernandez JA, Pastori G, del Rio LA, Sevilla F (1998) Role of the ascorbate-glutathione cycle of mitochondria and peroxisomes in the senescence of pea leaves. Plant Physiol 118:1327–1335

Kagan VE (1989) Tocopherol stabilizes membrane against phospholipase A, free fatty acids, and lysophospholipids. In: Diplock AT, Machlin J, Packer L, Pryor W (eds) Vitamin E: Biochemistry and Health Implications. Ann New York Acad Sci 570:121–135

Kamal-Eldin A, Appelqvist L (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31:671–701

Kangasjärvi S, Lepistö A, Hännikäinen K, Piippo M, Luomala EM, Aro EM, Rintamäki E (2008) Diverse roles for chloroplast stromal and thylakoid-bound ascorbate peroxidases in plant stress responses. Biochem J 412:275–285

Keles Y, Oncel I (2002) Response of antioxidative defence system to temperature and water stress combinations in wheat seedlings. Plant Sci 163:783–790

Kruk J, Hollander-Czytko H, Oettmeier W, Trebst A (2005) Tocopherol as singlet oxygen scavenger in photosystem II. J Plant Physiol 162:749–757

Larson RA (1988) The antioxidants of higher plants. Phytochemistry 27:969–978

Li CZ, Jiao J, Wang Gen-X (2004) The important roles of reactive oxygen species in the relationship between ethylene and polyamines in leaves of spring wheat seedlings under root osmotic stress. Plant Sci 166:303–315

Lee SH, Ahsan N, Lee KW, Kim DH, Lee DG, Kwak SS, Kwon SY, Kim TH, Lee BH (2007) Simultaneous overexpression of both Cu-Zn superoxide dismutase and ascorbate peroxidase in transgenic tall fescue plants confers increased tolerance to a wide range of abiotic stresses. J Plant Physiol 164:1626–1638

Li J, Chory J (1999) Brassinosteroid action in plants. J Exp Biol 50:275–282

Liu Y, Zhang S (2004) Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in Arabidopsis. Plant Cell 16:3386–3399

Li L, van Staden J, Jäger AK (1998) Effects of plant growth regulators on the antioxidant system in seedlings of two maize cultivars subjected to water stress. Plant Growth Regul 25: 81–87

Liu X, Hua X, Guo J, Qi D, Wang L, Liu Z, Jin Z, Chen S, Liu G (2008) Enhanced tolerance to drought stress in transgenic tobacco plants overexpressing *VTE1* for increased tocopherol production from *Arabidopsis thaliana*. Biotechnol Lett 30:1275–1280

Lu Z, Liu D, Liu S (2007) Two rice cytosolic ascorbate peroxidases differentially improves salt tolerance in transgenic *Arabidopsis*. Plant Cell Rep 26:1909–1917

Manivannan P, Jaleel CA, Kishorekumar A, Sankar B, Somasundaram R, Sridharan R, Panneerselvam R (2007) Changes in antioxidant metabolism of *Vigna unguiculata* (L.) Walp. by propiconazole under water deficit stress. Coll Surf B: Biointerf 57:69–74

Mathur PB, Vadez V, Sharma KK (2008) Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. Plant Cell Rep 27:411–424

Mehlhorn H (1990) Ethylene-promoted ascorbate peroxidase activity protects plants against hydrogen peroxide, ozone and paraquat. Plant Cell Environ 13:971–976

Metwally A, Safronova VI, Belimov AA, Dietz KJ (2005) Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. J Exp Bot 56:167–178

Meyer A, Hansen DB, Gomes CSG, Hobley TJ, Thomas ORT, Franzreb M (2005) Demonstration of a strategy for product purification by high-gradient magnetic fishing: recovery of superoxide dismutase from unconditioned whey. Biotechnol Prog 21:244–254

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Moller IM (2001) Plant mitochondria and oxidative stress. Electron transport, NADPH turnover and metabolism of reactive oxygen species. Annu Rev Plant Physiol Plant Mol Biol 52:561–591

Mullineaux PM, Rausch T (2005) Glutathione, photosynthesis and the redox regulation of stress-responsive gene expression. Photosynth Res 86:459–474

Munne-Bosch S (2005) The role of α-tocopherol in plant stress tolerance. J Plant Physiol 162: 743–748

Naya L, Ladrera R, Ramos J, González EM, Arrese-Igor C, Minchin FR, Becana M (2007) The Response of carbon metabolism and antioxidant defenses of alfalfa nodules to drought stress and to the subsequent recovery of plants. Plant Physiol 144:1104–1114

Nobuhiro S, Mittler R (2006) Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. Physiol Plant 126:45–51

Nordberg J, Arner ESJ (2001) Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radi Biol Med 31:1287–1312

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49:249–279

Núñez M, Mazzafera P, Mazorra LM, Siqueira WJ, Zullo MAT (2003) Influence of a brassinsteroid analogue on antioxidant enzymes in rice grown in culture medium with NaCl. Biol Plant 47: 67–70

Parida AK, Das AB, Mohanty P (2004) Defense potentials to NaCl in a mangrove, *Bruguiera parviflora*: Differential changes of isoforms of some antioxidative enzymes. J Plant Physiol 161:531–542

Pastori GP, Mullineaux, Foyer CH (2000) Post transcriptional regulation prevents accumulation of glutathione reductase protein and activity in the bundle sheath cells of maize. Implication on the sensitivity of maize to temperatures. Plant Physiol 122:667–675

Pietrini F, Iannelli MA, Pasqualini S, Massacci A (2003) Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav.) Trin. Ex Steudel. Plant Physiol 133:829–837

Pitcher LH, Zilinskas BA (1996) Overexpression of copper/zinc superoxide dismutase in the cytosol of transgenic tobacco confers partial resistance to ozone-induced foliar necrosis. Plant Physiol 110:583–588

Qadir S, Qureshi MI, Javed S, Abdin MZ (2004) Genotypic variation in phytoremediation potential of *Brassica juncea* cultivars exposed to Cd stress. Plant Sci 167:1171–1181

Qiu-Fang Z, Yuan-Yuan L, Cai-Hong P, Cong-Ming L, Bao-Shan W (2005) NaCl enhances thylakoid-bound SOD activity in the leaves of $C_3$ halophyte *Suaeda salsa* L. Plant Sci 168:423–430

Rausch T, Wachter A (2005) Sulfur metabolism: A versatile platform for launching defense operations. Trends Plant Sci 10:503–509

Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol 161:1189–1202

Sasse JM (2003) Physiological actions of brassinosteroids: An update. J Plant Growth Regul 22:276–288

Scandalios JG (1990) Response of plant antioxidant defense genes to environmental stress. Adv Genet 28:1–41

Scandalios JG (1993) Oxygen stress and superoxide dismutases. Plant Physiol 101:7–12

Schlagnhaufer CD, Arteca RN, Pell EJ (1997) Sequential expression of two 1-aminocyclopropane-1-carboxylate synthase genes in response to biotic and abiotic stress in potato leaves. Plant Mol Biol 35:683–688

Schutzendubel A, Polle A (2002) Plant responses to biotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1366

Schutzendubel A, Schwang P, Teichmann T, Gross K, Langenfeld-Heyer R, Godbold DL, Polle A (2001) Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in scots pine roots. Plant Physiol 127:887–898

Sekmen AH, Turkan I, Takio S (2007) Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritima* and salt-sensitive *Plantago media.* Physiol Plant 131:399–411

Semane B, Cuypers A, Smeets K, Van Belleghem F, Horemans N, Schat H, Vangronsveld J (2007) Cadmium responses in *Arabidopsis thaliana*: glutathione metabolism and antioxidative defence system. Physiol Plant 129:519–528

Serbinova EA, Packer L (1994) Antioxidant properties of α-tocopherol and α-tocotrienol. Methods Enzymol 234:354–366

Shalata A, Mittova V, Volokita M, Guy M, Tal M (2001) Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress: the antioxidative system. Physiol Plant 112:487–494

Shao HB, Chu LY, Wu G, Zhang JH, Lu ZH, Hu YC (2007) Changes of some anti-oxidative physiological indices under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at tillering stage. Coll Surf B: Biointerf 54:143–149

Shao HB, Liang ZS, Shao MA, Sun Q, Hu ZM (2005) Investigation on dynamic changes of photosynthetic characteristics of 10 wheat (*Triticum aestivum* L.) genotypes during two vegetative-growth stages at water deficits. Biointerfaces 43:221–227

Siefermann-Harms D (1987) The light-harvesting and protective functions of carotenoids in photosynthetic membranes. Physiol Plant 69:561–568

Singh B, Usha K (2003) Salicylic acid induced physiological and biochemical changes in wheat seedligs under water stress. Plant Grow Regul 39:137–141

Sirko A, Blaszczyk A, Liszewska F (2004) Overproduction of SAT and/or OASTL in transgenic plants: a survey of effects. J Exp Bot 55:1881–1888

Skadsen RW, Schulz-Lefert P, Herbt JM (1995) Molecular cloning, characterization and expression analysis of two classes of catalase isozyme genes in barley. Plant Mol Biol 29: 1005–1014

Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27–58

Smirnoff N (2000) Ascorbic acid: metabolism and functions of a multi-facetted molecule. Curr Opin Plant Biol 3:229–235

Srivalli B, Chinnusamy V, Khanna-Chopra R (2003) Antioxidant defense in response to abiotic stresses in plants. J Plant Biol 30:121–139

Srivastava M, Ma LQ, Singh N, Singh S (2005) Antioxidant responses of hyper-accumulator and sensitive fern species to arsenic. J Exp Bot 56:1335–1342

Sumithra K, Jutur PP, Carmel BD, Reddy AR (2006) Salinity-induced changes in two cultivars of *Vigna radiata*: responses of antioxidative and proline metabolism. Plant Growth Regul 50: 11–22

Sun Q, Ye ZH, Wang XR, Wong MH (2007) Cadmium hyperaccumulation leads to an increase of glutathione rather than phytochelatins in the cadmium hyperaccumulator *Sedum alfredii*. J Plant Physiol 164:1489–1498

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S (2005) Ethylene inhibits the abscisic acid-induced stomatal closure in Arabidopsis. Plant Physiol 138:2337–2343

Tausz M, Ircelj H, Grill D (2004) The glutathione system as a stress marker in plant ecophysiology: is a stress-response concept valid? J Exp Bot 55:1955–1962

Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr Opin Plant Biol 8:397–403

Tuna AL, Kaya C, Dikilitas M, Higgs D (2008) The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environ Exp Bot 62:1–9

Tuomainen J, Betz C, Kangasjärvi J, Ernst D, Yin ZH, Langebartels C, Sandermann H Jr. (1997) Ozone induction of ethylene emission in tomato plants: Regulation by differential accumulation of transcripts for the biosynthetic enzymes. Plant J 12:1151–1162

Turkan I, Bor M, Ozdemir F, Koca H (2005) Differential responses of lipid peroxidation and antioxidants in the leaves of drought tolerant *P. actifolius* Gray and drought sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Sci 168:223–231

Tuteja N, Singh MB, Misra MK, Bhalla PL, Tuteja R (2001) Molecular mechanisms of DNA damage and repair: progress in plants. Crit Rev Biochem Mol Biol 36:337–397

Tuteja N, Ahmad P, Panda BB, Tuteja R (2009) Genotoxic stress in plants: shedding light on DNA damage, repair and DNA repair helicases. Mutat Res 681:134–149

Upadhyaya H, Panda SK, Dutta BK (2008) Variation of physiological and antioxidative responses in tea cultivars subjected to elevated water stress followed by rehydration recovery. Acta Physiol Plant 30:457–468

Vahala J, Ruonala R, Keinänen M, Tuominen H, Kangasjärvi J (2003) Ethylene insensitivity modulates ozone-induced cell death in birch. Plant Physiol 132:185–195

Van Breusegem F, Vranová E, Dat JF, Inzé D (2001) The role of active oxygen species in plant signal transduction. Plant Sci 161:405–414

Van Heerden, PDR, Kruger GHJ (2002) Separately and simultaneously induced dark chilling and drought stress effects on photosynthesis, proline accumulation and antioxidant metabolism in soybean. J Plant Physiol 159:1077–1086

Vardhini BV, Rao SSR (2003) Amelioration of osmotic stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. Plant Growth Regul 41:25–31

Vital SA, Fowler RW, Virgen A, Gossett DR, Banks SW, Rodriguez J (2008) Opposing roles for superoxide and nitric oxide in the NaCl stress-induced upregulation of antioxidant enzyme activity in cotton callus tissue. Environ Exp Bot 62:60–68

Vogelli-Lange R, Wagner GJ (1990) Relationship between cadmium, glutathione and cadmium-binding peptides (phytochelatins) in leaves of intact tobacco seedlings. Plant Sci 114:701–710

Wang K, Li H, Ecker J (2002) Ethylene biosynthesis and signaling networks. Plant Cell 14(suppl):S131–S151

Wang FZ, Wang QB, Kwon SY, Kwak SS, Su WA (2005) Enhanced drought tolerance of transgenic rice plants expressing a pea manganese superoxide dismutase. J Plant Physiol 162: 465–472

Wang Y, Feng H, Qu Y, Cheng J, Zhao Z, Zhang M, Wang X, An L (2006) The relationships between reactive oxygen species and nitric oxide in ultraviolet-B-induced ethylene production in leaves of maize seedlings. Environ Exp Bot 57:51–61

Wu G, Wei ZK, Shao HB (2007) The mutual responses of higher plants to environment: physiological and microbiological aspects. Coll Surf B: Biointerf 59:113–119

Xia XJ, Wang YJ, Zhou YH, Tao Y, Mao WH, Shi K, Asami T, Chen Z and Yu ZQ (2009) Reactive Oxygen Species Are Involved in Brassinosteroid-Induced Stress Tolerance in Cucumber. Plant Physiol 150:801–814

Xiang C, Werner BL, Christensen EM, Oliver DJ (2001) The biological function of glutathione revisited in *Arabidopsis* transgenic plants with altered glutathione levels. Plant Physiol 126: 564–574

Yang Y, Han C, Liu Q, Lin B, Wang J (2008) Effect of drought and low light on growth and enzymatic antioxidant system of *Picea asperata* seedlings. Acta Physiol Plant 30:433–440

Zarembinski TI, Theologis A (1994) Ethylene biosynthesis and action: a case of conservation. Plant Mol Biol 26:1579–1597

Zhang Y, Yang J, Lu S, Cai J, Guo Z (2008) Overexpressing *SgNCED1* in tobacco increases ABA level, antioxidant enzyme activities, and stress tolerance. J Plant Growth Regul 27:151–158