Chapter 9
Antioxidant Defenses Against Drought Stress

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Abstract Plants possess a battery of structural, physiological, biochemical, and molecular mechanisms to withstand drought periods. During drought, stomatal limitation of photosynthesis, overreduction of the photosynthetic electron transport chain, enhanced photorespiration, and many other processes may result on enhanced formation of reactive oxygen species (ROS) and other oxidizing agents. One of the most important defense mechanisms against drought is the antioxidant system, which detoxifies prooxidants such as ROS and lipid peroxyl radicals, and keeps an adequate cellular redox balance. Antioxidants may be classified in enzymatic (e.g., ascorbate peroxidases, catalases, and superoxide dismutases) or nonenzymatic (syn. low molecular weight) antioxidants (e.g., ascorbate, glutathione, carotenoids, and tocopherols). Antioxidants may scavenge ROS directly or in co-operation with other antioxidants. This co-operation between antioxidants also allows re-cycling of oxidized antioxidants. Moreover, antioxidants are key sensors of the cellular redox status, so they trigger a number of signaling events intended to keep an adequate cellular redox balance. In this chapter, the function of the most important antioxidants in plants and the role of antioxidants in cellular redox homeostasis during drought stress will be reviewed.

Abbreviations

APx	Ascorbate peroxidase
CAT	Catalase
DHA	Dehydroascorbate
DHAR	DHA reductase
Fd_{red}	Reduced ferredoxin

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FTR	Ferredoxin-thioredoxin reductase
GRx	Glutaredoxin
GSH	Reduced glutathione
GSSG	GSH disulfide (oxidized GSH)
GST	GSH-*S*-transferase
MDHA	Monodehydroascorbate
MDHAR	MDHA reductase
NPQ	Non-photochemical quenching
NTR	NADPH-thioredoxin reductase
PRx	Peroxiredoxin
PSI/II	Photosystem I or II
ROS	Reactive oxygen species
qE	ΔpH-dependent NPQ
SOD	Superoxide dismutase
TRx	Thioredoxin

9.1 Introduction

Oxygen in aerobic organisms shows redox states between molecular oxygen (O_2) and water (H_2O). Reactive oxygen species (ROS), including $O_2^{-\cdot}$ (superoxide anion), H_2O_2 (hydrogen peroxide) and OH^- (hydroxyl radical), are partially reduced forms of oxygen that are extremely reactive and tend to completely reduce to H_2O very quickly (between milliseconds and picoseconds), thereby oxidizing lipids, proteins, sugars, nucleic acids, and other neighboring molecules. Another very reactive ROS is singlet oxygen (or $^1O_2$), the common name used foa an electronically excited state of molecular oxygen (O_2), that is usually used from light-harvesting chlorophyll molecules.

Among membrane components, poly-unsaturated fatty acids (PUFA) and proteins are the most common ROS targets. Lipid peroxides, which are products of lipid peroxidation, can in turn oxidize neighboring PUFAs, establishing a chain reaction that may lead to the dysfunction of biological membranes. Sulphur-containing amino acids, such as cysteine and methione, are also particularly prone to oxidation. The oxidation of these amino acids is sequential, from sulfhydryl to disulfide, sulfenic acid, sulfinic acid, and sulfonic acid, the first three oxidations (to disulfide, sulfenic, and sulfinic acids) being reversible. The oxidation to sulfinic acid is also reversible in some particular cases (Møller et al. 2007). After oxidation of thiol groups, protein carbonylation that occurs in lysine, arginine, proline, and threonine is the second most common protein oxidation reaction. Moreover, other aminoacids such as tryptophan and tyrosine are also common targets of ROS (Rinalducci et al. 2008; Spoel and Loake 2011). In plants, ROS additionally cause DNA base deletions, pyrimidin dimers, strand breaks, and base modifications such

Table 9.1 Main sources of ROS in mesophyll cells of drought-stressed plants

Cell compartment	Main sources of ROS	Brief description of the reaction	ROS formed
Chloroplast	PSII/PSI	Energy transfer from triplet state chlorophyll	$^1O_2$ $O_2^{-\bullet}$
	PSI (Mehler reaction)	Electron transfer to O_2 as alternative electron acceptor	
Peroxisome	Glycolate oxidase	Oxidation of glycolate from photorespiration	H_2O_2
Mitochondria	Respiratory electron transport chain	Electron transference from different complexes to O_2	$O_2^{-\bullet}$
Apoplast	NADPH oxidase	Oxidation of symplastic NADPH to generate O_2^-in the apoplast	$O_2^{-\bullet}$
	Polyamine oxidases		H_2O_2
	Class III peroxidases	Catabolism of polyamines	
		The catalytic cycle of class III peroxidases	$HOO^\bullet$, $O_2^{-\bullet}$
Ubiquitous	Fenton reaction	Generation of HO by the oxidation of transition metals	$HO^\bullet$
	Haber–Weiss cycle	Catalytic activity of transition metals in presence of O_2^- and H_2O_2	$HO^\bullet$
	SOD	Dismutation of O_2^-	H_2O_2

as alkylation and oxidation (Gill and Tuteja 2010). Moreover, products of the ROS-dependent PUFA peroxidation, such as malondialdehyde, can form adducts with DNA bases (preferentially guanine).

In addition to their deleterious effects leading to oxidative damage and destruction of several cellular components when found at high concentrations, ROS can also play an important role in cellular signaling in plant responses to environmental stresses, including drought (Møller et al. 2007). It is therefore essential that plants possess mechanisms that finely control ROS levels in various cellular compartments. The formation of ROS occurs in plants under optimum growth conditions in almost all subcellular compartments, but its production can be boosted during drought (Table 9.1). Plants contain a powerful antioxidant repertoire that is finely regulated in time and space in order to keep ROS levels under tight control.

An antioxidant is defined as a molecule that donates electrons or hydrogen atoms (i.e., has low reduction potential) to yield a radical that is either harmless or efficiently quenched by other electron donors, and the properties of which are displayed in a spatial and temporal correlation with oxidative stress (Hernández et al. 2009). Plants possess both nonenzymatic and enzymatic antioxidants. The formers donate electrons or hydrogen atoms to the oxidizing agent. The oxidized antioxidants may be converted to harmless products or they may be recycled back to the reduced antioxidant either spontaneously or through enzyme-catalyzed reactions. Enzymatic antioxidants are proteins that catalyze the scavenging of prooxidants using electrons provided by nonenzymatic antioxidants or other

electron donors such as water, NAD(P)H or ferredoxin (Fd). Also, one can distinguish between primary antioxidants, which are those that scavenge prooxidants; and secondary antioxidants, those that recycle primary antioxidants. We will discuss here the origin of oxidative stress, but also at the same time the antioxidant mechanisms operating in the different cellular compartments to avoid oxidative damage during drought stress.

9.2 Oxidative Stress and Antioxidants in Chloroplasts

Chloroplasts are quantitatively and qualitatively one of the most important sources of ROS in illuminated plant cells (Foyer and Noctor 2003). During drought stress, the stomatal closure prevents the diffusion of CO_2 to the carboxylation sites, which avoids its utilization by the enzyme RuBisCO. Under this condition, NADPH and ATP are not consumed in the Calvin cycle and can over-accumulate. If this occurs, drought results in the saturation of the photosynthetic electron transport, especially when it is combined with high light or other conditions that result on excess excitation energy in chloroplasts. At the level of the photosystem II (PSII) and some recent evidence suggests also this may also occur at the level of photosystem I (PSI, cazzaniga et al. 2012), energy can be transferred from triplet state chlorophyll (excited chlorophyll; ^{3}Chl*) directly to O_2 in its basal state (triplet; $^3O_2$) to yield $^1O_2$ (Table 9.1). At the reducing side of the photosystem I (PSI), in the so-called Mehler reaction, membrane-bound photosynthetic electron transporters such as reduced ferredoxin (Fd_{red}) can transfer one electron to O_2, generating O_2^-. This is quickly converted to H_2O_2, either spontaneously or in a reaction catalyzed by superoxide dismutases (SODs; Table 9.1). In order to cope with the enhanced formation of ROS that occurs during drought in chloroplasts plants has evolved a broad spectrum of antioxidants.

9.2.1 Carotenoids: Carotenes and Xanthophylls

Carotenoids are tetraterpenes unsaturated to a different extent, synthesized, and accumulated in plastids (Fig. 9.1). In chloroplasts, carotenoids are associated to both PSI and PSII, particularly to their respective light harvesting complexes, forming pigment-protein complexes. Carotenoids are the main factors responsible for the qE component of the nonphotochemical quenching (NPQ): the quenching of chlorophyll *a* fluorescence by processes other than photochemistry, which involves mainly energy dissipation as heat. During drought the factors limiting photosynthesis provoke the accumulation of singlet state excited chlorophylls (^{1}Chl*), increasing the probability of ^{3}Chl* formation by intersystem crossing, and subsequently that of $^1O_2$ by energy transfer to O_2. Lutein (and other carotenoids with nine or more conjugated double bounds; Fig. 9.1) is the main compound

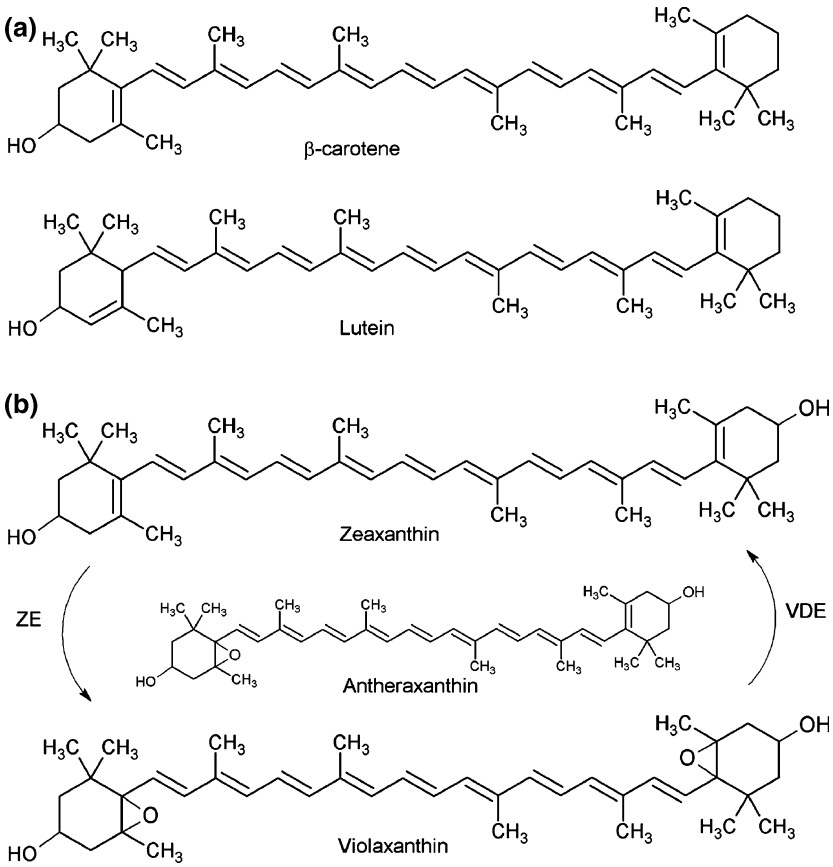

Fig. 9.1 Molecular formula of some representative nonenzymatic antioxidants in plant cells. First, some relevant carotenoids are shown (**a**). In **b**, the xanthophyll cycle is outlined: zeaxanthin is transformed into violaxanthin by the action of zeaxanthin epoxidase (*ZE*) through the intermediary antheraxanthin; the reverse reactions are catalyzed by violaxanthin de-epoxidase (*VDE*). Next, the molecular formula of tocopherols is depicted (**c**). In **d**, ascorbic acid and its oxidation products, monodehydroascorbic and dehydroascorbic acids, are shown. **e** shows the chemical formula of glutathione (γ-$_L$-glutamyl-$_L$-cysteinylglycine). Gallic acid is one of the most common hydrolysable tannins, while quercetin is also a representative flavonoid; both phenolics with high antioxidant capacity (**f**). Finally the most important polyamines, putrescine, spermine and spermidine, are shown in **g**

responsible for the quenching of ^{3}Chl* by harvesting its energy and releasing it as heat by thermal relaxation (Jahns and Holzwarth 2012), thereby preventing the formation of $^1O_2$. In addition, other carotenoids (especially the xanthophyll zeaxanthin) are able to quench ^{1}Chl*, yielding an excited singlet state carotenoid (for instance, ^{1}Zeaxanthin*). These excited singlet state carotenoid molecules return to their basal state by thermal relaxation as well. Similarly, most carotenoids, and particularly β-carotene (Fig. 9.1), quench $^1O_2$ via energy transfer and subsequent thermal relaxation. Plants have evolved a sophisticated method for the control of

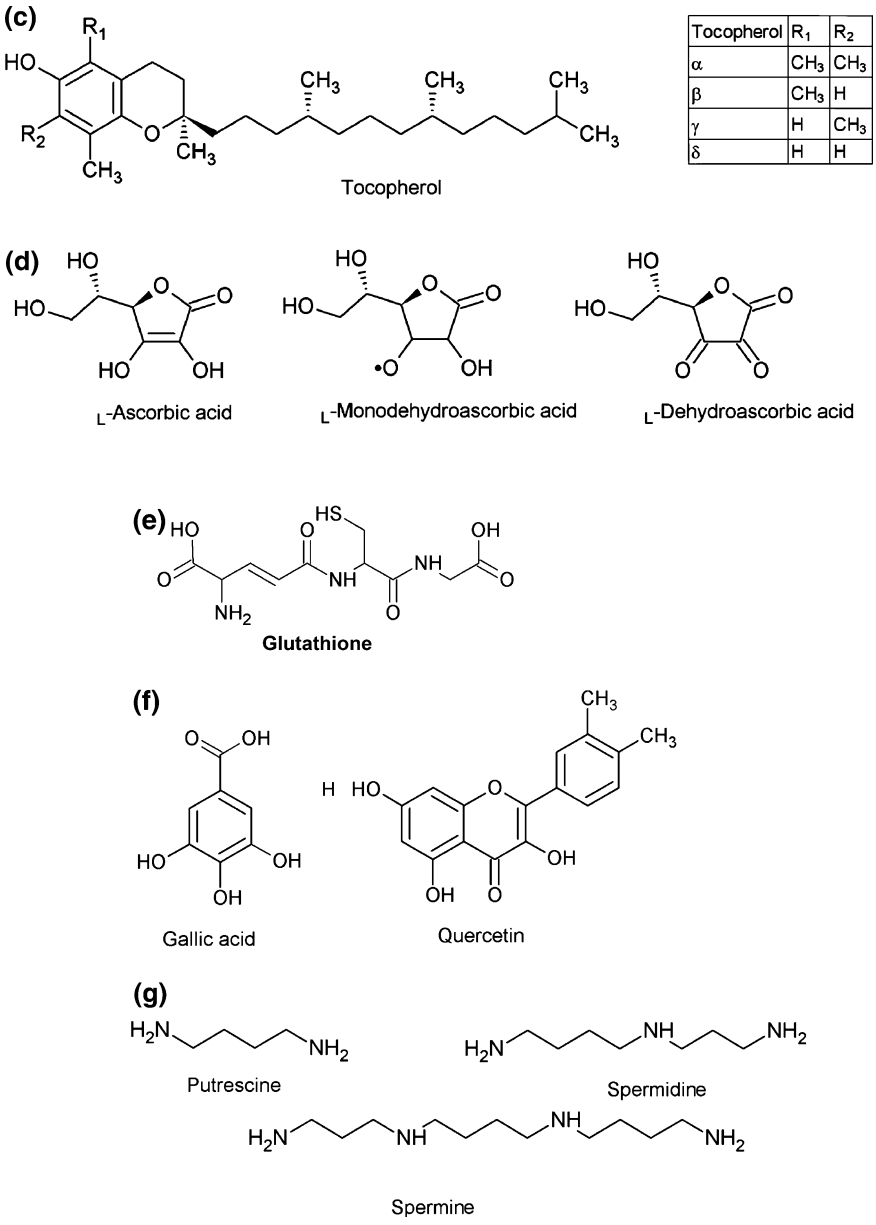

Fig. 9.1 (continued)

the qE consisting on a series of de-epoxidation and epoxidation reactions involving different xanthophylls, commonly called as the "xanthophyll cycle" (Fig. 9.1). The de-epoxidation reactions consist on the de-epoxidation of violaxanthin to zeaxanthin through an intermediary, antheraxanthin, by the action of violaxanthin

de-epoxidase (VDE) which uses electrons donated by ascorbate. The reverse reaction, the epoxidation reaction, is catalyzed by zeaxanthin epoxidase (ZE). Similar sets of reactions have been identified for lutein epoxide and lutein and for diadinoxanthin and datoxanthin as substrates for ZE and VDE, respectively, but these xanthophyll cycles are restricted to certain taxa (reviewed by Esteban et al. 2009). VDE shows highest activity when the pH in thylakoid lumen is lowest (i.e., when light is high), whereas ZE requires neutral pH in the thylakoid lumen (i.e. darkness). Violaxanthin is bound to PSII and PSI antenna complexes, and upon the activation of VDE it is replaced by zeaxanthin, which quenches ^{1}Chl* by energy transfer and subsequent thermal relaxation, preventing the formation of ^{3}Chl* and $^1O_2$ (reviewed by Demmig-Adams et al. 1996). Moreover, it has been also shown that carotenoids quench chemically, i.e. scavenge, free radicals such as $^1O_2$, O_2^- and lipid peroxyl radicals (Burton and Ingold 1984; Conn et al. 1992; Ramel et al. 2012). It has been generally shown that in drought-resistant plants, carotenoid levels increase under drought stress when expressed per chlorophyll unit, thus indicating an increased photoprotection per amount of light absorbed (Munné-Bosch and Alegre 2000). In addition, a number of *Arabidopsis* mutants, such as *npq1*, *npq4*, *lut2,* and *szl1*, have shown that carotenoids such as lutein and zeaxanthin are essential for the qE component of NPQ, so carotenoid-deficient plants are hypersensitive to photooxidative stress (Havaux and Kloppstech 2001). To our knowledge, the improvement of plant performance through engineering carotenoid levels or composition has not been reported so far. But enhanced tolerance to photooxidative stress, a condition tightly associated with drought stress, by such means have been achieved by many researchers. Plants with enhanced content in xanthophylls show enhanced resistance to photooxidative stress (Johnson et al. 2007). In addition, plants overexpressing β-carotene hydroxylase, which catalyzes the hydroxylation of β-type carotenes thus leading to the accumulation of zeaxanthin, are more tolerant to that stress factor (Davidson et al. 2002) and concomitantly, plants lacking simultaneously all β-type xanthophylls (such as zeaxanthin) are more sensitive to photooxidative stress than plants lacking only zeaxanthin or lutein (Pogson and Risler 2000). *Arabidopsis* mutants in which all leaf xanthophylls have been substituted by zeaxanthin show reduced levels of lipid peroxidation and enhanced tolerance to high light stress (Giuliano et al. 2008).

9.2.2 Tocopherols

Tocopherols (including α-, β-, γ-, and δ-tocopherol) are chloroplast synthesized and -located amphipathic molecules consisting on a hydrophobic prenyl chain and a hydrophilic chromanol ring (Fig. 9.1). The chromanol head is able to donate single electrons to various acceptors, yielding a resonance-stabilized tocopheroxyl radical. Some of the best electron acceptors of tocopherols in such reactions are lipid peroxyl radicals, which are converted into hydroperoxides, preventing the lipid peroxidation propagation in thylakoids. Tocopherols can also physically

quench (by resonance energy transfer) and chemically scavenge $^1O_2$ in PSII reaction centers, the latter resulting in the formation of tocopherol quinone or quinone epoxides. Moreover, O_2^{-}, $HOO,^{-}$ and OH^{-} can be scavenged by tocopherols in vitro, although whether these reactions occur in vivo is still unknown (Nishikimi et al. 1980; Fukuzawa and Gebicki 1983). Therefore, tocopherols can play a role in drought-stressed plants by dissipating excess excitation energy during photooxidative stress. In addition, since tocopherols modulate the levels of ROS and therefore the extent of lipid peroxidation, they also modulate the accumulation of PUFA oxidation products some of which —named oxylipins—play key signaling functions during drought and other stress factors (Sattler et al. 2006; Munné-Bosch et al. 2007; Cela et al. 2011). The most clear example of a PUFA peroxidation product involved in stress signaling, including drought, is the phytohormone jasmonic acid, which is formed by the peroxidation of linolenic acid and other tri-unsaturated fatty acids (Fonseca et al. 2009; Reinbothe et al. 2009; Munemasa et al. 2011). The levels of tocopherols, in agreement with their antioxidant function, increase in plants adapted to drought (e.g., Hernández et al. 2004; Munné-Bosch and Alegre 2003). Successful efforts to improve plant performance against drought through engineering tocopherol levels and composition have been reported in the literature. For instance, a recent study showed that tobacco plants overexpressing the *Arabidopsis VTE1* gene (tocopherol cyclase) accumulated 10–30 fold wild-type α-tocopherol levels, which led to reduced lipid peroxidation, electrolyte leakage, and H_2O_2 levels, and increased chlorophyll content, under water deficit (Liu et al. 2008).

9.2.3 Superoxide Dismutases

SODs (EC 1.15.1.1) are considered to be the first enzymatic antioxidant barrier of aerobic organisms; they are the fastest enzymes known and are present in all aerobic organisms as well as in some anaerobes (McCord and Fridovich 1969). The activity of SODs in chloroplasts (and in all other organelles in which they are present), aside of detoxifying $O_2^{-\cdot}$, is of capital importance to avoid the formation of $HO^{\cdot}$ by the Fenton reaction and the Haber–Weiss cycle, wich consist on the formation of OH from H_2O_2 in the presence of transition metals such as Fe or Cu (Fenton reaction), that may become catalytic in the presence of $O_2^{-\cdot}$ (Haber–Weiss cycle):

Fenton reaction (1):	$38; H_2O_2 + Fe^{2+} \rightarrow OH^{\bullet} + OH^{-} + Fe^{3+}$
Recycling the transition metal (2) :	$38; O_2^{-\bullet} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$
NetHaber - Weiss cycle $(1 + 2)$:	$38; H_2O_2 + O_2^{-\bullet} \rightarrow O_2 + OH^{\bullet} + OH^{-}$

Chloroplasts show Cu/Zn- and Fe-SODs (Alscher et al. 2002). The increment in endogenous levels of chloroplastic SODs during drought has been extensively reported (e.g., Salekdeh et al. 2002; Fulda et al. 2011), although the opposite trend

has been also reported (Cruz de Carvalho 2008). Furthermore, the overexpression of chloroplastic SODs has been proven a successful way to improve plant responses to different sources of oxidative stress (O_3, methyl viologen and chilling temperatures) (Perl et al. 1993, Van Camp et al. 1994, 1996). However, the overexpression of cytosolic SOD does not confer tolerance to drought, while the simultaneous overexpression of chloroplastic SODs and APX enhances drought stress tolerance (Lee et al. 2007; Faize et al. 2011). These reports highlight the complexity of the antioxidant machinery and the fact that the interaction between different antioxidants are still far from being fully understood.

9.2.4 Ascorbate, APxs, and Related Enzymes

$_L$-Ascorbate (ascorbate herein, vitamin C) is a small carbohydrate that is present in all plant species, tissues and organs, except in dormant seeds, showing the maximum concentrations, between 20 and 300 mM, in illuminated chloroplasts (Noctor and Foyer 1998). Ascorbate can be oxidized to monodehydroascorbate radical (MDHA; Fig. 9.1) by monovalent electron transfer, for instance, to H_2O_2. This reaction can be either spontaneous or catalyzed by ascorbate peroxidases (APxs; EC.1.11.1.11). APxs are class I heme-containing peroxidases present in most subcellular compartments. Chloroplasts bear, at least, three APx isoforms: thylakoidal APx is bound to the thylakoidal membrane, while stromal APx and lumen APx are soluble proteins. In some cases (for instance *Arabidopsis*) these isoforms are encoded by different genes, but in other cases (e.g., pumpkin and spinach) a single gene encodes the different APx isoforms by alternative splicing (Mano et al. 1997; Ishikawa et al. 1997). APxs have high affinity for H_2O_2 and ascorbate, which suggests that APxs not only detoxify H_2O_2 but also control H_2O_2 levels for signaling purposes (Mittler and Poulos 2005). Overexpression of APX has been shown to increase drought tolerance, while ascorbate deficient plants such as *Arabidopsis vtc1* mutants are known to be hypersensitive to drought stress (see for instance, Pastori et al. 2003; López-Carbonell et al. 2006; Faize et al. 2011). However, the overexpression of *Escherichia coli* catalase in tobacco chloroplasts led to the suppression of chloroplast APX gene expression while increased the tolerance of the transgenic plants to drought, thus indicating that the role of APxs is more complex than simply scavenging ROS (Shikanai et al. 1998).

Beside the total ascorbate amount, the redox state of the ascorbate pool (ascorbate/total ascorbate, where total ascorbate is the sum of reduced plus oxidized forms), has been shown to change differentially in resistant and sensitive plants in response to drought, so that drought-sensitive species usually have an ascorbate pool more shifted toward its oxidized forms, MDHA and particularly DHA (Jubany-Marí et al. 2010). MDHA, the primary product of ascorbate oxidation, is a relatively stable radical; but two MDHA molecules can yield ascorbate and DHA spontaneously in aqueous solutions or in a reaction catalyzed by monodehydroascorbate reductases (MDHARs; EC 1.6.5.4). MDHARs are FAD

enzymes that obtain the reduction equivalents from NADH or NADPH ($2\ \text{MDHA} + \text{NAD(P)H} + \text{H}^+ \rightarrow 2\ \text{Ascorbate} + \text{NADP}^+$). The reduction of MDHA to ascorbate may occur also nonenzymatically with electrons provided by Fd_{red} or MDHA itself ($2\ \text{MDHA} \rightarrow \text{ascorbate} + \text{DHA}$). Computational analyses though suggest that the majority of MDHA in chloroplasts are reduced to ascorbate by MDHARs (Polle 2001). If DHA is formed it can also be enzymatically converted back to ascorbate by DHA reductases (DHARs), which use glutathione (GSH) as an electron donor.

9.2.5 Glutathione and Glutathione-Related Enzymes

Glutathione is a tripeptide (γ-glutamylcysteinyl glycine or γ-ECG; Fig. 9.1) of enzymatic biosynthesis. Although ubiquitous, GSH shows its highest concentration, between 1 and 4.5 mM, in chloroplasts (Meyer 2008). GSH is well known to act as an antioxidant and redox buffer. When the sulfhydryl groups of the cysteine residues of two GSH molecules are oxidized they form a disulfide bond between each other to yield GSSG (GSH disulfide; *syn*. oxidized GSH). GSH can directly scavenge ROS, particularly peroxides, and NO (nitric oxide), but the best known electron acceptor for such reaction is DHA.

A number of studies have reported on the dynamics of GSH levels and GSH-utilizing enzymes under drought stress in different species (e.g., Pastori and Trippi 1992; Galle et al. 2009). The set of reactions in which ascorbate is used to detoxify H_2O_2 and recycled by GSH receives the name of ascorbate-GSH cycle (Dalton et al. 1993; Jiménez et al. 1997; Fig. 9.2), which has also been shown to be involved in drought stress tolerance in several species (reviewed by Jubany-Marí et al. 2010). Furthermore, great efforts have been devoted to understand the water–water cycle, which couples part of the linear photosynthetic electron transport with the ascorbate-GSH cycle to dissipate excess energy (reviewed by Asada 1999). This cycle starts with the photolysis of water in PSII reaction centers and ends with the water resulting from the ascorbate-GSH cycle. The key point of the cycle is the transfer of electrons in PSI from Fd_{red} to O_2 to yield $O_2^{\cdot -}$, so that NADPH resulting from complete linear electron transport is not formed. Instead, $O_2^{\cdot -}$ is formed, which by the action of SODs quickly disproportionates to H_2O_2, which is scavenged by ascorbate with the involvement of APxs to yield MDHA and H_2O. The oxidized forms of ascorbate are then recycled to reduced ascorbate by the means of GSH through the action of DHARs, MDHARs (see previous section), and GRs. GRs recycle GSSG to GSH with electrons provided by NADPH, which is reduced by water after its split at the level of PSII. Thus, GRs act as the link between the primary electron donor, water (NADPH), and antioxidant recycling. The ability of GRs to keep the GSH/GSSG redox balance under control has been shown to be determinant for the tolerance of the plant to drought stress (Torres-Franklin et al. 2007; Cruz de Carvalho and Contaour-Ansel 2008).

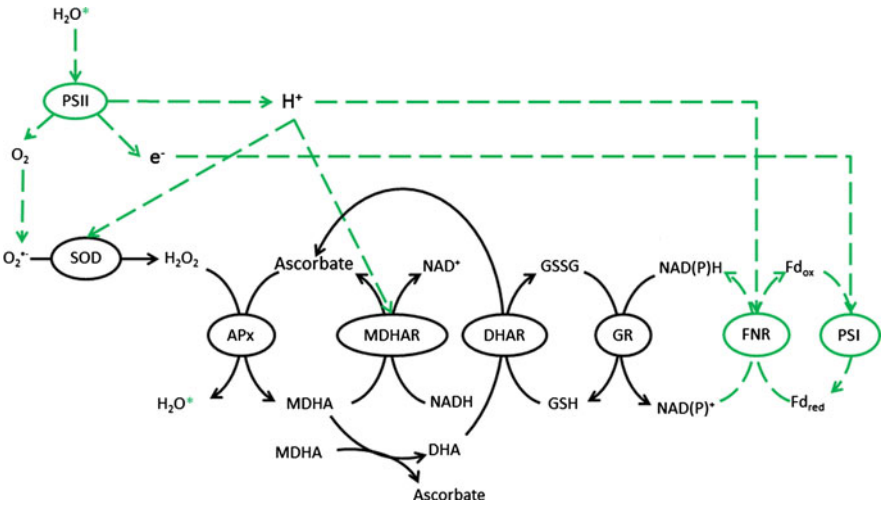

Fig. 9.2 The ascorbate–glutathione pathway and its variation, the water–water cycle (green dashed lines and circles). Water is split in the oxygen evolving complex of the PSII, yielding molecular oxygen (O_2), electrons (e^-), and protons (H^+). When the photosynthetic electron transport chain is over-reduced, the electrons may be transferred to O_2 yielding superoxide anion (O_2^-). O_2^- is quickly disproportionated to H_2O_2 (hydrogen peroxide) by the action of superoxide dismutases (*SOD*) in many different subcellular compartments. H_2O_2 is detoxified by ascorbate peroxidases (*APxs*) with electrons from ascorbate, yielding monodehydroascorbate (*MDHA*) and water. MDHA can be re-cycled to ascorbate by the action of MDHA reductases (MDHAR) or spontaneously yield dehydroascorbate (*DHA*), which is re-cycled to ascorbate by DHA reductases (*DHARs*), and ascorbate. DHARs obtain the reducing equivalents from glutathione (GSH; GSSG stands for oxidized GSH). GSSG is reduced back to GSH with reducing equivalents from NAD(P)H by the action of GSH reductases (GR), which obtain the reducing equivalents from NAD(P)H. In chloroplasts, $NAD(P)^+$ is reduced to NAD(P)H with electron from reduced ferredoxin (Fd_{red}), which obtains the electrons from the photosynthetic electron transport chain, i.e. from the electrons generated by splitting water. The stoichiometry of the reactions is not adjusted in this chart, but it is shown in Asada (1999) that the substrate of the whole set of reactions is water, and the only product of the net set of reactions is water (asterisks)

In addition, GSH participates in the posttranscriptional modification of several proteins. Glutathionylation is a type of posttranscriptional modification that consists on the formation of a mixed disulfide bond between a cysteine residue of a protein and GSH. Glutathionylation can occur spontaneously in the presence of GSSG, or in the presence of GSH and ROS. Thus, the enhanced formation of ROS, as it occurs in chloroplasts during drought, promotes glutathionylation (Fig. 9.3). The glutathionylation of proteins is a well-known regulatory mechanism, and a number of glutathionylation targets have been described in chloroplasts so far. These include the Calvin cycle enzymes triose phosphate isomerase, FBP aldolase, phosphoglycerate kinase, and ribose-5-phosphate isomerase (Dixon et al. 2002; Ito et al. 2003; Mohr et al. 1999; Michelet et al. 2008), and some thioredoxins (TRxs), which in turn are known to regulate the activity of several enzymes of the same cycle (reviewed by Rouhier et al. 2008).

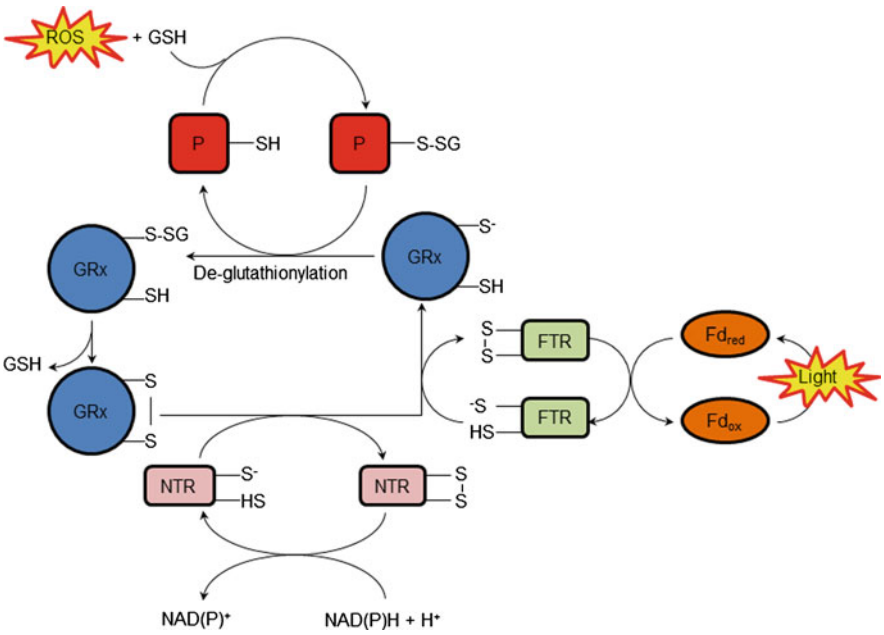

Fig. 9.3 De/glutathionylation of proteins and the role of the ferredoxin-thioredoxin and NADPH-thioredoxin systems in the reduction of oxidized glutaredoxins. Fd stands for ferredoxin (Fd_{red}, reduced ferredoxin; Fd_{ox}, oxidized ferredoxin); FTR, for Fd-thioredoxin reductase; GSH, for reduced glutathione; NTR, for NADPH-thioredoxin reductase; P indicates a protein susceptible of de/glutathionylation; ROS stands for reactive oxygen species

Moreover, GSH can be oxidized in reactions catalyzed by a number of enzymes including glutaredoxins (GRxs), GSH-*S*-transferases (GSTs), peroxiredoxins (PRxs), and TRxs. GSH peroxidase (GPx) is a general term for the enzymes that catalyze the reduction of H_2O_2 and organic hydroperoxides to water or their respective alcohols using GSH as electron donor ($H_2O_2 + 2$ GSH $\rightarrow 2$ H_2O + GSSG). However, plant GPxs show weak affinity for GSH, with K_m values over the physiological GSH concentration (Herbette et al. 2002). Thus, GPxs are nowadays included in the thiol peroxidase family of proteins (Sztajer et al. 2001, Herbette et al. 2002; Maiorino et al. 2007; Navrot et al. 2006). Still, GSH-dependent peroxidase activity exists in plant cells and is carried out by other enzymes such as GRxs, GSTs, or some PRxs (Rouhier et al. 2008). GRxs are small proteins that catalyze the reduction of other proteins or mixed disulfides and that are reduced nonenzymatically by GSH itself. Among other functions, GRxs can act as antioxidants by reducing directly peroxides, DHA, or TRxs, which in turn reduce H_2O_2 and alkyl hydroperoxides (Lee et al. 2002; Rouhier et al. 2001). Moreover, some GRxs are able to reduce methionine sulfoxide, the product of methionine oxidation by some ROS, back to methionine thus providing an antioxidant mechanism (Stadtman 2006). Oxidized GRxs can be also reduced back to their thiol form with electrons donated by Fd_{red} through the Fd-TRx (FTR;

Table 9.2 Overview of the antioxidant mechanisms in different subcellular compartments of plant cells under drought stress. Dots indicate major antioxidant mechanisms; dots in brackets indicate presence but not of major relevance, or the importance of which are unknown; crosses indicate the absence of the mechanism and zeros indicate data unknown; and asterisks indicate enzymatic activities rather than particular enzymes. CAT; catalase; SOD, superoxide dismutase; APx, ascorbate peroxidase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; ER, endoplasmic reticulum, and Golgi apparatus; GPx, glutathione peroxidase GR, glutathione reductase; GRx, glutaredoxin; GSH, glutathione; GST, glutathione-*S*-transferase; NADPH ox., NADPH oxidase; POx, peroxidase; PRx, peroxiredoxin; TRx, thioredoxin

	Chloroplast	Peroxisome	Mitochondria	Apoplast	Nucleus	Cytosol	ER
Tocopherols	•	**X**	**X**	**X**	**X**	**X**	**X**
Carotenoids	•	**X**	**X**	**X**	**X**	**X**	**X**
CAT	**X**	•	**X**	**X**	**X**	**X**	**X**
SOD	•	(•)	•	•	(•)	•	**O**
Ascorbate	•	(•)	•	•	(•)	•	(•)
APx	•	(•)	•	(•)	**X**	•	(•)
MDHAR	•	(•)	•	**X**	**X**	•	**X**
DHAR*	•	(•)	•	**X**	**X**	•	**X**
GSH	•	(•)	•	(•)	(•)	•	(•)
GR	•	(•)	•	**X**	**O**	•	**O**
GST	(•)	(•)	(•)	(•)	(•)	•	(•)
GPx*	(•)	(•)	(•)	(•)	**X**	(•)	(•)
GRx	(•)	**X**	(•)	**O**	(•)	(•)	(•)
TRx	(•)	**X**	(•)	(•)	(•)	(•)	(•)
PRx	(•)	**X**	(•)	**X**	(•)	(•)	**X**
Class III POx	**X**	**X**	**X**	•	**X**	**X**	(•)
NADPH ox.	**X**	**X**	**X**	•	**X**	**X**	**X**
Phenolics	(•)	(•)	(•)	•	(•)	(•)	**O**
Polyamines	(•)	**O**	(•)	•	(•)	(•)	**O**

Fig. 9.3, Dai et al. 2000). In addition, GRxs are involved in protein deglutathionylation. In support of a role of GRxs in plant resistance to drought, *Arabidopsis* plants devoid of a chloroplastic GRx, GRXS14, are hypersensitive to oxidative stress (Feng et al. 2006), but no study has reported up to date on enhanced tolerance to drought itself through engineering GRx levels. Finally, GSTs are a large family of proteins that catalyze the conjugation of GSH to electrophilic substrates. Although they are most common in the cytosol (see Sect. 9.6), they are also present in chloroplasts (Table 9.2). GSTs can transfer GSH to organic hydroperoxides such as lipid peroxides; thus exerting GSH-dependent peroxidase activity (GPx). Moreover, some GSTs have been shown to have GSH-dependent DHAR activity, thus catalyzing the recycling DHA back to its reduced form ascorbate. Two recent studies have shown that overexpressing GSTs of unknown function from soybean and *Prosopis juliflora* resulted on enhanced drought tolerance in tobacco plants (Suja et al. 2010; Ji et al. 2010). In agreement, it has been recently shown that the overexpression of a chloroplast-localized GST confers tolerance to osmotic stress to the transgenic plants (George et al. 2010).

9.2.6 Peroxiredoxins: A Diverse Subfamily of Thiol Peroxidases

Thiol peroxidases are a large family of nonheme peroxidases that catalyze the reduction of peroxides using catalytic cysteine residues and thiol-containing proteins as reductants (in contrast to heme-peroxidases, which use Fe in their catalytic mechanism). Thiol peroxidases include five types of peroxiredoxins (PRxs; EC 1.11.1.15) that vary in their sequence and mechanism of action; and nonselenium GPxs. PRxs are ubiquitous thiol-dependent peroxidases that reduce a wide range of peroxides from H_2O_2 to organic hydroperoxides. PRx lack a prosthetic group, so the oxidation of PRx thiols to disulfides, sulfenic, or even to sulfinic acids yield inactive forms of PRxs that need to be recycled back to their active, reduced form by additional electron donors such as thioredoxins (TRxs) or GRx. It is extremely difficult to determine unequivocally the reductant that recycles oxidized PRxs in vivo, since PRxs co-localize with a high number of possible electron donors (e.g., 26 TRxs and 31 GRxs). The mutation of an *Arabidopsis* 2-cysteine PRxs, a class of chloroplastic PRxs, results in impaired photosynthesis and accumulation of damaged proteins in chloroplasts. This suggests that these enzymes protect chloroplast proteins against photooxidative damage (Baier and Dietz 1999; Dietz et al. 2002; Baier et al. 2000). Potato mutants devoid of CDSP35, a chloroplastic TRx, show strong overoxidation of the 2-cysteine PRx pool. These plants are hypersensitive to drought stress or methyl viologen treatment (a promoter of O_2^- formation in chloroplasts), and show enhanced lipid peroxidation under those conditions which suggests that CDSP35 reduces oxidized 2-cysteine PRxs that are oxidized to terminate the lipid peroxidation reaction (Broin and Rey 2003). In addition, it has been suggested that 2-cysteine PRxs, with the involvement of TRxs, may take over ascorbate as primary antioxidant in the water–water cycle (Rey et al. 2005; Dietz et al. 2006, Vieira Dos Santos and Rey 2005).

9.2.7 TRxs: A Redox Regulatory Hub

TRxs are ubiquitous small proteins with a redox-active dithiol/disulfide group in their structure that reduce disulfide groups of other proteins to dithiols (TRx-$(SH)_2$ + Protein-S_2 → TRx-S_2 + Protein-$(SH)_2$). TRxs show a CxxC motif (being x any amino acid and C a cysteine), that forms an intermolecular disulfide bond when oxidized, but they do not show further sequence or structural homology between each other (Meyer and Hell 2005). Plants have a great amount of different TRxs (42 Trx genes in the *Arabidopsis thaliana* genome) that are found in plastids, cytosol, nucleus, mitochondria, and the apoplast (Meyer and Hell 2005). Chloroplasts bear four TRx types: TRx *f*, *m*, x and *y*, (Meyer and Hell 2005). In chloroplasts, oxidized TRxs are recycled by TRx reductases, which ultimately use the reduction equivalents from Fd_{red} (Fd-dependent TRx reductases, FTR; Fig. 9.3). Although TRxs do not play an antioxidant function sensu stricto, they

display important redox properties that enable them to fulfill a variety of functions. There have been identified over 500 TRx targets in oxygenic photosynthetic organisms, including enzymes capital for plant responses to drought such as Calvin cycle enzymes (e.g., RuBisCO, fructose-1,6-bisphosphatase, and glyceraldehyde-3-phosphate dehydrogenase), antioxidant enzymes (e.g. CATs, SODs, and MDHAR) and proteins involved in photosynthetic electron transport and light harvesting (e.g., LHCIIb, Fd and psaK) (Montrichard et al. 2009). TRxs, together with GSH and GRxs, are the major thiol-based regulatory systems in plants. The expression of some plastidic TRxs has been shown to be upregulated during drought (Rey et al. 1998). In agreement, transgenic plants lacking a plastidic TRx show enhanced sensitivity to photooxidative stress and in some cases this protective effect is exerted by recycling oxidized PRxs (Broin et al. 2002).

9.3 Photorespiratory H_2O_2 Production in Peroxisomes

Peroxisomes are quantitatively the most important source of ROS, particularly H_2O_2, in illuminated plant cells (Foyer and Noctor 2003). Under drought stress, the stomatal limitation of photosynthesis reduces the availability of CO_2 thus promoting the oxygenase activity of RuBisCO (in chloroplasts), which yields 2-phosphoglycolate. In the photorespiratory cycle, glycolate is oxidized to glyoxylate by the action of glycolate oxidase, producing H_2O_2 (Foyer and Noctor 2003; Table 9.1). In addition, there are other sources of ROS in peroxisomes (e.g., xanthine oxidase and fatty acid β-oxidation), but their relevance under drought stress, if any, is unknown. As in other subcellular compartments, the presence of O_2^{-} (for instance, generated by xanthine oxidase) together with transition metals in peroxisomes can lead to the formation of HO by the Fenton reaction and the Haber–Weiss cycle. To avoid the formation of $HO^{\cdot}$ or $O_2^{-\cdot}$ is quickly disproportionated to H_2O_2 by peroxisomal Mn-SODs (Sandalio et al. 1987). However, O_2^{-} is not the main ROS formed in peroxisomes during drought. Most of the photorespiratory H_2O_2 is scavenged by catalases (CATs). Still, it has been shown that this H_2O_2 can diffuse to other subcellular locations such as the nucleus and cytosol, and play a key role in intracellular signaling during acclimation to high light stress, a process that is tightly associated to drought (Vanderauwera et al. 2005).

9.3.1 Catalases: The Main Mechanism for Photorespiratory H_2O_2 removal

Catalases (CATs, *syn.* hydroperoxydases; EC 1.11.1.6) are enzymes that are ubiquitous among aerobic organisms (Feierabend 2005). CAT activity consists on reducing two H_2O_2 molecules to two molecules of H_2O and O_2 ($2\ H_2O_2 \rightarrow 2\ H_2O + O_2$). There are three main CAT types (nonheme Mn CATs, bifunctional

CATs (catalase-peroxidase), and monofunctional CATs), but only monofunctional CATs (they are not monofunctional strictly though) can be found in land plants (Klotz and Loewen 2003; Nicholls et al. 2001, Carpena et al. 2003). The mechanism of CAT activity lays on the heme group they bear, and since the heme group alone can do it, many heme-containing proteins such as methemoglobin, metmyoglobin, cytochrome *c* oxidase and chloroperoxidases exert CAT activity at very low rates (Keilin and Hartreef 1950, 1955; Bickar et al. 1982; Sun et al. 1994; Paco et al. 2009). Plant CATs show high turnover but low affinity toward H_2O_2, which makes them optimum for gross removal of H_2O_2 (Nicholls et al. 2001). It has been shown that downregulation of CAT gene expression leads to hypersensitivity to drought and other stress factors (reviewed by Smirnoff 2005).

9.3.2 Other Antioxidants in Peroxisomes

It has been also shown that the ascorbate-GSH cycle (Fig. 9.2), including ascorbate and GSH themselves as well as APxs, MDHARs, DHARs, and GRs, is fully active in peroxisomes, thereby providing an additional antioxidative protection to this organelle under drought stress (Jiménez et al. 1997). Furthermore, recent studies have shown that the expression of drought-responsive genes in plants with impaired root peroxisomal polyamine oxidase is altered, suggesting that polyamines (Fig. 9.1) may play a role in keeping ROS levels under control (Kamada-Nobusada et al. 2008).

9.4 Mitochondrial Respiration, Oxidative Stress, and Antioxidants

Aerobic metabolism leads to the production of ROS also in mitochondria, which may be the main ROS sources in plant cells in the dark. Complexes I and III of the mitochondrial electron transport chain are the main sources of ROS in these organelles (Table 9.1). The ubisemiquinone intermediary formed in these complexes can transfer a single electron to O_2 to yield O_2^- when the electrical and pH gradients are too steep and oxidized electron acceptors are not available. The main ROS formed in mitochondria is O_2^-, but it is quickly disproportionated to H_2O_2 by the action of mitochondrial Mn-SODs. Mitochondrial production of ROS under normal conditions is about 2–6 % of the consumed O_2, and in many cases increases during drought (Bartoli et al. 2004). It has been also shown that in some cases mitochondrial respiration decreases during drought, but, nevertheless, the ratio photosynthesis/respiration decreases almost invariably during drought (reviewed by Atkin and Macherel 2009). It is noteworthy that on the other hand, the respiration rates in roots of drought-stressed plants decrease (or transiently increase and then decrease) in most studies performed to date, a trend that has been ascribed to a substrate limitation (reviewed by Atkin and Macherel 2009).

Plant mitochondria possess several energy-dissipating mechanisms such as the ATP-sensitive plant mitochondrial potassium channel, the plant uncoupling proteins, the rotenone-insensitive type II NAD(P)H dehydrogenases, and the alternative oxidase (for review, see Atkin and Macherel 2009, and Millar et al. 2011). It is out of the scope of this chapter to review ROS formation avoidance mechanisms, but in mitochondria these mechanisms have been proven of capital importance in avoiding drought-induced oxidative stress. For instance, the overexpression of an *Arabidopsis* uncoupling protein (AtUCP) results in enhanced tolerance to drought (Begcy et al. 2011) and *Arabidopsis* plants devoid of alternative oxidase 1a (AOX1a) are hypersensitive to the combination of water deficit and excess light, and show altered expression of genes involved in the chloroplastic and mitochondrial antioxidant machineries (Giraud et al. 2008). Still, despite the ROS formation-avoidance mechanisms existing in mitochondria, this organelle is one of the most important sources of ROS, and it can be quantitatively the most important one in the dark.

9.4.1 Antioxidant Mechanisms Operating in Mitochondria Under Drought

As mentioned before, Mn-SODs disproportionate O_2^- to H_2O_2 thus preventing the Fenton reaction and the Haber–Weiss cycle, and mitochondria bear a fully operative ascorbate-GSH cycle, including ascorbate, GSH, APxs, MDHARs, DHARs, and GRs, that quenches H_2O_2 produced by the dismutation of O_2^-. In addition, as in chloroplasts, it has been suggested that PRxs, with the involvement of TRxs for the re-cycling of oxidized PRxs, may take over ascorbate in such cycle.

GRxs are also present in mitochondria. In other organelles, it is known that GRxs are able to reduce methionine sulfoxides and to catalyze protein de-glutathionylation, but it is so far unknown whether or not mitochondrial GRxs may fulfill these or any other function in plant responses to drought.

Polyamines (Fig. 9.1) have been also shown to be present in mitochondria, particularly bound to the membrane fraction (Votyakova et al. 1999). Liu et al. (2004) showed that during PEG-induced osmotic stress polyamine conjugation increases in wheat seedlings, and suggested that this phenomenon may be associated with an improved performance of mitochondrial membrane ATPase activity. Aside of this study there is no report on the role of mitochondrial polyamines in plant responses to drought.

9.5 Oxidative Stress and Antioxidant Defenses in the Apoplast

The production of ROS in the apoplast is boosted during plant responses to drought. The primary ROS formed in the apoplast under drought stress is O_2^-, formed by the action of plant NADPH oxidases (Table 9.1). H_2O_2 in the apoplast

induces the opening of plasma membrane Ca^{2+} channels in guard cells, which results in increased cytosolic Ca^{2+} levels and, ultimately, in stomatal closure. Thus, the production of H_2O_2 in the apoplast is key for ABA-mediated stomatal closure (Pei et al. 2000), and therefore for plant responses to drought, since stomatal function is the main mechanism for plants to regulate transpiration.

The catabolism of polyamines occurs mainly in the apoplast by the means of apoplastic polyamine oxidases (PAOs; Table 9.1), and the action of PAOs generates H_2O_2 (Moschou et al. 2008). It has been recently found that drought, by the means of ABA signaling, induces polyamines export to the apoplast, where these compounds are degraded producing H_2O_2 that may act as a cell signal for drought acclimation (Toumi et al. 2010). Plants possess also apoplastic SOD isoenzymes that dismutate O_2^- into H_2O_2.

9.5.1 Plant NADPH Oxidases

The mammalian respiratory burst oxidase multiproteic complex is a nonheme peroxidase composed of a membrane-bound NADPH binding flavocytochrome b_{558} and a number of cytosolic accessory proteins. The membrane-bound flavocytochrome b_{558} is comprised of two peptides: one of them, $gp91^{phox}$, contains all necessary elements to bind NADPH and to transfer one electron from it to O_2 thereby yielding O_2^-. Plant NADPH oxidases (also named Rboh after *respiratory burst oxidase homologue*) encode proteins homolog to $gp91^{phox}$ that are sufficient to transport electrons from NADPH to O_2 and generate O_2^- (1998; Torres et al. 1998; Foreman et al. 2003). Thus, plant NADPH oxidases are membrane proteins with the catalytic domain in the apoplast and a cytosolic N terminus end that contains two Ca^{2+} binding domains (EF-hand motifs) (Keller et al. 1998, Torres et al. 1998). It is well documented that ROS generation by plant NADPH oxidases in the apoplast increases during drought (see for example Duan et al. 2009). Plant NADPH oxidases are involved in plant responses to drought by generating the H_2O_2 in the apoplast necessary for the stomatal function and monolignol cross-linking (see Marjamaa et al. 2009 for review).

9.5.2 Lignification: A Structural Modification Depending on Free Radicals

Lignin is a highly branched polymer of phenylpropanoid units (monolignols; e.g., *p*-coumaryl, coniferyl, and sinapyl alcohols) cross-linked by oxidative coupling, which is deposited in secondary cell walls (for review, see Vanholme et al. 2010). The high evaporative demand that occurs during drought results in highly negative pressures in xylem cell walls that may result in cavitation or in the collapse

(implosion) of the xylem vessels (Hacke et al. 2001). It is widely accepted that lignification reduces xylem vulnerability by strengthening the secondary cell walls of xylem vessels (Raven 1987; Cochard et al. 2004).

The cross-linking of monolignols requires a monolignol radical that can be formed by the action of class III peroxidases (Vanholme et al. 2010). These enzymes are encoded by large multigene families (for instance, there are 73 class III peroxidases encoded in the *Arabidopsis* genome), that show variable sequence homologies that are highest in their active centers. For their regular peroxidase activity ("catalytic cycle"), class III peroxidases can use monolignols as substrates to reduce H_2O_2, yielding monolignol radicals that subsequently polymerize ($H_2O_2 + 2AH \rightarrow 2H_2O + 2A^{\bullet}$). Class III peroxidases, aside of their catalytic cycle, can catalyze a set of reactions so-called "hydroxylic cycle". In this cycle, the interconversion between several redox states of the heme group leads to the generation of $HO^{\bullet}$ and $HOO^{\bullet}$ (perhydroxyl radical): two of the most reactive ROS (Liszkay et al. 2003; Marjamaa et al. 2009). In addition, the downstream modification of peroxidase-oxidized products can generate O_2^{-} and subsequently H_2O_2. Thus, depending on the substrates and reaction conditions, class III peroxidases can scavenge H_2O_2 and $O_2^{-\cdot}$, or generate H_2O_2, $O_2^{-\cdot}$, $HO^{\cdot}$ or $HOO^{\cdot}$, aside of generating a substrate radical such as monolignol radicals (Møller and McPherson 1998; Caliskan and Cuming 1998; Barceló et al. 2002). Llorente et al. (2002) reported that AtPrx03, and *Arabidopsis* class III peroxidase, was induced by cold stress and that plants overexpressing this peroxidase showed increased tolerance to dehydration and salt stress, most likely due to improved lignification.

9.5.3 Other Antioxidants Operating in the Apoplast

Ascorbate is present in the apoplast, while GSH is either absent or at very low concentrations. The enzymes required for re-cycling oxidized ascorbate are also absent in the apoplast (Hernández et al. 2001; Table 9.2). Still, specific transporters exchange ascorbate from the symplast by DHA from the apoplast, which once in the symplast can undergo re-cycling by the ascorbate-GSH cycle, this way providing a mechanism for regenerating ascorbate oxidized in the apoplast (Horemans et al. 2000).

9.6 Other Organelles

It is obvious that the nucleus has an outstanding importance in plant responses to drought since it hosts DNA and gene expression machinery. ROS can induce a number of alterations in nucleic acids such as deletions, pyrimidin dimers, strand breaks, nucleic acid-protein crosslinks, nucleic-acid MDA adducts, sister chromatid exchange, and base modifications such as alkylation and oxidation (Sohal

and Weindruch 1996; Markesbery and Lovell 2006, Roldán-Arjona and Ariza 2009; Gill and Tuteja 2010). Several proteins (e.g., maturases, late embryogenesis abundant (LEA) proteins, and helicases) are involved in maintaining nuclear functions during desiccation by other means different from an antioxidative protection. However, the overexpression of a citrus LEA protein in tobacco led to the inhibition of lipid peroxidation of the transgenic plants under cold stress (Hara et al. 2003), so an antioxidant role of these proteins cannot be discarded. In nuclei one can find a broad repertoire of antioxidants (Table 9.2), including the complete set for the ascorbate-GHS cycle, GPx activity, GSTs, GRx, TRxs, PRxs, phenolic compounds (including flavonoids), and polyamines. Still, little is known about their possible antioxidant roles in nuclei of drought-stressed plants. The expression of a nuclear-located PRx (1-C PRx) from *Xerophyta viscosa*, a resurrection plant that keeps its viability at relative leaf water content as low as 5 %, was shown to be upregulated by dehydration, heat, high light stress, salt stress, and ABA (Mowla et al. 2002). Furthermore, the promoter of the *Arabidopsis* nuclear-located 1-C PRx shows ABA-responsive elements (Haslekås et al. 2003), so PRxs may pose an antioxidant mechanism in nuclei of drought-stressed plants.

It is worth to highlight that protein folding during drought stress is severely affected, and a number of chloroplast chaperones, including sulfhydryl oxidases, have been proven of capital importance in maintaining the correct functionality of many proteins under decreasing water potential (Wang et al. 2004; Stengel et al. 2010). The endoplasmic reticulum and Golgi apparatus host many antioxidant systems as well (see Table 9.1), but their role in plant responses to drought is still to be determined.

Finally, several sources of ROS, such as cytochromes P450 are well described in the cytosol of plant cells (Lewis 2002). However, it is largely unknown whether the production of ROS by these sources is enhanced during drought. Nevertheless, some of the ROS formed in many subcellular compartments (mainly H_2O_2 formed in peroxisomes, chloroplast, and mitochondria) can diffuse to the cytosol. Since ROS formation in most of these organelles is boosted during drought, ROS levels in the cytosol may increase as well during drought if ROS-scavenging mechanisms in the source organelles fail. Plant cell cytosol accounts for a broad array of antioxidants, including the complete set of elements of the ascorbate-GSH cycle (ascorbate, glutathione, APxs, MDHARs, DHARs, and GRs), GPx activity, GRxs, TRxs, PRxs, phenolic compounds, and polyamines (Table 9.2). Miao et al. (2006) showed that *Arabidopsis* cytosolic nonselenium GPx, AtGPX3, is essential for H_2O_2 scavenging and ABA-dependent and H_2O_2-mediated stomatal closure, and concomitantly plants overexpressing AtGPX3 are more tolerant to water deficit. But as mentioned in Sect. 9.2, plant GPxs show little affinity for GSH, and a higher affinity for other thiol-containing compounds such as TRxs, which might serve as an electron donor. Indeed, a number of TRxs have been reported in the cytosol of plant cells. They might reduce oxidized PRxs by using electrons from NADPH. Cytosolic PRxs are a target of GRxs in poplar (Rouhier et al. 2001). PRx in the cytosol of plant cells exert peroxidase activity by using reducing equivalents from GRxs or TRxs, although their importance in plant responses to drought is largely

unknown. Furthermore, most GSTs are localized in the cytosol (Dixon et al. 2009). The overexpression of GSTs has been successfully applied to improve plant performance during drought but, although predicted to be cytosolic in some cases, the subcellular localization of the ectopically overexpressed GST is not detailed in these reports (e.g., Ji et al. 2010; Jha et al. 2011). Similarly, other putative antioxidants such as polyamines and phenolic compounds such as flavonoids are known to be present in the cytosol of plant cells. However, their importance in plant responses to drought stress is still unknown.

9.7 Concluding Remarks

We have discussed here the origin of oxidative stress and the antioxidant mechanisms operating in different cellular compartments to avoid oxidative damage during drought stress. Most of the research performed thus far has been focused on better understanding oxidative stress and antioxidant protection mechanisms operating in chloroplasts, organelles with an extraordinary battery of antioxidants. It should be noted that several antioxidants, such as carotenoids and tocopherols, have been exclusively found in these organelles, which suggests that due to their photosynthetic function makes them require higher antioxidant protection compared to other organelles. Peroxisomes and mitochondria due to photorespiration and respiration, respectively, also appear to be organelles producing high amounts of ROS that contain at the same time an important set of antioxidant defenses. It should be noted, however, that the set of antioxidants required in these organelles will be more modest. This does not mean, however, that these organelles or others producing ROS at even lowest rates do not need antioxidants. The fact that ROS are used by aerobic organisms for cellular signaling forces these organisms, including plants, to finely modulate ROS levels in time and space with a complex array of antioxidants in all cellular compartments, as it has been summarized in this chapter.

Transgenic approaches to improve plant performance to drought through engineering antioxidant mechanisms have been proven useful. However, in many cases the output is far from what was expected, indicating our understanding of the antioxidant machinery as it works during drought stress is still far from being completely understood. A paradigmatic example is the case of the *Arabidopsis apx1/cat2* (CAT and cytosolic APx) double mutants. *apx1* and *cat2* single mutants are hypersensitive to oxidative stress, while *apx1/cat2* double mutants are more tolerant (Rizhsky et al. 2002). The double mutants show the DNA damage response constitutively activated, which contributes to the stress tolerance (Vanderauwera et al. 2011). Future research will undoubtedly be directed to better understand this fine tuning of ROS levels in different subcellular compartments and the relative contribution of different ROS in signaling and oxidative damage in drought-stressed plants.

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