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M.N.V. Prasad *Editors*

Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change

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Preface

Any external factor that imposes negative impact on growth and development of the plant is known as stress. Plants often experience abiotic stress like drought, salinity, alkalinity, temperature, UV-radiations, oxygen deficiency, etc. Abiotic stress is responsible for the huge crop loss and reduced yield more than 50% of some major crops. Ion imbalance and osmotic stress is the primary effect of abiotic stress. Prolonged exposure to primary stress causes secondary stress through the generation of reactive oxygen species (ROS). These are deleterious for the plants as it causes oxidative damage by reacting with biomolecules. Plants are able to perceive the external and internal signals and are then used by the plant to regulate various responses to stress. Plants respond the abiotic stress by up- and downregulation of genes responsible for the synthesis of osmolytes, osmoprotectants, and antioxidants. Stress-responsive genes and gene products including proteins are expressed and provide tolerance to the plant. To understand the physiological, biochemical, and molecular mechanisms for abiotic stress, perception, transduction, and tolerance is still a challenge before plant biologists.

The chapters in this book deal with the effect of different abiotic stresses on plant metabolism and responses of the plants to withstand the stress. Chapter 1 describes involvement of different osmolytes, osmoprotectants, and antioxidants during abiotic stress. Chapter 2 deals with the role of halophytes in understanding and managing abiotic stress. Chapter 3 addresses the effect and defense mechanisms in plants under UV stress. Chapter 4 throws light on the potassium uptake and its role under abiotic stress. Chapters 5–7 deal with the effect of temperature (heat, chilling) on plants and their responses. Chapter 8 deals with the formation and function of roots under stress. Chapter 9 is concerned with role of ROS and NO under abiotic stress. Chapter 10 throws light on nitrogen inflow and nitrogen use efficiency (NUE) under stress. Chapter 11 addresses Am symbiosis and soil interaction under abiotic stress. Chapter 12 deals with the role of small RNA in abiotic stress. Chapter 13 describes the involvement of transcription factors (TFs) under abiotic stress. Chapters 14–17 deal with the involvement of different signaling molecules (Ca^{2+}, H_2O_2, and phytohormones) under abiotic stress. Chapter 18 covers the role of ethylene and plant growth-promoting bacteria under environmental stress. Chapter 19 throws light on new approaches about metal-induced stress. Chapters 20 and 21 address the role of sulfur and salicylic acid in

alleviating heavy metal-induced stress. Chapters 22 and 23 cover the bioremediation of organic contaminants and utilization of different weeds in removal of heavy metals. We hope that this volume will provide the background for understanding abiotic stress tolerance in plants.

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Abiotic Stress Responses in Plants: An Overview

1

Hans-Werner Koyro, Parvaiz Ahmad, and Nicole Geissler

Abstract

Plants are more and more affected by environmental stresses, especially by the devastating consequences of desertification and water scarcity which can be seen and felt all over the world. About 3.6 billion of the world's 5.2 billion hectares of dryland used for agriculture have already suffered erosion, soil degradation, and salinization. Desertification can hinder efforts for sustainable development and introduces new threats to human health, ecosystems, and national economies. This problem is catalyzed by global climate change which exacerbates desertification and salinization. Therefore, solutions are desperately needed, such as the improvement of drought and salinity tolerance of crops, which in turn requires a detailed knowledge about tolerance mechanisms in plants. These mechanisms comprise a wide range of responses on molecular, cellular, and whole plant levels, which include amongst others the synthesis of compatible solutes/osmolytes and radical scavenging mechanisms. Regarding global change, elevated atmospheric CO_2 concentrations can enhance salt and drought tolerance because oxidative stress is alleviated and more energy can be provided for energy-dependent tolerance mechanisms such as the synthesis of compatible solutes and antioxidants, thus increasing the suitability of plants as crops in future. A detailed knowledge of the physiological and biochemical basis of drought and salt tolerance and its interaction with elevated CO_2 concentration can provide a basis for the cultivation of suitable plants in regions threatened by desertification and water scarcity under sustainable culture conditions. Even the drylands could offer tangible economic and ecological opportunities.

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The aim of this chapter is to uncover how compatible solutes and antioxidants alleviate environmental stress, especially drought and salt stress, and the role elevated CO_2 concentrations can play in this context, so that early indicators allowing successful breeding can be identified and the potential of plants as crops in a CO_2 rich world can be assessed.

Keywords
Abiotic stress • Antioxidants • Osmolytes • Oxidative stress

1 Introduction

Plants are continuously affected by a variety of environmental factors. Whereas biotic environmental factors are other organisms such as symbionts, parasites, pathogens, herbivores, and competitors, abiotic factors include parameters and resources which determine plant growth like temperature, relative humidity, light, availability of water, mineral nutrients, and CO_2, as well as wind, ionizing radiation, or pollutants (Schulze et al. 2002). The effect each abiotic factor has on the plant depends on its quantity or intensity. For optimal growth, the plant requires a certain quantity of each abiotic environmental factor. Any deviation from such optimal external conditions, that is, an excess or deficit in the chemical or physical environment, is regarded as abiotic stress and adversely affects plant growth, development, and/or productivity (Bray et al. 2000). Abiotic stress factors include, for example, extreme temperatures (heat, cold, and freezing), too high or too low irradiation, water logging, drought, inadequate mineral nutrients in the soil, and excessive soil salinity. As especially drought and salt stress are becoming more and more serious threats to agriculture and the natural status of the environment, this chapter will focus on these stress factors. They are recurring features of nearly all the world's climatic regions since various critical environmental threats with global implications have linkages to water crises (Gleick 1994, 1998, 2000). These threats are collaterally catalyzed by global climate change and population growth.

The latest scientific data confirm that the earth's climate is rapidly changing. Due to rising concentrations of CO_2 and other atmospheric trace gases, global temperatures have increased by about 1°C over the course of the last century, and will likely rise even more rapidly in coming decades (IPCC 2007). Scientists predict that temperatures could rise by another 3–9°C by the end of the century with far-reaching effects. Increased drought and salinization of arable land are expected to have devastating global effects (Wang et al. 2003b). Abiotic stress is already the primary reason of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Bray et al. 2000; Wang et al. 2003b). It will soon become even more severe as desertification will further increase and the current amount of annual loss of arable area may double by the end of the century because of global warming (Evans 2005; Vinocur and Altman 2005). Simultaneously, rapid population growth increasingly generates pressure on existing cultivated land and other resources (Ericson et al. 1999). Population migration to those arid and semiarid areas increases the problems of water shortage and worsens the situation of land degradation in the destination, and in turn causes severe problems of poverty, social instability, and population health threats (Moench 2002). Water scarcity and desertification could critically undermine efforts for sustainable development, introducing new threats to human health, ecosystems, and national economies of various countries. Therefore, solutions to these problems are desperately needed, such as the improvement of salt and drought tolerance of crops, which in turn

requires a detailed knowledge about salt and drought tolerance mechanisms in plants.

The viability of plants in both dry and saline habitats depends on their ability to cope with (I) water deficit due to a low water potential of the soil and (II) restriction of CO_2 uptake. Plants growing on saline soils are additionally confronted with (III) ion toxicity and nutrient imbalance.

Water deficit (I) causes detrimental changes in cellular components because the biologically active conformation and thus the correct functioning of proteins and biomembranes depends on an intact hydration shell. As a consequence, severe osmotic stress can lead to an impairment of amino acid synthesis, protein metabolism, the dark reaction of photosynthesis or respiration and can cause the breakdown of the osmotic system of the cell (Larcher 2001; Schulze et al. 2002). Water deficit can be counteracted by compatible solutes, organic compounds which are highly soluble and do not interfere with cellular metabolism. They serve as a means for osmotic adjustment and also function as chaperons by attaching to proteins and membranes, thus preventing their denaturation. This protective function of compatible solutes can also alleviate ion specific effects of salt stress caused by ion toxicity and ion imbalance such as the precipitation of proteins due to changes in charge or the destruction of membranes caused by alterations of the membrane potential.

Regarding the restriction of CO_2 uptake (II), the negative effects of osmotic stress described earlier force plants to minimize water loss; growth depends on the ability to find the best tradeoff between a low transpiration and a high net photosynthetic rate (Koyro 2006). However, various plant species show a clearly reduced assimilation rate under osmotic stress conditions due to stomatal closure (Huchzermeyer and Koyro 2005). A consequence can be an excessive production of reactive oxygen species (ROS) which are highly destructive to lipids, nucleic acids, and proteins (Kant et al. 2006; Türkan and Demiral 2009; Geissler et al. 2010). However, generated ROS can be scavenged by the antioxidative system which includes nonenzymatic antioxidants and antioxidative enzymes (Blokhina et al. 2003).

Ion toxicity (III) on saline habitats is caused by ion specific effects on membranes and proteins: On the one hand, changes of the ionic milieu lead to alterations of the membrane potential and thus to a destruction of biomembranes (Schulze et al. 2002). On the other hand, the hydration and charge of proteins are negatively influenced, so that their precipitation is promoted, but their activity is reduced (Kreeb 1996). These effects of salt stress can be alleviated by the protective chaperone function of compatible solutes, similarly as explained above for osmotic stress.

When looking at drought and salt tolerance of plants in the face of global climate change, another important aspect should be considered: Compared to salinity and drought, elevated atmospheric CO_2 concentrations have contrary effects on plants: They often improve photosynthesis while reducing stomatal resistance in C_3 plants, thus increasing water use efficiency, but decreasing photorespiration and oxidative stress (Urban 2003; Kirschbaum 2004; Rogers et al. 2004). Furthermore, more energy can be provided for energy-dependent tolerance mechanisms such as the synthesis of compatible solutes and antioxidants. Therefore, the salt and drought tolerance and the productivity of these plants can be enhanced under elevated CO_2 (Ball and Munns 1992; Wullschleger et al. 2002; Urban 2003), increasing their future suitability as crops. Against the background described earlier, this review uncovers how compatible solutes and antioxidants alleviate environmental stress, especially drought and salt stress, and the role elevated CO_2 concentrations can play in this context.

2 Compatible Solutes Which Can Prevent Detrimental Changes Under Environmental Stress

Severe osmotic stress can cause detrimental changes in cellular components. The best characterized biochemical response of plant cells to osmotic stress is the accumulation of high concentrations of either organic ions or other low

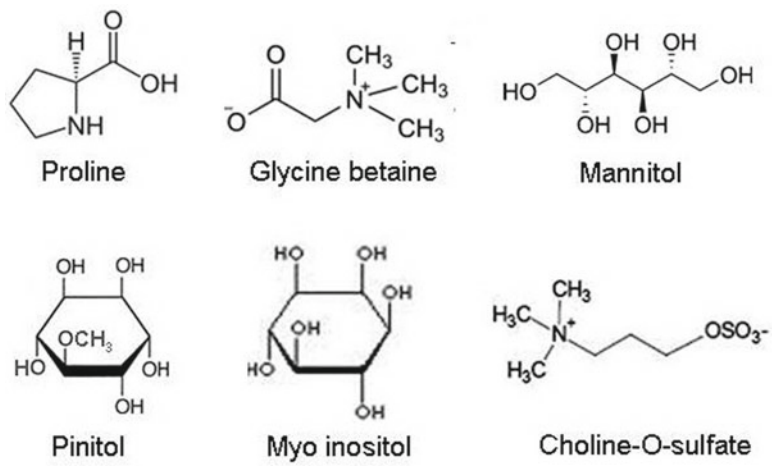

Fig. 1.1 Chemical structure of some important compatible solutes in plants

molecular weight organic solutes termed compatible solutes. These compounds are highly soluble in water, electrically neutral in the physiological pH range, and noninhibitory to enzymes even at high concentrations, so that they do not interfere with essential metabolic (enzymatic) reactions (Rhodes et al. 2002). The structure of some important compatible solutes is shown in Fig. 1.1.

Organic solutes play a crucial role in higher plants grown under dry or saline conditions. However, their relative contribution varies among species, cultivars, and even between different compartments within the same plant (Ashraf and Harris 2004). A wide range of metabolites which can prevent these detrimental changes in cellular components have been identified, including mono-, di-, oligo-, and polysaccharides (glucose, fructose, sucrose, trehalose, raffinose, and fructans), sugar alcohols (mannitol, glycerol, and methylated inositols), quaternary amino acid derivatives (Pro, GB, β-alaninebetaine and prolinebetaine), tertiary amines (1,4,5,6-tetrahydro-2-mehyl-4-carboxyl pyrimidine), and sulfonium compounds (choline-*O*-sulphate, dimethylsulphoniopropionate) (Flowers and Colmer 2008; Vinocur and Altman 2005). The primary function of compatible solutes is to reduce water potential, to maintain turgescent cells, and to ensure balanced water relations (Wang et al. 2003a). In addition, high concentration of compatible solutes exists primarily in the cytosol to balance the low water potentials achieved by high apoplasmic and vacuolar Na^+ and Cl^- concentration (Türkan and Demiral 2009). Recent studies indicate that compatible osmolytes also protect subcellular structures and mitigate oxidative damage caused by free radicals produced in response to salt stress (Slama et al. 2008; Smirnoff and Cumbes 1989). In many halophytes, organic osmolytes such as Pro or GB accumulate at suitably high concentrations to create osmotic potentials even below 0.1 MPa. In contrast to halophytes, in many glycophytes the concentrations of compatible solutes do not seem to be high enough to generate sufficiently low osmotic potentials (Türkan and Demiral 2009). This difference between halophytes and glycophytes can be used as an early indicator for salt resistance. Therefore, in the next chapters, the most important compatible solutes are described in detail.

2.1 Betaines

The quaternary ammonium compounds that function as effective compatible osmolytes in plants subject to salt stress are GB, β-alaninebetaine, prolinebetaine, choline-*O*-sulphate, hydroxyprolinebetaine, and pipecolatebetaine (Ashraf and

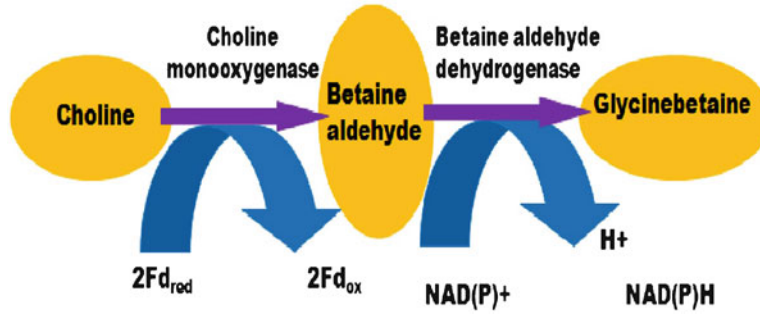

Fig. 1.2 Biosynthetic pathway of glycinebetaine (adopted from Ahmad and Sharma 2008)

Harris 2004). GB occurs most abundantly in response to a variety of abiotic stress conditions by numerous organisms including bacteria, cyanobacteria, algae, fungi, animals, and many plant families such as *Chenopodiaceae* and *Gramineae* (Türkan and Demiral 2009). This metabolite is mainly located in chloroplasts and plays a vital role in the stroma adjustment and protection of thylakoid membranes, thereby maintaining the photosynthetic activity (Jagendorf and Takabe 2001). GB protects the photosystem II (PS-II) complex at high salinity (Murata et al. 1992) and at extreme temperatures or pH (Mohanty et al. 1993). GB also protects membranes against heat-induced destabilization and enzymes, such as RUBISCO, against osmotic stress (Mäkelä et al. 2000). In higher plants, GB is synthesized from serine via ethanolamine, choline by two-step oxidation reactions that were catalyzed by choline monooxygenase and betaine aldehyde dehydrogenase, respectively (Russell et al. 1998; Ahmad and Sharma 2008; see Fig. 1.2). The insertion of serine and glycine can be taken as an indicator for the close relationship of the photorespiration (peroxisomes) to the synthesis of GB. Besides this, recently a biosynthetic pathway of GB from glycine with the involvement of two N-methyl transferase enzymes has been reported (Waditee et al. 2005). Highly tolerant genera such as *Spartina* and *Distichlis* accumulated the highest levels of GB, moderately tolerant species intermediate levels, and sensitive species hardly any GB (Rhodes and Hanson 1993). Genetic evidence that GB improves salinity tolerance has been obtained for many important agronomical crops such as tobacco, tomato, potato, barley, maize, and rice. These plants have long been a potential target for engineering GB biosynthesis pathway and thus for resistance against different environmental stress conditions (Sairam and Tyagi 2004; Türkan and Demiral 2009). The importance of N-methyltransferase for stress tolerance could also be shown for *Arabidopsis*. Genetically modified plants of this genus accumulated betaine to significant levels at different environmental stress conditions and hence improved seed yield (Waditee et al. 2005). A moderate stress tolerance was noted in some transgenic lines based on relative shoot growth in response to salinity, drought, and freezing. Huang et al. (2000) reported metabolic limitation in betaine production in transgenic plants. In fact, *Arabidopsis thaliana*, *Brassica napus,* and *Nicotiana tabacum* were transformed with bacterial choline oxidase cDNA, and their levels of GB were only between 5 and 10% of the levels found in natural betaine producers.

Beyond this, choline-fed transgenic plants synthesized substantially more GB. This result was taken as a hint that these plants require a distinct endogenous amount of choline to synthesize an adequate amount of GB (Sairam and Tyagi 2004).

The protective effect of GB at salinity or drought could also be demonstrated by exogenous application at rice seedlings, soybean, and common beans (Ashraf and Foolad 2007; Demiral and Türkan 2006). GB pretreatment also alleviated salinity-induced peroxidation (oxidative damage) of lipid membranes of rice cultivars. Besides rice,

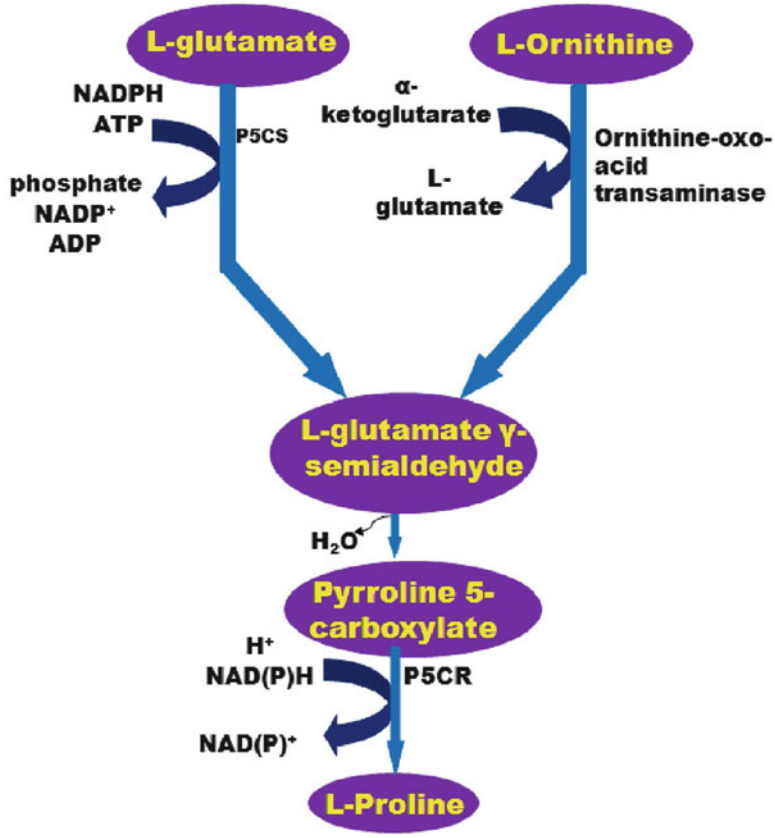

Fig. 1.3 Biosynthetic pathway of proline (adopted from Ahmad and Sharma 2008)

the correlation between the protective effect of GB and the antioxidative defense system has been observed in chilling-stressed tomato (Park et al. 2006), drought- or salt-stressed wheat (Raza et al. 2007), and salt-stressed suspension cultured tobacco BY2 cells (Hoque et al. 2007).

2.2 Amino Acids, Proline, and Amides

It has been reported that amino acids (such as alanine, arginine, glycine, serine, leucine, and valine, the nonprotein amino acids citrulline and ornithine (Orn)), together with the imino acid Pro, and the amides such as glutamine and asparagine are accumulated in higher plants under salinity and drought stress (Dubey 1997; Mansour 2000). Pro is known to occur widely in higher plants and can be accumulated in considerable amounts in response to salt stress, water deficit, and other abiotic stresses (Ali et al. 1999; Kavi Kishore et al. 2005; Koca et al. 2007; Ahmad and Sharma 2008). The Pro concentration is metabolically controlled. This imino acid is synthesized in plastids and cytoplasm while degraded to L-glutamate (Glu) in mitochondria. There are two different precursors of Pro in plants: Glu and Orn (Fig. 1.3). Pro is synthesized from Glu via glutamic-γ-semialdehyde (GSA) and Δ^1-pyrroline-5-carboxylate (P5C). P5C synthase (P5CS) catalyses the conversion of Glu to P5C, followed by P5C reductase (P5CR), which reduces P5C to Pro (Ashraf and Foolad 2007). The other precursor for Pro biosynthesis is Orn, which is transaminated to P5C by a mitochondrial Orn-γ-aminotransferase (OAT) enzyme (Verbruggen and Hermans 2008). In the reverse reaction, Pro is metabolized to Glu in a feedback manner, via P5C and GSA with the aid of Pro

dehydrogenase followed by P5C dehydrogenase (P5CDH) (Wang et al. 2003a).

The contribution of Glu and Orn pathways to stress-induced Pro synthesis differs between species, and it has been shown that stress-tolerant plants are able to accumulate Pro in higher concentrations than stress-sensitive plants. Slama et al. (2008) showed a positive correlation between Pro accumulation and tolerance to salt, drought, and the combined effects of these stresses. Osmotic stress (particularly mannitol stress) led to a considerable increase of the Pro concentration in the obligatory halophyte *Sesuvium portulacastrum*, while the contents in soluble sugars and in Na^+ remained unchanged. In drought-stressed plants, the concentration of K^+, Na^+, Cl^-, and Pro, as well as ornithine-δ-aminotransferase (δ-OAT) activity increased significantly. Inversely, Pro dehydrogenase activity was impaired. Re-watering leads to a recovery of these parameters at values close to those of plants permanently irrigated with 100% of field capacity. The presence of NaCl and mannitol in the culture medium (ionic and osmotic stress) led to a significant increase of the Na^+ and Pro concentration in the leaves, but it had no effect on leaf soluble sugar content. Slama et al. (2007a, b) assumed that the ability of NaCl to improve plant performance under mannitol-induced water stress is caused by an improved osmotic adjustment through Na^+ and Pro accumulation, which is coupled with the maintenance of the photosynthetic activity. Similarly, the Pro concentration in the roots of salt tolerant alfalfa plants rapidly doubled under salt stress and was significantly higher than in salt sensitive genotypes (Petrusa and Winicov 1997). In addition to its role as an osmolyte for osmotic adjustment, Pro contributes to stabilizing subcellular structures (membranes and proteins) by forming clusters with water molecules which attach to proteins and membranes and prevent their denaturation (Koca et al. 2007; Ashraf and Foolad 2007; Lee et al. 2008). Due to its protective function on membranes it can also improve cell water status and ion homeostasis (Gadallah 1999; Gleeson et al. 2005), and it can scavenge free radicals and buffer cellular redox potential under stress conditions (Koca et al. 2007; Ashraf and Foolad 2007; Lee et al. 2008). Pro is also involved in alleviation of cytoplasmic acidosis and sustaining $NADP^+$/NADPH ratios at required levels for metabolism (Hare and Cress 1997), thus supporting redox cycling (Babiychuk et al. 1995).

Transgenic approaches proved an enhancement of plant stress tolerance via overproduction of Pro. For instance, transgenic tobacco (*N. tabacum*), overexpressing the *p5cs* gene that encodes P5CS, produced 10- to 18-fold more Pro and exhibited better tolerance under salt stress (Kavi Kishor et al. 2005). In *Arabidopsis*, the overexpression of an antisense Pro dehydrogenase cDNA resulted in an increased accumulation of Pro and a constitutive tolerance to freezing and a higher salt tolerance (Nanjo et al. 2003). Similarly, Borsani et al. (2005) reported that the *Arabidopsis* P5CDH (Δ^1-pyrroline-5-carboxylate dehydrogenase) and SRO5, an overlapping gene of unknown function in the antisense orientation, produced two types of siRNAs, 24-nt siRNA and 21-nt siRNA. In fact, they compared the levels of salt stress-induced Pro accumulation in various mutant plants (dcl2, sgs3, rdr6, and nrpd1a) which lacked SRO5-P5CDH nat-siRNAs and cleavage of the P5CDH transcript, Pro accumulation was not significantly induced by salt stress or was induced to a lesser extent than in the corresponding wild type. This result is consistent with their inability to downregulate P5CDH under stress, thereby causing a continued Pro catabolism and reduced Pro accumulation. In contrast, the dcl1 and rdr2 mutants, which were able to degrade P5CDH mRNA, had the same Pro level as the wild type under salt stress. The wild-type level of Pro accumulation in dcl1 indicates that although the 21-nt P5CDH nat-siRNAs were not produced, the 24-nt SRO5-P5CDH nat-siRNA alone was sufficient to cause the downregulation of P5CDH (Fig. 1.4).

An alternative approach to improve plant stress tolerance is the exogenous application of Pro, which can lead to either osmoprotection or cryoprotection. For example, in various plant species growing under salt stress, among them the halophyte *Allenrolfea occidentalis*, exogenous application of Pro led to a higher osmoprotection and an increased growth (Yancey 1994).

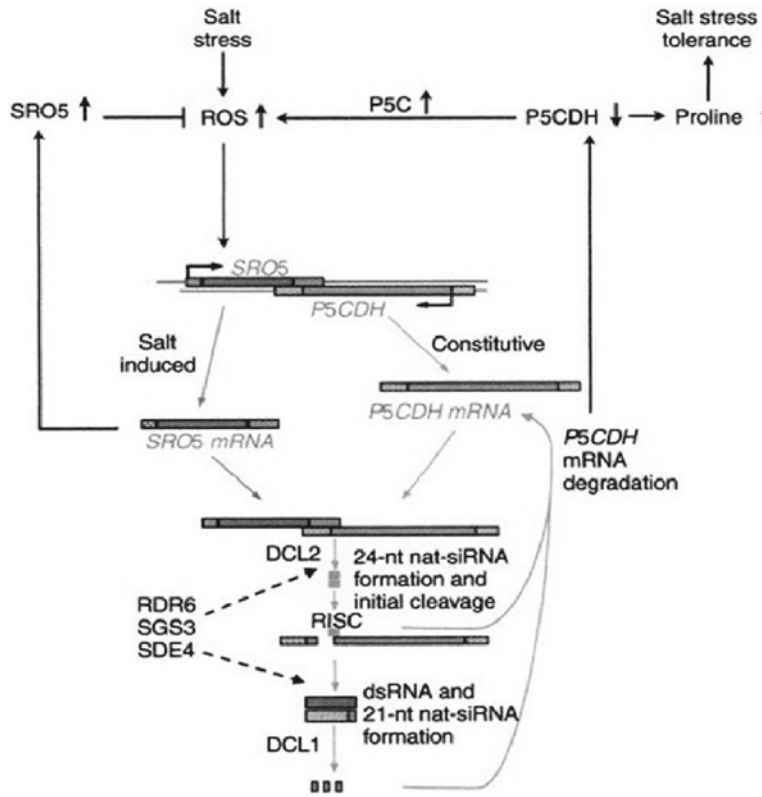

Fig. 1.4 Diagram of phased processing of SRO5-P5CDH nat-siRNAs and its role in a salt-stress regulatory loop (Borsani et al. 2005)

2.3 Sugars and Sugar Alcohols

Several studies have been attempted to relate the magnitude of changes in soluble carbohydrates to salinity tolerance. Parida and Das (2005) found out that carbohydrates such as sugars (glucose, fructose, sucrose, and fructans) and starch are accumulated under salt stress. Furthermore, Megdiche et al. (2007) and Geissler et al. (2009a) proved that *Cakile maritima* and *Aster tripolium* plants accumulate high amounts of total soluble carbohydrates and Pro at high salinity (400 and 500 mM NaCl, respectively). The major functions of sugars and sugar alcohols are osmoprotection, osmotic adjustment, carbon storage, and radical scavenging (Adams et al. 2005; Ashraf et al. 2006; Messedi et al. 2006; Lee et al. 2008; Ahmad and Sharma 2008). Furthermore, there is a discussion about that they serve as molecular chaperones (Hasegawa et al. 2000; Liu et al. 2006).

There is a difference between starch and sugar accumulation in short- and long-term reaction (da Silva and Arrabaca 2004). In short-term water stress experiments, a decrease in sucrose and starch content was observed for *Setaria sphacelata*, a naturally adapted C_4 grass while in long-term experiments, a higher amount of soluble sugars and a lower amount of starch were found. da Silva and Arrabaca (2004) assumed that the shift of metabolism towards sucrose might occur because starch synthesis and degradation are more affected than sucrose synthesis.

Trehalose, a rare, nonreducing sugar, is present in several bacteria and fungi and in some desiccation-tolerant higher plants (Vinocur and Altman 2005). Under various abiotic stresses, the disaccharide trehalose accumulates in many organisms as an osmolyte and osmoprotectant, protects membranes and proteins in cells, and reduces the aggregation of denatured proteins

(Ashraf and Harris 2004). In the transgenic plants, a comparatively moderate increase in trehalose levels lead to a higher photosynthetic rate and to a decrease in photooxidative damage during stress. Trehalose is thought to protect biological molecules from environmental stress (such as desiccation-induced damage), as suggested by its reversible water-absorption capacity (Penna 2003). It was shown that the contents of reducing and nonreducing sugars and the activity of sucrose phosphate synthase increase under salt stress, whereas starch phosphorylase activity decreases (Dubey and Singh 1999).

In general, the sugar alcohols are divided in acyclic (e.g., mannitol) and cyclic (e.g., pinitol) polyols. Polyols can make up a considerable percentage of all assimilated CO_2 and can have several functions such as compatible solutes, low molecular weight chaperones, and scavengers of stress-induced oxygen radicals (Bohnert et al. 1995). Polyols act in two indistinguishable ways, namely, osmotic adjustment and osmoprotection (Parida and Das 2005). In osmotic adjustment they act as osmolytes, facilitating the retention of water in the cytoplasm and enabling the sequestration of sodium into the vacuole or apoplast (cell wall). These osmolytes protect cellular structures by interacting with membranes, protein complexes, or enzymes. For instance, mannitol, a sugar alcohol that accumulates upon salt and water stress can alleviate abiotic stress. Transgenic wheat expressing the mannitol-1-phosphatase dehydrogenase gene (mtlD) of *Escherichia coli* was significantly more tolerant to water and salt stress (Abebe et al. 2003). Consequently, the transgenic wheat plants showed an increase in biomass, plant height, and number of secondary stems (tillers). The cyclic sugar alcohols pinitol and ononitol were accumulated in tolerant species such as the facultative halophyte *Mesembryanthemum crystallinum* when exposed to salinity or water deficit (Bohnert and Jensen 1996). Pinitol can be synthesized from myoinositol by the sequential catalysis of inositol methyl transferase and ononitol epimerase. An inositol methyl transferase (Imt) cDNA was isolated from transcripts in *M. crystallinum* growing under saline conditions (Vernon and Bohnert 1992), and transgenic tobacco for Imt has been obtained (Vernon et al. 1993).

2.4 Polyamines

Under stressful conditions, different plant species respond differently towards levels of polyamines. Some might accumulate polyamines in response to stress, while others do not increase or even decrease their endogenous polyamine contents when exposed to harsh environments. It is proposed that PA play a defensive role during biotic stress responses (Walters 2003a, b). One of the examples is the hypersensitive response (HR) which consists of rapid cell death at the sight of pathogen entry, typically develops in the interaction between tobacco mosaic virus (TMV) and *N* resistance gene carrying *N. tabacum* and leads to enhanced polyamine synthesis and accumulation (Kusano et al. 2008). It is also believed that stress-induced polyamines tend to modulate the activity of a certain set of ion channels to adapt ionic fluxes in response to environmental changes. Many more examples of responses to biotic stress have been quoted by Kusano et al. (2008).

Various abiotic stress conditions have been reported to alter the concentration of polyamines (Bouchereau et al. 1999; Walters 2003a). Exogenous polyamine application and/or inhibitors of enzymes involved in polyamine biosynthesis pointed out a possible role of these compounds in plant adaptation/defense to several environmental stresses (Bouchereau et al. 1999; Alcázar et al. 2006; Groppa and Benavides 2008; Alcázar et al. 2010). More recent studies using either transgenic overexpression or loss-of-function mutants support this protective/adaptive/defensive role of PAs in plant response to abiotic stress (Alcázar et al. 2006; Kusano et al. 2008; Gill and Tuteja 2010). For example, *Arabidopsis* plants overexpressing *Cucurbita ficifolia* Spd synthase gene were tolerant of multistresses (chilling, freezing, salinity, drought, and paraquat toxicity) (Kusano et al. 2007; Tassoni et al. 2010). According to Rhee et al. (2007), the basic principle underlying polyamine adaptive responses appears to be shared by the prokaryotic stringent response and the eukaryotic unfolded protein response (UPR). UPR is triggered when unfolded proteins and uncharged tRNAs accumulate in the endoplasmic reticulum (ER) due to ER stress or nutrient starvation.

As a result of this, cap-dependent translation of many mRNAs is suppressed and the expression of a certain set of genes including the luminal binding protein gene *BiP* is induced. The underlying mechanisms of UPR in yeasts and mammals have been well researched (Rutkowski and Kaufman 2004), although those in plants have not (Kamauchi et al. 2005; Urade 2007; Kusano et al. 2008). Recently, nitric oxide (NO), an endogenous signaling molecule in plants and animals, has gained considerable importance in the PA studies. It is known to mediate responses to biotic and abiotic stresses. It has been reported by Tun et al. (2006) that spermine and spermidine are potent inducers of NO in *Arabidopsis*, but putrescine and its biosynthetic precursor arginine are not. There are many more examples of NO affecting the concentrations of PAs and over the past few years studies on polyamines and NO are gaining attention (Kusano et al. 2008).

3 Oxidative Stress and Antioxidative Responses to Environmental Stress

3.1 Production of ROS

Environmental stresses are responsible for the production of ROS. The production and removal of ROS is thought to be at equilibrium under normal conditions, whereas environmental stress disturbs this equilibrium by enhancing the production of ROS. ROS are very toxic for the organism as they affect the structure and function of the biomolecules. The main source of ROS production in plants is chloroplasts, mitochondria, and peroxisomes (Fig. 1.5).

Mitochondria are responsible for the generation of oxygen radicals and hydrogen peroxide due to the overreduction of the electron transport chain.

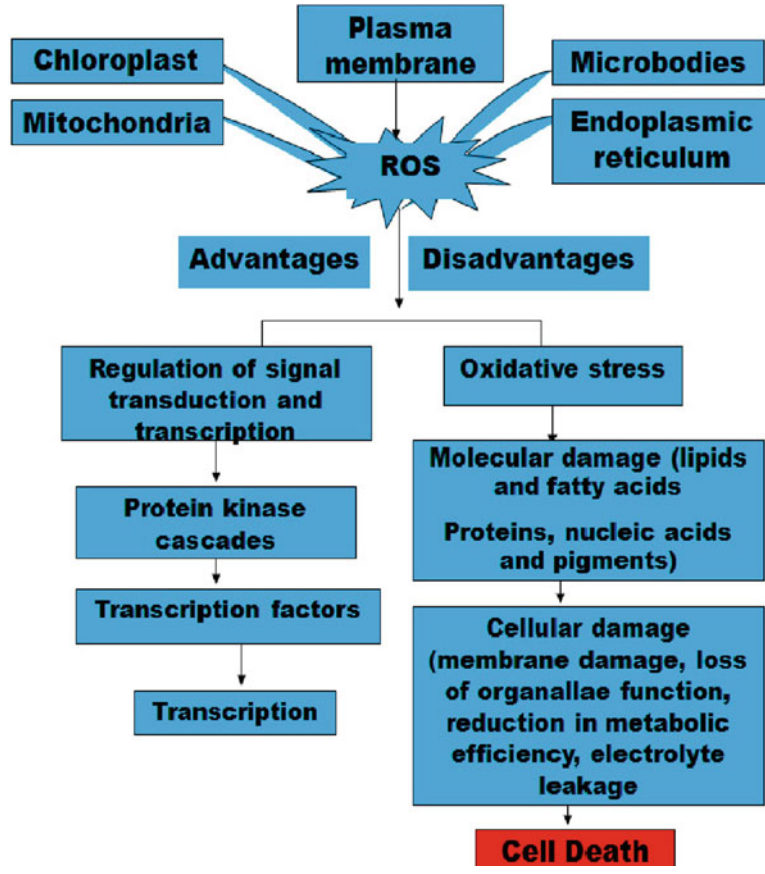

Fig. 1.5 Sites of reactive oxygen species (ROS) and the biological consequences leading to a variety of physiological dysfunctions that can lead to cell death (adopted from Ahmad et al. 2008)

Chloroplasts are found to be the major producer of O_2 and H_2O_2 (Davletova et al. 2005). This is because the oxygen pressure in the chloroplast is higher than in other organelles. Photoreduction of O_2 to $O_2^{\bullet-}$ during the photosynthetic electron transport takes place and is called Mehler reaction. The production of superoxides is due to the reduction of molecular oxygen in the plastoquinone pool. This reduction may be due to the plastosemiquinone, by ferredoxin (Fd) or by sulfur redox centers in the electron transport chain within PSI (Dat et al. 2000). These superoxides are converted to hydrogen peroxide either spontaneously or by the action of the enzyme SOD. Hydrogen peroxide is also responsible for the production of hydroxyl radicals ($OH^{\bullet}$).

The major producer of H_2O_2 in plant cells are peroxisomes. It has been reported that peroxisomes are also responsible for the production of superoxides (O_2^-). In peroxisomes, the production of O_2^- occurs in the peroxisomal matrix and the peroxisomal membrane. In the peroxisomal matrix, the oxidation of xanthine and hypoxanthine to uric acid in the presence of the enzyme xanthine oxidase generates O_2^- radicals (Halliwell and Gutteridge 2000). Peroxisomes have got two pathways for the production of H_2O_2. One is the disproportionation of O_2^- generated in this organelle and the other is a direct pathway. During photorespiration glycolate is catalyzed by glycolate oxidase, yielding H_2O_2. Fatty acid β-oxidation, the enzymatic reaction of flavin oxidases, can also produce H_2O_2 (Baker and Graham 2002; del Rio et al. 2002).

ROS include $^1O_2$, $O_2^{\bullet-}$, $H_2O^{\bullet}$, H_2O_2, $OH^{\bullet}$, $RO^{\bullet}$ organic hydroperoxide (ROOH), excited carbonyl ($RO^{\bullet}$), etc. They cause damage to biomolecules like proteins, lipids, carbohydrates, and DNA, which ultimately results in cell death (Foyer and Noctor 2005). Fortunately, plants are equipped with an antioxidant machinery that scavenges the ROS and helps the plant to tolerate the stress conditions. The antioxidants include enzymatic antioxidants, viz., superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), etc., and nonenzymatic antioxidants like ascorbic acid (AsA), vitamin E (α-tocopherol), reduced glutathione (GSH), etc.

3.2 Enzymatic Antioxidants

3.2.1 Superoxide Dismutase

SOD is one of the ubiquitous enzymes in aerobic organisms and plays a key role in cellular defense mechanisms against ROS. Within a cell, the SODs constitute the first line of defense against ROS. Its activity modulates the reactive amounts of $O_2^{\bullet-}$ and H_2O_2, the two Haber–Weiss reaction substrates, and decreases the risk of OH radical formation, which is highly reactive and may cause severe damage to membranes, proteins, and DNA (reviewed by Ahmad et al. 2010b). SOD was for the first time reported by Cannon et al. (1987) in maize and it catalyzes the dismutation of superoxide into hydrogen peroxide and molecular oxygen.

$$2H^+ + 2^{\bullet}O_2^- \rightarrow 2H_2O_2 + O_2$$

Different types of SOD isoforms have been observed in plants on the basis of metal cofactors attached to the active site. The isozyme containing Mn II at its active site is known as Mn-SOD. Similarly, the isozyme the active site of which contains Cu II and Zn II is known as Cu/Zn-SOD. The third isozyme contains Fe III and is referred to as Fe-SOD. The fourth SOD isoform contains Ni at the active site, is called Ni-SOD and is found in several *Streptomyces* species (Youn et al. 1996) and cyanobacteria (Palenik et al. 2003). Ni-SOD has not been reported in plants yet. Whereas only one type of SOD is found in most organisms, plants have multiple form of each type, which are encoded by more than one gene, indicating that plants have more complex antioxidant defense systems than other organisms.

Several studies have reported enhanced stress tolerance related to overproduction of chloroplastic SOD (Pastori and Foyer 2002). In maize leaves, GR and DHAR were exclusively localized in mesophyll cells whereas most of the SOD and APX were localized in mesophyll and bundle sheath cells. Increased SOD activity was reported in *Radix astragali* under water deficit stress, which varied in three different genotypes (Tan et al. 2006). Chilling stress has a significant effect in the enhancement of SOD activity in cucumber

seedlings (Feng et al. 2003). The increase in SOD activity under drought stress was about 25% in soybean plants (Zhang et al. 2007). SOD activity was doubled in water stressed citrus plants when compared to well-watered control plants during seedling stage (Wu et al. 2006).

SOD activity increased under drought stress in *Euphorbia esula* (Davis and Swanson 2001), maize (Pastori et al. 2000), *Cassia angustifolia* (Agarwal and Pandey 2003), wheat (Singh and Usha 2003), rice (Wang et al. 2005), *P. acutifolius* (Turkan et al. 2005), and *Camellia sinensis* (Chen et al. 2011), and the SOD activity was higher under salinity stress in *C. roseus* (Jaleel et al. 2008) and *Morus alba* (Ahmad et al. 2010a). While subjecting higher plants to water deficit stress SOD activity increases (Reddy et al. 2004). Koca et al. (2007) have shown that elevated SOD activity is accompanied with an increase in the activity of major H_2O_2 scavenging enzymes like APX, CAT, and POX in salt tolerant sesame cultivar *Cumhuriyat* as compared to cultivar *Orhangazi*. SOD activity increased by 1.6-fold in a salt tolerant mutant of *Chrysanthemum* compared to a non-tolerant one under NaCl stress (Hossain et al. 2006). An increased activity of SOD enzyme has also been reported under different abiotic stresses in *Catharanthus roseus* (Jaleel et al. 2007), *Pisum sativum* (Ahmad et al. 2008), *M. alba* (Ahmad et al. 2010a), and *Brassica juncea* (Ahmad 2010; Ahmad et al. 2011). SOD activity has also been observed to increase by the application of heavy metals such as cadmium (Shah et al. 2001; John et al. 2009; Ahmad et al. 2011), lead (Verma and Dubey 2003; John et al. 2009), and copper (Lombardi and Sebastiani 2005). Canola overexpressing Mn-SOD confers tolerance to aluminum stress (Basu et al. 2001). Overexpression of Mn-SOD in transgenic *Arabidopsis* showed a twofold increase in Mn-SOD activity and higher tolerance to salt as compared to nontransgenic plants (Wang et al. 2004). Tanaka et al. (1999) demonstrated that expression of yeast mitochondrial Mn-SOD in rice chloroplasts led to a 1.7-fold increase in Mn-SOD as compared to nontransgenic plants. Transgenic *Arabidopsis* with Mn-SOD confers tolerance to heat (Im et al. 2009). Wang et al. (2005) demonstrated that transgenic rice plants expressing Mn-SOD have shown reduced injury and sustained photosynthesis under PEG stress. Overexpression of Cu/Zn-SOD and APX in transgenic tobacco enhanced seed longevity and germination rates after various environmental stresses (Lee et al. 2010). Transgenic tobacco expressing Cu/Zn-SOD have been shown to tolerate chilling and heat stress (Gupta et al. 1993) and enhanced tolerance to salt, water, and PEG stress (Badawi et al. 2004). Prashanth et al. (2008) have also demonstrated that Cu/Zn-SOD confers tolerance to salinity in rice plants.

3.2.2 Catalase

Plant catalases are tetrameric iron porphyrins and play a role in stress tolerance against oxidative stress. Catalases are produced in peroxisomes and glyoxysomes. Catalases are involved in eliminating hydrogen peroxide generated by different environmental stresses (Kim et al. 2008; Ahmad et al. 2010b). Catalases decompose hydrogen peroxide to water and molecular oxygen without consuming reductants and may thus provide plant cells with an energy efficient mechanism to remove hydrogen peroxide (reviewed by Ahmad et al. 2010b). The enzyme is abundant in the glyoxysomes of lipid-storing tissues in germinating barley, where it decomposes H_2O_2 formed during the β-oxidation of fatty acids (Jiang and Zhang 2002) and in the peroxisomes of the leaves of C_3 plants, where it removes H_2O_2 produced during photorespiration by the conversion of glycolate into glyoxylate (Kiani et al. 2008). This is also due to the fact that there is a proliferation of peroxisomes during stress, which might help in scavenging H_2O_2, which can diffuse from the cytosol (Lopez-Huertas et al. 2000; Kusaka et al. 2005).

High temperatures affect the structure of most proteins and thus the activity of many enzymes. Hertwig et al. (1992) have demonstrated that the translation of catalase was hampered at 40°C. Anderson (2002) showed that high temperature is responsible for the decrease in catalase activity in pepper plants. In comparison, the desert plant *Retama raetam* exposed to heat shock temperature showed only a minor inactivation of catalase activity (Streb et al. 1997). Scandalios et al.

(2000) have also observed a reduced catalase activity in maize on exposure to temperatures of 35–40°C.

Sublethal doses of NaCl induce catalase activity in *Nicotiana plumbaginifolia* through activation of *cat2* and *cat3* genes (Savoure et al. 1999). However, catalase activity was found to decrease due to the salt stress because of accumulation of salicylic acid (Shim et al. 2003). Vaidyanathan et al. (2003) have demonstrated that salt tolerant rice cultivars contain higher levels of catalase activity compared to susceptible cultivars. Increase in catalase activity during salt stress has also been shown by other workers in maize (Azevedo-Neto et al. 2006; Arora et al. 2008), in sesame (Koca et al. 2007), and in mulberry (Ahmad et al. 2010a).

Catalase activity has also been found to decrease in presence of heavy metal stress (Mishra et al. 2006; Khan et al. 2007; Mobin and Khan 2007; Ahmad et al. 2011). Verma and Dubey (2003) also demonstrated that the activity of catalase declines in rice plants with increasing concentration of Pb. John et al. (2009) also reported that an increase in Cd and Pb concentrations decreases the catalase activity in mustard. Decrease in catalase may be due to the inhibition of enzyme synthesis or change in assembly of enzyme subunits (Shah et al. 2001).

3.2.3 Ascorbate Peroxidase

APX is an important antioxidant enzyme mainly found in higher plants and algae (Raven 2003). APX helps to detoxify H_2O_2 in the ascorbate-glutathione (= Halliwell-Asada) pathway. APX utilizes ascorbic acid and reduces H_2O_2 to water and monodehydroascorbate (MDA).

$$2\text{AsA} + \text{H}_2\text{O}_2 \rightarrow 2\text{MDA} + 2\text{H}_2\text{O}$$

APX was first isolated from chloroplasts and algae (Shigeoka et al. 1980; Nakano and Asada 1981). Different isoforms of APX which include thylakoid (tAPX), glyoxisomal (gmAPX), stromal (sAPX), and cytosolic (cAPX) have been reported (Shigeoka et al. 2002; Mittler et al. 2004). In comparison to other antioxidants, APX and guaiacol peroxidase (GPX) have a high affinity towards H_2O_2 (Mittler and Poulos 2005). APX isozymes have been found to be most stress responsive among the APX gene family during environmental stress (Mittler and Poulos 2005). APX1 has been found to enhance in response to environmental stress (Mittler 2002; Shigeoka et al. 2002). APX2 is expressed under stressful conditions and its expression is elevated in response to light stress or heat shock (Mullineaux and Karpinski 2002; Panchuk et al. 2002). Cytosolic APX1 has been found to protect *Arabidopsis* plants from a combination of stresses (Koussevitzky et al. 2008). Lu et al. (2007) demonstrated that cAPX improves salt tolerance in transgenic *Arabidopsis*.

Mittler et al. (1999) have demonstrated that suppression of APX1 in tobacco leads to a higher sensitivity of the plant to pathogen attacks. Overexpression of APX1 resulted in enhanced tolerance to oxidative stress in tobacco (Yabuta et al. 2002). Biologists have demonstrated the importance of APX1 by using APX1 knockout mutants. The plants lacking APX1 have showed delayed growth, no response of guard cells towards light, and light stress resulted in an induction of catalase and heat shock proteins (Pnueli et al. 2003). The accumulation of H_2O_2 is responsible for the abnormal closure of stomata in knockout APX1 plants (Pnueli et al. 2003). The induction of heat shock proteins in knockout APX1 plants may be due to an enhanced level of H_2O_2 which is considered as an essential signaling molecule during abiotic stress (Mittler 2002; Neill et al. 2002).

3.2.4 Glutathione Reductase

GR is a flavo-protein oxidoreductase and is found in both prokaryotes and eukaryotes (Romero-Puertas et al. 2006). GR is an important enzyme of the ascorbate–glutathione system and maintains the balance between reduced glutathione (GSH) and the ascorbate pool (reviewed by Ahmad et al. 2010b). Meldrum and Tarr (1935) for the first time reported GR in eukaryotes and yeast, and in 1951 it was also observed in plants (Conn and Vennesland 1951; Mapson and Goddard 1951). Later on GR has been isolated