Chapter 8
Plant Response and Tolerance to Abiotic Oxidative Stress: Antioxidant Defense Is a Key Factor

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Abstract In a persistently changing environment, plants are constantly challenged by various abiotic stresses such as salinity, drought, temperature extremes, heavy metal toxicity, high-light intensity, nutrient deficiency, UV-B radiation, ozone, etc. which cause substantial losses in the yield and quality of a crop. A key sign of such stresses at the molecular level is the accelerated production of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•). ROS are extremely reactive in nature because they can interact with a number of cellular molecules and metabolites, thereby leading to irreparable metabolic dysfunction and death. Plants have well-developed enzymatic and non-enzymatic scavenging pathways or detoxification systems to counter the deleterious effects of ROS that include the enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione *S*-transferase (GST), glutathione peroxidase (GPX) and peroxidases (POX) as well as non-enzymatic compounds such as ascorbate (AsA), glutathione (GSH), carotenoids and tocopherols. In plant cells, specific ROS-producing and scavenging systems are found in

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different organelles and the ROS-scavenging pathways from different cellular compartments are coordinated. Recent studies in plants have shown that relatively low levels of ROS act as signaling molecules that induce abiotic stress tolerance by regulating the expression of defense genes. Additionally, numerous results have shown that plants with higher levels of antioxidants, whether constitutive or induced, showed greater resistance to different types of environmental stresses. In this chapter we attempt to summarize recent researches on the mechanisms and possible regulatory roles of ROS in abiotic stress tolerance. Further, we discuss the progress made during the last few decades in improving the oxidative stress tolerance of plants through genetic engineering by different components of ROS detoxification systems in plants.

Abbreviations

ABA	abscisic acid
APX	ascorbate peroxidase
AsA	ascorbic acid
ATP	adenosine triphosphate
CAT	catalase
DHA	dehydroascorbate
DHAR	dehydroascorbate reductase
ETC	electron transport chain
GAP	glycerinaldehyde-3-phosphate
GO	glycolate oxidase
GPX	glutathione peroxidase
GR	glutathione reductase
GSH	reduced glutathione
GSSG	oxidized glutathione
GST	glutathione *S*-transferase
HM	heavy metal
LOOH	lipid hydroperoxides
MDA	malondialdehyde
MDHA	monodehydroascorbate
MDHAR	monodehydroascorbate reductase
NADPH	nicotinamide adenine dinucleotide phosphate
NADPHox	NADPH oxidases
NO	nitric oxide
PC	phytochelatins
PCD	programmed cell death
PEG	polyethylene glycol
POX	peroxidases
ROOH	organic hydroperoxides
ROS	reactive oxygen species
RuBisCO	ribulose-1,5-bisphosphate carboxylase/oxygenase
Se	selenium
SNP	sodium nitroprusside

TG	total glutathione
XO	xanthine oxidase

8.1 Introduction

Plants are frequently exposed to a plethora of unfavorable or even adverse environmental conditions, termed abiotic stresses (such as salinity, drought, heat, cold, flooding, heavy metals, ozone, UV radiation, etc.) and thus they pose serious threats to the sustainability of crop yield (Bhatnagar-Mathur et al. 2008). Abiotic stresses remain the greatest constraint to crop production worldwide. It has been estimated that more than 50% of yield reduction is the direct result of abiotic stresses (Rodríguez et al. 2005; Acquaah 2007). Abiotic stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang et al. 2001). However, the rapidity and efficiency of these responses may be decisive for the viability of the given species.

Oxygen supports aerobic life of land plants granting them great energetic benefits but on the other hand challenges them through an endless formation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•). However, certain environmental stresses or genetic defects cause the production of ROS to exceed the management capacity. ROS play two divergent roles in plants: at low concentrations, they act as signaling molecules for the activation of defense responses under stresses, whereas at high concentrations, they cause exacerbating damage to cellular components. If prolonged, abiotic stresses, through enhanced production of ROS, can pose a threat to cells by causing the peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of the programmed cell death (PCD) pathway and ultimately cell death (Mittler 2002; Sharma and Dubey 2005, 2007). Oxidative stress is essentially a regulated process, and the equilibrium between ROS and antioxidative capacity determines the fate of the plant. The enhanced production of ROS is, however, kept under tight control by versatile and cooperative ROS-scavenging antioxidant mechanisms that modulate intracellular ROS concentration (Apel and Hirt 2004). These mechanisms can be conveniently divided into two groups, viz. non-enzymatic and enzymatic antioxidants (Fig. 8.4). Under control conditions the antioxidant defense system provides adequate protection against active oxygen and free radicals (Asada and Takahashi 1987). However, under stressful situations the equilibrium between the production and scavenging of ROS may be perturbed and the response becomes moderate or low (Gill and Tuteja 2010). Several reports confirmed that enhanced antioxidant defense combats oxidative stress induced by abiotic stressors like salinity (Hasanuzzaman et al. 2011a, b; Hossain et al. 2011), drought (Selote and Khanna-Chopra 2010; Hasanuzzaman and Fujita 2011), heat (Chakrabortty and Pradhan 2011; Rani et al. 2011), cold (Zhao et al. 2009; Yang et al. 2011), flooding (Li et al. 2011a), heavy metal toxicity (Hossain et al. 2010; Gill et al. 2011a), UV-radiation (Kumari et al. 2010; Li et al. 2010b; Ravindran et al. 2010) and ozone (Yan et al. 2010a, b). As increasingly extreme environmental factors are having an ever greater effect on agriculture, plant

biologists are facing with the urgent task of developing genotypes capable of tolerating environmental changes with the least possible damage. Hence it is first necessary to obtain knowledge on the defense and regulatory processes of plants. Developing plants with higher antioxidative potential provides an opportunity to develop plants with enhanced tolerance to abiotic stresses.

This chapter attempts to present an overview of our recent understanding on the physiology and molecular biology of plant tolerance mechanisms in response to abiotic stress factors. Special emphasis has been given to abiotic stress-induced ROS metabolism and differential regulation of the antioxidative defense system (both enzymatic and non-enzymatic) in inducing abiotic stress tolerance.

8.2 Abiotic Stressors in Plants

Most crops grown under field conditions are frequently exposed to various abiotic stresses. The complex nature of the environment, along with its unpredictable conditions and global climate change, are increasing gradually, which is creating a more adverse situation (Mittler and Blumwald 2010). A number of abnormal environmental parameters are collectively termed abiotic stress (Fig. 8.1). Abiotic stresses modify plant metabolism leading to harmful effects on growth, development and productivity. If the stress becomes very high and/or continues for an extended period it may lead to an intolerable metabolic load on cells, reducing growth, and in severe cases, result in plant death. However, plant stress may vary depending on the types of stressor and on the prevailing period. In nature, plants may not be completely free from abiotic stresses. They are expected to experience some degree of stress by any factor(s). Some environmental factors, such as air temperature, can become stressful in just a few minutes; others, such as soil water content, may take days to weeks, and factors such as soil mineral deficiencies can take months to become stressful (Taiz and Zeiger 2006).

8.3 Production of Reactive Oxygen Species in Plants

In plant cells, ROS are continuously produced as a consequence of aerobic metabolism in all the intracellular organelles, in particular in the chloroplast, mitochondria and peroxisomes (Apel and Hirt 2004). The chloroplast is the main source of ROS in plants. Insufficient energy dissipation during photosynthesis can lead to the formation of a chlorophyll triplet state that can transfer its excitation energy onto O_2 to make $^1O_2$ (Logan 2005). $O_2^{\bullet -}$ is produced by the photosynthetic electron transport chain (ETC) via the reduction of O_2 (Mehler reaction) (Apel and Hirt 2004), which is subsequently converted to H_2O_2 by superoxide dismutase (SOD) (Foyer and Noctor 2000). The photoproduction of ROS is largely affected by physiological and environmental factors, including high light intensity and drought stress (Asada 2006). Under conditions those impair CO_2 fixation in the chloroplast, the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) increases and the glycolate that is produced moves from chloroplasts to peroxisomes (Takahashi and Murata 2008;

Fig. 8.1 Different types of abiotic stressors in plants

Fig. 8.2). In peroxisomes, the generation of H_2O_2 involves glycolate oxidation catalyzed by glycolate oxidase (GO), the β-oxidation of fatty acids and catabolism of lipids (Halliwell 2006). On the other hand, the generation of $O_2^{\bullet-}$ involves both the reaction of xanthine oxidase (XO) in the organelle matrix and a small electron transport chain at the peroxisomal membrane level. The plant mitochondrial electron transport chain is also an important source of ROS production in plant cells (Fig. 8.2) and consists of several dehydrogenase complexes that reduce a common pool of ubiquinone (Q). ROS production is likely to occur mainly in complex I (NADH dehydrogenase) and the Q zone (Møller 2001; Blokhina et al. 2003; Fig. 8.2). Although mitochondrial ROS production is much lower compared to chloroplasts, mitochondrial ROS are important regulators of a number of cellular processes, including stress adaptation and PCD (Robson and Vanlerberghe 2002). In glyoxysomes, acyl-CoA oxidase is the primary enzyme responsible for the generation of H_2O_2. Plasmamembrane-bound NADPH oxidases (NADPHox) as well as cell-wall associated peroxidases (POX) are the main sources of $O_2^{\bullet-}$ and H_2O_2 producing apoplastic enzymes activated by various forms of stress (Mittler 2002; Mhamdi et al. 2010).

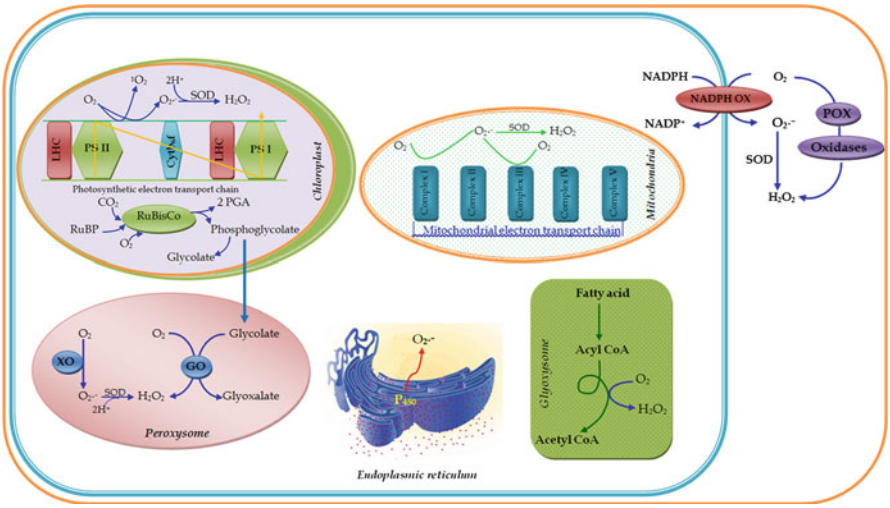

Fig. 8.2 Mechanisms of ROS production in different cell system (Adapted from Mhamdi et al. 2010)

Additional sources of ROS in plant cells include the detoxifying reactions catalyzed by cytochromes in both the cytoplasm and the endoplasmic reticulum (Urban et al. 1989).

8.4 Detoxification of ROS by the Antioxidant Defense System

In general, plant cells are adequately equipped to keep ROS within the limits that are generated as a consequence of normal cellular metabolic activities. Under different stress conditions, however, ROS generation often exceeds the overall cellular antioxidative potential leading to stress-induced adverse effects on plant growth and physiology. A steady state balanced is required to protect plant cells from oxidative damage (Fig. 8.3). Plants possess an efficient non-enzymatic (ascorbate, AsA; glutathione, GSH; α-tocopherol; phenolic compounds, alkaloids and non-protein amino acids) and enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione reductase, GR; glutathione peroxidase, GPX; glutathione *S*-transferase, GST and peroxidases, POX) antioxidant defense systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging ROS (Mittler et al. 2004; Gill and Tuteja 2010; Fig. 8.4). These antioxidant defense systems are found in almost all cellular compartments (Table 8.1, Fig. 8.5), demonstrating the importance of ROS detoxification for cellular survival (Mittler et al. 2004). These defenses are not restricted to the intracellular compartment, but are also found in the apoplast to a limited extent (Mittler 2002; Gill and Tuteja 2010).

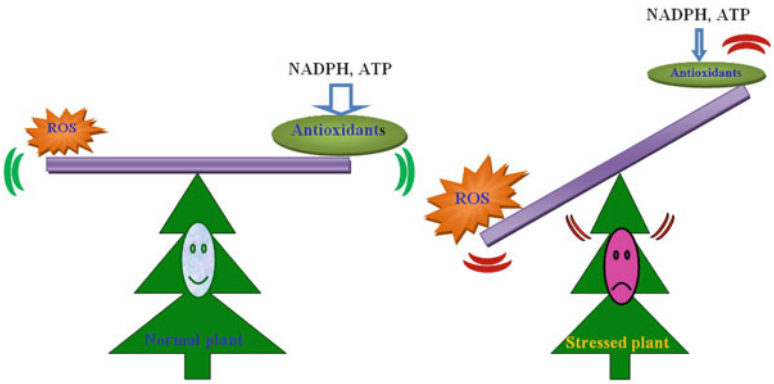

Fig. 8.3 The equilibrium and imbalance between ROS and antioxidants. Energy support also plays an important role in this equilibrium

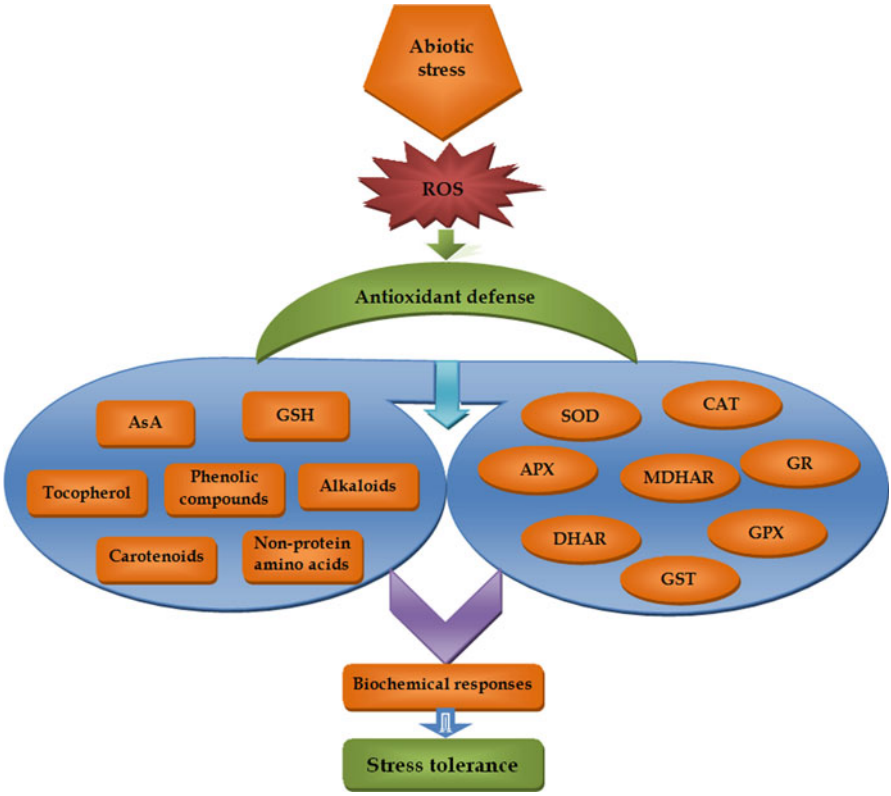

Fig. 8.4 Major non-enzymatic and enzymatic antioxidants involved in antioxidant defense system

Table 8.1 Different ROS-scavenging antioxidants and catalyzed reactions involved

Antioxidants	Enzyme code	Major reactions catalyzed	Site of reaction[a]
SOD	EC 1.15.1.1	$2O_2^{-} + 2H^+ \rightarrow H_2O_2 + O_2$	Chl, Cyt, Apo, Mit, Per
CAT	EC 1.11.1.6	$H_2O_2 \rightarrow H_2O + ½O_2$	Per, Chl, Mit
APX	EC 1.11.1.11	H_2O_2 + 2AsA → $2H_2O$ + 2MDHA	Chl, Cyt, Apo, Mit, Per
MDHAR	EC 1.6.5.4	NADPH + H^+ + 2MDHA → 2AsA + $NADP^+$	Chl, Cyt, Mit
DHAR	EC 1.8.5.1	DHA + 2GSH → AsA + GSSG	Chl, Cyt, Mit
GR	EC 1.6.4.2	NADPH + H^+ + GSSG → 2GSH + $NADP^+$	Chl, Mit, Cyt
GPX	EC 1.11.1.9	2GSH + ROOH (H_2O_2) → GSSG + ROH + H_2O ($2H_2O$)	Cyt, Mit
GST	EC 2.5.1.18	H_2O_2 + 2GSH → $2H_2O$ + GSSG RX + GSH→ HX + GS-R	Chl, Cyt, Mit
AsA	–	Scavenges $O_2^{\cdot-}$, H_2O_2, OH·, and $^1O_2$	Chl, Cyt, Apo, Mit, Per
GSH	–	Scavenges H_2O_2, OH·, and $^1O_2$	Chl, Cyt, Apo, Mit, Per
Tocopherols	–	Scananges $^1O_2$, OH·, ROO· and ROOH	Membranes

Adapted from Mittler (2002), Blokhina et al. (2003), Ashraf (2009), and Gill and Tuteja (2010)
[a]*Chl* chloroplast, *Cyt* cytosol, *Mit* mitochondria, *Apo* apoplast, *Per* peroxisome, *R* may be an aliphatic, aromatic or heterocyclic group, *X* may be a sulfate, nitrite or halide group

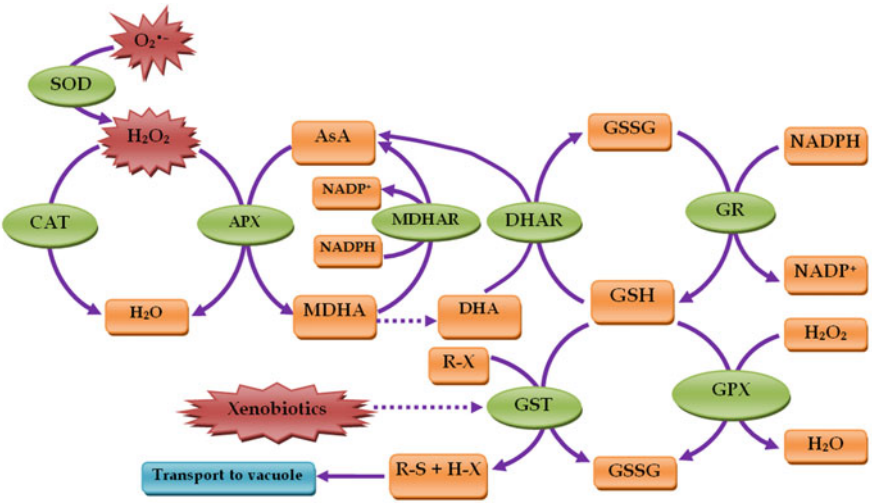

Fig. 8.5 Mechanisms of ROS detoxification by different antioxidant enzymes. *Dotted lines* denote non-enzymatic conversions. *R* may be an aliphatic, aromatic or heterocyclic group; *X* may be a sulfate, nitrite or halide group

8.4.1 Non-enzymatic Components

8.4.1.1 Ascorbate (AsA)

Ascorbate (AsA) is an important antioxidant in plant tissues which is synthesized in the cytosol of higher plants primarily from the conversion of D-glucose to AsA. It reacts with a range of ROS such as H_2O_2, $O_2^{\bullet-}$ and $^1O_2$, which are the basis of its antioxidant action. AsA, the terminal electron donor in these processes, scavenges free radicals in the hydrophilic environments of plant cells. It also scavenges OH• at diffusion-controlled rates (Smirnoff 2005). In the AsA-GSH cycle, two molecules of AsA are utilized by APX to reduce H_2O_2 to water with the concomitant generation of MDHA. MDHA is a radical with a short life span that can disproportionate into DHA and AsA. The electron donor is usually NADPH and the reaction is catalyzed by MDHAR or ferredoxin in a water–water cycle in the chloroplasts (Gapper and Dolan 2006). In plant cells, the most important reducing substrate for the removal of H_2O_2 is AsA (del Río et al. 2006; Wu et al. 2007). AsA is also thought to maintain the reduced state of the chloroplastic antioxidant, α-tocopherol. AsA in plants may be involved in the synthesis of zeaxanthin, which dissipates excess light energy in the thylakoid membranes, preventing oxidative damage (Conklin et al. 1996). AsA is also responsible for keeping prosthetic metal ions in a reduced form, thereby maintaining the activity of various antioxidant enzymes (De Tullio 2004). AsA plays an important role in plant stress tolerance (Sharma and Dubey 2005; Hossain et al. 2010; Hasanuzzaman et al. 2011a; Hossain et al. 2011). Exogenous application of AsA influences the activity of many enzymes and minimizes the damage caused by oxidative processes through synergic function with other antioxidants (Shalata and Neumann 2001).

8.4.1.2 Glutathione (GSH)

Glutathione (GSH) acts as an antioxidant and is involved directly in the reduction of most ROS (Noctor and Foyer 1998). Additionally, GSH plays a key role in the antioxidative defense system by regenerating other potential water-soluble antioxidants like AsA via the AsA-GSH cycle (Foyer and Halliwell 1976). It also plays an indirect role in protecting membranes by maintaining α-tocopherol and zeaxanthin in a reduced state. GSH prevents the denaturation of proteins caused by the oxidation of protein thiol groups under stress. In addition, GSH is a substrate for GPX and GST, which are also involved in the removal of ROS (Noctor et al. 2002a). Other functions for GSH include the formation of phytochelatins (PCs), which have an affinity to HM and are transported as complexes into the vacuole, thus allowing plants to have some level of resistance to HM (Sharma and Dietz 2006). GSH also takes part in the detoxification of xenobiotics and acts as a storage and transport form of reduced sulfur (Srivalli and Khanna-Chopra 2008). The role of GSH in the antioxidant defense system provides a strong basis for its use as a stress marker. The change

in the ratio of its reduced (GSH) to oxidized (GSSG) form during the degradation of H_2O_2 is important in certain redox signaling pathways (Li and Jin 2007). GSH acts as a redox sensor of environmental cues, and an increase in GSH provides resistance to plants against oxidative stress. Recent reports suggest that an increase in GSH content enhances protection to various abiotic stresses (Hossain and Fujita 2010; Hossain et al. 2010; Hasanuzzaman et al. 2011a, b; Hasanuzzaman and Fujita 2011).

8.4.1.3 Tocopherol

Tocopherols is very abundant in the thylakoid membranes, which contain polyunsaturated fatty acids (PUFA) and are in close proximity to ROS produced during photosynthesis (Fryer 1992) and circumstantial and correlative evidence strongly suggest an antioxidant role for tocopherol (Munne-Bosch and Alegre 2003). There are four tocopherol and tocotrienol isomers (α, β, γ and δ). Relative antioxidant activity of the tocopherol isomers in vivo is α > β > γ > δ and hence α-tocopherol has the highest antioxidant activity (Garg and Manchanda 2009). Tocopherols contribute to reduce ROS levels (mainly $^1O_2$ and OH•) in photosynthetic membranes and limits the extent of lipid peroxidation by reducing lipid peroxyl radicals (LOO•) to their corresponding hydroperoxides (Maeda et al. 2005). Tocopherols can physically quench and therefore deactivate $^1O_2$ in chloroplasts. Before being degraded, one molecule of α-tocopherol can deactivate up to 120 $^1O_2$ molecules by resonance energy transfer (Munné-Bosch 2007). Furthermore, tocopherols are part of an intricate signaling network controlled by ROS, antioxidants, and phytohormones, and are therefore good candidates to influence cellular signaling in plants (Munné-Bosch 2007).

8.4.2 Enzymatic Components

Antioxidant enzymes are located in different sites of plant cells and work together to detoxify ROS. The major antioxidant enzymes are SOD, CAT, GPX, GST and AsA-GSH cycle enzymes. The AsA–GSH cycle involves four enzymes (APX, MDHAR, DHAR and GR) as well as AsA, GSH and NADPH which work together to detoxify H_2O_2 in a series of cyclic reactions and further regenerate AsA and GSH (Fig. 8.5).

8.4.2.1 Superoxide Dismutases (SOD)

In plant cells, SODs constitute the frontline of defense against ROS. It removes $O_2^{\bullet-}$ by catalyzing its dismutation, one $O_2^{\bullet-}$ being reduced to H_2O_2 and another oxidized to O_2. SODs are classified based on the metal ion in their active site, namely copper

and zinc (Cu/ZnSOD), manganese (MnSOD), and iron (FeSOD). Cu/ZnSOD is localized in the cytosol and chloroplasts, MnSOD in the matrix of mitochondria and peroxisomes, and FeSOD in the chloroplasts of some higher plants, but they are also generally found in prokaryotes (Scandalios 1993). The enhanced activity of SODs minimizes abiotic oxidative stress and has a significant role in the adaptation of a plant to stressed environments (Mobin and Khan 2007; Singh et al. 2008).

8.4.2.2 Catalases (CAT)

Catalases (CATs) are tetrameric heme-containing enzymes that use H_2O_2 as a substrate and convert it to H_2O and O_2, thus preventing cells from oxidative damage (Sanchez-Casas and Klesseg 1994). CATs are present in peroxisomes, glyoxysomes, and related organelles where H_2O_2-generating enzymes are located (Agarwal et al. 2009). CAT has one of the highest turnover rates of all enzymes: one molecule of CAT can convert around six million molecules of H_2O_2 to H_2O and O_2 per minute. Thus, CAT is important in removing H_2O_2, which is generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration, and purine catabolism (Gill and Tuteja 2010). It has also been reported that apart from its reaction with H_2O_2, CAT also reacts with some hydroperoxides (Ali and Alqurainy 2006). CAT activity shows variable trends under different abiotic stresses (Singh et al. 2008; Hasanuzzaman et al. 2011a, b; Hasanuzzaman and Fujita 2011).

8.4.2.3 AsA-GSH Cycle Enzymes

The AsA–GSH cycle is the major defense system against ROS in chloroplasts, cytosol, mitochondria, peroxisomes and apoplasts. The AsA–GSH cycle involves four enzymes (APX, MDHAR, DHAR and GR) as well as AsA, GSH and NADPH which work together to detoxify H_2O_2 in a series of cyclic reactions and further regenerate AsA and GSH (Fig. 8.5). In this cycle APX catalyses the reduction of H_2O_2 to H_2O with the simultaneous generation of monodehydroascorbate (MDHA), which is converted to AsA by the action of NADPH-dependent MDHAR or disproportionates nonenzymatically to AsA and dehydroascorbate (DHA) (Asada 1992). DHA undergoes irreversible hydrolysis to 2, 3-diketogulonic acid or is recycled to AsA by DHAR, which uses GSH as the reductant (Chen et al. 2003). This results in the generation of GSSG, which is regenerated to GSH by GR.

Ascorbate Peroxidases (APX)

The scavenging of H_2O_2 by APX is the first step of the AsA-GSH cycle and may play the most essential role in scavenging ROS and protecting cells in higher plants (Asada 1994). APXs are heme-containing enzymes involved in scavenging H_2O_2 in water-water and AsA-GSH cycles using AsA as the substrate, catalyzing the transfer

of electrons from AsA to H_2O_2, producing DHA and water (Raven 2000; Pang and Wang 2010). The APX family consists of at least five different isoforms including mitochondrial (mAPX), thylakoid (tAPX) and glyoxisome membrane forms (gmAPX), as well as chloroplast stromal soluble form (sAPX), cytosolic form (cAPX) (Noctor and Foyer 1998). APX activity is enhanced in plants in response to during different abiotic stress conditions (Singh et al. 2008; Hossain et al. 2010; Hasanuzzaman and Fujita 2011).

Monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR)

The univalent oxidation of AsA leads to the formation of MDHA. If MDHA is not reduced again to AsA by MDHAR, it will spontaneously disproportionate into AsA and DHA. DHA is then reduced to AsA by DHAR in a reaction requiring GSH (Chen et al. 2003). Rapid regeneration is necessary in order to maintain the antioxidative capacity of AsA. The regeneration of AsA could be regulated in this cycle mainly by NADPH-dependent MDHAR activity (Mittova et al. 2000) and thus it is crucial for AsA regeneration and essential for maintaining a reduced pool of AsA (Martínez and Araya 2010). Although there are also a few reports about MDHAR activity in other physiological processes that are related to oxidative stress, research on different crops under environmental stresses revealed the regulatory role of MDAHR during oxidative stress tolerance and acclimation (Mittova et al. 2003; Hossain et al. 2010, 2011). MDHAR and DHAR are equally important in regulating the level of AsA and its redox state under oxidative stress (Eltayeb et al. 2006, 2007). DHAR is also a key component of the AsA recycling system (Martínez and Araya 2010) which regenerates AsA from the oxidized state (DHA) and regulates the cellular AsA redox state. It is thus crucial for tolerance to various abiotic stresses leading to the production of ROS. Increased DHAR activity was reported in response to various ROS-inducing stresses (Lee et al. 2007; Hossain et al. 2010; Hasanuzzaman et al. 2011a).

Glutathione Reductase (GR)

Glutathione reductase (GR) is a potential enzyme of the AsA-GSH cycle and plays an essential role in the defense system against ROS. Increased GR activity confers stress tolerance and has the ability to alter the redox state of important components of the ETC. This enzyme catalyzes the reduction of GSH, involved in many metabolic regulatory and antioxidative processes in plants where GR catalyses the NADPH-dependent reduction of disulphide bond of GSSG and is thus important for maintaining the GSH pool (Chalapathi Rao and Reddy 2008). Thus GR also maintains a high ratio of GSH/GSSG in plant cells, also necessary for accelerating the H_2O_2 scavenging pathway, particularly under stress conditions (Pang and Wang 2010). GR plays a crucial role in determining the tolerance of a plant under various stresses by maintaining the antioxidant machinery of the cell, confering stress tolerance (Sumithra et al. 2006; Hossain et al. 2011; Hasanuzzaman et al. 2011a).

8.4.2.4 Glutathione Peroxidases

Glutathione peroxidases (GPXs) are a large family of diverse isozymes that use GSH to reduce H_2O_2 and organic and lipid hydroperoxides (LOOHs), and therefore protect plant cells from oxidative stress (Noctor et al. 2002a). GPX is also a principal cellular enzyme capable of repairing membrane lipid peroxidation and is an important protectant against oxidative membrane damage (Kühn and Borchert 2002). In recent years, a number of GPXs genes have been identified from plant species (reviewed by Kumar et al. 2010). Apart from H_2O_2-detoxifying activity, GPX functions as an oxidative signal transducer (Miao et al. 2006).

8.4.2.5 Glutathione *S*-Transferases (GST)

Plant GSTs are a superfamily of multifunctional enzymes which catalyse the conjugation of electrophilic xenobiotic substrates with GSH (Dixon et al. 2010). Among the enzymes related to GSH metabolism, GST isoenzymes account for approximately 1% of a plants total soluble protein (Marrs 1996). GSTs catalyse the binding of various xenobiotics (including numerous pesticides) and their electrophilic metabolites with GSH to produce less toxic and more water-soluble conjugates (Edwards et al. 2000). Besides catalyzing the conjugation of electrophilic compounds to GSH, GST isoenzymes also exhibit POX activity (Gullner and Kömives 2001). Various abiotic stresses are powerful inducers of GST activity in plants (Dixon et al. 2010). Plant GSTs are also associated with responses to various forms of abiotic stress (Hossain et al. 2006; Dixon et al. 2010; Hossain and Fujita 2010; Hossain et al. 2010, 2011) and confer stress tolerance in plants.

8.5 Plant Responses and Antioxidant Defense Under Major Abiotic Stresses

8.5.1 Salinity

Soil salinity, one of the most severe abiotic stresses, limits the production of nearly over 6% of the world's land and 20% of irrigated land (15% of total cultivated areas) and negatively affects crop production worldwide. On the other hand, increased salinity of agricultural land is expected to have destructive global effects, resulting in up to 50% land loss by the middle of the twenty-first century (Mahajan and Tuteja 2005). Some of the adverse effects of salinity have been attributed to an increase in sodium (Na^+) and chloride (Cl^-) ions and hence these ions produce the critical conditions for plant survival by intercepting different plant mechanisms. Both Na^+ and Cl^- produce many physiological disorders in plants but Cl^- is the most dangerous. Due to the accumulation of Cl^-, relative salt tolerance has been linked

to plant growth water use efficiency and transpiration. In addition to upper plant parts, salinity also effects root growth and physiology and ultimately their function in nutrient uptake.

8.5.1.1 Plant Responses to Salt Stress

A plant's response to salt stress depends on the genotype, developmental stage, as well as the intensity and duration of the stress. Increased salinity has diverse effects on the physiology of plants grown in saline conditions and in response to major factors like osmotic stress, ion-specificity, nutritional and hormonal imbalance, and oxidative damage. The outcome of these effects may cause the disorganization of cellular membranes, inhibit photosynthesis, generate toxic metabolites and decline nutrient absorption, ultimately leading to plant death (Mahajan and Tuteja 2005). In general, the response of a crop plant to salinity is reduced growth (Tavakkoli et al. 2011). Osmotic stress due to salinity leads to a slow growth rate and developmental characteristics such as vegetative development, net assimilation capacity, leaf expansion rate and leaf area index (Zheng et al. 2008; Hasanuzzaman et al. 2009). A reduction in photosynthesis is also one of the most conspicuous effects of salinity stress (Leisner et al. 2010; Raziuddin et al. 2011). To cope with salt stress, plants exhibit some morphological, anatomical, and physiological, or biochemical adaptive features which help them to sustain and thrive under saline conditions. Physiologically, a common mechanism in plants is the accumulation of certain compatible solutes such as glycerol, sucrose, trehalose, pinitol, proline and quaternary ammonium compounds such as glycinebetaine (Ashraf and Harris 2004). Generally, these compatible solutes protect plants from stress injury through different means, including the protection of cytoplasm and chloroplasts from salt-induced damage and scavenging ROS, stabilization of proteins and general maintenance of physiological stability of plants under stressful conditions (Galinski and Truper 1994; Ashraf and Harris 2004).

8.5.1.2 Oxidative Stress in Plants Under Salinity

In plants, salt stress can lead to the reduction of CO_2 availability and inhibit carbon fixation, exposing chloroplasts to excessive excitation energy which in turn could increase the generation of ROS (Gill and Tuteja 2010). Under salt stress, stomatal conductance in plants decreases to avoid excessive water loss which leads to a decrease in the internal CO_2 concentration (Ci) and slows down the reduction of CO_2 by the Calvin cycle. This response causes the depletion of oxidized NADP+, which acts as a final acceptor of electrons in photosystem I, and alternatively increases the leakage of electrons to O_2 forming $O_2^{\bullet-}$. In addition, Na^+/Cl^- toxicity resulting from salt stress could disrupt photosynthetic electron transport and provoke electron leakage to O_2. The decrease in Ci slows down the reactions of the Calvin cycle and induces photorespiration, resulting in the generation of more H_2O_2

in the peroxisome. The cell membrane-bound NADPHox and apoplastic diamine oxidase are also activated during salt stress and contribute to the generation of ROS (Ashraf 2009; Abogadallah 2010). In fact, it is not possible to determine the contribution of all sources to the generation of ROS under salt stress. Enhanced ROS production under salt stress induces phytotoxic reactions such as lipid peroxidation, protein degradation, and DNA mutations (Tanou et al. 2009). Several reports showed the overproduction of ROS in plants under saline conditions and ROS-induced membrane damage is a major cause of cellular toxicity by salinity (Mittova et al. 2004; Hossain et al. 2011; Hasanuzzaman et al. 2011a, b).

8.5.1.3 Antioxidant Defense in Plants Exposed to Salt Stress

When ROS increases in response to salinity, plants use a scavenging mechanism involving non-enzymatic and enzymatic antioxidants (Demiral and Turkan 2005). In tomato (*Lycopersicon esculentum*) seedlings, exogenous AsA increased the capacity to recover from salt stress (Shalata and Neumann 2001). The addition of exogenous AsA to the root medium remarkably increased seedling survival under NaCl stress (300 mM for 7, 8 or 9 h). In addition, exogenous AsA also partially inhibited increases in lipid peroxidation. Exogenously applied AsA partially or completely countered the inhibitory effects of salt stress in maize (Hamada and Al-Hakimi 2009). Under salt stress, the AsA-deficient *Arabidopsis* mutant *vtc-1* contained 30–60% of the AsA content of wild-type (WT) plants and accumulated a much higher level of H_2O_2 than WT (Huang et al. 2005), which coincides with a greater decrease in the ratio of reduced AsA to total AsA and with reduced activity of the AsA–GSH cycle enzymes. Likewise, GSH also plays a protective role in salt tolerance by maintaining the redox state. Investigation on the enzymatic pathways leading to GSH synthesis during the response to salt stress of WT and salt-tolerant *Brassica napus* L. (canola) plants showed that salt stress induced the assimilation of sulfur and the biosynthesis of cysteine and GSH in order to mitigate salt-induced oxidative stress (Ruiz and Blumwald 2002; Hussain et al. 2008). Sumithra et al. (2006) found that GSH concentration in the salt-stressed mung bean leaves of cv. Pusa Bold was higher than cv. CO 4, whereas GSSG concentration was higher in the leaves of CO 4 than in those of Pusa Bold, indicating that Pusa Bold was more tolerant than CO 4 as the levels of lipid peroxidation and H_2O_2 concentration in Pusa Bold was lower than in CO 4 under salt stress. In addition, maintaining a high ratio of GSH/GSSG plays an important role in salt tolerance (Hossain et al. 2011). Salt-tolerant cultivars of cotton had a higher GSH/GSSG ratio than salt-sensitive lines under saline conditions (Gossett et al. 1996).

The activity of ROS-scavenging enzymes is highly correlated with antioxidant defense and salt stress tolerance. However, the activities vary with plant cultivar, stress duration and dose. The generation of ROS and increased activity of many antioxidant enzymes during salt stress have been reported in different plant studies with several reports indicating that the activity of antioxidant enzymes of salt-tolerant genotypes increased in response to salinity whereas salt-sensitive species failed to

do so (Mittova et al. 2002; Heidari 2009; Ghosh et al. 2011; Hasanuzzaman et al. 2011a, b; Hossain et al. 2011). El-Bastawisy (2010) concluded that salt tolerance was related to the endogenous levels of the enzymatic and the non-enzymatic antioxidants in wheat seedlings. Among the three wheat cultivars (H 168, Gimmeza 7 and Beni swif 1) under observation, the activities of SOD, CAT, APX and GR as well as the non-enzymatic antioxidants (AsA and GSH) increased mostly in H 168, but declined in Gimmeza 7 and particularly in Beni swif 1. H 168 had a superior antioxidant defense system and was more tolerant to NaCl than the other two cultivars due to the higher enzymatic and non-enzymatic antioxidants. Mittova et al. (2002) reported that, compared with cultivated tomato (L. *esculentum*), the better protection of wild salt-tolerant tomato (L. *pennellii*) root plastids from salt-induced oxidative stress was correlated with increased activities of SOD, APX and GPX. In another study, Vaidyanathan et al. (2003) investigated the immediate responses to salinity-induced oxidative stress in two major rice (Oryza sativa L.) cultivars, salt-sensitive Pusa Basmati 1 (PB) and salt-tolerant Pokkali (PK). Upon exposure to NaCl stress, PK showed higher activity of ROS-scavenging enzymes as well as enhanced levels of AsA and GSH than PB. Although SOD activity was lower in PK, it showed less lipid peroxidation and lower levels of H_2O_2 than PB under stress. Mandhania et al. (2006) observed that the activities of CAT, POX, APX and GR increased with an increase in salt stress in both sensitive and tolerant wheat cultivars, although SOD activity declined. Upon desalanization, partial recovery of the activity of these enzymes was observed in the salt-tolerant cultivar but a very slow recovery in the sensitive cultivars. Azooz et al. (2009) reported that the activity of CAT, POX, APX and SOD in salt-tolerant maize cultivars increased markedly during salinity stress but mostly decreased after salinity stress in the salt-sensitive cultivar. In another study, Dai et al. (2009a) indicated that the NaCl-induced gene expression and increased activities of SOD, CAT and POX enhanced the tolerance of oilseed rape plants against NaCl stress. In our study, mung bean seedlings, salt tolerance was correlated with higher activities of AsA-GSH cycle enzymes, including CAT and GPX (Hossain et al. 2011). GR, GPX and GST activities increased in response to salt stress (200 mM, 48 h), while the activities of MDHAR, DHAR and CAT decreased sharply with an associated increase in H_2O_2 and lipid peroxidation (expressed as malondialdehyde, MDA) level. Importantly, proline or betaine pre-treated salt-stressed seedlings showed an increase in the activities of APX, DHAR, GR, GST, GPX and CAT involved in the ROS detoxification system compared to the untreated control and mostly salt-stressed plants with a simultaneous decrease in H_2O_2 and MDA level. Hasanuzzaman et al. (2011a) confirmed that the antioxidative system was enhanced by the application of exogenous selenium (Se), which induced oxidative stress in rapeseed (*Brassica napus*) seedlings subjected to salt stress (100 and 200 mM NaCl for 48 h). The AsA content of the seedlings decreased significantly with an increase in salt stress. The amount of GSH and GSSG increased with an increase in the level of salt stress, while the GSH/GSSG ratio decreased. In addition, APX and GST activity increased significantly with increased salt concentration (both at 100 and 200 mM NaCl), while GPX activity increased only at moderate salt stress (100 mM NaCl). GR activity remained unchanged at 100 mM NaCl,

while it decreased under severe (200 mM NaCl) salt stress. The activity of MDHAR, DHAR and CAT decreased after salt stress was imposed whereas a sharp decrease in their activities was observed under severe salt stress (200 mM NaCl). A concomitant increase in the levels of H_2O_2 and MDA was also observed. More importantly, Se treatment in the salt-stressed seedlings increased the contents of AsA and GSH, the GSH/GSSG ratio, and the activities of APX, MDHAR, DHAR, GR, GST, GPX and CAT which led to a reduction in the levels of H_2O_2 and MDA compared to salt stress alone. The application of exogenous Se rendered the plants more tolerant to salt stress-induced oxidative damage by enhancing their antioxidant defense. In wheat seedlings, Hasanuzzaman et al. (2011b) further showed that modulation of ROS detoxification systems by exogenously applied SNP (an NO donor) improved oxidative stress tolerance of wheat seedlings subjected to salt stress (150 and 300 mM NaCl, 4 days). Salt-stressed seedlings pretreated with NO (1 mM SNP, 24 h) showed an increase in the AsA and GSH contents and the GSH/GSSG ratio as well as the activities of MDHAR, DHAR, GR, GST and GPX. Although different studies have established that the antioxidant defense system plays a crucial role in salt-stress tolerance in plants, defining salt tolerance has been quite difficult until now because of the complex nature of salt stress and the wide range of plant responses.

8.5.2 Drought

Drought is one of the most devastating environmental stresses that affects the growth and development of plants. The effects of drought stress are expected to increase with climate change and a growing water crisis (Harb et al. 2010). Thus, a better understanding of the effects of drought on plants is vital for improved management practices and breeding efforts in agriculture and for predicting the fate of natural vegetation under climate change. A plant suffers from drought stress due to the unavailability of water to the root zone or excessive transpiration rate. However, the adverse effects of drought stress on growth and development of crop plants are multifarious in nature.

8.5.2.1 Plant Responses to Drought

Plant responses to drought differ considerably depending on the intensity and duration of stress as well as plant species, cultivar and growth stage (Jaleel et al. 2008a, b). In general, drought stress affects the growth, dry matter production and economic yield of plants. Drought stress is characterized by a reduction of water content, decreased leaf water potential, turgor loss, stomatal closure and decrease in cell elongation and expansion (Jaleel et al. 2009; Mingchi et al. 2010; Din et al. 2011). However, water stress inhibits cell enlargement more than cell division (Jaleel et al. 2009). Reduced growth under drought stress is attributed to the impairment of various physiological and biochemical processes, such as photosynthesis, respiration,

translocation of nutrients, ion uptake, and carbohydrate metabolism (Jaleel et al. 2008a, b, c). Drought stress followed by desiccation can potentially lead to gross disruption of metabolism and cell structure and eventually to the cessation of enzyme-catalyzed reactions (Smirnoff 1993). A reduction in chlorophyll content was reported in different crops grown under drought stress (Kiani et al. 2008). The leaf photosynthetic rate of higher plants under drought stress decreases due to the lower relative water content and leaf water potential (Lawlor and Cornic 2002). Drought stress also negatively affects dry matter partitioning and temporal biomass distribution (Petropoulos et al. 2008; Wu et al. 2008). In drought tolerance, plants are able to tolerate water deficiency by manipulating the biochemical and physiological parameters and thus avoiding the injurious effects of drought. Adaptation to drought is a complex process involving numerous changes including attenuated growth, the activation/increased expression or induction of genes, transient increase in abscisic acid (ABA), accumulation of compatible solutes and protective proteins, increased level of antioxidants and suppression of energy-consuming pathways (Bartels and Sunkar 2005).

8.5.2.2 Oxidative Stress in Plants Under Drought

Drought stress may lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of ROS and induce oxidative stress (Mittler 2002; de Carvalho 2008). The excess production of ROS during drought stress results from impaired electron transport processes in the chloroplasts and mitochondria (Smirnoff 1993). Down-regulation of PSII results in a disproportion between the generation and utilization of electrons, resulting in changes in quantum yield. These changes in the photochemistry of chloroplasts in the leaves of drought-stressed plants result in the dissipation of excess light energy in the PSII core and antenna, thus generating free radicals like $O_2^{\cdot-}$, $^1O_2$, H_2O_2 and OH•, which are potentially dangerous under drought stress (Li et al. 2010a; Faize et al. 2011). In fact, under drought stress, ROS production is enhanced in different ways. However, it is quite complicated to assess the part of ROS generated by the Mehler reaction to that generated by photorespiration. Photorespiration is one of the major causes of ROS production under drought stress; more than 70% of total H_2O_2 is produced due to photorespiration (Noctor et al. 2002b). ROS accumulation and oxidative stress increase under drought stress (Li et al. 2010a; Faize et al. 2011; Sorkheha et al. 2011) and drought-induced oxidative stress significantly increases lipid peroxidation (Pandey et al. 2010; Hasanuzzaman and Fujita 2011).

8.5.2.3 Antioxidant Defense in Plants Exposed to Drought Stress

The enhancement of antioxidant defense mechanisms is considered to be an adaptive mechanism of plants to drought stress and the strengthening of these defense

mechanisms, through the enhanced functions of antioxidant components (enzymatic and non-enzymatic), may reduce or prevent oxidative damage and improve the drought resistance of plants (Sharma and Dubey 2005; de Carvalho 2008; Jaleel et al. 2009). AsA is one of the strongest non-enzymatic antioxidants that provides better protection against drought stress (Yazdanpanah et al. 2011). Increased AsA content in mung bean seedlings supplemented with proline and glycinebetine conferred better protection against drought stress (Hossain and Fujita 2009). Under drought stress, the protective action of the GSH system against oxidation of sulfhydryl groups of soluble proteins is established (Loggini et al. 1999). Selote and Khanna-Chopra (2006) demonstrated that mild water deficit stress did not change the GSH pool but the GSH/GSSG ratio was altered in the leaves of drought-acclimated wheat seedlings. Exposure to severe drought stress, however, resulted in a drastic decline in GSH redox pool in the leaves of non-acclimated plants than that of drought-acclimated ones (Selote and Khanna-Chopra 2006). This might be due to either higher biosynthesis or regeneration of GSH accompanied by the enhanced activities of the AsA–GSH cycle enzymes in the leaves of drought-acclimated wheat seedlings during severe water stress. In addition to AsA and GSH, several reports showed that drought stress resulted in an increase in α-tocopherol levels that enhanced stress tolerance (Munné-Bosch et al. 2009).

In parallel to non-enzymatic antioxidants, the activity of antioxidant enzymes also play a significant role in drought stress tolerance of many plants (Sharma and Dubey 2005; Hossain and Fujita 2009; Selote and Khanna-Chopra 2010; Hasanuzzaman and Fujita 2011). SOD and the enzymes of the AsA-GSH cycle (APX, MDHAR, DHAR and GR) appear to function as important components of the antioxidative defense system under drought-induced oxidative stress in rice (Sharma and Dubey 2005). Hossain and Fujita (2009) also observed short-term enhanced drought tolerance with increased activities of APX, MDHAR, DHAR, GR, GPX, GST and CAT in mung bean seedlings, confirmed by lower levels of H_2O_2 and MDA. Mohammadkhani and Heidari (2007) observed a positive and strong correlation between antioxidant enzymes and drought stress while investigating the responses of *Zea mays* L. var. 704 (drought-tolerant) and var. 301 (drought-sensitive). With 40% polyethylene glycol (PEG), the activity of GPX, APX and CAT in the roots and shoots of 704 plants was higher than in the control. However, enzyme activity decreased in 301 sensitive plants under drought than in 704 tolerant plants. Shehab et al. (2010) reported an increase in the activity of various antioxidant defense enzymes (SOD, APX, GR and CAT) in rice, representing protective activity to counteract the oxidative injury caused by drought. Selote and Khanna-Chopra (2010) demonstrated that drought acclimation induces oxidative stress tolerance of wheat seedlings, attributed to a well-coordinated induction of the ROS detoxification system. Direct exposure of severe water stress to non-acclimated seedlings caused greater water loss, excessive accumulation of H_2O_2 followed by elevated lipid peroxidation due to the poor response of antioxidant enzymes, particularly APX, MDHAR, DHAR, GR and the AsA–GSH redox balance. Drought-acclimated wheat roots during subsequent severe water stress conditions enhanced systematic up-regulation of SOD, APX, CAT, POX, and AsA–GSH cycle components

at both the whole cell level as well as in mitochondria and maintained a higher relative water content and lower level of H_2O_2. Furthermore, termination of stress followed by rewatering led to a rapid enhancement of all the antioxidant defense components in non-acclimated roots, suggesting that the excess levels of H_2O_2 during severe water stress conditions might have inhibited or down-regulated the antioxidant enzymes. In a study with ten cultivars of oilseed rape (*B. napus*), Abedi and Pakniyat (2010) reported that the oilseed rape variety with the highest level of enzyme activity under both optimum and limited irrigation regimes (drought) was considered to be the most tolerant cultivar while the varieties with the lowest enzymes activities were considered to be sensitive to drought stress. Filippou et al. (2011) suggested that CAT has a primary role in H_2O_2 detoxification in Medicago plants: CAT was significantly induced in leaves after imposing water stress as well as in roots after 3 days of water stress in comparison with cAPX, which was differentially regulated (suppressed expression). Sečenji et al. (2010) demonstrated that the expression levels of cAPX and tAPX variants increased significantly in a drought-tolerant wheat cultivar while cytosolic and stromal APX-coding transcripts were higher in a drought-sensitive cultivar after a 4 week-long water deficit stress. However, Sofo et al. (2005) suggested that CAT may be less important than APX in scavenging H_2O_2 in roots in long-term stress. Sánchez-Rodríguez et al. (2010) further demonstrated that drought tolerance of a tolerant tomato cultivar (*L. esculentum* cv. Zarina) is attributed to a higher antioxidant defense system. Five tomato cultivars were subjected to mild water stress (50% field capacity) and maintained for 22 days. Drought stress significantly increased the H_2O_2 and MDA level in all the cultivars except for Zarina. Analysis of antioxidants revealed that the activities of APX, DAHR, MDHAR and GR increased sharply in the tolerant cultivar more than in others. Importantly, Zarina maintained a higher level of AsA under drought stress. Therefore, appropriate induction of both enzymatic and non-enzymatic antioxidant defense systems allowed cv. Zarina to be tolerant to drought-induced oxidative stress.

The importance of well coordinated antioxidant defense in inducing drought tolerance was also observed in a study with rapeseed seedlings (*B. napus* cv. BINA sharisha 3) by using exogenous Se (Hasanuzzaman and Fujita 2011). Drought stress (mild or severe) caused a significant increase in GSH and GSSG content; however, the AsA content increased only under mild stress. The activity of APX was not affected by drought stress. MDHAR and GR activities increased only under mild stress. The activities of DHAR, GST and GPX significantly increased under any level of drought stress, while CAT activity decreased. Importantly, the Se-pretreated (25 μM Na_2SeO_4, 48h) seedlings exposed to drought stress showed a rise in AsA and GSH content, maintained a high GSH/GSSG ratio, and evidenced increased activities of APX, DHAR, MDHAR, GR, GST, GPX and CAT accompanied by lower levels of H_2O_2 and MDA. Coordinated induction of AsA and GSH and their metabolizing enzymes, a consequence of the application of exogenous Se, rendered the plant tolerant to drought-induced oxidative stress. Based on the above reports we concluded that better antioxidant protection is vital for plant growth and development under drought stress conditions.

8.5.3 High Temperature

High temperature or heat stress results from temperatures high enough to damage plant tissues, substantially influencing the growth and metabolism of plants (Balla et al. 2009). Although variable for different plant species, temperatures in the range of 35–45°C produced heat stress effects on tropical plants (Hall 1992). However, the extent to which this occurs in specific climatic zones depends on the probability and period of high temperatures occurring during the day and/or at night. Different global circulation models predict that greenhouse gases will gradually increase the world's average ambient temperature and lead to global warming (Meehl et al. 2007). Therefore, plants' responses and adaptation to elevated temperature and the mechanisms to develop heat-tolerant cultivars should be examined.

8.5.3.1 Plant Responses to High Temperature

Plant responses to high temperatures vary with the degree of temperature, duration and plant type. At very high temperatures, cellular damage or cell death may occur within minutes, which may lead to a catastrophic collapse of cellular organization (Schöffl et al. 1999). However, at moderately high temperatures, cell injury or death may occur only after long-term exposure. These injuries ultimately lead to starvation, inhibited growth, reduced ion flux, and the production of toxic compounds and ROS (Howarth 2005). The main symptoms of high temperature stress on plants may include scorching of leaves and twigs, sunburn on plant organs, leaf senescence and abscission (Guilioni et al. 1997; Ismail and Hall 1999). High temperature causes a delay in seed germination and a loss of vigor (Egli et al. 2005) as well as reduced plant emergence. Shoot dry mass, relative growth rate and net assimilation rate are significantly reduced by high temperature (Wahid 2007). High temperatures can cause fruit discoloration and damage, and reduce yield (Wahid et al. 2007). More importantly, heat stress, singly or in combination with drought, is a common constraint during anthesis and grain-filling stages in many cereal crops of temperate regions. Heat stress reduces kernel growth, ultimately causing the loss of kernel weight and density (Guilioni et al. 2003; Monjardino et al. 2005). A familiar consequence of high temperature in plants is the heat-induced imbalance in photosynthesis and respiration (Wahid et al. 2007). Normally, photosynthetic activity remains stable up to 30°C but decreases sharply above this temperature to reach complete inhibition at about 40°C (Bar-Tsur et al. 1985). However, high temperature influences the photosynthetic capacity of C3 plants more strongly than in C4 plants. High temperature stress is often associated with reduced water availability in field conditions (Simões-Araújo et al. 2003). Under heat stress, different plant species may accumulate a variety of osmolytes such as sugars and sugar alcohols (polyols), proline, tertiary and quaternary ammonium compounds, and tertiary sulphonium compounds. Accumulation of such solutes may contribute to enhanced high temperature stress tolerance of plants (Wahid et al. 2007).

8.5.3.2 Oxidative Stress in Plants Under High Temperature

High temperature stress impaired mitochondrial functions and resulted in induced oxidative damage (Suzuki and Mittler 2006). Extreme temperature stress accelerates the generation and reactions of ROS including $^1O_2$, $O_2^{\bullet-}$, H_2O_2 and OH•, thereby inducing oxidative stress (Mittler 2002; Yin et al. 2008). Under high temperature, RuBisCO can lead to the production of H_2O_2 as a result of its oxygenase reactions (Kim and Portis 2004). The main effects of ROS include autocatalytic peroxidation of membrane lipids and pigments, modification of membrane permeability and functions (Xu et al. 2006). A number of research works has revealed high temperature-induced oxidative damages in plants (El-Shintinawy et al. 2004; Yin et al. 2008). The drastic increase in lipid peroxidation due to high temperature stress was reported by many scientists (Mo et al. 2010; Wu et al. 2010).

8.5.3.3 Antioxidant Defense in Plants Exposed to High Temperature Stress

Tolerance to high temperature stress in crop plants is associated with an increase in antioxidants (Almeselmani et al. 2006; Babu and Devraj 2008; Almeselmani et al. 2009) Studies on heat-acclimated versus non-acclimated turfgrass species suggested that the former had lower production of ROS as a result of enhanced synthesis of AsA and GSH (Xu et al. 2006). In wheat, heat stress induced the accumulation of GSH levels and increased the activity of the enzymes involved in GSH synthesis and the GSH/GSSG ratio (Kocsy et al. 2002). Chauhan (2005) observed a heat stress-induced increase in the levels of GSH in the flag leaf of two wheat genotypes with contrasting behavior in heat tolerance at all stages of grain development. Almeselmani et al. (2006) found a significant increase in the activity of SOD, APX and CAT in late and very late planting and at all growth stages of wheat; however, GR and POX activity decreased in late and very late planting (exposed to high temperature) compared to normal planting. Later on, Almeselmani et al. (2009) observed that the activities of SOD, APX, CAT, GR and POX increased significantly at all stages of growth in heat-tolerant cultivars (C 306) in response to heat stress while the susceptible cultivar (PBW 343) showed a significant reduction in CAT, GR and POX activities in the high temperature treatment. A significant increase in the APX-mRNA level under heat stress at the vegetative and anthesis stages was also observed; expression was greater in C 306. Several authors also reported the involvement of SOD in temperature stress tolerance (Lu et al. 2008; Zhao et al. 2009). Badawi et al. (2007) reported higher GR activity in heat-tolerant wheat cultivars compared to sensitive cultivars. Babu and Devraj (2008) observed that heat stress drastically reduced the activities of GR and CAT in French bean (*Phaseolus vulgaris*). However, no variations were observed in APX, POX, and CAT isozymes. Zhao et al. (2009) reported that the co-expression of GST and CAT in heat-induced plants had an important effect on the antioxidant system, in particular, the whole AsA–GSH cycle. Rani et al. (2011) exposed a 5-day-old thermo-tolerant genotype, namely BPR-542-6, and a thermo-susceptible genotype, namely NPJ-119, of *Brassica juncea* to high

temperature stress (45.0 ± 0.5°C) and observed that the activities of SOD, POX, CAT, APX and GR increased, although the increase was significant only in the tolerant genotype. On revival, SOD and CAT began to decrease but the activities of POX and GR continued to increase in both the genotypes. APX, however, continued to increase in the tolerant genotype but started to decrease in the susceptible genotype. Chakraborty and Pradhan (2011) observed that CAT, APX and SOD showed an initial increase before declining at 50°C, while POX and GR activities declined at all temperatures ranging from 20 to 50°C. In addition, total antioxidant activity was maximum at 35–40°C in the tolerant varieties and at 30°C in the susceptible ones. Clearly, an increase in temperature leads to the increased expression of these antioxidative enzymes until a pre-determined temperature after which they decline, this temperature varying in tolerant and susceptible varieties. Tolerant varieties could maintain increased activities at higher temperatures than susceptible ones (Chakraborty and Pradhan 2011).

8.5.4 Low Temperature

About two-thirds of the world's land is annually subjected to temperatures below freezing point and about half of it suffers from temperatures below −20°C (Larcher 2001). Thus, in most regions around the world, plants are exposed to low temperature at least part each year. Among the abiotic stresses, low temperature stress is a serious threat to the sustainability of crop yield. Chilling stress results from temperatures cool enough to produce injury without the formation of ice in plant tissues whereas in freezing stress ice forms in plant tissues. Both chilling and freezing stresses are together termed cold stress. Chilling stress usually occurs at temperature between 0 and 10°C but a few tropical species such as rice and sugarcane are exceptionally sensitive to chilling and show injury signs up to 15°C (Thomashow 1999).

8.5.4.1 Plant Responses to Low Temperature Stress

There are various effects of low temperature stress depending on the species, plant age, and the duration of exposure. Low temperature may impose stress on a plant in two ways: By the effects of low temperature alone, and by dehydration of the cells and tissues when cellular water freezes. Low temperature stress affects seedlings more than mature plants with noticeable symptoms on plants including surface lesions, a water-soaked appearance, desiccation, discoloration, tissue breakdown, accelerated senescence and faster decay due to leakage of plant metabolites (Sharma et al. 2005; Solanke and Sharma 2008). Another major negative effect of low temperature stress is that it induces severe membrane damage which is largely due to acute dehydration associated with freezing (Yadav 2010). Low temperature-sensitive plants show a physical transition of the cell membrane from a flexible liquid-crystalline to a solid gel phase thereby affecting the cellular function in a number of ways. Thus the

immediate effect is higher membrane permeability and ion leakage (Farooq et al. 2009). In extreme cases, chilling stress results in accelerated senescence and eventually plant death (Sharma et al. 2005). Low temperature stress also severely hampers the reproductive development of plants which may cause floral sterility (Nahar et al. 2009; Yadav 2010). Chilling stress also affects the root growth of plants (Einset et al. 2007; Farooq et al. 2009). These changes limit the roots' capacity for water and mineral uptake and ultimately overall plant growth (Ercoli et al. 2004; Farooq et al. 2009). Low temperature reduces dry matter production and partitioning in crop plants (Verheul et al. 1996).

8.5.4.2 Oxidative Stress in Plants Under Low Temperature

With decreasing temperature, the solubility of a gas increases, which leads to a higher concentration of O_2 and thus enhances the risk of oxidative stress at low temperature which leads to the increased production of $O_2^{\bullet-}$, H_2O_2, $^1O_2$, and OH• (Guo et al. 2006). Low temperature conditions aggravate the imbalance between light absorption and light use by inhibiting the activity of the Calvin–Benson cycle. In addition, enhanced photosynthetic electron flux to O_2 and the over-reduction of respiratory ETC causes ROS accumulation during chilling (Hu et al. 2008). During cold treatment, the enzymes of the Calvin-Benson cycle are slowed by simple thermodynamics, thus limiting the supply of $NADP^+$ for reduction and ADP and Pi for phosphorylation. Incoming light energy continues to be channeled into ETC as long as the pigment beds remain intact and connected to the photosystems (PS I and PS II). These two factors, a slowing of the dark reactions and continuing energy absorption, over-reduce the photosynthetic ETC leading to the leakage of absorbed energy in an uncontrolled manner from the thylakoid membrane. As the light-independent reaction of photosynthesis is very temperature sensitive, the energy leaked during chilling in light causes the formation of ROS (Wise 1995), whose increased concentration causes damage to membrane lipids, proteins and nucleic acids, leading to PCD (Apel and Hirt 2004). Low temperature increases MDA content as a result of oxidative stress (Mo et al. 2010).

8.5.4.3 Antioxidant Defense in Plants Exposed to Low Temperature Stress

The improvement of low temperature stress tolerance is often related to the enhanced activities of enzymes of antioxidant systems in plants. Plants exposed to low temperatures use several non-enzymatic and enzymatic antioxidants to cope with the harmful effect of oxidative stress; higher contents of antioxidant defense enzymes are correlated with higher chilling tolerance (Kang and Saltveit 2002; Huang and Guo 2005). Antioxidant enzymes have higher activity in chilling-tolerant cultivars than in susceptible ones (Guo et al. 2005).

Zhang et al. (2008) observed a significant increase in AsA and GSH levels in maize plants during low temperature stress. In addition, increases in GSH levels

and/or GR activity during low temperature stress have been reported in different plant studies (Kocsy et al. 2000, 2001; Bhowmik et al. 2008). Guo et al. (2006) tested four rice cultivars under chilling conditions and concluded that chilling tolerance was well correlated with the enhanced antioxidant capacity of the cultivars, which was attributed to the higher AsA content and increased activity of antioxidant enzymes like APX and GR. Several studies reported enhanced TG levels in low temperature-tolerant plants like maize, tomato, and turf grass (Bhowmik et al. 2008) more than in sensitive plants. During low temperature acclimation, the maintenance of a high GSH/GSSG ratio is very important to ensure the functionality of GSH in the AsA-GSH cycle and other physiological processes (Kocsy et al. 2000). During cold-acclimation, ROS scavenging enzyme systems are activated, which help to detoxify ROS and increase tolerance to cold stress. A number of experiments comparing different species have reported that low temperature-tolerant plants showed greater activities in antioxidant enzymes than in sensitive ones. Huang and Guo (2005) found the higher efficiencies of antioxidant enzymes in chilling-tolerant rice cultivars than in chilling-susceptible cultivars. They observed that the activities of SOD, CAT, APX and GR, as well as AsA content of tolerant cultivar (Xiangnuo-1) remained high, while those of a chilling susceptible cultivar (IR-50) decreased under chilling. Zhao et al. (2009) observed a strong relationship between chilling sensitivity and the activities of antioxidant enzymes of postharvest tomato fruits exposed to short-term (24 h) or long-term (20 days) chilling stress. They observed that the chilling tolerance of tomato cultivars could obviously be indicated by higher activities of CAT, APX, POX and SOD. Dai et al. (2009b) reported that the elevated activities of APX and GR allow the cell to cope with oxidative stress due to chilling. Yang et al. (2011) observed that the enhanced activities of SOD, CAT, APX and POX in *Cucumis sativus* plants reflected better tolerance to chilling injury. Prasad (1997) observed higher GR and GPX activities in acclimated seedlings compared with non-acclimated seedlings during low-temperature stress and recovery. Gechev et al. (2010) reported that CAT and DHAR are most strongly affected by chilling (5°C) and may be the rate-limiting factor of the antioxidant system at low temperatures. In chickpea cultivars the activities of SOD, APX, GR and POX increased in cold-acclimated plants and subsequent chilling stress (2 and 4°C for 12 days), which indicated the enhanced chilling tolerance capacity of this cultivar to protect plants from oxidative damage (Turan and Ekmekçi 2011).

8.5.5 Waterlogging

Due to the increased frequency of extreme climate events, waterlogging has become an important constraint to crop production globally, causing a significant reduction in yield (Wollenweber et al. 2003). Waterlogging stress may develop due to several direct (improper irrigation practices) and indirect (global warming) anthropogenic and natural consequences (meteorological) leading to altered plant metabolism, architecture and ecogeographical distribution depending upon a plant's responses.

Waterlogging induces the progressive reduction in soil O_2 concentration and in redox potential (Ruiz-Sánchez et al. 1996), which contribute to the appearance of several reduced compounds of either chemical or biochemical origin (Kozlowski 1997). Alarming changes in the earth's average temperature, erratic rainfall, and rise in sea level due to increasing melting glaciers could exaggerate waterlogging or flooding problems in the near future.

8.5.5.1 Plant Responses to Waterlogging

The growth and development of most of the higher plant species are hampered by soil flooding, and particularly by complete submergence, both of which can result in death (Jackson and Colmer 2005). During waterlogging, the gas exchange between soil and the upper atmosphere decreases, and as gas diffusion in water decreases many fold, O_2 in the soil declines rapidly, and the soil may become hypoxic or anoxic within a few hours (Malik et al. 2002). One of the initial responses to waterlogging stress appears to involve the closing of stomata to avoid water loss, with a subsequent down-regulation of the photosynthetic machinery (García-Sánchez et al. 2007). Under submerged conditions, there is a decrease in total chlorophyll content in plants (Damanik et al. 2010), which sometimes respond to waterlogging by reducing leaf water potential, stomatal conductance, gas exchange and plant growth (Arbona et al. 2008). During long-term soil submergence, root hydraulic conductance decreases, which impairs water uptake and eventually leads to leaf wilting and chlorosis in citrus (Arbona et al. 2008). However, many wetland plant species can sustain themselves in flood-prone areas, achieved by a combination of genetic potential and some major physiological adaptations and acclimation such as physical 'escape' from a submerged environment (Voesenek et al. 2003), avoidance of O_2-deficiency through effective internal aeration (Jackson and Armstrong 1999), tolerance to anoxia (Gibbs and Greenway 2003), and the capacity to prevent or repair oxidative damage (Blokhina et al. 2003).

8.5.5.2 Oxidative Stress in Plants Under Waterlogging

Waterlogging, like other abiotic stresses, also leads to oxidative stress through an increase in ROS, such as $O_2^{\bullet-}$, $^1O_2$, H_2O_2 and OH• (Arbona et al. 2008). ROS are produced at the transition when a plant or any of its parts either enters to hypoxia/anoxia from normoxic conditions or returns to an aerobic environment (Irfan et al. 2010). Kumutha et al. (2009) and Sairam et al. (2009) showed that hypoxia-induced ROS are due to induction of membrane-linked NADPH oxidase. Higher accumulation of H_2O_2 and increased lipid peroxidation under anaerobic conditions has been reported by several researchers (Hossain et al. 2009; Kumutha et al. 2009; Sairam et al. 2011).

8.5.5.3 Antioxidant Defense in Plants Exposed to Waterlogging Stress

In many plant systems, the involvement of oxidative stress in flooding-induced damage and the antioxidant response was studied and a direct relationship between an increase in antioxidant activity and stress tolerance was observed (Arbona et al. 2008; Bin et al. 2010). Waterlogging stress resistance may depend, at least in part, on the enhancement of the antioxidative defense system which includes antioxidant enzymes such as SOD, CAT, APX, MDHAR, DHAR, GR, GPX as well as other non-enzymatic antioxidant compounds such as AsA, GSH, carotenoids and α-tocopherol (Arbona et al. 2008; Hossain et al. 2009; Bin et al. 2010).

Under waterlogging, leaves of citrus showed a significant increase in total ascorbic acid, AsA, DHA, and AsA/DHA ratio in stressed plants than in control conditions (Arbona et al. 2008). Likewise, TG, GSH and GSH/GSSG ratio also increased with a concomitant decrease in GSSG content. High levels of some antioxidant enzymes were important to survive oxidative stress after plants were subjected to different levels of waterlogging. Waterlogging stress increased SOD, CAT, APX and GR activities, although some differences were observed among genotypes. In general, the stress-sensitive cultivars showed lower activities than tolerant cultivars (Arbona et al. 2008). Hossain et al. (2009) demonstrated that coordinated antioxidant activity involving increased activities of SOD and CAT, together with a modulation of the AsA–GSH cycle, allowed citrus plants to cope with flooding-induced oxidative stress up to a certain point. Among the different antioxidant enzymes, SOD and CAT showed early responses whereas APX exhibited a late response with the de novo synthesis of AsA under walerlogging which was maintained by unaltered or decreased MDHAR and DHAR activities during the entire period of anoxia and post-anoxia; there was no positive correlation between DHAR activity and AsA/DHA ratio (Hossain et al. 2009). Kumutha et al. (2009) showed that an increase in the activity of antioxidant enzymes during waterlogging of pigeonpea (*Cajanus cajan*) is required to scavenge not only the post-hypoxic ROS build up, but also to detoxify the cellular system of ROS produced during hypoxia itself. The activity of antioxidant enzymes such as SOD, APX, GR and CAT increased under waterlogging. The comparatively greater antioxidant enzyme activities resulting in less oxidative stress in ICP 301 (waterlogging tolerant) could be one of the factors determining its higher tolerance to flooding than Pusa 207 (susceptible to waterlogging). The higher expression of SOD, POX and APX, predictive of waterlogging tolerance, reduced the level of ROS in Chrysanthemum (Yin et al. 2009). The more effective expression of antioxidant mechanisms in the tolerant cultivar also contributed to its lower level of lipid peroxidation (Yin et al. 2009). In rice seedlings, Damanik et al. (2010) suggested that tolerance to submergence stress might be proven by increasing the capacity of the antioxidative system. Following 8 days of complete submergence, they observed higher activities of antioxidative enzymes (SOD, CAT, APX, and GR) in waterlogging-tolerant varieties. Bin et al. (2010) suggested that in maize seedlings, increased POX, APX, GR, CAT and SOD activities led to an efficient H_2O_2 scavenging system and enhanced protection against oxidative stress caused by waterlogging. However, the activities were higher in waterlogging-tolerant genotypes. Sairam et al. (2011)

observed that under waterlogging, the activity of three antioxidative enzymes (SOD, APX and GR) showed a continuous increase up to 8 days of waterlogging in waterlogging-tolerant mung bean genotypes; in susceptible genotypes the increase in the activity of these enzymes was observed only following 2–4 days of waterlogging. In all subsequent stages there was a decline in activity of all three enzymes compared to the control and plants waterlogged for 2–4 days. Li et al. (2011a) showed that waterlogging pretreatment or hardening applied before anthesis can effectively improve the tolerance of wheat to waterlogging occurring during the generative growth stage, also effectively alleviating the oxidative damage to flag leaf cells by maintaining relatively higher activities of ROS-scavenging enzymes (SOD, CAT and APX) than the non-hardening treatment.

8.5.6 Heavy Metals

Heavy metals (HMs) are defined as metals with a density higher than 5 g cm^{-3}. Among the 90 naturally occurring elements, 53 are HMs (Weast 1984), only 17 HMs are available to living cells and are of importance for organisms and ecosystems based on their solubility under physiological conditions (Weast 1984). Although some elements have an importance as micronutrients, at higher concentrations they are toxic to plants and other organisms (Nies 1999). In nature, there are two main sources of HMs: the underlying parent material and the atmosphere. The HM content in soils depends on the weathering of rocks and on atmospheric metallic pollution. In addition to natural sources, viz. volcanoes and dusts, anthropogenic activities like mining, metal industries, agrochemicals, waste dumping, power houses, combustion of fossil fuels, etc., cause the emission of HMs and the accumulation of these compounds in ecosystems (Galloway et al. 1982; Angelone and Bini 1992). In recent years, substantial amounts of HMs have been released by geological activities or by accelerated anthropogenic impacts causing serious environmental problems (Sun et al. 2008). Since HMs are often found both in soil and water as contaminants, studies on complex HM toxicity in different plant species have come into focus.

8.5.6.1 Plant Responses to Heavy Metal

Making a generalization about the effect of HMs on plants is difficult due to the multidimensional variations in parameters under different concentrations, types of HMs, duration of exposure, target organs of plants, plant age, etc. Several physiobiochemical processes in plants cells are affected by HMs (Dubey 2011). Direct phytotoxic effects of HMs include their direct interactions with proteins, enzymes, displacement of essential cations from specific binding sites, causing altered metabolism, inhibiting the activities of enzymes, etc. (Sharma and Dubey 2007;

Sharma and Dietz 2008). Initially, a HM interacts with other ionic components present at the entry point of a plant root system. Later, the HM ion reacts with all possible interaction partners within the cytoplasm, including proteins, other macromolecules and metabolites. After that, HMs influence homeostatic events, including water uptake, transport and transpiration and thus symptoms start to develop and become visible, eventually leading to the death of plant cells (Fodor 2002; Poschenrieder and Barceló 2004). The most obvious plant reaction under HM toxicity is the inhibition of growth rate (Sharma and Dubey 2007). HMs also cause chlorosis, necrosis, leaf rolling, inhibition of root growth, stunted plant growth, altered stomatal action, decreased water potential, efflux of cations, alterations in membrane functions, inhibition of photosynthesis, altered metabolism, altered activities of several key enzymes, etc. (Sharma and Dubey 2007; Dubey 2011). Seed germination is also severely affected by HMs (Ahsan et al. 2007). Higher levels of HMs usually decrease photosynthesis (Heckathorn et al. 2004). HM inhibit the rate of photosynthesis and respiration (Llamas et al. 2000; Vinit-Dunand et al. 2002) and also inhibit carbohydrate metabolism and their partitioning in growing plants. Physiological adaptation of plants in response to HM stress also involves the production of different types of organic solutes, which includes small molecules such as proline, betaine which protect plants from stresses by cellular adjustment through the protection of membrane integrity and enzyme stability (Hossain et al. 2010). In addition, some signaling molecules like NO also increase under HM stress (Hsu and Kao 2004). Plant responses to HM also caused significant induction of sulfur assimilation (Gill and Tuteja 2011)

8.5.6.2 Oxidative Stress Under Heavy Metal Toxicity

There is enough evidence that exposure of plants to excess concentrations of redox active HM results in oxidative injury. HM uptake by transporters and distribution to organelles is followed by ROS generation, stimulated either by HM redox activity or by the effects of an HM on metabolism in a subcellular site-specific manner. HM-dependent activation of plasma-membrane-localized NADPH oxidase also contributes to the release of ROS. In contrast to the physiologically non-redox-active HM, such as Zn^{2+} and Cd^{2+}, the redox-active HMs Fe, Cu, Cr, V and Co enable redox reactions in the cell. They are involved in the formation of OH• from H_2O_2 via Haber-Weiss and Fenton reactions and initiate non-specific lipid peroxidation (Sharma and Dietz 2008). Lipid peroxidation is also specifically induced by HM-dependent activation of lipoxygenases (LOX) (Montillet et al. 2004). Among the HMs, Cd is the most widely studied in plants. The presence of Cd led to excessive production of ROS causing cell death due to oxidative stress such as membrane lipid peroxidation, protein oxidation, enzyme inhibition and damage to nucleic acids (Gill and Tuteja 2010; Hossain et al. 2010; Gill et al. 2011b). Hossain et al. (2010) observed a 130% increase in H_2O_2 and a 103% increase in MDA content in mung bean seedlings when exposed to Cd stress (1 mM $CdCl_2$, 48 h).

8.5.6.3 Antioxidant Defense in Plants Exposed to Heavy Metals Stress

To repair the HM-induced inhibitory effects of ROS, plants employ a ROS-detoxifying antioxidant defense machinery which includes non-enzymatic (GSH, AsA, α-tocopherol and carotenoids) and enzymatic (SOD, CAT, APX, GR, MDHAR, DHAR, GPX and GST) antioxidants (Gill and Tuteja 2010; Hossain et al. 2010; Gill et al. 2011a). Exposure to HMs provoke prominent responses of antioxidative systems, but the direction of the response dependends on the plant species, plant organ, the HM used and the intensity of the HM stress (Schützendübel and Polle 2002).

In a comprehensive study on mung bean, Anjum et al. (2011) observed a decrease in AsA, the AsA/DHA ratio, GSH, the GSH/GSSG ratio in both tolerant and sensitive cultivars exposed to Cd treatment (100 mg kg^{-1} soil). However, the decreases were lower in the tolerant cultivar compared to the sensitive cultivar. This suggests the protective role of the AsA and GSH pools toward Cd stress tolerance. Under HM stress, GSH serves in bio-reductive reactions as an important defense line against ROS to protect cells from oxidative stress damage, and to modify metal toxicity by altering the rates of metal uptake, elimination and by chelating metal ions in cells (Lima et al. 2006). GSH have a stronger ability to directly scavenge metal-induced ROS (Gill and Tuteja 2010, 2011). There are several possible ways for GSH to be involved in HM tolerance and sequestration (Wójcik and Tukiendorf 2011). Cai et al. (2010) studied the effect of exogenously applied GSH on the performance of rice cultivars under Cd stress and noted that exogenous GSH significantly alleviated Cd-induced growth inhibition and markedly reduced Cd uptake in both genotypes. In contrast, Wójcik and Tukiendorf (2011) indicate that the natural content of endogenous GSH in WT *Arabidopsis* plants is sufficient for Cd-tolerance. This decrease in GSH content led to lower Cd-tolerance of plants although an increase in GSH content could not enhance Cd tolerance, even showing toxicity.

As for non-enzymatic antioxidants, the protection against oxidative stress caused by toxic HMs is also greatly achieved by the production of enzymatic antioxidants such as SOD, CAT, enzymes of the AsA-GSH cycle (APX, MDHAR, DHAR and GR), GST and GPX; cumulatively, these biochemical attributes serve as an index of HM sensitivity or tolerance in different plant species (Hossain et al. 2010; Anjum et al. 2011; Gill et al. 2011a, b). Gill et al. (2011b) reported that higher tolerance to Cd is due to better coordination between the antioxidative enzymes, which help to protect the photosynthetic machinery. The enzymatic antioxidant system involves the sequential and simultaneous action of a number of enzymes for the removal of ROS under HM stress (Gill et al. 2011b). El-Beltagi et al. (2010) observed substantial increases in antioxidant enzymes, such as CAT, GST and POX in Cd-stressed plants in comparison with the control. The specific activity of CAT increased by increasing the Cd concentration, and reached a maximum value with 25 ppm of Cd in leaves, while at the highest concentration of Cd (50 ppm), CAT activity decreased relative to 25 ppm Cd in both leaf and root tissues. Cd treatment caused a significant increase in GST-specific activity in both roots and

leaves. GST activity at the highest concentration of Cd (50 ppm) reached 459% in leaves and 756% in roots, relative to control plants (El-Beltagi et al. 2010). Domínguez et al. (2010) confirmed that the antioxidant system efficiently achieves tolerance to Cd toxicity, allowing normal plant development, even in the presence of the highest Cd concentration. They showed that the activation of GPX, CAT, APX and SOD, coupled with the activation of AsA-GSH cycle enzymes (APX, MDHAR, DHAR and GR), was sufficient to reduce Cd-induced ROS accumulation and oxidative damage caused by the lower Cd concentrations (10 and 100 μM), but not by the highest Cd concentration (1 mM). Anjum et al. (2011) observed a protective role of AsA–GSH cycle metabolism in two mung bean cvs. Pusa 9531 (Cd–tolerant) and PS 16 (Cd–susceptible) under Cd stress. The changes in the AsA–GSH redox state and an increase in AsA–GSH–regenerating enzymes, such as APX, MDHAR, DHAR and GR and other antioxidant enzymes, such as SOD strongly supported over-utilization of AsA–GSH in Cd–treated plants. They observed that the oxidative stress caused by Cd toxicity was partially overcome by an AsA–GSH–based detoxification mechanism in the two genotypes studied because increases in lipid peroxidation and H_2O_2 content were accompanied by a corresponding decrease in reduced AsA and GSH pools. APX has an important role in the scavenging of H_2O_2 under stressed conditions but its activity depends on the Cd concentration applied (Gill et al. 2011a). Hossain et al. (2010) observed that Cd stress caused a significant increase in GSH and GSSG content in mung bean seedlings, while the AsA content decreased significantly with a sharp increase in H_2O_2 and MDA. APX, GST and GPX activities increased in response to Cd stress (1 mM $CdCl_2$, 24 h), while the activities of CAT, MDHAR, DHAR and GR were sharply decreased. Exogenous application of betaine or proline, resulting in an increase in GSH and AsA contents, maintenance of a high GSH/GSSG ratio and increased activities of APX, DHAR, MDHAR, GR, GST, GPX and CAT involved in the ROS detoxification system compared to the control and mostly also Cd-stressed plants, with a concomitant decrease in the levels of GSSG, H_2O_2 and MDA. They concluded that up-regulation of the antioxidant machinery provided protection against Cd-induced oxidative stress.

Kachout et al. (2009) reported that Atriplex plants cultured in soil polluted with HMs (Cu, Ni, Pb, Zn) showed varietal differences in HM tolerance, correlated with differences in antioxidant enzyme activities. Ahmed et al. (2010) reported that enhanced activity of POX, CAT and SOD may be of great significance for scavenging oxidative stress caused by excessive Cu in safflower plants and that these antioxidant enzymes served as good predictors for the evaluation of HM tolerance. It was suggested that the antioxidative activity seems to be of fundamental importance for the adaptive response of Atriplex plants to HM stress. Verma and Dubey (2003) indicated that SOD, POX and GR could serve as important components of the antioxidative defense mechanism against Pb-induced oxidative injury in rice seedlings. They observed a 1.9–2.0-fold increase in SOD activity, a 1.2–5.6-fold increase in GPX activity and a 1.2–1.9-folds increase in APX activity in the roots of rice seedlings exposed to 1 mM Pb for 15 days. GR activities showed an about 128–196%

increase in roots and 69–196% in shoots compared to control seedlings, while Pb treatment resulted in a decline in CAT activity in roots. Qureshi et al. (2007) observed that AsA content declined under Pb stress in a dose-dependent manner while DHA content increased. Similarly Pb-treated plants showed a rapid decline in GSH content, while the GSSG and total glutathione contents increased rapidly. Pb-treated plants showed a dose-dependent increase in SOD and APX activities, while CAT activity declined under severe stress (500 μM Pb-acetate) (Qureshi et al. 2007). Singh et al. (2006) observed that the levels of AsA and GSH, AsA/DHA and GSH/GSSG ratios in the fronds of *P. vittata* were much greater than in *P. ensiformis* under As stress indicating that *P. vittata* has a greater antioxidant capacity than P. ensiformis. The lower levels of antioxidant compounds (AsA, GSH and carotenoids) in *P. ensiformis* than in *P. vittata* are correlated with its greater exposure to ROS and lower scavenging ability. In wheat, Li et al. (2007) reported that APX and SOD activities decreased at low concentrations of As, and increased at high concentrations of As, while CAT activity displayed an increasing trend when the concentration of As was lower than 1 mg kg^{-1}, and then decreasing trend. Gupta et al. (2009) observed a significant increase in the activities of SOD, GPX and CAT in two varieties (Varuna and Pusa Bold) of *B. juncea* at lower concentrations of As stress (50 μM). The increased tolerance in Pusa Bold may be due to the higher activity of antioxidant enzymes. Shri et al. (2009) observed enhanced activity of antioxidant enzymes and isozymes of SOD, APX, POX and GR in rice seedlings subjected to As exposure. Contradictorily, Sun et al. (2008) reported that the activities of SOD and POX in rice leaves were significantly decreased under As stress (40 mg kg^{-1}) which resulted in a higher accumulation of ROS in As-stressed leaves, causing lipid peroxidation. In rice seedlings, the activities of all isoforms of SOD (Cu-ZnSOD, MnSOD and FeSOD), GPX and APX increased in Ni-treated (200 and 400 μm $NiSO_4$) seedlings, while no clear induction of CAT was observed (Maheshwari and Dubey 2009). The activity of AsA-GSH cycle enzymes (MDHAR, DHAR and GR) significantly increased in Ni-treated seedlings. In another study, Wang et al. (2010) observed significant increases in SOD, CAT and GPX activity of cotyledons, stems and roots of *Luffa cylindrica*, suggested that treatment with different levels of Ni may enhance the activity of these antioxidants, thus alleviating Ni-induced oxidative damage and enhancing Ni tolerance. Shanker et al. (2004) demonstrated the role of ROS-scavenging enzymes in plant parts under chromium (Cr) stress. Scavenging enzymes were not induced by a lower concentration of Cr because there is controlled ROS production. However, the combined action of SOD and CAT showed a major role in minimizing the effects of oxidative stress due to their capacity to scavenge $O_2^{\bullet-}$ and H_2O_2 under Cr stress. Shiyab et al. (2009) reported that Indian mustard (*Brassica juncea*) showed an efficient metabolic defense and adaptation system to mercury (Hg)-induced oxidative stress due to antioxidant defense. A lower level of H_2O_2 was observed in shoots with higher Hg concentrations due to the effective generation of an enzymatic antioxidant defense system (especially CAT) to scavenge H_2O_2.

8.5.7 UV Radiation

In the past few decades there has been a depletion of the stratospheric ozone (O_3) layer due to emissions of halogen-containing compounds of anthropogenic origin. This has resulted in a concomitant increase in solar ultraviolet-B radiation (Mpoloka 2008) because a 1% loss of O_3 leads to a 2% increase in UV radiation. This increase is predicted to increase in the near future, which may cause a negative impact on plants and other biological organisms. Extended exposure to UV-B radiation is especially harmful to all photosynthetic organisms due to their requirement for light (Sinha et al. 2003). Plants use solar radiation for photosynthesis and accordingly are also exposed to UV-B radiation.

8.5.7.1 Plant Responses to UV Radiation

Plants exhibit tremendous variability in their sensitivity to UV-B radiation (Mpoloka 2008). Under exposure to UV-B radiation, different kinds of morphological, biochemical and physiological responses of plants have been reported. UV-B radiation has detrimental effects such as reduced photosynthesis, biomass reduction, decreased protein synthesis, impaired chloroplast function, damage to DNA, etc. (He et al. 2003; Zhang et al. 2003). Enhanced UV-B radiation significantly decreases plant height and leaf area, and increases leaf thickness (Ren et al. 2007). Increased leaf thickness suggests the possibility of a lower penetration of UV-B radiation into the deeper mesophyll layer (Bornman and Vogelmann 1991). The photosynthetic system is also a sensitive component to increased exposure to UV-B (Sharma et al. 1998). However, the response of plants to changes in UV-B radiation also depends upon associated stresses e.g., low light, temperature extremes, atmospheric pollutants, metal toxicity, drought and nutrient deficiencies (Correia et al. 2005).

8.5.7.2 Oxidative Stress in Plants Under UV Radiation

Exposure to UV-B leads to the generation of ROS such as $^1O_2$, $O_2^{\bullet-}$, H_2O_2 and OH• (Moldau 1999). An increase in ROS by UV-B radiation has been observed in several plant species (Agrawal and Rathore 2007; Du et al. 2011; Singh et al. 2011), leading to the oxidative destruction of cell components through oxidative damage of nucleic acids, membrane lipids, proteins and enzymes (Roleda et al. 2006a, b). Uncontrolled generation of ROS in plant cells induced by UV-B also causes detrimental effects on enzymatic activities and gene expression, which eventually leads to cellular injury and PCD (Mackerness et al. 2001).

8.5.7.3 Antioxidant Defense in Plants Exposed to UV Radiation-Induced Stress

Plants contain a complex biochemical defense system which is considered to play a major role in protecting plants from UV-B damage (Liang et al. 2006). However, the available reports on the effect of UV-B radiation and their antioxidant response indicate considerable differences between plant tissues and/or plant species (Rao et al. 1996; Mackerness et al. 2001). The antioxidant defense system includes non-enzymatic antioxidants and antioxidant enzymes. Jain et al. (2003) found that UV-B enhanced the level of AsA in cucumber cotyledons; however, treatment of cotyledons with UV-B radiation reduced the α-tocopherol content. Enhancement of the AsA level under UV-B stress was observed in wheat leaves (Sharma et al. 1998) and *Arabidopsis thaliana* (Rao et al. 1996). UV-B radiation evidently induces a signal transduction that enhances the in vivo level of AsA (Jain et al. 2003). Costa et al. (2002) reported that UV-B radiation induced an antioxidant defense system in sunflower cotyledons and plant survival was higher, despite the oxidative stress. They observed that the GSH/GSSG ratio was significantly increased in response to UV-B treatments (15.0 and 30.0 kJ m^{-2}) while the AsA/DHA ratio was not affected. The activity of the antioxidant enzymes CAT and GPX increased under UV-B radiation while the activities of APX and GR were not altered. Tocopherols are involved in the reduction of PUFA radicals that are formed in plants during UV-B stress. Acute exposure of UV-B led to a decrease in α-tocopherol levels in plants (Jain et al. 2003; Agrawal et al. 2009) reflecting reactions with lipid radicals. α-tocopheroxyl radicals are immediately produced in UV-B-irradiated liposomes and thus result in the instant breakdown of the antioxidantive role of α-tocopherol which is not regained without the presence of AsA/thiols (Agrawal et al. 2009). Activation of antioxidant enzymes (SOD, CAT, APX, POX and GR) by UV-B has been reported in several plant species (Rao et al. 1996; Sharma et al. 1998). Ren et al. (2007) concluded that *Populus* trees respond to enhanced UV-B radiation with changes in the levels of antioxidant enzymes, especially APX and SOD. Recently, Ravindran et al. (2010) observed that CAT, POX and SOD activities were inhibited under supplemental UV-B radiation treatment in *Indigofera tinctoria* L. seedlings. Kumari et al. (2010) observed that the activities of SOD, CAT, APX and GR in *Acorus calamus* (sweet flag) plants were stimulated under elevated UV-B, while no definite trend of change was observed for AsA. They suggested that UV-B radiation may stimulate the enzymatic and non-enzymatic defense systems of Acorus plants, showing its better adaptation at a lower dose of UV-B (+1.8 kJ m^{-2} day^{-1}). Li et al. (2010b) reported the increased activities of SOD and POX in *Corallina officinalis* L. exposed to UV-B, while APX and CAT activities remained stable. Those enzymes worked together as ROS scavengers at a low dose of UV-B. However, at a high dose of UV-B, the antioxidant capacity decreased. In cucumber cotyledons, UV-B enhanced the activity of SOD, APX and GPX (Tekchandani and Guruprasad 1998; Jain et al. 2003).

8.5.8 Ozone

Ozone (O_3) is a secondary air pollutant formed by photochemical oxidation of primary pollutants such as nitrogen oxides, hydrocarbons and carbon monoxide (Lelieveld and Crutzen 1990) that are released into the atmosphere mainly by fossil fuel combustion, biomass burning, and biogenic emissions (Kesselmeier and Staudt 1999). It is predicted that significant crop losses due to O_3 damage will increase by 25% in background O_3 concentration over the next 30–50 years (Meehl et al. 2007). In many industrialized countries, tropospheric ozone (O_3) reaches to such high concentrations which is harmful for the plant species (Schraudner et al. 1997). Therefore, considering the predicted effect of O_3, it is necessary to explore the multifarious responses of plants and their adaptation under elevated O_3.

8.5.8.1 Plant Responses to O3

Ozone is the most damaging air pollutant to plants (Ashmore 2005). Ozone stress has been characterized as either acute or chronic, depending on the O_3 concentration and the duration of exposure (Sandermann 1996). While the actual concentration and duration threshold for O_3 damage varies from species to species and even among genotypes of the same species (Burkey et al. 2000), it is commonly accepted that acute damage is caused by a very high concentration of O_3 (>150 ppb) within a short period of time while chronic O_3 damage occurs by a lower concentration of exposure over a longer period of time (Gillespie et al. 2011). Many reports indicate that O_3 leads to a general reduction of growth and competitive fitness of plants (Gillespie et al. 2011) in which elevated O_3 concentrations cause oxidative injury in living tissues and may result in negative long-term effects on the vitality of plants, leaf damage, biomass reduction, altered metabolism and accelerated senescence, which lead to losses in yield (Ashmore 2005; Li et al. 2010c; Feng et al. 2011).

8.5.8.2 Oxidative Stress in Plants Induced by O_3

Being a strong oxidant, O_3 can interact with constituents of the apoplast to generate ROS such as H_2O_2, $O_2^{\bullet-}$, OH• and HOO• (Yan et al. 2010a, b). Significant increases in protein carbonylation, increased lipid peroxidation and changes in cellular permeability are a consequence of O_3 exposure (Gillespie et al. 2011). Wohlgemuth et al. (2002) reported that blocking H_2O_2 and $O_2^{\bullet-}$ accumulation markedly reduced O_3-induced cell death in various plant species. Different studies indicated that elevated O_3 remarkably increased the levels of H_2O_2 levels and MDA in plants (Li et al. 2010c; Yan et al. 2010a, b; Feng et al. 2011).

8.5.8.3 Antioxidant Defense in Plants Exposed to O_3 Stress

The antioxidant system that is responsible for controlling the level of ROS in plant tissues plays an important role in conferring O_3 tolerance to plants (Tausz et al. 2007). Increasing the level of endogenous antioxidants such as AsA or flavonoids could limit the deleterious effects of oxidative stress caused by O_3 (Vickers et al. 2009). Application of chemical antioxidants for protecting vegetation from O_3 injury has been extensively studied over the past four decades. Apoplastic AsA forms the first line of defense against O_3 (Didyk and Blum 2011), which participates in a series of cell wall reactions targeted to prevent O_3 from generating free radicals. At least in some crops, O_3 tolerance has been shown to be a heritable trait involving the antioxidant system and high apoplastic AsA content (Fiscus et al. 2005) and apoplastic AsA was found to detoxify up to 30–50% of O_3 taken up by leaves (Turcsanyi et al. 2000). Burkey et al. (2000) observed that in *Phaseolus vulgaris* L., AsA was the only variable identified as a potential factor in O_3 tolerance in which tolerant genotypes contained more AsA than sensitive lines. In addition, *Arabidopsis* mutants containing diminished concentrations of AsA were more susceptible to O_3 than WT plants (Conklin et al. 1996), suggesting that a minimum level of AsA is required to protect plants against O_3 stress. Sen Gupta et al. (1991) observed higher GSH and GSSG levels in poplar leaves following a 3-h exposure to 180 nmol mol^{-1} of O_3 compared to the control. Ozone fumigation of clover leaves induced a decrease of TG content, mainly due to the strong increase in GSSG, while GSH decreased (Scebba et al. 2003). The increase in GSSG could, therefore, be an index of O_3-induced oxidative stress. Borowiak et al. (2009) showed that SOD activity increased with an increase in O_3 concentration in sensitive as well as resistant cultivars. However, O_3-sensitive and O_3-resistant poplar clones exhibited different patterns of SOD activity. Bandurska et al. (2009) reported a positive correlation between O_3 level and APX activity in a resistant tobacco cv. Bel B, which did not reveal visible symptoms, indicating that this enzyme may contribute to the detoxification of H_2O_2 and alleviation of O_3-induced oxidative damage. Strohm et al. (2002) demonstrated that in poplar leaves, the total ascorbate contents and the activities of APX, MDAR and DHAR were not significantly affected by acute O_3 exposure in all poplar lines. They also showed that in developing leaves of transgenic plants, over-expressing GR in the cytosol or chloroplasts, APX and DHAR activities were higher than in WT plants. Scebba et al. (2003) demonstrated that in clover leaf, the activities of POX, APX and MDHAR increased in both clover species, but always more than in tolerant species, confirming again its higher level of protection against oxidative-induced stress. Pukacka and Pukacki (2000) found GPX to play a regulatory role in scavenging H_2O_2 under O_3 stress. Both resistant and sensitive cultivars showed a positive and highly significant correlation between GPX activity and the degree of leaf damage. Gillespie et al. (2011) indicated that soybean grown at chronic elevated O_3 concentrations (90 ppb) increased the total antioxidant capacity of plants which were matched by changes in AsA content, but not phenolic content. In their study, DHAR activity more than doubled in plants grown at elevated O_3 levels than in controls, whereas GR activity was significantly lower.

8.6 Transgenic Approaches to Enhance Oxidative Stress Tolerance

Recently, understanding of the role of ROS-scavenging systems in plant stress tolerance has increased through the use of gene transfer technology to manipulate the antioxidative capacity of plants. Several studies clearly demonstrated that enhancement of ROS-scavenging systems in plants through transgenic approaches can provide partial protection from oxidative damage, indicating that this strategy could be used to improve plant stress tolerance (Roxas et al. 2000; Ruan et al. 2011). Several successful approaches to achieve tolerance through the genetic engineering of specific genes have been studied and the improvement of the antioxidant defense system, enhanced abiotic stress tolerance and increased productivity. Often, non-enzymatic and enzymatic components of antioxidative defense systems are significantly up-regulated in transgenic plants compared to non-transformed or WT plants.

Hemavathi et al. (2010) showed that transgenic potato (*Solanum tuberosum* L. cv. Taedong Valley) over-expressing the L-gulono-c-lactone oxidase (*GLOase*) gene showed enhanced basal levels of AsA content (141%) than non-transgenic tubers and showed better survival under various abiotic stresses caused by methyl viologen, NaCl and mannitol. There was also a direct correlation between elevated levels of AsA accumulation in transgenics and their ability to withstand abiotic stresses. Alteration in GSH levels by transgenic approaches also conferred enhanced stress tolerance in plants. *B. juncea* (mustard) plants overexpressing *GS* or γ-*ECS* showed enhanced tolerance to a variety of HMs (Cd, Zn, As and Pb) due to the higher capacity of GSH synthesis as well as PC synthesis (Reisinger et al. 2008). Liu et al. (2008) suggested that the overexpression of α-tocopherol can increase the tolerance of plants to oxidative stress caused by abiotic stresses. Tocopherol cyclase (*VTE1*, encoded by *VTE1* gene) catalyzes the penultimate step of tocopherol synthesis. Transgenic tobacco plants overexpressing *VTE1* from *Arabidopsis* showed decreased lipid peroxidation, electrolyte leakage, and H_2O_2 content compared to the WT when exposed to drought conditions (20% PEG) (Liu et al. 2008).

Faize et al. (2011) showed that simultaneous overexpression of Cu/Znsod and *apx*, or at least *apx*, in the cytosol of transgenic tobacco plants alleviated the damage produced by water stress (3–5 days). In general, oxidative stress parameters such as lipid peroxidation, electrolyte leakage, and H_2O_2 levels, were lower in transgenic plants than in non-transformed plants suggesting that, at least, overexpression of cytapx protects tobacco membranes from water stress. Moreover, an increase in the activity of some antioxidant enzymes was also observed in the chloroplasts of transgenic plants overexpressing cytsod and/or cytapx. Artlip et al. (2009) observed that SOD overexpression (*SOD-OX*) leaves exhibited improved resistance to both acute (30 min) and longer-term exposure (2 to 24 h) to elevated temperatures (40 and 45°C) compared to the non *SOD-OX* lines. In sweet potato (*Ipomoea batatas*) plants, expression of Cu/ZnSOD and APX in chloroplasts enhanced drought resistance and the capacity to recover from drought stress (Lu et al. 2010). Compared with

non-transgenic plants, the expression of antioxidant enzymes (SOD, APX and CAT) in transgenic plants was profoundly increased under drought stress and rewatering periods resulted in low levels of MDA and electrolyte leakage. Transgenic plants also exhibited better growth, photosynthetic activity (Fv/Fm) and water status under drought stress compared with non-transgenic plants. Zhao and Zhang (2006) showed that co-expression of the GST and *CAT1* genes resulted in a greater increase of CAT and SOD activity in transgenic compared to non-transgenic rice seedlings exposed to both salt (200 mM NaCl) and paraquat while a significant increase of GST activity in transgenics occurred only in paraquat-stressed plants. The generation of H_2O_2 and MDA decreased in the transgenics than in non-transgenics under the same conditions. Moreover, the transgenic seedlings showed markedly enhanced tolerance to salt stress upon 200 mM NaCl treatment compared with non-transgenics. They concluded that enhancement of the ROS-scavenging system that led to increased oxidative stress protection in GST + *CAT1*-transgenic rice plants could result not only from increased GST and CAT activity but also from the combined increase in SOD activity. CAT activity of transgenic *Brassica juncea* plants overexpressing the *BjCAT3* gene was approximately 2-fold higher than that of WT which was correlated with enhanced tolerance under Cd stress (Guan et al. 2009). Transgenic rice plants (cv. Nipponbare and cv. BR5) overexpressing a CAT gene from *Escherichia coli, katE*, were more tolerant to NaCl (100 mM) than WT plants (Nagamiya et al. 2007; Moriwaki et al. 2008).

Rice plants overexpressing *OsAPXa* showed increased APX activity under cold stress (Sato et al. 2011). In their study, the levels of H_2O_2 and MDA increased by 1.5- and 2-fold, respectively in WT plants subjected to a 12°C treatment for 6 days. In contrast, transgenic lines showed significantly lower levels of H_2O_2 and MDA than WT plants. While studying different ROS signals, Miller et al. (2007) generated a double mutant lacking thylakoid ascorbate peroxidase (*tylapx*) and cytosolic ascorbate peroxidase 1 (*apx1*) genes. Two different signals were likely generated in plants lacking cytosolic *APX1* or *tylAPX*. The absence of a chloroplastic H_2O_2-removing enzyme triggered a specific signal in cells that resulted in enhanced tolerance to heat stress (38°C) (Miller et al. 2007). Sun et al. (2009) found that the thylakoid-bound APX gene (*LetAPX*) from tomato, when overexpressed in tobacco, improved salt (200 mM NaCl) tolerance.

The simultaneous expression of multiple antioxidant enzymes, such as CuZnSOD, APX, and DHAR, in chloroplasts was more effective than single or double expression for developing transgenic plants with enhanced tolerance to multiple environmental stresses (Lee et al. 2007). Transgenic tobacco plants expressing both CuZnSOD and APX in the chloroplast (CA plants), or DHAR in chloroplast showed enhanced tolerance to oxidative stresses such as paraquat and salt. Later, they introduced the gene encoding DHAR into CA transgenic plants and observed that mature leaves of transgenic plants expressing all three antioxidant genes (CAD plants) had approximately 1.6–2.1-fold higher DHAR activity, and higher ratios of reduced AsA/DHA, and GSH/GSSG compared to CA plants. Thus CAD plants were more resistant to paraquat-induced stress, exhibiting only an 18% reduction in membrane damage relative to CA plants. In addition, seedlings of CAD plants had enhanced tolerance

to NaCl (100 mM) compared to CA plants. The manipulation of DHAR expression is important for the genetic engineering of stress-tolerant plants (Amako and Ushimaru 2009). The results obtained by Yin et al. (2010) indicate that plants overexpressing DHAR showed better root growth than WT plants and showed lower H_2O_2 content, less lipid peroxidation and a lower level of oxidative DNA damage than WT plants under Al stress (300–500 μM). Compared with WT plants, DHAR-overexpressing plants showed a higher AsA level and APX activity which contributed to their higher antioxidant capacity and higher tolerance to Al stress. The overexpression of MDHAR minimizes the deleterious effects of environmental stresses (Eltayeb et al. 2007). In their study, transgenic tobacco plants overexpressing the *A. thaliana* MDHAR gene (*AtMDAR1*) in the cytosol exhibited up to a 2.1-fold higher MDHAR activity and a 2.2-fold higher level of reduced AsA compared to non-transformed control plants. The transgenic plants showed enhanced stress tolerance under O_3, salt, and PEG stresses and greater PSII effective quantum yield under O_3 and salt stresses. Furthermore, these transgenic plants exhibited significantly less H_2O_2 when tested under salt stress. Thus, overexpressed MDHAR confers enhanced tolerance to O_3 (2 ppm), salt (300 mM NaCl), and drought (10% PEG) stress (Eltayeb et al. 2007).

Martret et al. (2011) observed that tobacco chloroplast transformants expressing genes encoding DHAR, GR, and GST exhibit altered anti-oxidant metabolism and improved tolerance to salinity and chilling. This improved protection could be explained by synergistic effects of DHAR with GR or GST with GR. The expression of these combinations of transgenes also increased the regeneration of AsA (1.6-fold) and GSH (2.4-fold) and participated in a more rapid scavenging of $O_2^{\bullet-}$ and H_2O_2 prior to their interaction with target molecules. In both chilling and salt stresses, the protective effect could be observed when DHAR or GST levels were enhanced independently and in the case of chilling stress, a further improvement was observed when these were combined with increased GR activity. Plants overexpressing DHAR and GST did not differ from WT in their tolerance to methyl viologen, while DHAR:GR and GST:GR did. These different results illustrate the fact that in some cases, overexpression of a single antioxidant enzyme does not provide protection against oxidative stress and simultaneous expression of multiple antioxidant enzymes is more effective than single expression for enhancing tolerance to environmental stresses. Roxas et al. (2000) showed that overexpression of a tobacco GST with GPX activity in transgenic tobacco (*Nicotiana tabacum* L.) enhanced seedling growth under heat and salt stress. In addition to increased GST and GPX activity, transgenic seedlings expressing GST/GPX had elevated levels of MDHAR activity and higher levels of AsA and GSH than WT seedlings. When stress was imposed, overexpression of GST/GPX in transgenic tobacco seedlings provided increased GSH-dependent peroxide scavenging ability and alterations in AsA and GSH metabolism that led to reduced oxidative damage, indicated by a decrease in lipid peroxidation. Jha et al. (2011) showed the significant induction of the plant-specific *Tau* class *GSTU* genes by different abiotic stresses provided better protection of plants against oxidative damage. A transcript study of *SbGST* gene expression under salt, cold, drought with time period point and concentration point revealed

that the expression of the *SbGST* gene was up-regulated under all stress conditions. Consequently, the transgenic lines showed higher seed germination and survival than WT, confirming that over-expression of the *tau* class *SbGST* gene in transgenic tobacco plays a vital role in abiotic stress tolerance. Herbette et al. (2011) reported that transgenic tomato showing higher GPX activity was more resistant to an abiotic stress (mechanical injury) but more susceptible to biotic stresses (such as pathogen attack). They also suggested that overexpression of GPX provoked opposite effects in biotic and abiotic challenges, suggesting a key role for this scavenger enzyme in controlling both types of stress responses.

Plant resistance to abiotic stresses is genetically complex and multigenic, and thus more difficult to control and engineer. Plant engineering strategies for abiotic stress tolerance rely on the expression of genes that are involved in signaling and regulatory pathways or genes that encode proteins conferring stress tolerance or enzymes present in pathways leading to the synthesis of functional and structural metabolites (Vinocur and Altman 2005). Though it is possible to confer partial tolerance to a certain abiotic stress by overexpressing a single component of the antioxidant defense system, only limited improvement in stress tolerance has been achieved (Lee et al. 2009). As the ROS detoxification system is a coordinated process, overexpressing one enzyme is not sufficient to counterbalance the ROS levels. Therefore, increases in one component might not result in an overall increase in protection against abiotic stresses. Transgenic plants will, however, continue to be extremely useful tools in biotechnology and will lead to an improved understanding of the gene networks and molecular physiology of plant responses to abiotic stresses.

8.7 Conclusion and Future Perspectives

The unquestionable importance of abiotic stress in world agriculture is demonstrated by the fact that abiotic factors cumulatively account for major limitations in crop production worldwide. Therefore, further steps to understand the molecular and physiological mechanisms of abiotic stress tolerance and to find the ways that would increase stress tolerance in plants are crucial in agriculture. It is possible to minimize losses in agricultural production due to abiotic stresses by a judicious blend of knowledge in crop physiology and crop husbandry procedures. The production, metabolism and detoxification of ROS are essential processes in plant growth, adaptation and survival. The generation and scavenging of ROS are vital parts of plant defense mechanisms and regulation, and over-expression of novel isoforms of genes coding for ROS-detoxifying enzymes increase tolerance against environmental stresses. Although ROS were initially recognized as toxic by-products of aerobic metabolism, recently, it has become apparent that ROS also play an important signaling role in plants' processes such as growth, development, and responses to adverse environmental conditions. However, to evaluate the negative effects caused by potential stressors, it is important to understand mechanisms of

resistance and tolerance. The potential of engineering plants that overexpress genes for antioxidants provides an opportunity to develop plants with enhanced tolerance to abiotic stresses. With advancements in molecular biology and the availability of advanced genetic tools, considerable progress has been made in improving stress-induced oxidative stress tolerance in crop plants by developing transgenic lines with altered levels of antioxidants (Lee et al. 2007; Ashraf et al. 2008). Use of exogenous chemical protectants like proline, glycinebetaine, Se and signaling molecules like NO has also showed significant up-regulation of antioxidative defense and thus better alleviation of oxidative stress due to efficient co-regulation of both enzymatic and non-enzymatic antioxidant defense systems (Hossain et al. 2010; Hasanuzzaman et al. 2011a, b; Hasanuzzaman and Fujita 2011). In fact, the ROS detoxification system is very complex and multileveled controlled, and changing one component of the antioxidative defense system might not modify the capacity of the pathway as a whole defense system (Lee et al. 2009). Furthermore, overexpression of combinations of antioxidant enzymes in transgenic plants has been shown to have synergistic effects on stress tolerance. Therefore, increased emphasis is being placed on producing transgenic plants overexpressing genes – i.e., gene stacking – associated with more than one antioxidant in order to achieve tolerance to multiple environmental stresses.

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