Chapter 17
Transgenic Plants for Higher Antioxidant Content and Drought Stress Tolerance

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17.1 Introduction

Stress, by definition, is any external factor that exerts a detrimental impact on the plant growth and development. Therefore, it is vital to understand the basics of plant responses to various abiotic stresses associated with climate change. Abiotic stresses are the extreme restriction for crop production worldwide and account for yield reductions of as much as 50–60 % [2, 164]. Crop plants, as sessile organisms, encounter unavoidable abiotic stresses during their life cycles, including salinity, drought, extreme temperatures, metal toxicity, flooding, UV-B radiation, ozone, and so on, which all pose a serious threat to plant growth and development, metabolism, and productivity [83, 84, 86, 88]. Among these abiotic stresses, drought is supposed to be the most complex and devastating on a global scale [156], and its frequency is expected to increase as a consequence of climate change [33, 109]. According to the *Water Initiative* report of The World Economic Forum at Davos [99], water shortages are expected to deplete global crop production losses of up to 30 % by 2025, compared to current yields. Therefore, drought stress represents a major threat for sustaining food security under current conditions and will be more of a danger in the future, as climate change is projected to induce more frequent and more intense higher temperatures and drier conditions in many regions of the world [59, 129, 171, 177].

Drought is one of the most important limiting factors for agricultural crop production all around the world. Drought stress both during vegetative and early

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reproductive growth reduces yield. The assimilation of vegetative parts contributes to yield and that is why vegetative-stage drought is important; more emphatically, drought in the reproductive stage diminishes the yield directly by hampering the reproductive parts or developmental stages, which are very susceptible to all kinds of stress, including drought. Drought hampers the yield by obstructing the panicle, peduncle, rachis, tiller growth, and development; reducing the number of seeds, seed size, and seed quality. The complex nature of the morphological, physiological, and phonological traits of plant genotypes are influenced by the soil moisture, which determines the yield. Effects of drought on crop plants also depend on their developmental stages. Although the pattern of damage may differ, the result is the same (i.e., reduction of yield). The primary effect of drought stress is largely a reduction in plant growth, which depends on cell division, cell enlargement, and differentiation, and involves genetic, physiological, ecological, and morphological events, and their complex interactions. These events are seriously inhibited by drought stress, which adversely affects a variety of vital physiological and biochemical processes in plants, including stomatal conductance, membrane electron transport, carbon dioxide (CO_2) diffusion, carboxylation efficiency, water-use efficiency (WUE), respiration, transpiration, water loss, photosynthesis, and membrane functions (Fig. 17.1). Disruption of these key functions limits growth and developmental processes, and leads to reductions in final crop yield [161].

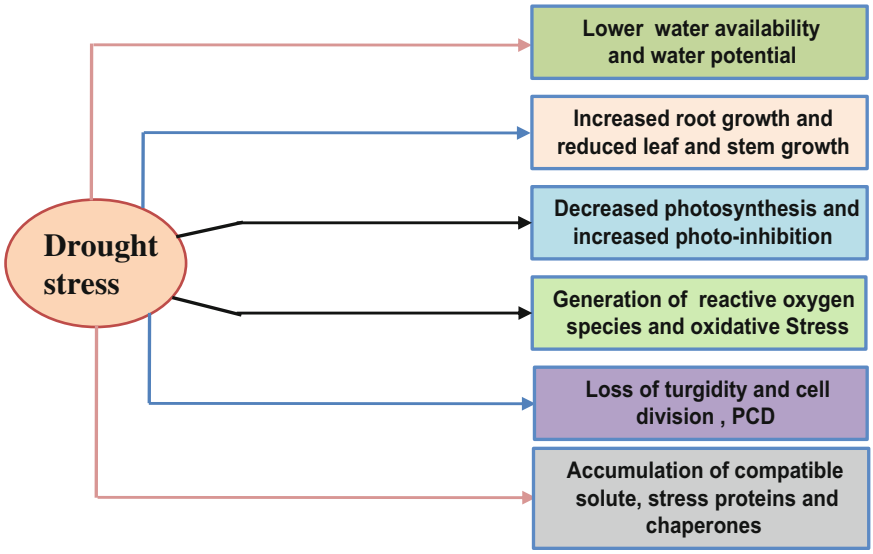

Fig. 17.1 Possible effects of drought stress in plants. Reduced water uptake results in a decrease in tissue water content and reduction in turgidity due to drought. Under drought stress conditions, cell elongation in higher plants is inhibited by reduced turgor pressure. Drought stress also inhibit cell elongation, and expansion result in growth reduction. Severe drought conditions limit photosynthesis due to a decrease in the enzyme activities required for photosynthesis. Drought stress disturbs the balance between the production of ROS and antioxidant defense, causing oxidative stress

Drought stress, like other kinds of abiotic stresses, aggravates the production of ROS such as $O_2^{\bullet -}$, $^1O_2$, H_2O_2, and $OH^{\bullet}$ to levels that are often beyond the plant's scavenging capacity. This causes oxidative stress that damages cells and cellular components, disrupts the physiological and biochemical life processes, and even leads to cell death [58, 85, 109, 120, 182]. Improvements in tolerance and resistance to drought stress will require intensive studies, and researchers are now focusing on oxidative stress as one of the basic damage responses in almost all kinds of stress. In this chapter, we review the generation of ROS under drought stress. We also review recent reports on development of drought stress tolerance transgenic plants via the antioxidant mechanism or removal of ROS from different cellular organelles.

17.2 Oxidative Stress and Reactive Oxygen Species

The O_2 molecule is a free radical, contain two impaired electrons with the same spin quantum number. This spin makes O_2 prefer to accept its electrons one at a time, leading to the generation of the so-called ROS. Reactive oxygen intermediates (ROIs) are partially reduced forms of atmospheric oxygen (O_2). They typically result from the excitation of O_2 to form $^1O_2$ or from the transfer of one, two, or three electrons to O_2 to form a $O_2^{\bullet -}$, H_2O_2, or a $OH^{\bullet}$. In contrast to atmospheric oxygen, ROIs are capable of unrestricted oxidation of various cellular components which in turn lead to the oxidative damage of the cell [12, 14, 42, 80]. It has been estimated that 2 % of O_2 consumption leads to the formation of ROS in plant tissues [26]. Increase of ROS as a result of numerous environmental stresses is the key to the loss of crop productivity worldwide [135]. ROS affects several cellular functions via damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation [66]. Gratao et al. [75] established that whether ROS will act as protective, damaging, or signaling factors depends on the subtle equilibrium between ROS production and scavenging at the proper cellular site and time. ROS are also produced continuously as by-products of several metabolic pathways that are localized in different cellular compartments such as chloroplast, mitochondria, and peroxisomes [46, 149]. In higher plants and algae, photosynthesis takes place in chloroplasts, which contain a highly organized thylakoid membrane system that contains components of the light-capturing photosynthetic apparatus and provides all structural properties for optimal light harvesting. Oxygen generated in the chloroplasts during photosynthesis can accept electrons passing through the photosystems, thus forming $O_2^{\bullet -}$. Under steady-state conditions, the ROS molecules are scavenged by various antioxidative defense mechanisms [66, 77]. The equilibrium between the production and the scavenging of ROS may be perturbed by various biotic and abiotic stress factors such as salinity, UV radiation, drought, heavy metals, extreme temperature, nutrient deficiency, air pollution, herbicides, and pathogen attacks. These disturbances in equilibrium lead to a sudden increase in intracellular levels of

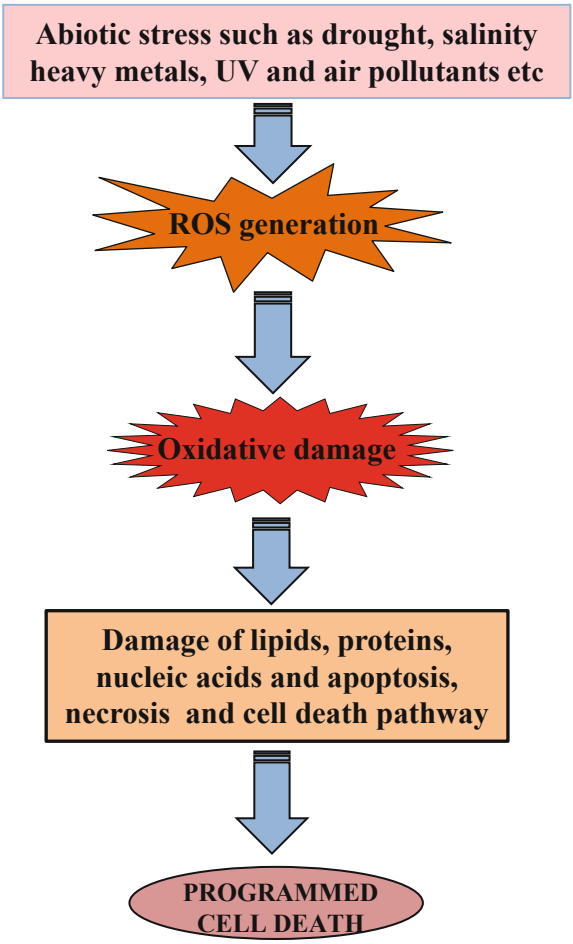

Fig. 17.2 Reactive oxygen species induced oxidative damage to proteins, lipids, and nucleic acids. Oxidative stress induced by the accumulation of ROS can bring out a range of stress responses. Exposure of cells to severe oxidative stress can elicit lethal response pathways such as apoptosis and possibly other forms of cell death pathways which can ultimately lead to programmed cell death

ROS that can cause significant damage to cell structures (Fig. 17.2). Exposure of cells to severe oxidative stress can elicit a lethal response pathway such as apoptosis and possibly other forms of cell death pathway that can ultimately lead to programmed cell death.

Plants, being sedentary, are inept to escape from stress and often display exceptional adaptive responses to overcome these stresses. A rapid transient production of huge amounts of reactive oxygen species called an oxidative burst is supposed to be the most common feature associated with plant responses to stress. Leshem and Kuiper [118] proposed a hypothesis termed the "General Adaptation Syndrome (GAS) response" according to which different types of stress evoke a similar adaptive response and implicating the role of ROS in the underlying

adaptive response mechanism. The sites of oxidative burst are the cell wall and the plasma membrane, involving the enzymes NADH peroxidase (NADH-PX) and NADPH oxidase (NADPH-OX or RBOH (respiratory burst oxidase homologue), respectively [1, 28, 152]. Interesting facts were also reported by some researchers including Gapper and Dolan [70] and Moller et al. [138]. Their experiments proved that ROS generated in response to environmental stress are necessary for cell function, regulation, and development. For instance, plant cells use ROS for polymerization of lignin during cell wall formation. Several other enzymes associated with the cell wall also contribute to the generation of ROS including lipoxygenase, oxalate oxidase, xanthine oxidase, amine oxidases, and peroxidases [3, 24, 197]. Plant cells generate H_2O_2 during normal metabolism via the Mehler reaction in chloroplasts, electron transport in mitochondria, and photorespiration in peroxisomes. ROS generated by chloroplasts as by-products of photosynthesis include $^1O_2$ and the $O_2^{\bullet -}$, whereas the main species generated by peroxisomes are $O_2^{\bullet -}$ and H_2O_2 [15, 46]. In the dark, most ROS are generated in mitochondria, which mainly form $O_2^{\bullet -}$, by over reduction of the electron transport chain [188]. Superoxide can be converted into H_2O_2 in a reaction catalyzed by superoxide dismutase (SOD) and H_2O_2 serves as an inert diffusible species that can give rise to reactive $OH^{\bullet}$ through the catalysis by free transition metal ions [78]. H_2O_2 has a property to diffuse freely, and this movement is facilitated through peroxiporin membrane channels. Although H_2O_2 is relatively stable and found in the plant cell at µmolar concentration range, the residual ROS have short half-lives [36]. It has now been a well-established fact that out of several primary ROS, $OH^{\bullet}$ is the most reactive and is capable of oxidizing all known biomolecules at diffusion-limited rates of reaction. According to estimates, the average diffusion distance before $OH^{\bullet}$ reaction with a cellular component is only 3 nm (i.e., approximately the average diameter of a typical protein) [98]. The degree of cytotoxic damage induced by ROS ultimately depends on the redox homeostasis or the balance between ROS detoxification and ROS production mechanisms in the cell.

An antioxidative system consisting of antioxidant molecules and enzymes is in place in different compartments of plant cells that scavenges or detoxifies the ROS and keeps cellular redox homeostasis. Under physiological steady-state conditions, ROS are scavenged by different antioxidants. However, the balance between production and scavenging of ROS is disturbed by a number of adversative environmental stress factors, resulting in a rapid increase of intracellular ROS levels. Although high concentrations of ROS can cause irreversible damage to the macromolecules leading to cell death, at the same time they can also influence signaling and gene expression, indicating that cells have evolved strategies to utilize ROS to their advantage in various cellular programs and functions. The role of ROS is increasingly implicated in cell signaling processes involving the induction of stress-related genes regulated by a network of transcription factors. Transcription factors are proteins that act together with other transcriptional regulators, including chromatin-modifying proteins, for binding or obstruction of RNA polymerases with

the DNA template [10, 114]. The antioxidant enzymes include SOD, catalase (CAT), glutathione peroxidase (GPX), and the ascorbate–glutathione cycle (Halliwell-Asada pathway) enzymes: ascorbate peroxidase (APX), glutathione reductase, monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR) [74, 135] (Table 17.1). In the case of animals, the GPXs function as key enzymes that scavenge H_2O_2; in plants this function mainly belongs to CAT and the enzymes of the ascorbate–glutathione cycle. On the other hand, studies have indicated that GPXs or GST/GPXs become the main H_2O_2-scavenging enzymes under extreme/persistent stress conditions [79]. In addition to these antioxidant enzymes that scavenge ROS, plants synthesize antioxidant molecules that include L-ascorbic acid (vitamin C), glutathione, α-tocopherol (vitamin E), and carotenoids [4, 7].

17.3 ROI Signal Transduction Pathway

Recent studies have identified several components involved in the signal transduction pathway of plants that senses ROIs. These include the mitogen-activated protein (MAP) kinase kinase kinases AtANP1 and NtNPK1, and the MAP kinases AtMPK3/6 and Ntp46MAPK [109, 169]. In addition, calmodulin has been implicated in ROI signaling [48, 82]. A hypothetical model depicting some of the players involved in this pathway is shown in Fig. 17.3. H_2O_2 is sensed by a sensor that might be a two-component histidine kinase, as in yeast [42]. Calmodulin and a MAP-kinase cascade are then activated, resulting in the activation or suppression of several transcription factors. These regulate the response of plants to oxidative stress [48, 127]. Crosstalk with the pathogen-response signal transduction pathway also occurs and might involve interactions between different MAP-kinase pathways, feedback loops, and the action of NO and SA as key hormonal regulators. This model (Fig. 17.3) is simplified and is likely to change as research advances our understanding of this pathway.

ROIs act as signals that mediate the systemic activation of gene expression in response to pathogen attack [9], wounding [153], and high light [145]. They were suggested to act in conjunction with a compound that travels systemically and activates their production in distal parts of the plant, where they mediate the induction of gene expression. The involvement of ROIs in the regulation of stomatal closure [155] and in other cellular responses involving auxin [213] might suggest that more signaling pathways involving ROIs as inducers of systemic signals await discovery. It is unlikely that ROIs can travel systemically because they are highly reactive and would be scavenged along the way by the many antioxidative mechanisms and antioxidants present in the apoplast. However, it is possible that a wave of activity similar to the “oxidative burst” is activated in cells

Table 17.1 Important antioxidants and their localization in plant cells

Antioxidant	Localization of antioxidant*	Respective ROS	References
Enzymatic			
Superoxide dismutase (SOD) (EC 1.15.1.1)	Chl, Mit, Per, Cyt, Apo	$O_2^{\bullet-}$	[23, 124]
Catalase (CAT) (EC 1.11.1.6)	Per, Gly	H_2O_2	[135, 202]
Ascorbate peroxidase (APX) (EC 1.11.1.11)	Chl, Mit, Per, Cyt, Apo, Gly	H_2O_2	[14, 32]
Peroxidase (POX) (EC 1.11.1.7)	Vac, Cyt, CW	H_2O_2,	[12, 135]
Glutathione reductase (GR) (EC 1.6.4.2)	Chl, Cyt, Mit	Reduction of glutathione	[39, 53]
Glutathione peroxidase (GPX) (EC 111.1.9)	Cyt	H_2O_2, Lipid peroxyl radicals (ROO), organic hydroperoxide (ROOH)	[51, 94, 135]
Glutatjione S-transferase (GST) (EC 2.5.1.18)	Cyt, Mit, ER	Organic hydroperoxide (ROOH)	[68, 166]
Dehydroascorbate reductase (DHAR) (EC 1.8.5.1)	Chl, Mit, Per	Regeneration of ascorbate from dehydroascorbate (DHA)	[13, 133]
Monodehydroascorbate reductase (MDHAR) (EC 1.6.5.4)	Chl, Cyt, Mit, Per	Reduction of monodehydroascorbate (MDA) to give rise to ascorbate	[13, 20, 100, 133]
Nonenzymatic			
Glutathione	Chl, Mit, Per, Cyt, Apo	H_2O_2,$^{\bullet}OH$, $^1O_2$, $O_2^{\bullet-}$	[14, 101, 135, 149]
Ascorbic acid	Chl, Mit, Per, Cyt, Apo	H_2O_2,$^{\bullet}OH$, $^1O_2$, $O_2^{\bullet-}$	[14, 135, 149, 180]
α-tocopherol	Membrane	•OH, $^1O_2$, ROO, ROOH	[12, 93, 135, 144]
Carotenoids	Chloroplast	$^1O_2$	[12, 135]
Flavonoids	Vac	$^{\bullet}OH$, $^1O_2$, $O_2^{\bullet-}$, ROO and peroxinitrice	[30, 198]

*Chl-chloroplast; Mit-mitochondrion; Per-peroxisome; Gly-glyoxisome; Cyt-cytosol; Apo-apoplast; CW-cell wall; Vac-vacuole; ER-endoplasmic reticulum

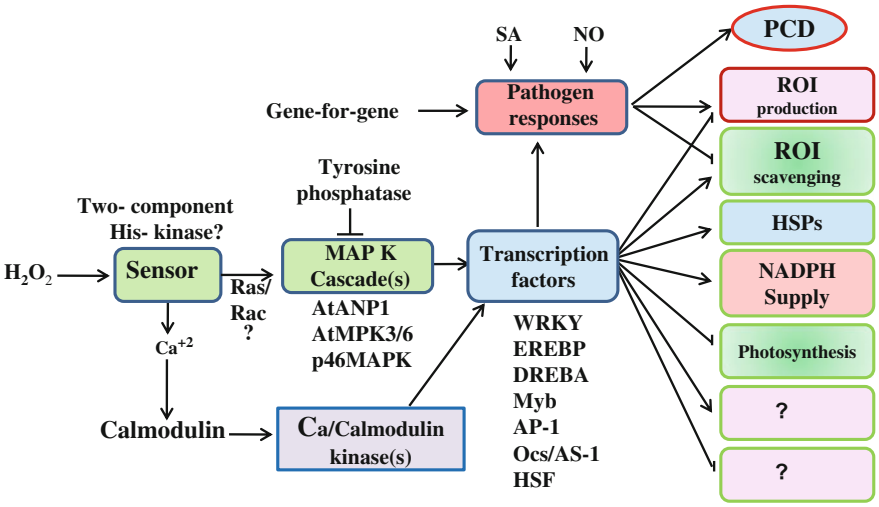

Fig. 17.3 A suggested model for the activation of signal transduction events during oxidative stress. Adopted from Mittler [135] H_2O_2 is detected by a cellular receptor or sensor. Its detection results in the activation of a mitogenactivated-protein kinase (MAPK) cascade and a group of transcription factors that control different cellular pathways. H_2O_2 sensing is also linked to changes in the levels of Ca_2^+ and calmodulin, and to the activation or induction of a Ca_2^+–calmodulin kinase that can also activate or suppress the activity of transcription factors. The regulation of gene expression by the different transcription factors results in the induction of various defense pathways, such as reactive oxygen intermediate (ROI) scavenging and heat-shock proteins (HSPs), and in the suppression of some ROI-producing mechanisms and photosynthesis. There is also crosstalk with the plant–pathogen signal transduction pathway, which might depend on pathogen recognition by the gene-for-gene mechanism and can result in an inverse effect on the regulation of ROI-production and ROI-scavenging mechanisms, as well as on the activation of programmed cell death (PCD). The plant hormones nitric oxide (NO) and salicylic acid (SA) are key regulators of this response [135]

along the systemic path and in distal tissues, resulting in the accumulation of ROIs. Future studies using plants with altered levels of ROI-scavenging and/or ROI-producing mechanisms might resolve this question.

17.4 Drought-Induced ROS and Effect on Plant Growth and Metabolism

Almost all the abiotic stresses result in increased ROS production in the plant cell [166]. Among all the abiotic stresses, drought-induced ROS production has been documented in several plant species growing under different environments [21, 89, 104, 172]. Under normal conditions, most cellular compartments of plants keep a

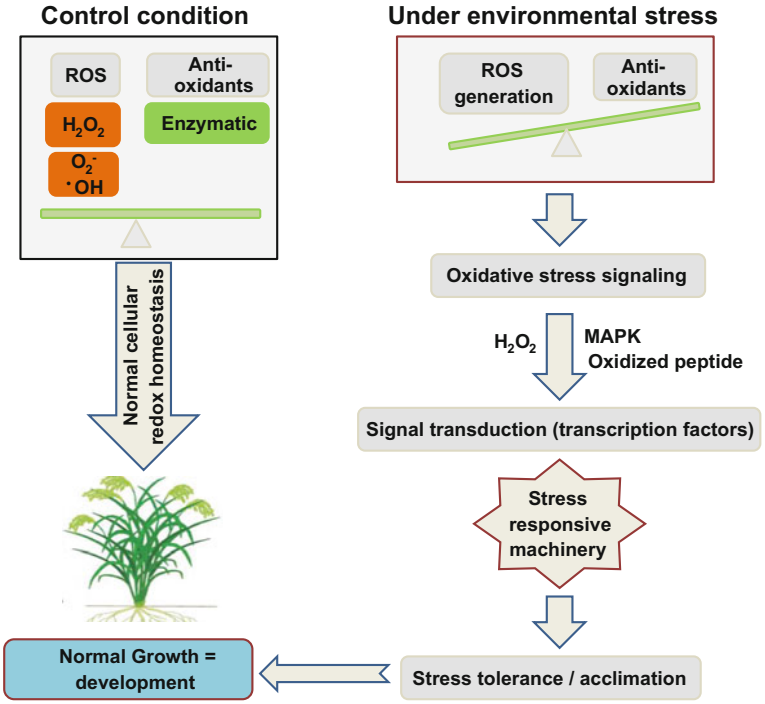

Fig. 17.4 Plant growth and development under normal and drought stress conditions. ROS production beyond the plant's quenching capability is often defined as a disruption of redox signaling and redox control. During the drought stress, an increased ROS level is prominent which can cause oxidative stress by damaging membrane proteins, lipids, photosynthetic pigments, and nucleic acids through the oxidation process and these are significantly augmented under drought stress. However ROS at optimum level orchestrate plant adaptive responses to drought stress

reducing environment and maintain a steady homeostatic state. However, during the stress, an increased ROS level is prominent. ROS production beyond the plant's quenching capability is often defined as a disruption of redox signaling and redox control [103], which can cause oxidative stress by damaging membrane proteins, lipids, photosynthetic pigments, and nucleic acids through the oxidation process and these are significantly augmented under drought stress [85, 109, 120, 182] (Fig. 17.4).

Drought stresses damage the photosynthetic pigments and photosynthetic apparatus and other enzymatic processes, resulting in the production of ROIs dangerous beyond the plant's scavenging capacity and imposing oxidative stress [148]. Water deficit makes the cellular content more viscous, causing the denaturation of proteins, thus the membrane of the photosynthetic apparatus as well as the cell membrane denatures, and at the same time the enzymes of the Calvin cycle are inactivated, the efficiency of carboxylation reaction and CO_2 fixation by RuBisCO

is reduced, and this results in increased photorespiration, which is one of the key reasons of ROS production [141].

Drought-induced photorespiration is reported to cause more than 70 % of total H_2O_2 generation in the plant cell [150, 151]. The rate of regeneration of NADP is also reduced under drought, therefore, the electrons of the electron transport chain cannot be accepted properly and finally surplus reduction of the electron transport chain causes leakage of electrons to O_2 and the production of ROS ($O_2^{\bullet -}$, $^1O_2$, H_2O_2, and $^{\bullet}OH$). Drought-induced stomatal closure is another common phenomenon that reduces the CO_2 availability in the fixation site of the Calvin cycle [193, 194]. In addition, stressful conditions also cause the chloroplasts to receive excessive excitation energy beyond their capability to combine it and thus ferredoxin remains in the over reduced condition during photosynthetic electron transfer; the electrons having a high state of energy are transferred from Photosystem I to molecular oxygen. During this transfer, the $O_2^{\bullet -}$ is generated through the Mehler reaction and this superoxide radical leads to the production of more harmful oxygen radicals such as $^{\bullet}OH$ [43, 135]. They also proposed that the drought stress response occurs in three successive phases. Normal ROS steady-state level is disturbed by drought stress where initially the enhancement of ROS production due to stomatal closure shifts the equilibrium upwards and this triggers defense signal transduction pathways. Prolonged drought stress results in aggravated ROS production that cannot be counterbalanced by the antioxidant system, leading to toxic oxidative events that ultimately result in cell death. Several other studies have shown that drought stress causes imbalance between light capture and its utilization in Photosystem II changes the photochemistry of chloroplasts, which causes excess production of highly reactive ROS species [64, 157]. Lipid peroxidation is thought to be the most common and obvious damaging process for all living organisms, including animals and plants [74]. Lipid peroxidation affects normal cellular functioning and the lipid-derived radicals accelerate oxidative stress [139]. There are several reports of enhanced electrolyte leakage under drought stress due to oxidative stress, and subsequent lipid peroxidation and plasmalemma injury such as in maize [47], rice [76], and rapeseed [85]. Drought stress produces ROS that subsequently impair the photosystem and reaction center of the photosynthetic apparatus. Singlet oxygen damages light-harvesting complexes [121]. The highly reactive $^1O_2$ can react with proteins, lipids, and pigments [190]. Lu and Zhang [124] reported that Photosystem II is more susceptible to drought as compared to Photosystem I, and a decline in D1 and D2 proteins causes the destruction of Photosystem II. Again, due to differences in the antioxidative capacity, oxidative damage was not found to be uniform in the different cells or tissues in C4 plants [52, 65]. Interestingly, the oxidative stress causes additional damage to bundle sheath tissue as compared to the mesophyll tissue [106]. Higher leakage of electrons to O_2 occurred during photosynthesis under drought stress and as compared to unstressed wheat seedlings the drought stress caused approximately 50 % higher leakage of photosynthetic electrons through the Mehler reaction [27, 179]. Sgherri et al. [173] also reported similar results in the case of sunflower. The production of hydroxyl radicals in

thylakoids under drought was considered as an additional menace because of their oxidizing potential to react with almost all biological molecules [42]. Their accumulation leads to a chain of deleterious reactions, and exerts harmful effects by damaging thylakoidal membranes and the photosynthetic apparatus; several reports state that no enzymatic reactions were found to remove or reduce the highly reactive $^{\cdot}OH$ [207]. Reactive nitrogenous species were also found to increase under drought. An increased nitric oxide (NO) level of water-stressed grapevine leaves was observed by Patakas et al. [154]. Arasimowicz-Jelonek et al. [11] also reported a higher level of NO in cucumber roots during water stress. The physiology and cellular status of root and leaf tissues of *Medicago truncatula* were observed under 11 days of drought stress [61]. Cellular damage, membrane damage, enhanced levels of reactive oxygen and nitrogen species, and reduced stomatal conductance were key features of that stress in *Medicago* and rewatering resulted in partial or complete alleviation of the stress-induced damage.

Drought stress responses towards the production of H_2O_2 and lipid peroxidation differ by genotype, and sometimes with the variety of genotypes, duration of water stress and the age of the plant are also important factors. Uzilday et al. [193, 194] compared the effects of drought among two C3 (*Cleome spinosa*) and C4 (*Cleome gynandra*) plants. These plants were exposed to drought stress for 5 and 10 days. Lipid peroxidation as represented by MDA and H_2O_2 contents remarkably increased in *C. spinosa* as compared to *C. gynandra* under drought stress, which proved the higher sensitivity of the C3 plant towards drought stress. Selote and Khanna-Chopra [172] also reported that increased H_2O_2 and lipid peroxidation *T. aestivum* (20 days old and 9–11 days water stress), *Populus przewalskii* (water stress at 2 months old) [117], and *Pinus densata*, *Pinus tabulaformis*, and *Pinus yunnanensis* (water stress for 1 month in 1-year-old plants) [69]. It is well established that as a result of drought stress the generated harmful ROS damage the cell structures, proteins, lipids, carbohydrates, and nucleic acids, and disrupt cellular homeostasis, in severe cases leading to cell death. However, in spite of the detrimental effects of ROS, they also play major physiological roles in intracellular signaling, cellular regulation, and as secondary messengers, which have been studied in several reports. In addition, the role of ROS as signals for gene expression has also been confirmed by several authors [96, 74, 132].

17.5 Antioxidant Defense System in Plants Under Drought Stress: Transgenics Approach

Water is the most important to complete the plant life cycle with most plant cells consisting of at least 70 % water on the basis of fresh weight. When there is scarcity of water, plant water status is disturbed causing imbalance in osmotic and ionic homeostasis, loss of cell turgidity, and damage to functional and structural cellular

membranes and proteins. Therefore, water-stressed plants show wilting, loss of photosynthetic capacity, and are unable to sequester assimilates into the appropriate plant organs. Severe drought conditions result in reduced yield and ultimately the plant death. The overall aim of genetically improving crops for drought resistance is to develop plants able to obtain water and use it to produce sufficient yields for human needs under drought conditions. Although advances have been made in developing crops that are genetically improved with traits such as herbicide and pesticide resistance, attempts to improve plant drought resistance have been hindered by the complexity of plant drought resistance mechanisms at the whole plant, cellular, metabolic, and genetic levels. Interaction between these mechanisms and the complex nature of drought itself adds another layer of difficulty to this problem. Plants acquire well-organized enzymatic and nonenzymatic defense systems that function together to control the flow of uncontrolled oxidation under various stress condition and protect plant cells from oxidative damage by scavenging ROS. The well-documented antioxidant enzymes in plants are SOD, catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPX), guaicol peroxidase (GPOX), glutathione-*S*-transferase (GST), and so on. Among the nonenzymatic antioxidant components, ascorbic acid, glutathione (GSH), phenolic compounds, alkaloids, nonprotein amino acids, and α-tocopherols are commonly found in plants [74] (Fig. 17.5). The improvement of the antioxidant defense system via the transgenic approach is considered to be effective in the development of resistance and adaptive features in plants against drought stress. It has been supported by many research findings that the enhanced activities of components of the antioxidant system such as antioxidant enzymes and nonenzymatic compounds via the transgenics approach decrease oxidative damage, and develop and improve the drought tolerance and resistance of plants [43, 174]. Enzymatic antioxidants include SOD, CAT, APX, MDHAR, DHAR, and GR and nonenzymatic antioxidants are GSH, ascorbic acid, tocopherols, and proline.

17.5.1 Enzymatic Components

Plants have different antioxidant enzymes that are compartment-specific and present in various cell organelles, including chloroplasts, mitochondria, peroxisomes, cytosol, and stroma, by which ROS production remains under control via highly efficient scavenging mechanisms. The major oxidative enzymes and nonenzymatic components, their respective ROS, cellular localization, are presented in Table 17.1.

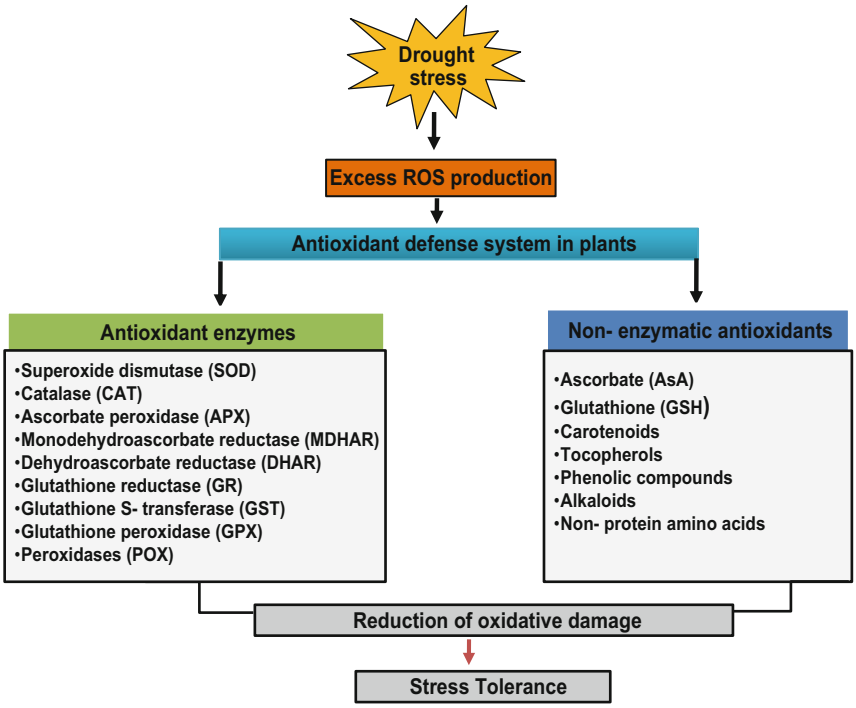

Fig. 17.5 Antioxidant defense system in plants under drought stress-induced oxidative stress. Plants acquire well-organized enzymatic and nonenzymatic defense systems that function together to control the flow of uncontrolled oxidation under various stress conditions and protect plant cells from oxidative damage by scavenging ROS. Antioxidant enzymes and nonenzymatic antioxidant components, commonly found in plants protect the cellular homeostasis level and the ROS is scavenged

17.5.1.1 Superoxide Dismutase (SOD)

SOD is a kind of metalloenzyme and is the most effective intracellular enzymatic antioxidant that is ubiquitous in all aerobic organisms and in all subcellular compartments prone to ROS-mediated oxidative stress. It is well established that various environmental stresses often lead to the increased generation of ROS where SOD has been proposed to provide the first line of defense against the toxic effects of elevated levels of ROS, important for plant stress tolerance. The SODs remove $O_2^{\bullet -}$ by catalyzing its dismutation: one $O_2^{\bullet -}$ being reduced to H_2O_2 and another oxidized to O_2. It removes $O_2^{\bullet -}$ and hence decreases the risk of $OH^{\bullet}$ ion formation via the metal catalyzed Habere Weiss-type reaction. This reaction has a 10,000-fold faster rate than spontaneous dismutation. SODs are classified on the basis of their metal cofactors into three known types: the copper/zinc (Cu/Zn-SOD), the manganese (Mn-SOD), and the iron (Fe-SOD), which are localized in different cellular compartments [135]. In the *A. thaliana* genome, three Fe-SOD genes (FSD1, FSD2, and

FSD3), three Cu/ZnSOD genes (CSD1, CSD2, and CSD3), and one MnSOD gene (MSD1) have been reported [108]. The Mn-SOD is found in the mitochondria of eukaryotic cells and in peroxisomes [45]; some Cu/Zn-SOD isozymes are found in the cytosolic fractions, and also in chloroplasts of higher plants [44]. The Fe-SOD isozymes, often not detected in plants [60] are usually associated with the chloroplast compartment when present [8]. All forms of SOD are nuclear-encoded and targeted to their respective subcellular compartments by an amino terminal targeting sequence. Several forms of SOD have been cloned from a variety of plants [170].

There have been many reports of the production of abiotic stress-tolerant transgenic plants overexpressing different SODs (Table 17.2). *Cu/Zn-SOD* overexpressing transgenic tobacco plants showed multiple stress tolerance [22]. The transgenics plants were able to show tolerance to salt and polyethylene glycol (PEG)-induced drought stresses and enhancement in the chloroplast antioxidant system. They showed that overexpression of rice cytosolic *Cu/Zn-SOD* in chloroplasts of tobacco plant improved their photosynthetic performance during photooxidative stresses such as high salt, drought, and PEG treatment when compared to untransformed plants. Although in both plants, net photosynthesis was steadily decreased, the rate of reduction was lower in the transgenic plants. The constitutively expressed *Cu/Zn-SOD* acts as an early scavenger, protecting the transgenic plants from the initial damage before their endogenous defense system is activated, and the higher SOD activity in the transgenic plants during the stress keeps the superoxide radical at a lower level leading to reduced oxidative damage and higher photosynthetic rate. When they match the result of net photosynthesis and antioxidant enzyme activities during salt stress, water deficiency, and PEG-induced stress, it was interpreted that the enhanced tolerance is caused by the overexpressed *Cu/Zn-SOD* and increased cellular antioxidant enzymes. Prashanth et al. [159] also showed the overexpression of *Avicennia marina* Cu/Zn SOD in transgenic rice and reported that the transgenic plants were more tolerant to MV-mediated oxidative stress, salinity stress, and drought stresses. Interestingly, Faize et al. [58] aimed to test whether overexpression of cytosolic SOD or cytosolic APX, alone or in combination, could enhance the tolerance of tobacco to drought stress and if the expression of these transgenes could affect the antioxidant metabolism in the soluble and chloroplastic fractions. To accomplish this goal, transgenic tobacco overexpressing each or both (SOD/APX) transgenes was generated and tolerance of the plants to a mild water stress was evaluated by analyzing the antioxidant metabolism in the soluble and chloroplastic fractions. The effect of the transgenes on the photosynthesis rate and on chlorophyll fluorescence parameters was also studied. The results showed that the overexpression of at least cytosolic APX protects tobacco plants from water stress and affects the antioxidant metabolism in the cytosol as well as in the chloroplast. High levels of cyt SOD and cyt APX gene transcripts as well of their respective activities suggested that the transgenes were constitutively and functionally expressed. Interestingly, some of the plant lines harboring only the cyt SOD transgene also had high APX activity. This was the case for at least six transgenic lines. This result was not surprising inasmuch as the reaction product of the SOD activity (H_2O_2) is the substrate for APX activity.

Table 17.2 ROS scavenging enzymatic antioxidants and their role in transgenic plants for drought stress tolerance

Gene	Target transgenic	Gene source	Response in transgenic plants	References
Superoxide dismutase (SOD)				
Cu/Zn SOD	*Nicotiana tabacum*	*Oryza sativa* L.	Enhanced tolerance to salt, water, PEG-induced drought stresses and enhancement in chloroplast antioxidant system	[22]
Cu/Zn SOD	*Oryza sativa* cv. Pusa Basmati 1	*Avicennia marina*	Transgenic plants were more tolerant to MV-mediated oxidative stress, salinity stress, and drought stress	[159]
Mn SOD	*Triticum aestivum cv* Oasis	*Nicotiana plumbaginifolia*	Photooxidative stress tolerance, lower oxidative damage, higher H_2O_2, and significant increase in SOD and GR activity	[130, 136]
Cu/Zn SOD + APX	*Nicotiana tabacum*	*Pisum sativum*	Increase in SOD and APX activity, drought stress tolerance	[58]
MnSOD	*Oryza sativa*	*Pisum sativum*	Enhanced tolerance to drought, and enhancement in cellular antioxidant system	[199]
Mn SOD + Fe SOD	*Medicago sativa* L.	*Nicotiana plumbaginifolia* and *Arabidopsis thaliana*	Mild water stress tolerance with high photosynthetic activity	[168]
Catalase (CAT)				
CAT-2	*Lycopersicon esculentum*	*Escherichia coli*	Transgenic plants have higher CAT activity as compared to wild-type plants. The transgenic plants showed increased tolerance to the oxidative damage caused by drought stress or chilling stress under high light intensity (1000 µmol m^{-2} s^{-1})	[206]
Ascorbate peroxidase (APX)				
c APX	*Lycopersicon esculentum cv.* Zhongshu No.5	*Pisum sativum*	Enhanced tolerance to drought, UV-B heat, and chilling stresses, increase in APX activity	[200, 201]
APX3	*Nicotiana tabacum*	*Arabidopsis thaliana*	Water deficit tolerance with higher photosynthesis	[204]
APX 2	*Nicotiana tabacum*	*Cucumber*	Increased drought tolerance due to reduced stomatal conductance	[62]

(continued)

Table 17.2 (continued)

Gene	Target transgenic	Gene source	Response in transgenic plants	References
POD	*Nicotiana tabacum*	*Ipomoea batatas*	Resistance to various abiotic (salt, mannitol, MV and H_2O_2) and biotic stress (*Phytophthora parasitica*)	[107]
Glutathione reductase (GR)				
GR	*Triticum Aestivum cv. Oasis*	*Escherichia coli*	Higher GSH content and GSH/GSH + GSSG ratio than control; no increase in SOD and GR activity	[130, 136]
GR	*Nicotiana tabacum*	*Arabidopsis thaliana*	Several oxidative stress tolerances including MV, water, and chilling stress tolerance.	[50]
GR + DHAR + GSH	*Nicotiana tabacum*	Brassica	Increased tolerance of the transgenic plants to a variety of abiotic stresses like MV-induced oxidative stress, salt, cold, and drought stresses	[115, 116]
GR	*Gossypium hirsutum* L.	*Nicotiana tabacum*	No oxidative stress tolerance	[125]
Monodehydroascorbate reductase (MDHAR)				
MDHAR1	*Nicotiana tabacum*	*Arabidopsis thaliana*	Ozone, salt, and PEG-induced drought stress tolerance due to higher MDAR activity and higher level of reduced AsA	[55]
Dehydroascorbate reductase (DHAR)				
DHAR	*Nicotiana tabacum*	*Arabidopsis thaliana*	Ozone and drought tolerance with higher DHAR activity and reduced AsA content	[54]
DHAR	*Solanum tuberosum*	*Arabidopsis thaliana*	Enhanced DHAR activity with faster growth, even under drought and salt stress conditions	[56]
Glutathione S-Transferase (GST)				
GST	*Nicotiana tabacum*	*Prosopis juliflora*	Survived better than control plants fewer than 15 % PEG-induced water stress	[72]
GST	*Nicotiana tabacum*	*Pyrus pyrifolia*	Transgenic tobacco lines showed relatively normal growth under drought, NaCl, and cadmium (Cd) stresses activity	[123]

(continued)

Table 17.2 (continued)

Gene	Target transgenic	Gene source	Response in transgenic plants	References
GST + GPX	*Nicotiana tabacum*	*Nicotiana tabacum*	Transgenic tobacco seedlings provide increased GSH-dependent peroxide scavenging that leads to reduced oxidative damage	[167]
GST	*Nicotiana tabacum*	Cotton	Increased activities of GST and GPX which strengthen the antioxidant defense of transgenic plants to resist the oxidative stress	[209]
GST	*Arabidopsis*	*Lycopersicon esculentum*	Enhanced resistance to salt and drought stress	[102]
Glutathione peroxidase (GPX)				
GPX2	*Arabidopsis thaliana*	*Synechcystis PCC 6803*	Tolerance to H_2O_2, Fe^{+2}, MV, chilling, high salinity or drought stresses	[67]

In conclusion, they found that coexpression of cytosolic antioxidant genes (SOD and APX) has only minor effects during drought, similar to those exhibited when expressing cytosolic APX alone. However, a partial protection of photosynthesis and membrane integrity was observed.

17.5.1.2 Catalases (CAT)

CATs are a tetrameric heme-containing enzyme which plays a pivotal role in directly dismutating H_2O_2 into H_2O and O_2 and is critical for ROS detoxification during stressed conditions [71]. CAT has one of the highest turnover rates of all enzymes: one molecule of CAT can convert 26 million molecules of H_2O_2 to H_2O and O_2 per minute. CAT is important in the removal of H_2O_2 generated in peroxisomes by oxidases involved in beta-oxidation of fatty acids, photorespiration, and purine catabolism. The CAT isozymes have been studied extensively in higher plants [158]. It has also been reported that apart from reaction with H_2O_2, CAT also react with some hydroperoxides such as methyl hydrogen peroxide [6]. Azpilicueta et al. [19] reported that incubation of *H. annuus* leaf discs with 300 and 500 mM $CdCl_2$ under light conditions increased the CATA3 transcript level but this transcript was not induced by Cd in etiolated plants. Moreover, in roots of the transgenic CAT-deficient tobacco lines (CAT 1AS), the DNA damage induced by Cd was higher than in wild-type tobacco roots [73]. There are reports of the development of drought-tolerant transgenic plants overexpressing different CAT (Table 17.2). Transgenic tomato plants overexpressing *CAT2* showed an increase in CAT activity and thus enhanced tolerance to drought [206].

17.5.1.3 Ascorbate Peroxidase (APX)

APX plays an essential role in scavenging ROS and protecting cells in higher plants, algae, euglena, and other organisms. APX is involved in scavenging of H_2O_2 in water–water and AsA-GSH cycles and utilizes AsA as the electron donor. The APX enzyme family consists of at least five different isoforms including thylakoid (t-APX) and glyoxisome membrane forms (gm-APX), as well as chloroplast stromal soluble form (s-APX), cytosolic form (c-APX) [149]. APX has a higher affinity for H_2O_2 (mM range) than CAT and peroxidase (POD) and it may have a more crucial role in the management of ROS during stress. It has also been noted that overexpression of APX in *Nicotiana tabacum* chloroplasts enhanced plant tolerance to salt and water deficit [22]. Wang et al. [199–201] demonstrated the effect of increased cytosolic ascorbate peroxidase (cAPX) on drought, water deficit, chilling, and UV-B stress tolerance using transformed tomato (*Lycopersicon esculentum* cv. Zhongshu No. 5) plants. They showed in laboratory or field tests, the potential to enhance tolerance to drought, UV-B, and sunscald stress by gene transfer. Overexpression of *cAPX* in transgenic tomato enhanced resistance to heat (40 °C) and UV-B stress compared to wild-type plants. APX activity in leaves of *cAPX* transgenic plants was several-fold higher than in leaves of wild-type plants when exposed to heat, UV-B, and drought stresses. Tobacco (*Nicotiana tabacum* L.) plants were transformed to constitutively overexpress the *Arabidopsis thaliana* gene for *APX3* [204]. Following repeated water-deficit cycles, fruit number and seed mass of transgenic tobacco were significantly higher than those of control plants. Although these data did not support the idea that overexpression of the gene for APX3 enhances protection of the photosynthetic apparatus during water deficit, overexpression of *APX3* may affect other cellular metabolisms that result in higher CO_2 assimilation under moderate water-deficit conditions and therefore higher seed mass after repeated water-deficit treatments.

Kim et al. [107] demonstrated that transgenic tobacco plants overexpressing *swpa4* (sweet potato POD c-DNA) showed increased H_2O_2 production followed by the upregulation of multiple apoplastic acidic PR genes. The *swpa4* transgenic plants manifested significantly enhanced tolerance to a variety of abiotic and biotic stresses in the H_2O_2-regulated stress-response signaling pathway. In order to assess the effects of *swpa4* expression on drought stress tolerance in soil-grown whole plants, 2-month-old plants were not watered for 8 days, and then watered for 8 days for recovery. More bleaching and a greater loss of PSII photosynthetic efficiency was observed in the control plants compared to the transgenic plants. In addition, the tobacco leaf discs from transgenic lines 1 and 2 exhibited higher levels of tolerance in the presence of mannitol (drought inducing) and NaCl, as seen by assessments of lipid peroxidation and total Chl. On the basis of the above results, it was evident that the *swpa4* transgenic plants are more tolerant to drought H_2O_2, dehydration, and high salinity than the control plants. Overexpression of APX in transgenic plants conferred drought stress tolerance is presented in Table 17.2.

17.5.1.4 Glutathione Reductase (GR)

Glutathione reductase, also known as GSR or GR (EC 1.8.1.7), belongs to the family of NADPH-dependent oxidoreductase and occurs in both prokaryotic and eukaryotic organisms. Although GR is located in chloroplasts, cytosol, and mitochondria, more than 80 % of its activity in photosynthetic tissues was reported to be of chloroplastic isoform [16]. GR plays an essential central role in cell defense against reactive oxygen metabolites by efficiently maintaining the cellular reduced GSH pool through catalyzing the reduction of GSSG to GSH with the accompanying oxidation of NADPH [38]. GR is a flavo-protein oxidoreductase, found in both prokaryotes and eukaryotes [165]. It is a potential enzyme of the AsA–GSH cycle and plays an essential role in the defense system against ROS by sustaining the reduced status of GSH. It is localized predominantly in chloroplasts, but a small amount of this enzyme has also been found in mitochondria and cytosol [39, 53]. GR catalyzes the reduction of GSH (glutathione), a molecule involved in many metabolic regulatory and antioxidative processes in plants where GR catalyzes the NADPH-dependent reaction of the disulphide bond of GSSG and is thus important for maintaining the GSH pool [35, 163]. GSH plays an important role within the cell system, which includes participation in the AsA–GSH cycle, maintenance of the sulfhydryl (-SH) group, and a substrate for GSTs. GR and GSH play a crucial role in determining the tolerance of a plant under various stresses [35].

In addition to reports of differential modulation of GR in metal metalloids, salinity and drought stresses, increased GR activity has been widely observed in many plant species including *T. aestivum* [87], *Z. mays* [110], *Cucumis sativus* [41], *N. tabacum* [186], and *Phaseolus aureus* [111] under high temperature (HT) stress. In *N. tabacum*, coexpression of GR resulted in the increased tolerance of the transgenic plants to a variety of abiotic stresses such as MV-induced oxidative stress, salt, cold, and drought stresses [116]. This was due to the changes in enzyme activities, and levels or redox state of AsA and GSH. Contour-Ansel et al. [38] investigated the variations in GR gene expression in two cowpea (*Vigna unguiculata*) cultivars viz. EPACE-1 (drought-resistant) and 1183 (drought-sensitive) using reverse-transcription where two new cDNAs encoding a putative dual-targeted and a cytosolic GR gene were cloned and sequenced. Drought stress induced an upregulation of the expression of the cytosolic GR gene directly related to the intensity of the stress in both cultivars. Although a noticeable activation of the antioxidant metabolism was observed in both cultivars, the drought-tolerant cultivar responded faster than the sensitive cultivar under a fast desiccation. Moreover, exogenous ABA enhanced significantly the activity and expression levels of GR in both cultivars after treatment for 24 h. In *Proteus vulgaris*, two cDNAs of the enzyme GR encoding a dual targeted isoform (dtGR) and a cytosolic isoform (cGR) were cloned and their expression under drought stress was observed [189]. Moderate drought stress induced an upregulation of the expression of cGR in the susceptible cultivars, whereas dtGR expression decreased. However, in tolerant cultivars the expression remained stable suggesting that that moderate drought stress may lead to a hardening process and tolerance.

Overexpression of a eukaryotic GR from *B. campestris* (*BcGR*) and *E. coli* GR (*EcGR*) was studied in *E. coli* in pET-28a. It was found that BcGR overproducing *E. coli* showed better growth and survival rate than the control but far better growth was noted in *E. coli* strain transformed with the inducible EcGR in the presence of oxidative stress, water stress, and Cd [208]. In an interesting study, transgenic *N. tabacum* with 30–70 % less GR activity were used to find out the possible mechanism of GR against oxidative stress. Transgenic plants with less GR activity showed enhanced sensitivity to oxidative stress. It was suggested that GR plays an important role in the regeneration of GSH and thus protects against oxidative stress also by maintaining the AsA pool [50]. Shu et al. [175] isolated the tomato chloroplast GR gene (*LeGR*) and produced antisense transgenic tomato lines that showed depletion of tomato chloroplast GR. Further investigation revealed that transgenic plants accumulated more H_2O_2, GSSG whereas GSH content decreased in transgenic plants with no changes in total GSH. These seedlings also showed worse performance in terms of physiological parameters. On the other hand, WT plants showed higher activities of enzymes and synthesis of metabolites. Transgenic plants that produce GR have been found to be abiotic stress tolerant (Table 17.2).

17.5.1.5 Monodehydroascorbate Reductase (MDHAR) and Dehydroascorbate Reductase (DHAR)

MDHAR is a flavin adenin dinucleotide (FAD) enzyme that is present as chloroplastic and cytosolic isozymes. MDHAR exhibits a high specificity for monodehydroasorbate (MDHA) as the electron acceptor, preferring NADH rather than NADPH as the electron donor. Asada [14] studied the multistep reduction of FAD in detail. The first step is the reduction of the enzyme-FAD to form a charge transfer complex. The reduced enzyme donates electrons successively to MDHA, producing two molecules of ascorbate via a semiquinone form [E-FAD-NADP(P)]. It is well established that the disproportionation by photoreduced ferrodoxin (redFd) in the thylakoids is of great importance. Because red Fd can reduce MDHA more effectively than NADP, MDHAR cannot participate in the reduction of MDHA in the thylakoidal scavenging system. Therefore, MDHAR only function in the presence of NAD(P)H [14]. Accompanying APX, MDHAR is also located in peroxisomes and mitochondria, where it scavenges H_2O_2 [44]. Similarly, DHAR regenerates ASH from the oxidized state and regulates the cellular ASH redox state (which is crucial for tolerance to various abiotic stresses) leading to the production of ROS. Eltayeb et al. [55] reported the overexpression of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt, and polyethylene glycol-induced drought stresses. To examine whether an overexpressed level of MDAR could minimize the deleterious effects of environmental stresses, they developed transgenic tobacco plants overexpressing the *Arabidopsis thaliana* MDAR gene (*AtMDAR1*) in the cytosol. Incorporation of the transgene in the genome of tobacco plants was confirmed by PCR and Southern-blot analysis and its expression was confirmed by Northern and Western-blot analyses. These transgenic

plants exhibited up to 2.1-fold higher MDAR activity and a 2.2-fold higher level of reduced AsA compared to nontransformed control plants. The transgenic plants showed enhanced stress tolerance in term of significantly higher net photosynthesis rates under ozone, salt, and polyethylene glycol stresses and greater PSII effective quantum yield under ozone and salt stresses. Furthermore, these transgenic plants exhibited a significantly lower hydrogen peroxide level when tested under salt stress. These results demonstrate that an overexpressed level of MDAR properly confers enhanced tolerance against ozone, salt, and PEG stress. In addition, Eltayeb et al. [54] also showed that the transgenic tobacco overexpressing dehydroascorbate reductase in cytosol showed enhanced tolerance to ozone and drought stresses. In order to examine the protective role of DHAR against oxidative stress, we developed transgenic tobacco plants overexpressing the cytosolic *DHAR* gene from *Arabidopsis thaliana*. Incorporation of the transgene in the genome of tobacco plants was confirmed by polymerase chain reaction and Southern blot analysis, and its expression was confirmed by Northern and Western blot analyses. These transgenic plants exhibited 2.3–3.1-fold higher DHAR activity and 1.9–2.1-fold higher level of reduced AsA compared with nontransformed control plants. The transgenic plants showed maintained redox status of AsA and exhibited an enhanced tolerance to ozone, drought, salt, and polyethylene glycol stresses in terms of higher net photosynthesis. In this study, we report for the first time that the elevation of the AsA level by targeting DHAR overexpression in cytosol properly provides a significantly enhanced oxidative stress tolerance imposed by drought and salt. Similarly, transgenic potato plants overexpressing the *Arabidopsis* DHAR gene in the cytosol exhibited enhanced DHAR activity with faster growth, even under drought and salt stress conditions [56]. Overexpression of MDAR and DHAR in transgenic plants has been summarized in Table 17.2.

17.5.1.6 Glutathione S-Transferases (GST)

The plant glutathione transferases, formerly known as glutathione S-transferases (GST, EC 2.5.1.18) are a large and diverse group of enzymes that catalyze the conjugation of electrophilic xenobiotic substrates with the tripeptide glutathione. Glutathione S-transferases (GSTs) are ubiquitous enzymes in animals and plants, and they are multifunctional proteins encoded by a large gene family. GSTs are involved in response to the oxidative stress including drought, salt, heavy metals, and so on. Under oxidative stress, the excessive ROS induce an increase in GST levels, and then the GSTs metabolize the toxic products of lipid peroxidation, damaged DNA, and other molecules [51].

Overexpression of GST in transgenic plants has been summarized in Table 17.2. It has also been found that GST overexpression also enhances plant tolerance to various abiotic stresses. Liu et al. [123] isolated and characterized a full-length cDNA of a novel zeta GST gene, *PpGST*, from fruit of *Pyrus pyrifolia* Nakai cv. Huobali and the same gene was successfully integrated into the genome of the transgenic tobacco lines and expressed. Growth of T1 generation plants of *PpGST*

transgenic lines and WT under nonstressful conditions was similar, however, the transgenic tobacco lines showed relatively normal growth under drought, NaCl, and cadmium (Cd) stresses. Furthermore, the T1 transgenic tobacco lines showed a significantly slower superoxide anion production rate than the WT under abiotic stress. Simultaneously, the MDA content of each T1 transgenic tobacco plant was only slightly increased and significantly less than that of the WT under drought, salt, and Cd stress. Together with the GST activity of the transgenic tobacco lines, which was significantly increased under stressful conditions, as compared with that in WT, overexpression of *PpGST* in tobacco enhanced the tolerance of transgenic tobacco lines to oxidative damage caused by drought, NaCl, and Cd stresses. Transgenic tobacco seedlings overexpressing GST and GPX showed enhanced seedling growth under a stressed environment. Additionally, a significant increase in MDHAR activity, GSH, and ASH content along with GST and GPX has also been noted in transgenic GST/GPX expressing (*GSTþ*) seedlings than WT. These results indicated that overexpression of GST/GPX in transgenic tobacco seedlings provides increased GSH-dependent peroxide scavenging and alterations in GSH and ASH metabolism that lead to reduced oxidative damage [167]. Transgenic tobacco plants overexpressing Gstcr1 showed significant increase in the activities of GST and GPX which strengthen the antioxidant defense of transgenic plants to resist oxidative stress [209]. Transgenic tobacco plants overexpressing *Prosopis juliflora* GST (*PjGSTU1*) survived better than control plants with fewer than 15 % undergoing PEG stress. Furthermore, GFP fusion studies revealed the presence of PjGSTU1 in the chloroplast of transgenic plants that was correlated with its role in ROS removal [72, 64]. Very recently, Jing et al. [102] generated transgenic *Arabidopsis* overexpressing tomato glutathione S-transferase designated *LeGSTU2* that showed enhanced resistance to salt and drought stress. The increased tolerance of transgenic plants was correlated with the changes in proline, malondialdehyde, and antioxidative emzyme activities.

17.5.1.7 Glutathione Peroxidase (GPX)

GPXs (EC 1.11.1.9) are a large family of diverse isozymes that use GSH to reduce H_2O_2 and organic and lipid hydroperoxides, and therefore help keep plant cells from oxidative stress [150, 151]. Millar et al. [131] identified a family of seven related proteins in cytosol, chloroplast, mitochondria, and endoplasmic reticulum, named AtGPX1–AtGPX7 in *Arabidopsis*. Recently, Yang et al. [205] introduced the radish phospholipid hydroperoxide GPX gene (RsPHGPx) into a yeast PHGPx-deletion mutant and found that it significantly rescued the growth of the recombinant cell exposed to linolenic acid, indicating a similar role to the yeast PHGPx3 gene (ScPHGPx3) in protection of membrane. Glutathione peroxidase (GPX)-like proteins (GPX-1 and GPX-2) of Synechocystis PCC 6803 (S. PCC 6803) reduce unsaturated fatty acid hydroperoxides using NADPH, but not reduced glutathione (GSH), as an electron donor. Gaber et al. [67] generated transgenic *Arabidopsis* plants overexpressing S. PCC 6803 GPX- 2 in the cytosol (AcGPX2)

or chloroplasts (ApGPX2). Both transgenic lines (AcGPX2 and ApGPX2) showed enhanced tolerance to oxidative damage caused by treatment with H_2O_2, Fe ions, or methylviologen, and environmental stress conditions, such as chilling with high light intensity, high salinity, or drought. The degree of tolerance of the transgenic plants to all types of stress was correlated with the levels of lipid peroxide suppressed by the overexpression of S.PCC 6803 GPX-2. Under conditions of oxidative stress due to the H_2O_2 treatment, the NADPH/(NADP:NADPH) ratio in the transgenic plants was lower than that in the wild-type plants. This indicated that the expression of S. PCC6803 GPX-2 contributes to the reduction in unsaturated fatty acid hydroperoxides using NADPH in situ under stress conditions in the transgenic plants. Overexpression of GPX has been found to enhance drought stress tolerance in transgenic plants (Table 17.2).

17.5.2 Nonenzymatic Antioxidants

17.5.2.1 Glutathione (GSH)

Tripeptide glutathione (glu-cys-gly; GSH) is one of the crucial metabolites in plants that are considered to be the most important intracellular defense against ROS-induced oxidative damage. It occurs abundantly in reduced form (GSH) in plant tissues and is localized in all cell compartments such as cytosol, endoplasmic reticulum, vacuole, mitochondria, chloroplasts, and peroxisomes, as well as in apoplast [101, 134] and plays a central role in several physiological processes, including regulation of sulfate transport, signal transduction, conjugation of metabolites, detoxification of xenobiotics [146], and the expression of stress-responsive genes. It is well established that GSH also plays an important role in several growth and development related events in plants, including cell differentiation, cell death and senescence, pathogen resistance, and enzymatic regulation [162]. GSH provides a substrate for multiple cellular reactions that yield GSSG (i.e., two glutathione molecules linked by a disulfide bond). The balance between the GSH and GSSG is an indispensable component in maintaining a cellular redox state. GSH is necessary to maintain the normal reduced state of cells so as to counteract the inhibitory effects of ROS-induced oxidative stress [66]. It is a potential scavenger of $^1O_2$, H_2O_2 [31] and the dangerous ROS such as $OH^{\bullet}$ [103]. Moreover, GSH plays a key role in the antioxidative guard system by regenerating another potential water-soluble antioxidant such as AsA, via the ASH–GSH cycle [63]. It has been reported that when the intensity of a stress increases, GSH concentrations usually decline and the redox state becomes more oxidized, leading to deterioration of the system [188]. GSH is particularly important in plant chloroplasts because it helps to protect the photosynthetic apparatus from oxidative damage. Overexpression of a chloroplast-targeted γ-glutamylcysteine synthetase (*γ-ECS*) in transgenic tobacco plants resulted in a threefold increase in GSH level [40].

17.5.2.2 Ascorbic Acid (AsA)

Ascorbic acid is the most abundant, powerful, and water-soluble antioxidant and it acts to prevent or minimize the damage caused by ROS in plants [18, 181]. It occurs in all plant tissues, usually being higher in photosynthetic cells and meristems (and some fruits). Its concentration is reported to be highest in mature leaves with fully developed chloroplast and highest chlorophyll. It has been reported that AsA mostly remain available in reduced form in leaves and chloroplast under normal physiological conditions [180]. About 30–40 % of the total ascorbate is in the chloroplast and stromal concentrations as high as 50 mM have been reported [66]. In plants, mitochondria play a central role in the metabolism of AsA. Plant mitochondria do not only synthesize AsA by L-galactono-g-lactone dehydrogenase but also take part in the regeneration of AsA from its oxidized forms [185]. The regeneration of AsA is extremely important because fully oxidized dehydroascorbic acid has a short half-life and would be lost unless it is reduced back. AsA is considered as a most powerful ROS scavenger because of its ability to donate electrons in a number of enzymatic and nonenzymatic reactions. It can provide protection to membranes by directly scavenging the $O_2^{\cdot-}$ and $OH^{\cdot}$ and by regenerating α-tocopherol from the tocopheroxyl radical. In chloroplast, AsA acts as a cofactor of violaxantin de-epoxidase thus sustaining dissipation of excess excitation energy [169]. In addition to the importance of AsA in the AsA–GSH cycle, it also plays an important role in preserving the activities of enzymes that contain prosthetic transition metal ions [149]. The AsA redox system consists of L-AsA, MDHA, and DHA. Both oxidized forms of AsA are relatively unstable in aqueous environments whereas DHA can be chemically reduced by GSH to AsA [63].

Plants with higher ascorbate content can effectively scavenge the excessive ROS generated during stress conditions, and confer increased tolerance to abiotic stresses. Increased abiotic stress sensitivity of the *Arabidopsis* vtc mutant is attributed to the low intrinsic ascorbate levels and impaired ascorbate–glutathione cycle, which resulted in an enhanced ROS activity and a significant decrease in the CO_2 assimilatory capacity [97]. Moreover, deficiency of ascorbate may limit the recycling of α-tocopheroxyl radicals to α-tocopherol, which may, in turn, increase the oxidation of thylakoid membrane lipids in drought conditions [143]. Several transgenic plants overproducing ascorbate showed an enhanced salt and drought tolerance with reduced membrane lipid peroxidation and chlorophyll content loss. These plants also exhibited a higher survival rate under stress conditions and a significantly higher seed germination rate, fresh weight, and root length (Wang et al. [199, 200]; Sun et al. [184, 211]. Transgenic potato plants expressing the strawberry *GalUR* gene and rat *GLOase* gene with several-fold increased biosynthesis of AsA also exhibited a better survival under salinity and drought stress conditions including a reduction in the level of lipid peroxidation [90, 91, 92, 192].

17.5.2.3 Proline (Pro)

Proline (an osmolyte) is considered to be a potent antioxidant and potential inhibitor of programmed cell death. Hence, Pro are now regarded as nonenzymatic antioxidants that plant microbes and animals, require to moderate the adverse effects of ROS [37]. The synthesis of L-Pro from L-glutamic acid via D1-pyrroline-5-carboxylate (P5C) is catalyzed by the activities of the enzymes D1-pyrroline-5-carboxylate synthetase (P5CS) and D1-pyrroline-5-carboxylate reductase (P5CR) in plants [196]. On the other hand, mitochondrial enzymes Pro dehydrogenase (oxidase; ProDH) and P5C dehydrogenase (P5CDH) metabolize L-Pro into L-Glu via P5C. It has been established that following salt, drought, and metal stress, the enhanced accumulation of Pro may be due to increased synthesis or decreased degradation. Free Pro has been anticipated to play a role as an osmoprotectant, a protein stabilizer, a metal chelator, an inhibitor of lipid peroxidation, and/or $OH^{\bullet}$ and $^{1}O_2$ scavenger [17, 95, 191]. Pro, mannitol, sorbitol, and myo-inositol have been tested for $OH^{\bullet}$ scavenging capacity and out of these, the Pro seemed to be more effective scavenger of $OH^{\bullet}$ [178]. Thus, Pro is not only a vital molecule in redox signaling, but also an operative quencher of ROS formed under salt, metal, and dehydration stress conditions in plants and algae [5]. It was suggested that the ability of Pro to scavenge ROS and ability to inhibit ROS-mediated apoptosis can be an important function in response to cellular stress. Increased accumulation of Pro has been correlated with improved tolerance to various abiotic stresses especially salt and drought. Enhanced synthesis of Pro under drought or salt stress has been implicated as a mechanism to alleviate cytoplasmic acidosis and maintain NADP:NADPH at values compatible with metabolism [81]. An additional advantage of the refilling of NADP supply by Pro synthesis may be to support redox cycling, which is especially important in plant antioxidant defense mechanisms during stress. There is now enough evidence suggesting the important role for Pro synthesis in potentiating pentose–phosphate pathway activity, as this pathway is a most important component of antioxidative defense mechanisms, which need NADPH to maintain GSH and ASH in the reduced state.

It has also been found that overexpression of Pro biosynthetic pathway genes enhance abiotic stress tolerance in transgenic plants. Su and Wu [183] reported that both constitutive expression and stress-inducible expression of the P5CS cDNA in transgenic *O. sativa* have led to the accumulation of P5CS mRNA and Pro which resulted in higher salt and water deficiency stress tolerance. Vendruscolo et al. [195] reported the effects of water deficit on wheat plants transformed with the *Vigna aconitifolia* D1-pyrroline-5-carboxylate synthetase (P5CS) cDNA that encodes the key regulatory enzyme in proline biosynthesis, under the control of a stress-induced promoter complex-AIPC. Transgenic wheat plants submitted to 15 days of water shortage presented a distinct response. They found that drought resulted in the accumulation of proline. The tolerance to water deficit observed in transgenic plants was mainly due to protection mechanisms against oxidative stress and not caused by osmotic adjustment. Molinari et al. [135] reported the evaluation of the stress-inducible production of proline in transgenic sugarcane. After 9 days without

irrigation, proline content in transgenic events was on the average 2.5-fold higher than in controls. However, no osmotic adjustment was observed in plants over-producing proline during the water-deficit period. The photochemical efficiency of PSII observed was higher (65 %) in the transgenic events at the end of the water-deficit experiment. The effects of proline on lipid peroxidation as MDA levels and on the decline of Chl in leaf discs along the drought period suggest that proline protected the plants against the oxidative stress caused by the water deficit. The overall capacity of transgenic plants to tolerate water-deficit stress could be assessed by the significantly higher biomass yields 12 days after withholding water. These results suggested that stress-inducible proline accumulation in transgenic sugarcane plants under water-deficit stress acts as a component of an antioxidative defense system rather than as an osmotic adjustment mediator. Yamada et al. [203] transformed petunia plants with Δ^1-pyrroline-5-carboxylate synthetase genes (*AtP5CS* from *Arabidopsis thaliana* L. or *OsP5CS* from *Oryza sativa* L.). The transgenic plants accumulated Pro and their drought tolerance was tested. The Pro content amounted to 0.57–1.01 % of the total amino acids in the transgenic plants, or 1.5–2.6 times that in wild-type plants grown under normal conditions. The transgenic plant lines tolerated 14 d of drought stress, which confirms that both *P5CS* transgenes had full functionality. Exogenous L-Pro treatment caused the plants to accumulate Pro; plants treated with 5 mM L-Pro accumulated up to 18 times more free Pro than untreated plants. Exogenous L-Pro restricted the growth of wild-type petunias more than that of *Arabidopsis* plants. The capacity for free Pro accumulation might depend on the plant species. The growth of petunia plants was influenced not only by the Pro concentration in the plants, but by the ratio of the Pro content to the total amino acids, because the growth of the transgenic petunia plants appeared normal. Simon-Sarkadi et al. [176] reported stress-induced changes in the free amino acid composition in transgenic soybean plants having increased proline content. Following drought stress at supraoptimal temperature the increase in proline content in transgenic (T) soybean [*Glycine max* (L.) Merr. cv. Ibis] plants overexpressing the gene coding for the last enzyme of Pro biosynthesis, L-Δ^1-pyrroline-5-carboxylate reductase, was much greater than in wild-type (W) plants (105-fold vs. 19-fold after 7 d). The results indicate that manipulating the content of a single amino acid influences the whole free amino acid composition in soybean.

17.5.2.4 α-Tocopherols (Vitamin E)

Tocopherols are lipid-soluble antioxidants and considered as potential scavengers of ROS and lipid radicals [93]. Tocopherols are also considered as a major antioxidant in biomembranes, where they play both antioxidant and nonantioxidant functions. Tocopherols are considered general antioxidants for protection of membrane stability, including quenching or scavenging ROS like $^1O_2$. Tocopherols are confined in plants in the thylakoid membrane of chloroplasts. Out of four isomers of tocopherols (α, β, γ, £) found in plants, α -tocopherol has the highest antioxidative activity due to the presence of three methyl groups in its molecular structure [105]. It is synthesized

from β-tocopherol in chloroplasts by g-tocopherol methyltransferase (*γ-TMT*; VTE4). A high level of α-tocopherol has been found in the leaves of many plant species including Arabidopsis but these are low in γ-tocopherol. It has been found that nitration of γ-tocopherol is considered to be an important mechanism for the regulation and detoxification of NOx in animal tissues. Germinating seeds of *Brassica napus*, *N. tabacum*, and *A. thaliana* also showed the presence of 5-NγT. It can be said that γ-tocopherol or 5-NγT prolongs early development by reducing NOx concentration [49]. Tocopherol has been shown to prevent the chain propagation step in lipid auto-oxidation which makes it an effective free radical trap. Additionally, it has been estimated that one molecule of α-tocopherol can scavenge up to 120 $^1O_2$ molecules by resonance energy transfer [144]. Different plant studies have indicated the positive relationship between tocopherol biosynthesis/accumulation and water stress [142]. Several reports have shown there is a remarkable elevation of α-tocopherol under water-deficit conditions in pea [139, 187], wheat and cereals [25, 160], rosemary [142], and lavender. Liu et al. [122] observed that transgenic tobacco plants overexpressing the vte1 gene enhanced α-tocopherol synthesis resulting in better protection to water deficiency; this was also associated with upregulated antioxidant defense such as decreased lipid peroxidation, electrolyte leakage, and H_2O_2 levels. However, severe drought stress (30 % PEG) resulted in a loss of α-tocopherol in rice chloroplast [29].

Successful efforts to improve plant performance against drought through engineering tocopherol level and composition have been reported in the literature. Liu et al. [122] reported enhanced tolerance to drought stress in transgenic tobacco plants overexpressing VTE1 for increased tocopherol production from *Arabidopsis thaliana.* Yusuf et al. [210] and Kumar et al. [112] observed that α-tocopherol-enriched transgenic *B. juncea* plants constitutively overexpressing the *γ-TMT* gene showed enhanced tolerance to drought stress (200 mM mannitol) compared to wild-type plants. Transgenic plants showed enhanced activities of SOD, CAT, APX, and GR and decreased the levels of MDA, H_2O_2, and electrolyte leakage compared to wild type. Their findings implicated the role of higher α-tocopherol levels in conferring better tolerance against salt, heavy metal, and osmotic stresses and also established the existence of interplay between this lipid-soluble antioxidant and other water-soluble components of plant antioxidant defense. In the investigation with Arabidopsis plants Cela et al. [34] observed a 3.6- and 13.5-fold increase in the level of α- and γ-tocopherol, respectively, under water deficiency. However, some important genes (e.g., VTE2, VTE1, and VTE4) responsible for tocopherol biosynthesis did not change significantly [34]. Espinoza et al. [57] reported that tobacco seedlings overexpressing VTE2.1, which encodes the enzyme HPT, can catalyze the prenylation step in tocopherol biosynthesis under drought stress. The elevated level of α- tocopherol may enhance photosynthetic efficiency and lower lipid peroxidation leading to better oxidative protection.

17.6 Conclusion and Future Perspectives

Drought stress is a complex phenomenon and plants cope with drought stress via several morphological, anatomical, and biochemical adaptations at the cellular and organelle levels. A better understanding of the effects of drought on plants is therefore essential in order to establish improved management practices and breeding efforts in agriculture, and to allow prediction of the fate of natural vegetation under severe climate change. Coordinated approaches involving traditional plant breeding, along with molecular approaches, should be followed to identify and cultivate drought-tolerant varieties. It is well documented that drought stress leads to the overproduction of ROS in plants which is highly reactive and toxic and ultimately results in oxidative stress. Overall, the involvement of ROS in various metabolic processes in plant cells might have general implications. Oxidative stress is a condition in which ROS or free radicals are generated at the cellular level, which can exert their toxic effects on the cells. These species may affect cell membrane properties and cause oxidative damage to nucleic acids, lipids, and proteins that may make them nonfunctional. Different exogenous protectants, such as osmoprotectants (proline, glycine betaine, trehalose, etc.), plant hormones (gibberellic acids, jasmonic acid, brassinosteroids, salicylic acid, etc.), antioxidants (AsA, GSH, tocopherols, etc.), signaling molecules (NO, H_2O_2, etc.), and polyamines (spermidine, spermine, putrescine, etc.), have been found effective in mitigating drought-induced damage in plants. Crop plants that are tailored to have improved capacity for biosynthesis or bioaccumulation of these protectants might show enhanced drought tolerance. It is well known that plant cells and their organelles such as chloroplasts, mitochondria, and peroxisomes employ antioxidant defense systems to protect themselves against ROS-induced oxidative stress. A great deal of research has also established that the induction of the cellular antioxidant machinery is important for protection against ROS and ROS-induced cellular damage. Overexpression of ROS scavenging enzymes including isoforms of SOD (Mn-SOD, Cu/Zn-SOD, Fe-SOD), CAT, APX, GR, DHAR, GST, and GPX resulted in drought stress tolerance in various crop plants due to efficient ROS scavenging capacity. However, pyramiding of ROS scavenging enzymes may also be used to obtain durable resistance to drought stress. Therefore, transgenic plants have tremendous potential in the near future to mitigate drought stress as well as to increase the quality of plant products.

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