Tariq Aftab
Khalid Rehman Hakeem *Editors*

Antioxidant Defense in Plants

Molecular Basis of Regulation

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Preface

Environmental stresses, such as drought, salinity, or floods, induce the generation of reactive oxygen species (ROS) which causes severe damage to cell membrane integrity by accelerating lipid peroxidation. Growing evidence has suggested that ROS play a critical role as the signaling molecules throughout the entire cell death pathway. Though ROS act as a signaling molecule, they can cause oxidative burst if there is an imbalance between ROS generation and their scavenging. Oxidative stresses also decrease the efficiency of PSI and PSII by disrupting the electron transport chain and chloroplast integrity. Moreover, under severe stress conditions, the generation of ROS often exceeds beyond the antioxidant potential of the plants, resulting in oxidative damages. To counteract the detrimental effect of ROS, plants are inherited with an intricate and vibrant antioxidant defense system, composed of enzymatic (catalase, peroxidase, superoxide dismutase, glutathione reductase, glutathione S-transferase, guaiacol peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, etc.) and nonenzymatic (glutathione, ascorbate, α-tocopherol, carotenoids, flavonoids, etc.) antioxidants, which scavenge and/or reduce excess ROS and improve plant tolerance to abiotic stresses. Stress tolerance in most crop plants is positively correlated with an efficient oxidative system. Therefore, studying the efficiency of antioxidant defense systems in plants is necessary for facilitating the plant's nature of adaptation against abiotic stresses.

Knowledge about the oxidative mechanisms in plants may contribute to the development of plants, adapted to the environment and resistant to pathogens. During the last decades, antioxidant enzymes have been used to develop transgenic plants that have increased tolerance to several stresses. The ROS production, major antioxidant enzymes as well as nonenzymatic antioxidants involved in detoxification, and defense under stresses are the major areas to be elucidated.

The book comprises 20 chapters (review articles) written by experts, highlighting the various enzymatic and nonenzymatic antioxidants, defense mechanisms, and adaptation strategies employed by plants to avoid the stressful conditions. We are hopeful, this volume would furnish the need of all researchers who are working or have interest in this particular field.

We are highly grateful to all our contributors for accepting our invitation and for not only sharing their knowledge and research but also venerably integrating their expertise in dispersed information from diverse fields in composing the chapters and enduring editorial suggestions to finally produce this venture. We also thank Springer-Nature team for their generous cooperation at every stage of the book production.

Lastly, thanks are also due to well-wishers, research students, and editors' family members for their moral support, blessings, and inspiration in the compilation of this book.

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Tariq Aftab received his Ph.D. in the Department of Botany at Aligarh Muslim University, India, and is currently an Assistant Professor there. He is the recipient of a prestigious Leibniz-DAAD fellowship from Germany, Raman Fellowship from the Government of India, and Young Scientist Awards from the State Government of Uttar Pradesh (India) and Government of India. After completing his doctorate, he has worked as Research Fellow at National Bureau of Plant Genetic Resources, New Delhi, and as Post-doctorate Fellow at Jamia Hamdard, New Delhi, India. Dr. Aftab also worked as Visiting Scientist at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, and in the Department of Plant Biology, Michigan State University, USA. He is a member of various scientific associations from India and abroad.

He has edited 15 books with international publishers, co-authored several book chapters, and published over 75 research/review papers in peer-reviewed international journals. His research interests include physiological, proteomic, and molecular studies on medicinal and crop plants.

Khalid Rehman Hakeem, Ph.D. is Professor at King Abdulaziz University, Jeddah, Saudi Arabia. After completing his doctorate (Botany; specialization in Plant Eco-physiology and Molecular Biology) from Jamia Hamdard, New Delhi, India, in 2011, he worked as a lecturer at the University of Kashmir, Srinagar, for a short period. Later, he joined Universiti Putra Malaysia, Selangor, Malaysia, and worked there as Post-doctorate Fellow in 2012 and Fellow Researcher (Associate Prof.) from 2013 to 2016. Dr. Hakeem has more than 10 years of teaching and research experience in plant eco-physiology, biotechnology and molecular biology, medicinal plant research, plant–microbe–soil interactions as well as in environmental studies. He is the recipient of several fellowships at both national and international levels; also, he has served as the visiting scientist at Jinan University, Guangzhou, China. Currently, he is involved with a number of international research projects with different government organizations.

So far, Dr. Hakeem has authored and edited more than 70 books with international publishers. He also has to his credit more than 140 research publications in peer-reviewed international journals and 60 book chapters in edited volumes with international publishers.

At present, Dr. Hakeem serves as an editorial board member and reviewer of several high-impact international scientific journals.

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An Overview of Roles of Enzymatic and Nonenzymatic Antioxidants in Plant

1

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Abstract

The postindustrial age radically altered global climate conditions, posing a difficult task for plants and crops to thrive under stress environments like high temperatures, salt, waterlogging, heavy metals, drought, and so on. A small period of poor weather had a substantial impact on the development and growth of plants, eventually influencing crop quality, yield, and agricultural sustainability as a whole. Plant cells produce free oxygen (O_2) radicals and their derivatives, known as reactive oxygen species (ROS), as by-products of other reactions in such hostile environments. Furthermore, these ROS molecules are used as signaling molecules in plants for signal transduction in response to changing environmental conditions. The cytoplasmic balance that triggers the antioxidant defense mechanisms is disrupted as a result of the excessive accumulation of ROSs inside the cell. Plants have developed a complicated ROS scavenging system to avoid sensitive cellular components from being damaged

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by reactive oxygen species. Enzymatic antioxidants, like catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), glutathione peroxidases (GPX), and ascorbate peroxidases (APX), and nonenzymatic antioxidants, like glutathione, ascorbate, tocopherols, and phenolic compounds, are important antioxidants that play key roles in eliminating superoxide (O_2^-) and (H_2O_2). The antioxidant capacity of plants is the sum of the activities of all enzymatic and nonenzymatic antioxidant systems. This chapter seeks to provide fundamental information on enzymatic and nonenzymatic antioxidants, their occurrence, characteristics, and the antioxidant defense system involved in reactive oxygen species (ROS) detoxification under various stresses, as well as their interactions with cellular components.

Keywords

ROS · Abiotic stress · Oxidative stress · Enzymatic antioxidants · Nonenzymatic antioxidants

1.1 Introduction

Based on their biochemical nature, antioxidants are divided into two categories: enzymatic and nonenzymatic. While substantial anabolic and catabolic reactions are occurring, these chemicals are engaged in the detoxification of free radicals or reactive oxygen species (ROS). Both classes of antioxidants are capable of efficiently neutralizing ROS and converting them into relatively stable nontoxic molecules, preventing oxidative damage to cellular apparatuses. As a result, antioxidants are the most important first line of defense against the oxidative stress-induced cell damage. Antioxidants, both enzymatic and nonenzymatic, are electron-rich compounds that readily share electrons with highly energetic ROS and free radicals, stabilizing cellular randomness.

Furthermore, they may interfere with the oxidizing chain reaction in order to reduce free radical damage (Apel and Hirt 2004). Antioxidants are also known as ROS scavengers because they use dynamic and synergistic processes to keep the intracellular concentration of ROS in check. It (antioxidant) is a substance that may scavenge reactive oxygen species (ROS) without being converted into a harmful radical (Noctor and Foyer 1998). As a result, antioxidant enzymes are crucial for sustaining good cellular and systemic health and well-being. All highly reactive, oxygen-containing molecules, including the free radicals, are referred to as ROS. The hydroxyl radical (OH^-), singlet oxygen, superoxide anion radical, H_2O_2 (hydrogen peroxide), hypochlorite radicals, lipid peroxides, and nitric oxide radical are all examples of reactive oxygen species. All have the ability to react with membrane lipids, enzymes, and other molecules, resulting in the loss of critical cellular structures and functions and a variety of negative consequences for plants and animals. As previously stated, free radicals or ROS are highly reactive compounds that are released directly or as a by-product during normal metabolic processes in

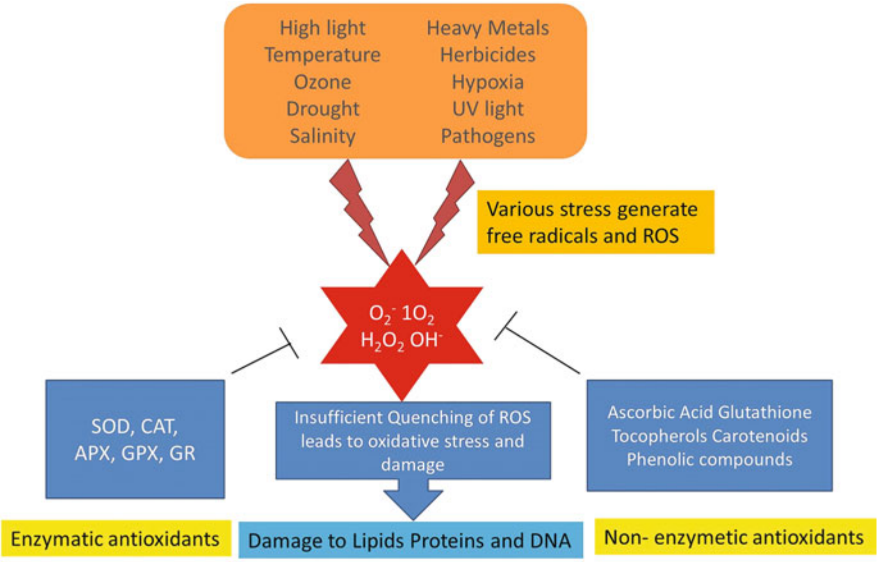

Fig. 1.1 Illustrative representation of different agents generating free radicals and reactive oxygen species in plants and different antioxidants scavenging agents

various cellular compartments like mitochondria, chloroplast, peroxisomes, and apoplast (Panieri and Santoro 2015), but their synthesis is accelerated during extreme conditions. Because these compounds have a lone pair of electrons, they are very unstable and thus highly reactive (Del Río and López-Huertas 2016). ROS-induced oxidative stress is a well-controlled process, and the balance in-between the ROS and its quenching define a plant's and any other organism's well-being. If antioxidants reach a condition of disequilibrium, meaning they are unable to destroy free radicals, the cell and tissue suffer oxidative damage. The degree of oxidative stress caused by free radicals/ROS is determined by their concentration, kind, synthesis site, and developmental stage (Møller et al. 2007). Plants exposed to ROS for a long time period can suffer considerable damage to their cell machinery and biomolecules, including protein oxidation, lipid peroxidation (Mittler 2002), PCD (programmed cell death), and inhibition of the enzymes and also damages nucleic acid, which can lead to tissue necrosis or plant death (Fig. 1.1) (Pérez-Pérez et al. 2012).

1.2 Enzymatic Antioxidants

Several enzymes, like GPX, SOD, glutathione reductase, APX, and CAT (catalase), are the enzymatic components of the antioxidant defense system found in diverse subcellular compartments. In plants under oxidative stress, these enzymatic antioxidant molecules are critical for maintaining cellular homeostasis.

1.2.1 SOD (Superoxide Dismutases)

SODs are enzymes which can catalyze the dismutation/partitioning of O_2^- to H_2O_2 and molecular oxygen (O_2). Because O_2^- is a typical consequence of oxygen metabolism, SOD is thought to be the first line of defense against the reactive O_2 species-induced damage. By neutralizing the superoxide radical, the Haber–Weiss reaction eliminates the possibility of hydroxyl ion production. These enzymes are classed as metalloenzymes based on metal cofactors, subcellular distribution, and protein folds. Cu-/Zn-containing SODs have been found in prokaryotic and eukaryotic organisms, and in plant cells, they can be found in the cytoplasm, extracellular space, or chloroplasts and can also be found in lysosomes, cytoplasm, and nuclear compartments in mammalian cells. Plant cytoplasm and chloroplasts have been found to have Fe-containing SODs. MnSODs are found in all kingdoms but particularly in eukaryotic mitochondria, where they play a critical role in preserving mitochondria by scavenging ROS (Pilon et al. 2011) and initiating cellular differentiation (Moller 2001, 2012). According to Feng et al. (2016), SODs are found in different organisms, such as mammals, yeast, bacteria, and plants. Multiple genes producing SODs exist in plants, and their expression can be influenced by developmental stage, tissue type, and environmental cues (Scandalios 2005; Menezes-Benavente et al. 2004). There are nine SOD genes in tomatoes, which are unevenly distributed across 12 chromosomes and include four Cu/ZnSODs, one MnSOD, and three FeSODs (Feng et al. 2016). Various investigations revealed that different types of SOD genes had varied levels of expression under harsh environmental conditions. Underwater stress, for example, the expression patterns of the banana genes like MaCSD1B and MaMSD1A, which are involved in SOD production, were utterly incompatible (Feng et al. 2015).

Furthermore, SODs with the same cofactor may not necessarily have the same role in various species. MnSOD expression was not affected by oxidative stress in *Arabidopsis*, but it was affected significantly by drought and cold stress in wheat and salt stress in pea (Baek and Skinner 2003). Additionally, alternative splicing and miRNAs have been implicated in the regulation of SOD gene expression (Lu et al. 2010). Various plant species, such as *Populus trichocarpa*, *Sorghum bicolor*, *Musa acuminata*, and *Arabidopsis thaliana*, have been found to contain the SOD genes (Srivastava et al. 2009).

1.2.2 CAT (Catalases)

These are tetrameric enzymes, with iron as a prosthetic group attached to each monomer; they catalyze the energy-efficient disproportionation of H_2O_2 into water molecules (H_2O) and molecular oxygen (Regelsberger et al. 2002; Zamocky et al. 2008). In contrast to H_2O_2, it has a lower affinity for R-O-O-R (organic peroxides) and also has a higher turnover rate among antioxidant enzymes and is unique among antioxidant enzymes because it does not need a reducing counterpart. Unfavorable conditions necessitate increased energy generation, and expenditure by plants and other organisms leads to an increase in catabolic reactions, which yields H_2O_2. CATs are predominantly attached to peroxisomes, where they execute β-oxidation of fatty acid, photorespiration, and purine catabolism, all of which produce a considerable amount of H_2O_2 (Mittler 2002). Hydrogen peroxide (H_2O_2) is also a key signaling molecule in plant development and plant response to the environment (Mhamdi et al. 2010). Plants have numerous CATs, each produced by a separate gene, that respond differently to different stressors that are known to cause ROS. Recent investigations reveal that CAT is also prevalent in other subcellular compartments like the mitochondria, chloroplast, and cytosol, albeit no evidence of substantial CAT activity has been observed (Mhamdi et al. 2010). CAT1 is mostly expressed in plant pollen and seeds; on the other side, CAT2 is not only expressed in photosynthetic tissues (mostly) but also expressed in seeds and roots, while CAT3 is predominantly expressed in vascular tissues and leaf in angiosperms (McClung 1997; Frugoli et al. 1996).

1.2.3 APX (Ascorbate Peroxidases)

Ascorbate peroxidases are the heme peroxidase superfamily that is involved in the recycling of AsA and the response to environmental stress in plants (Ishikawa and Shigeoka 2008; Lazzarotto et al. 2011). These enzymes catalyze the conversion of H_2O_2 to water and monodehydroascorbate (MDHA) using ascorbate (AsA) as an electron donor (Caverzan et al. 2012). Plants have five different isoforms of APX, which are found in diverse subcellular compartments like the cytosol, mitochondria, peroxisomes, and chloroplast (Sharma and Dubey 2005). These enzymes are divided into groups based on the amino acids they contain and the cell compartments in which they are found. Several abiotic stressors in plants have varied effects on the APX genes (Rosa et al. 2010; Caverzan et al. 2014). APXs, CATs, and SODs must be in balance to determine the effective intracellular level of H_2O_2 and oxygen, and variations in this equilibrium appear to trigger compensation processes (Apel and Hirt 2004; Scandalios 2002, 2005). Under a normal and stressful environment, APX is a key component of the ASC–GSH (ascorbate–glutathione) cycle, which eliminates excess H_2O_2 from plant cells (Mittler and Zilinskas 1991). Because APX is more broadly distributed and also has a higher affinity for hydrogen peroxide than CAT, it is a more effective H_2O_2 scavenger during stressful situations. APX is a chloroplastic isoenzyme expressed by a single gene in higher plants (apx1).

1.2.4 GPx (Glutathione Peroxidases)

The term glutathione peroxidases collectively describe the group of phylogenetically related nonheme and thiol-containing peroxidase enzymes. It was called after GPx-1, the mammalian tetrameric seleno enzyme, which was the first to be defined and reported. More than 700 members of the GPx family have been identified so far, overall domains of life. G. C. Mills discovered its activity in red blood cells in 1957, where it protected hemoglobin from oxidative degradation. The biological function of GPxs is to catalyze the H_2O_2 reduction or lipid hydroperoxides into H_2O or the corresponding alcohols by using GSH (glutathione) as a reducing agent. The catalytic moiety of selenium was later identified as selenocysteine, the 21st naturally occurring amino acid. Selenocysteine was recommended as a way to ensure a rapid reaction with the hydroperoxide and glutathione reducibility. GPx1 is found in the mitochondria, chloroplasts, and cytosol of a wide range of plants and animals, and it serves as an antioxidant in several cellular compartments. In human beings, there are eight distinct glutathione peroxidase isoforms (GPx1-8) that have been found. The mechanism of reaction comprises oxidation of the selenol of a selenocysteine residue by H_2O_2. The RSeOH (selenenic acid) group is formed as a result of this action. A two-step mechanism converts selenenic acid back to selenol, starting with a reaction with GSH to create the GS-SeR and H_2O. The GS-SeR intermediate is reduced back to selenol by a second GSH molecule, releasing a by-product GS-SG.

1.3 Nonenzymatic Antioxidants

Antioxidants that are generally nonenzymatic in nature are found in all subcellular parts of plants. These antioxidants help to detoxify reactive O_2 species and free radicals and can also help to reduce the substrates from antioxidant enzymes (Mittler 2002). The primary cellular redox buffers GSH and ascorbate, along with carotenoids, tocopherol, and polyphenolic compounds, are different forms of nonenzymatic defense pathways (Scandalios 2002).

1.3.1 Vitamin C (Ascorbic Acid)

Ascorbate is a well-known vitamin having anti-oxidizing properties that have been found in a variety of organelles and even apoplast. It occurs in different reduced and oxidized forms, as ascorbic acid (90% of the ascorbate pool) and mono- and dehydroascorbic acid (Smirnoff 2011). The ratio of oxidized to reduced ascorbate is a key element that influences plant oxidative stress resistance (Conklin et al. 2000; Cruz-Rus et al. 2012). Glutathione reductase, dehydroascorbate reductase, and monodehydroascorbate reductase are among the NAD(P)H-dependent enzymes that keep AsA in its reduced state (Mittler 2002; Foyer and Noctor 2011). According to the Horemans et al. (2000), the mitochondria are the major site for ascorbate production, from which it is transferred to other cell organs via a proton-electron gradient. Due to its ability to transfer electrons in enzymatic and nonenzymatic processes, AsA is a critical component for ROS detoxification in the aqueous phase. AsA can quickly remove O_2^-, hydroxide ions, and $1O_2$, and therefore, it can reduce H_2O_2 to H_2O by the ascorbate peroxidase mechanism, protecting membranes (Blokhina et al. 2003). In the ascorbate–glutathione cycle, APX utilizes two AsA molecules to decrease H_2O_2 to water, with the intermediate monodehydroascorbate, which is a short-lived radical that is further disproportionated into dehydroascorbate

(DHA) and AsA. Furthermore, it plays a crucial role in the dynamic and control of the cell cycle, the advancement of the G1 to S stage of the cell division pathway, and cellular elongation, as well as many photosynthetic functions (Smirnoff 2011). Although the exact biosynthetic process for ascorbate is unknown, it is speculated that D-glucose may be used as a precursor.

1.3.2 Glutathione

Glutathione (GSH) oxidation by ROS results in the formation of GSSG, which is present in all plant cell compartments. In cellular compartments, GSH, together with its oxidized counterpart, GSSG, maintains a redox balance. It has been revealed that the GSH/GSSH pair is involved in gene regulation and cell cycle pathways (Mittler 2002). The antioxidant glutathione and ascorbic acid are prolific and stable, and they have the right redox potential to react with different varieties of substrate and compounds. Glutathione is a tripeptide molecule made up of three amino acids, glutamine, cysteine, and glycine, that are found in all plant cell parts, including the cytoplasm, vacuoles, chloroplasts, mitochondria, and endoplasmic reticulum (Millar et al. 2003). In most plant cells, glutathione is the important source of nonprotein thiols. Glutathione is appropriate for a wide range of metabolic actions in all organisms due to the presence of a thiol group and its reactivity. The reduced form of glutathione (GSH) is found at higher concentrations in chloroplasts. Unlike the ascorbate biogenesis system, the glutathione biosynthetic pathway is well-established and identical throughout all domains of life. The amino acids are linked to produce the full tripeptide in two ATP-dependent stages catalyzed by GSHS and γ-ECS. These events take place in chloroplastic and non-chloroplastic segments, and glutathione concentrations and redox status play a crucial role in different pathways. GSH is used to reduce DHA both in an enzymatic and nonenzymatic way in the ascorbate–glutathione pathway, and it is then oxidized to GSSG. Glutathione reductase catalyzes the regeneration of GSH from GSSG, with NADPH as the reducing agent. The cysteine residue in GSH's tripeptide has a high reactivity potential. GSH scavenges harmful H_2O_2 by reacting nonenzymatically with O_2^-, OH^-, and $1O_2$. The ability of GSH to replenish another potent antioxidant, ascorbic acid, via the ascorbate–glutathione cycle gives it a crucial role in antioxidative defense (Millar et al. 2003). It has been observed that the antioxidant property of glutathione was utilized to achieve transgenic lines of tobacco (Foyer and Noctor 2005), which can withstand oxidative stress (Del Río and López-Huertas 2016). GSH has been involved in sensing alterations in redox equilibrium and transferring these alterations to appropriate target proteins, in addition to being a co-substrate and reducing agent in defense against reactive oxygen species.

1.3.3 Vitamin E

All types of tocopherols are methylated fat-soluble phenols that have similar to vitamin E (Sharma et al. 2012). They primarily function as antioxidants in plants, algae, and mammals, but they may also have additional functions. In lipid bilayers, α-tocopherol is the most effective for the removal of peroxyl radicals. Tocopherols are the most powerful scavengers of free radicals. At the energy of 323 kJ mol^{-1}, the hydroxyl bond present in vitamin E becomes weaker than in the majority of phenols and readily liberates hydrogen atom and thereby helps in quenching peroxyl radicals and other free radicals, decreasing their harmful effect (Lide 2006). The produced tocopheryl radical is generally nonreactive, and through redox interaction with a hydrogen donor such as ascorbate or other antioxidants, it reverts to tocopherol (Igamberdiev and Hill 2004; Traber and Stevens 2011). Tocopherols are integrated into cell membranes and thereby protect the chloroplast membrane from oxidative damage due to their fat-soluble nature (Blokhina et al. 2003). α-Tocopherols are significantly bioactive and prominent antioxidants in the chloroplast lamina and are primarily utilized for defending them against the photooxidative effect. It has been observed that a single α-tocopherol molecule may neutralize one 20 singlet oxygen and also act as reusable chain reaction terminators for PUFA radicals synthesized during oxidation of lipids (Hare et al. 1998; Wu and Tang 2004; Ledford and Niyogi 2005). Photosynthesis and other metabolic processes in chloroplasts produce ROS, which causes lipid peroxidation in plant cells. To cope up with a range of abiotic stress conditions, the content of α-tocopherol in photosynthetic plant tissues increases dramatically (Noctor 2006). The ability of α-tocopherols to scavenge and quench ROS aids in the modulation of signal transduction and the stabilization of membranes (Kruk et al. 2005; Noctor 2006). They work as a free radical capturing process by preventing the chain extension stage in lipid autooxidation. Plants respond to oxidative stress by expressing genes involved in tocopherol production (Table 1.1) (Giacomelli et al. 2007; Wu and Tang 2004).

1.3.4 Carotenoids

Carotenoids, also known as tetra terpenoids, are pigments that naturally occur in plants and microorganisms (Otles and Cagindi 2008). To date, more than 750 natural carotenoids have been discovered. These chemicals give different vegetables and fruits their distinct colors. Xanthophylls and carotenes are the two broad categories of carotenoids. Carotenoids are classified into two broad subclasses, xanthophylls and carotenes; the former class contains oxygen, whereas the latter class is purely hydrocarbons and contains no oxygen (Ngamwonglumlert et al. 2017). Carotenoids found in the human diet may help to prevent cancer, age-related muscle degeneration, atherosclerosis, and other disorders. These pigments are lipid-soluble and are absorbed together with fats through the gut tract. Carotenoids have a variety of beneficial activities in plants, including attracting pollinators, indicating fruit development, assisting in photosynthesis, and protecting cells from light-induced damage

Table 1.1 ROS and their characteristics

ROS	Site of generation	Mode of action	Interaction with protein	Interaction with DNA	Scavenging systems
O_2^-	Membranes, mitochondria, chloroplast	The reaction occurs with a double bond having molecules such as iron–sulfur proteins	Through Fe-center	Excessively low	Super oxide dismutase
OH^-	Membranes, chloroplast, mitochondria	Highly reactive with different biomolecules	Fast reacting	Quick reacting	Flavonoid and proline
H_2O_2	Membranes, mitochondria, chloroplasts peroxisomes	Oxidizes proteins and help in the generation of OH^- through O_2^-	Reacts with Cys residue	Excessively low	CAT and flavonoid
O_2	Membranes, mitochondria chloroplasts	Oxidizes proteins, polyunsaturated fatty acid, and deoxyribonucleic acid	Highly reactive for Trp, Met, His, Tyr, and Cys amino acids moties	Polyunsaturated fatty acid	Carotenoids and vitamin E

in plants and in photosynthetic bacteria and algae (Lerfall 2016). The ability of β-carotene to quench free radicals such as O_2^-, OH^-, and $1O_2$ without undergoing any changes or degradation is largely due to its highly delocalized polyene backbone or conjugated double bond structure, which is primarily responsible for its antioxidant behavior. Carotenoids found in higher concentrations in particular tissues and organs can protect lipids against oxidative damage.

1.3.5 Phenolic Compounds

Tannins, flavonoids, lignins, and stilbenes are examples of phenolic compounds, which constitute a varied group of naturally occurring secondary metabolites common in plants. Multiple phenol rings distinguish these compounds, making them suited for free radical scavenging in both de novo and in vitro conditions. Over 8000 phenolic bioactive substances have been detected in different plant families. Phenylalanine/shikimic acid is the common intermediate precursor for all plant phenolic compounds. Under in vitro conditions, polyphenols have been shown to be more potent antioxidants than ascorbate and tocopherols. Phenols are the most significant dietary elements for humans, providing bitterness, color, astringency, flavor, odor, and oxidative stability in food (Schroeter et al. 2002). Antioxidative activities of polyphenols are characterized by the presence of readily available donor hydrogen or electron (Rice-Evans et al. 1997). In another way, phenols alter the peroxidation kinetics of lipid membrane and packaging, thereby protecting the membrane integrity (Schroeter et al. 2002). Furthermore, phenolics have been implicated in the H_2O_2 scavenging cascade in plant cells. Polyphenols play different functions in plants, which include pigmentation to plants; increase and decrease of plant growth regulators, for example, auxin; UV protectants against ionizing light; deterrence to herbivores; phytoalexins; and signaling compounds in ripening and other plant developmental activities (Huber et al. 2003; Lattanzio et al. 2006).

1.4 Conclusion

Antioxidants are created normally, but they are triggered and upregulated in stressful situations, which help to retain the structural firmness of cell organelles while probably reducing oxidative damage. Plant defense is aided by a number of antioxidant enzymes. The production and activation of ROS scavenging enzyme systems in transgenic plants to increase their tolerance to a variety of stress conditions. Furthermore, because numerous enzymes and their different isoforms are involved and reactive oxygen species is only one of the major factors of plant resistance to unfavorable environmental and biotic stimuli, further research is needed in this field. The increasing number of articles addressing superoxide dismutase, common antioxidant enzyme, ascorbate peroxidase, glutathione peroxidase enzyme, and glutathione reductase enzymes demonstrates these enzymes' favorable responses to biotic and abiotic stressors. These findings highlight the need to investigate these enzymes in order to better understand their role in the scavenging of hazardous cell products in a variety of species and the relationship between biological processes and oxidative stress.

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Functional Characterization of the Antioxidant Enzymes in Plants Exposed to Environmental Stresses

2

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Abstract

In alarming condition (stressful), the metabolic imbalances cause an excess production of reactive oxygen species (ROS) and lead to oxidative imbalances. Every plant's first line of defense amid stress is the ROS generation. ROS serve as messengers in initiating plant defense signaling. Large quantities of ROS have negative impacts on plants survival, but low amounts of ROS are necessary and act as signaling molecule to protect plant from death. Plants have a complex antioxidant system that protects cells from ROS damage. Superoxide dismutase (SOD), guaiacol peroxidase (GPOX), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR) are the main enzymatic components of the defense system. Nonenzymatic antioxidants include carotenoids, glutathione (GSH), proline, ascorbate (ASA), tocopherols, phenolics, etc. Most effective intracellular enzyme antioxidants is the superoxide dismutase (SOD); it catalyzes the conversion of superoxide anions to oxygen and hydrogen peroxide, thus avoiding damage. Catalase works catalytically, i.e., it catalyzes the energy-efficient conversion of H_2O_2 into water and oxygen. Glutathione reductase (GR) is a flavoprotein enzyme that converts oxidized glutathione (GSSG) to

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reduced glutathione (GSH) via the oxidation of NADPH to $NADP^+$ whereas glutathione peroxidase is a free radical and hydrogen peroxide elimination enzyme that catalyzes glutathione oxidation in the presence of a hydroperoxide. Guaiacol peroxidase is a heme protein that oxidizes aromatic electron donors like guaiacol and pyragallol by using H_2O_2. Ascorbate peroxidase (APX) is also a heme peroxidase that carries out the reduction of H_2O_2 into water by employing ascorbate as an electron source.

Keywords

Abiotic stress · Antioxidant system · Ascorbate peroxidase · Guaiacol peroxidase · Catalase · Glutathione reductase · Superoxide dismutase

2.1 Introduction

Plants are affected from both biotic and abiotic stress environments. In stressful environment, water and nutrient uptake, membrane permeability, and normal growth and development are all hampered (Pitman and Lauchli 2002). Such alterations have a deleterious influence on hormone metabolism and gas exchange and in turn accelerate the formation of reactive oxygen species, compromising cell expansion and division. As a result, plant development is altered, and finally, plant senescence and death occur (Rossatto et al. 2017). However, in alarming condition (stressful), the metabolic imbalances cause an excess production of ROS and lead to oxidative imbalances. Among the different forms of ROS that can be formed include the singlet oxygen, superoxide radicals, hydrogen peroxide, and radical hydroxyl (Gupta and Huang 2014). Every plant's first line of defense amid stress is the ROS generation. Despite the fact that plants lack specialized cells for immune function to pathogens, ROS serve as messengers in initiating plant defense signaling. Large quantities of ROS have been shown to have negative impacts on plants survival, growth, and production, but low amounts of ROS are necessary and acts as signaling molecule to protect plant from death. When the amount of ROS produced exceeds the amount that can be digested and scavenged, they may react with a variety of cellular components and cause damage to cellular components and molecules like DNA, lipids, proteins, and carbohydrates. Such damage results in structural and function changes or inhibition. Plants, on the other hand, have a complex antioxidant system that protects cells from ROS damage. Superoxide dismutase (SOD), guaiacol peroxidase (GPX), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR) are the main enzymatic components of the defense system (Mushtaq et al. 2021). SOD emerges first in the line of defense when it comes to ROS elimination, dismutating superoxide radicals into hydrogen peroxide. The CAT and the APX convert hydrogen peroxide into water and oxygen, with the help of the GR (Rossatto et al. 2017). Nonenzymatic antioxidants such as carotenoids, glutathione (GSH), proline, ascorbate (ASA), tocopherols, phenolics, and others are also present in addition to enzymatic antioxidants. In this chapter, we will try to explain the roles of various antioxidant enzymes in plants under stress.

2.2 Generation of Reactive Oxygen Species (ROS) in Plant Cells

ROS are produced by normal cellular processes, and the primary generating organelles are peroxisomes chloroplasts and mitochondria. Superoxide radicals are formed in chloroplasts at the thylakoid membrane and photosystem II. Complexes I and III are the primary producers in mitochondria, whereas matrix and membranes are the primary producers in peroxisomes (Corpas et al. 2015). These ROS are produced as a result of electron leakage onto O_2 from the electron transport system or as a result of numerous metabolic reactions in cellular compartments. Oxygen-centered radicals and oxygen-centered non-radicals are the two types of reactive oxygen species. Hydroxyl radical, superoxide anion, peroxyl radical, and alkoxyl radical are oxygen-centered radicals, whereas hydrogen peroxide and singlet oxygen are oxygen-centered non-radicals. Other reactive include nitrogen bearing molecules like nitric oxide and peroxynitrite (Huang et al. 2005a, b). Under lighting circumstances, O_2 is continually given in photosystem I (PSI) by the water autolysis done in PSII, as stated in reaction [A]; thus light would encourage the superoxide radical generation reaction [B] at the PSI site. Under conditions of abundant reduced ferredoxin and limited NADP availability, autooxidation of this iron sulfur protein occurs, resulting in the generation of $O_2^{\bullet -}$, as shown in reaction [C] (Corpas et al. 2015).

$$\mathbf{Reaction\ [A] : 2H_2O \rightarrow 4e^- + O_2 + 4H^+}$$

$$\mathbf{Reaction\ [B] : 2O_2 + 2e^- \rightarrow 2\ O_2^{\cdot -}}$$

$$\mathbf{Reaction\ [C] : Fdred + O_2 \rightarrow Fdox + O_2^{\cdot -}}$$

2.2.1 Beneficial Role of ROS in Plants

Plant growth rate is negatively impacted by abiotic and biotic factors like salt, drought, temperature, infections, and water stress. Plants have a unique capacity to cope with these pressures and thrive in a variety of demanding environments. Plants have created several endogenous defensive mechanisms to resist such pressures in order to protect themselves. ROS have been implicated as a second messenger in intracellular signaling cascades because they stimulate numerous responses in plant cells, including tolerance to biological and chemical stresses. The signals are firstly detected by numerous membrane-bound sensors, which are subsequently amplified and transduced to the nucleus in order to drive the chain of responses that surely results in saving an organism. Such signal transduction routes are incredibly selective and sensitive. Interactions between signaling and receptor elements are driven by weak or non-covalent forces (Yan et al. 2007). ROS, notably superoxide, H_2O_2, and creation of bursts of superoxide at the plasma membrane, are some primary responses of plants under environmental changes (Foyer and Noctor 2005; Foreman et al. 2003; Garg and Manchanda 2009). Aside from controlling growth, they also affect organ quantity and commencement, as well as the formation of rhizobia symbiotic association in legumes (Sagi et al. 2004; Pauly et al. 2006). The production of superoxide or its products by apoplast has a function in the detection of a number of diseases causing agents (Torres et al. 2006). ROS can trigger host cell wall thickening in plants by cross-linking glycoproteins (Lamb and Dixon 1997). ROS are signaling molecules that mediate the establishment of defense genes (Levine et al. 1994). Additional defense-related regulatory activities for ROS cooccur in which chemical molecules like NO and salicylic acid takes place. Potato nodal explants subcultured with H_2O_2 were found to be heat shock tolerant for 15 h at about 42 °C temperature (Foyer et al. 1997). Prevention of light-induced photo bleaching was seen in *Arabidopsis* leaves when injected with H_2O_2 (Karpinski et al. 1999). Similarly, H_2O_2 application on maize coleoptiles prevented them from chilling stress (Prasad et al. 1994).

2.2.2 ROS Scavenging Antioxidant Enzymes

Controlling the consistent amount of ROS in cells is vital. ROS signaling mechanisms during freezing, temperature, and other stresses might offer an added potent technique for increasing crop resistance to these adverse environmental conditions. The word antioxidant refers to a large family of substances that offer protection from harm induced by encounter to certain highly reactive substances. An antioxidant is a molecule that may prevent other molecules from oxidizing. Free radicals are produced during oxidation events, which can set off chain reactions that harm cells. Antioxidant enzymes stop such chemical reaction by scavenging free radicals and inhibiting other oxidative damage. They accomplish it by being oxidized, which is why antioxidants are frequently used as reducing agents. Plants have various antioxidant enzymes that are found in small quantities in plant cell partitions and considerably decrease or prevent oxidation of the oxidizable substrate (Kurutas 2015; Dat et al. 2000). The following is a full description of antioxidant enzymes:

2.2.3 Superoxide Dismutase (EC 1.15.1.1)

One of the most effective intracellular enzymatic antioxidants is the superoxide dismutase (SOD); it catalyzes the conversion of superoxide anions to oxygen and hydrogen peroxide, thus avoiding damage. Catalase or glutathione peroxidase removes the hydrogen peroxide. There are various forms of superoxide dismutase that differ in their kind of active metal centers, cofactor, and the sequence of amino acids. In plants, Mn-SOD, Cu,Zn-SOD, and Fe-SOD are found (Sawada et al. 1972; Asada et al. 1973). Mn-SOD was first isolated and biochemically described in pea leaves (Fernandez et al. 1982). Till year 1981, it was considered that Fe-SODs were only found in prokaryotes. However, a group led by Marvin L. Salin found SOD that contained iron, and eventually, iron-containing SOD was described in various families (Bridges and Salin 1981). At its active site by consecutive oxidation and reduction reactions, superoxide dismutase neutralizes superoxide ions (Kurutas 2015). This enzyme is made up of two identical subunits, and both units have own active sites, Cu and Zn ions. Dismutation of superoxide anion to O_2 and H_2O is carried out by this enzyme. The 96 kDa homotetramer Mn type SOD is found in mitochondria, which contain 1 Mn per subunit. Copper- and zinc-containing tetrameric SOD is also found extracellular. This SOD has strong attraction with specific glycosamino glycans (Sheng et al. 2014). The reaction catalyzed by SOD is shown in reaction D (McCord and Fridovich 1969):

$$\textbf{Reaction D}: -\mathbf{O_2}^{\cdot -} + \quad \mathbf{O_2}^{\cdot -} + \mathbf{2H}^{+} \rightarrow \mathbf{H_2O_2} + \mathbf{O_2}$$

2.2.3.1 SOD Under Environmental Stress

Stress causes an increase in ROS production, which activates the enzyme activity (DelRo 2015). In most of the plants investigated, cultivars' higher SOD activity was correlated with higher abiotic stress tolerance (Wang et al. 2016). Upregulation of iron SOD and downregulation of Mn and Cu, Zn SOD were seen under Cd stress (Rodríguez-Serrano et al. 2009). The activity of defense enzymes was examined in pepper plant cultivars with varying sensitivity to cadmium. Under 0.5 mM cadmium stress, decreased activity of Cu, Zn-SODs was found, whereas increased activity of Mn and Fe-SODs, as well as glutathione reductase and guaiacol peroxidase was seen (León et al. 2002).

An enhancement in defensive antioxidant systems toward ROS was seen in tolerant pea plants when they were treated with NaCl. Cu, Zn-SOD activity was observed to be increased (Hernandez et al. 1995). Under long-term NaCl concentration of 110 mM, enhancements in all chloroplastic SODs were found in tolerant pea cultivar (Gomez et al. 1999). The activity of Fe-SOD was reduced, whereas Cu, Zn-SODs were elevated under the application of salt (400 mM NaCl), demonstrating that Cu, Zn SODs protect halophyte *Cakile maritima* from salt-mediated stress (Houmani et al. 2016). Salt stress increased the activity of antioxidant enzymes like SOD, MDAR, APX, and CAT in NaCl-tolerant *Lycopersicon pennellii*, but decreased activities in salt responsive plants (Mittova et al. 2003). Salt stress reduced H_2O_2 concentration and lipid per oxidation in roots of salt tolerant *Lycopersicon pennellii*, while increasing the activity of peroxisomal SOD, APX, MDAR, and catalase in salt-sensitive plants (Mittova et al. 2004). Under potassium deficiency, salinity increased SOD activity in *Hordeum maritimum* L. (Hafsi et al. 2010).

Water shortages and flooding decrease photosynthesis and increase ROS production in chloroplasts. Several studies have reported the production of oxidative stress by water deficiency, and positive correlation of enzymatic antioxidant system is associated with it (Wang et al. 2016). Under dryness, the activities of Cu, Zn SODs with APX improved in plants like maize and pea (Mittler and Zilinskas 1994; Malan et al. 1990). Resistant varieties of *Sorghum* have enhanced strength when compared with drought prone variants (Dat et al. 2000). Researchers have found that low temperatures cause differential expression of multiple genes, resulting in an increase in several metabolites that defend against the effects of low temperature stress (Winfield et al. 2010). When plants are subjected to temperature stress, accumulation of ROS and activation of defense system occur. In low temperature condition, ROS are generated by mitochondria and chloroplasts (Dat et al. 2000). In plant species like *Arabidopsis thaliana* and *Capsicum annuum* L., temperature stress resulted in oxidative and nitrosative damage (O'Kane et al. 1996; Airaki et al. 2012). Increased chloroplastic and cytosolic SOD were found in tobacco under low temperature stress (Tsang et al. 1991).

2.2.4 Catalase (CAT 1.11.1.6)

Catalase is an antioxidant in all aerobic organisms. It is a tetrameric enzyme consisting of four identical 60 kDa subunits organized tetrahedrally, each with a heme and NADPH in its active core. The enzyme activity of catalase varies with H_2O_2 levels. When the H_2O_2 level is high, the catalase works catalytically; i.e., it catalyzes the energy-efficient conversion of H_2O_2 into water and oxygen. However, at low levels of $H_2O_{2,}$ it operates peroxidically and eliminates H_2O_2, but oxidizes its substrates in the presence of an appropriate hydrogen donor (Scibior and Czeczot 2006). In cellular surroundings, catalase is found at all significant H_2O_2 production sites such as peroxisomes, mitochondria, cytosol, and chloroplast. Catalase isozymes have several molecular forms, indicating their varied involvement in the plant system. *Cat1*, *Cat2*, *and Cat3* encode CAT-1, CAT-2, and CAT-3, respectively. Times, type of species, and quantity of stress are some factors that are responsible for differential gene expression. Plants with deficiency in catalase develop abnormalities like chlorosis and photo respiratory sensitivity (Sharma and Ahmad 2014). One molecule of catalase can convert around six million hydrogen peroxide molecules into water and oxygen each minute, and this makes catalase the highest turnover rate enzyme (Kurutas 2015).

2.2.4.1 Catalase Under Environmental Stress

Various studies have demonstrated the importance of catalase in the plant defense system (Beulah and Ramana 2013). Enhanced CAT activities are critical to survive under stress condition; on the other hand, high stress levels can result in irreparable damage to the enzyme (Youssef and Azooz 2013). Catalase activity was increased in *Brassica rapa* L. when 50 μM $CdCl_2$ was applied for 2 weeks (Zhong et al. 1994). Spray of 50 mg chitosan to Cd-treated plants increased catalase activity to levels higher than in Cd-treated plants alone. *Brassica napus* L. treated with 1.5 mM cadmium ($CdCl_2$) for 1 week showed reduced catalase activity when compared with control. Superoxide dismutase (SOD) activity, on the other hand, was greater treated plants when compared with control. From the study, we concluded that the activity of catalase is not all times improved with SOD (Karam et al. 2017).

Drought increased CAT and SOD activities in both tolerant and sensitive varieties of *Amaranthus tricolor*, but the activities were much higher in the tolerant plant than sensitive, implying that CAT and SOD play a significant role in drought tolerance by detoxifying H_2O_2 and activating the dismutation reaction (Sarker and Oba 2018).

Zeeshan et al. analyzed wheat and barley cultivars and determined that greater antioxidant enzyme activities, especially CAT, are substantially connected with greater salt tolerance, thus indicating a definite role for antioxidant activities in salt-induced oxidative stress mitigation. In the same way, elevated levels of SOD and CAT activities were found in faba bean genotypes, indicating the control of antioxidant response and its moderation under salt stress (Alzahrani et al. 2019). In *Nicotiana plumbaginifolia*, mutants lacking CAT genes are more susceptible to oxidative stress than control plants when exposed to salt, ozone, and H_2O_2 (Willekens et al. 1997).

2.3 Glutathione Reductase (E.C. 1.6.4.2)

Glutathione reductase (GR) is a flavoprotein enzyme that converts oxidized glutathione (GSSG) to reduced glutathione (GSH) via the oxidation of NADPH to $NADP^+$. Glutathione, in its reduced form, performs critical functions in the cellular regulation of ROS. This enzyme is extremely well preserved in nature. GR has been isolated and identified from the leaves of numerous plant species, including *Spinacia oleracea* (Halliwell and Foyer 1978) and *Pisum sativum* (Kalt-Torres et al. 1984). There are three forms of GR in higher plants, viz., cytosolic, chloroplastic, and mitochondrial. Eighty percent of GR activity is seen in plant chloroplasts and photosynthetic cells (Edwards et al. 1990). Glutathione availability has a significant impact on developmental processes ranging from seed formation to germination to blooming (Zuccarelli et al. 2017). γ-glutamylcysteine synthetase, abbreviated as (GSH1), is the primary enzyme involved in synthesis of GSH; in *Arabidopsis*, lack of this enzyme results in an embryo-lethal phenotype (Cairns et al. 2006). Similarly, embryo development is interrupted at globular stage in AtGR2-knockout *Arabidopsis* mutants (Ding et al. 2016), and thus plays an important role in the normal developmental processes of plant (Zuccarelli and Freschi 2018). Figure 2.1 describes the overall mechanism and role of antioxidant system under stress.

2.3.1 Glutathione Reductase Under Environmental Stress

Like other enzymes of antioxidant system, environmental strains also boost the activity of GR (Maheshwari and Dubey 2009). Recent research showed increases in GR activity in pea and *Arabidopsis* (Huang et al. 2005a, b; Hernandez et al. 2000). In maize drought, strains have enhanced the activities of GR (Pastori and Trippi 1993). However, observations in rice show a gradual decline in GR activity with

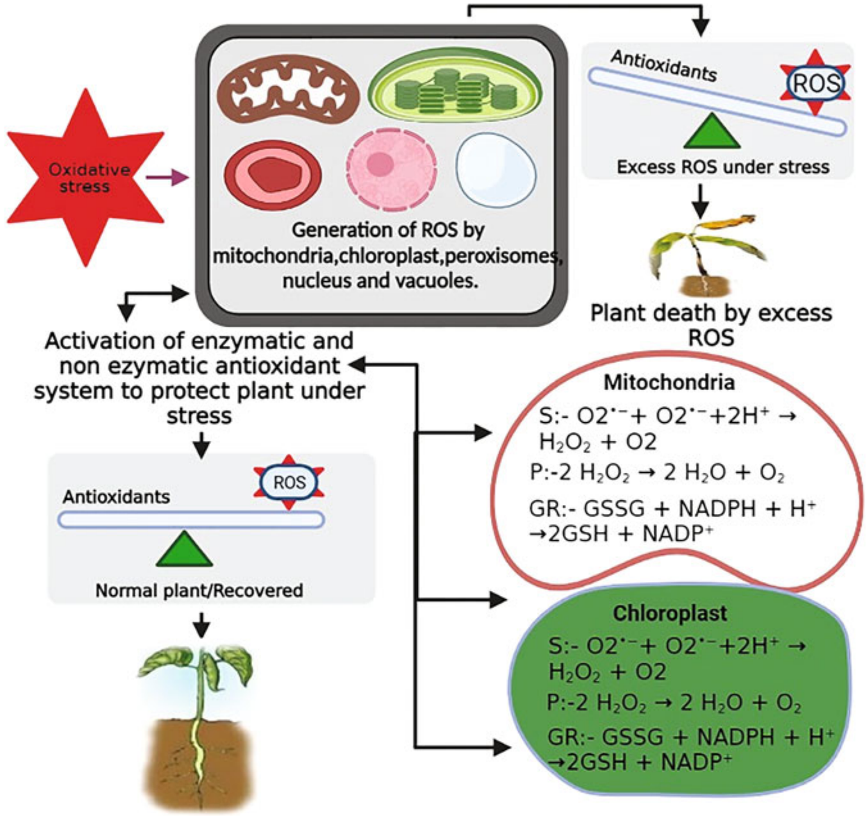

Fig. 2.1 Overview of different process under plant stress. Activation of antioxidant enzymes under excessive ROS generation, S mean reaction catalyzed by SOD, P by peroxidase, and GR by glutathione reductase

increasing salt stress exposure, implying a gradual decline in GSH regeneration capability under high salt stress conditions (Demiral and Türkan 2005). In Cuccumber, maize and wheat chilling induced increases in GR activity have been seen (Kocsy et al. 2002; Lee et al. 2001). Short-term heat stress increases GR, but long-term exposure reduces GSH regeneration (Nagesh Babu and Devaraj 2008). Heat stress of 40 °C for 4–24 h was found to enhance GR expression in *Phalaenopsis*, in contrast to the rapid reduction found after 48 h of same heat exposure (Ali et al. 2005). Heavy metals stimulate the activity of this enzyme as well. Cd treatment enhances GR activity in wheat (Yannarelli et al. 2007), Sugarcane, *Arabidopsis*, and soybean (Skórzyńska-Polit et al. 2003; Fornazier et al. 2002; Ferreira et al. 2002). Application of Cd and Hg in *Arabidopsis* also enhanced the expression of GR (Sobrino-Plata et al. 2014).

2.4 Glutathione Peroxidase

Plant contains glutathione peroxidases (GPXs) in a wide range of tissues, partitions, and during various developmental stages (Yang et al. 2006). Chloroplasts are the most common source of glutathione peroxidase. However, several of the enzyme isoforms have been discovered in mitochondria, cytosol, and peroxisomes (Yousuf et al. 2012). Glutathione peroxidase is a free radical and hydrogen peroxide elimination enzyme that catalyzes glutathione oxidation in the existence of hydroperoxide, i.e., hydrogen peroxide or lipid hydroperoxide (Kurutas 2015). GPx is an antioxidant enzyme that contains selenium and actively transforms H_2O_2 and lipid peroxides to water and lipid alcohols, respectively, using GSH as a cofactor, and in turn oxidizing reduced glutathione (GSH) to the disulfide form (GSSG). Unlike vertebrate GPXs, plants' GPXs have cysteine in their active site rather than selenocysteine (Bela et al. 2015). As a result, selenium-dependent (GPx, EC1.11.1.19) and selenium-independent (GST, EC 2.5.1.18) forms of this enzyme are present.

2.4.1 Glutathione Peroxidase Under Environmental Stress

Multiple plant GPX coding cDNAs were isolated and characterized, confirming the enzyme family's importance in stress responses. GPX mRNA levels frequently rise in response to numerous biotic and abiotic stresses (Milla et al. 2003; Herbette et al. 2007). Oxidative stress, cold, metal treatments salt, drought, etc. have all been shown to activate GPX genes (Yang et al. 2005; Milla et al. 2003; Kang et al. 2004; Navrot et al. 2006). Salinity stress caused a continuous rise in PgGPx transcript until 12 h of observation, whereas dryness caused an over expression of PgGPx transcript till 48 h of observation from 6 h. In response to dryness, a higher level of PgGPx gathering was identified at very early stage (3 h), indicating plant GPxs have versatility in stress sensing, signaling, and adaptation pathways (Islam et al. 2015). Exogenous treatments like salt, mannitol, heat, or cold have all been shown to increase AtGPx expression (Milla et al. 2003). AtGPx mRNA expression was also discovered to be stimulated by plant hormones such as salicylic acid, indole acetic acid, etc. In response to heavy metal poisoning, OsGPx transcripts were shown to be upregulated (Ramos et al. 2009). The saline and drought stressors both resulted in significant upregulation of the PgGPx transcript.

2.5 Guaiacol Peroxidase (EC 1.11.1.7)

Guaiacol peroxidase is a heme protein that oxidizes aromatic electron donors like guaiacol and pyragallol by using H_2O_2. It is found in a wide range of animals, plants, and microbes. Two structural Ca^{2+} ions and four conserved disulfide bridges are present in these enzymes (Schuller et al. 1996). Plant tissues contain numerous isoenzymes of guaiacol peroxidase, which are present in vacuoles, cell wall, and the cytoplasm (Asada 1992). Guaiacol peroxidase is engaged in several important biosynthetic processes, including cell wall lignification, IAA breakdown, ethylene synthesis, wound healing, and defense against abiotic and biotic stresses (Kobayashi et al. 1996). The guaiacol peroxidase enzyme is commonly known as a stress enzyme (Sharma et al. 2012). Guaiacol peroxidase activity in plants is rapidly activated by stresses (Moussa and Abdel-Aziz 2008). Guaiacol peroxidase has been revealed to account for nearly 90% of peroxidase activity in plants (Foyer et al. 1994).

2.5.1 Guaiacol Peroxidase Under Environmental Stress

Various environmental conditions, such as heavy metals (Srivastava and Dubey 2011), salt stress (Jakovljević et al. 2017), and ozone (Li et al. 2013), have been shown to boost guaiacol peroxidase activity. When compared with salt-sensitive cultivars, it was observed that salt-tolerant safflower plants are more resistant to salt-induced oxidative damage due to higher guaiacol peroxidase activity, catalytic efficiency, and induction of certain isoenzymes (Tayefi-Nasrabadi et al. 2011). Guaiacol peroxidase activity in *Betula pendula* increased considerably (up to 2.8 times than control) under increased traffic (pollutants), resulting in a biphasic paradoxical influence on lipid peroxidation rate. When compared with the control, the quantity of lipid peroxidation increased in the first phase, but it was reversed in the second phase by enhanced guaiacol peroxidase activity. The effects of traffic pollution on guaiacol peroxidase activity and lipid peroxidation rate in *Tilia cordata* were contradictory. However, no relationship was found between changes in guaiacol peroxidase activity and lipid peroxidation rate under middle- and high-level pollution (Erofeeva 2015). Other studies also revealed role of guaiacol peroxidase in eliminating oxidative stresses under metal toxicity (Radotić et al. 2000).

2.6 Ascorbate Peroxidase (EC 1.1.11.1)

Ascorbate peroxidase (APX) is a heme peroxidase that carries out the reduction of H_2O_2 into water by employing ascorbate as an electron source (Welinder 1992). The method uses two molecules of ascorbate to convert H_2O_2 to water while also producing two molecules of monodehydroascorbate (MDHA). In MDAR reaction, NAD(P)H is used as an electron donor to convert two molecules of monodehydroascorbate (MDHA) back into ascorbate. Sometimes, these monodehydroascorbate molecules escape the MDAR reaction, then they spontaneously disproportionate and produce ascorbate and dehydroascorbate (DHA). The reduction of DHA is catalyzed by DHARs by utilizing two molecules of GSH, resulting in one molecule of oxidized glutathione that is reduced again to two GSH through GR. This reaction is NADPH-dependent. To smooth organelle and cellular levels of H_2O_2, APX isoforms are distributed across the cytosol, mitochondria, chloroplasts, and peroxisomes in higher plants (Maruta and Ishikawa 2018).

2.6.1 Ascorbate Peroxidase Under Environmental Stress

CAT reduces the effectiveness of APX in facilitating scavenging activity to remove H_2O_2; however, isoforms of APX have a significantly greater reactivity for H_2O_2 and are renowned peroxide-scavenging enzymes in the plant cells (Maruta and Ishikawa 2018). Water shortage, salinity, freezing, metal toxicity, and ultraviolet radiation all have a significant impact on APX activity (Hefny and Abdel-Kader 2009). In sensitive chickpea genotype, the activity of APX and GR was considerably reduced under high temperature conditions in comparison with tolerant genotypes (Kumar et al. 2013). In *Jatropha macrocarpa*, higher APX activity was found in response to high H_2O_2 levels, which improved resistance to low-temperature stress, while lower APX activity with *J. curcas* was linked with increased sensitivity under low temperature (Spano et al. 2017). In wheat and barley cultivars, it was found that greater antioxidant activities (SOD, POD, APX, GR, and CAT) are substantially connected with better salt tolerance, indicating that antioxidant activities play a significant role in mitigating salt-induced oxidative stress (Zeeshan et al. 2020). Drought exposed *Vigna radiata* seedlings had lower AsA/DHA and GSH/GSSG ratios, but higher APX, GR, GPX, and GST rates, which attributed to drought, induced oxidative damage tolerance (Nahar et al. 2017).

2.7 Conclusion

Plants have fine-tuned network of ROS generation and scavenging system, which aids in proper growth and development under various abiotic and biotic stresses. All enzymatic and nonenzymatic antioxidant network acts to save plant under harmful situations. In about all the plants, the expression of antioxidant machinery is upregulated under various stress conditions. In the future, we can use plants that have the ability to tolerate extreme environmental stress and have the ability to scavenge and fine-tune the levels of ROS. Researchers have already achieved successful results, but still, some doubts and unawareness about ROS, their impact, and nature need to be addressed. Future researches should focus on the development of transgenics with over expressed genes for ROS scavenging enzymes.

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3

Gene Expression and Role of Antioxidant Enzymes in Crop Plants Under Stress

Mohd Yasin Bhat, Mir Zahoor Gul, and Jehangir Shafi Dar

Abstract

Plants encounter a variety of stresses in the field due to their stationery and fixed lifestyle. An important outcome during the exertion of all the stresses is a large-scale creation and buildup of reactive oxygen species (ROS) in their cells, which has physiological, biochemical, and morphological repercussions. In crop plants, especially, it leads to a tremendous loss in terms of quality and quantity of crop yield. The defense arsenal of plants in response to ROS apart of nonenzymatic components includes certain important enzymes, which provide defense against the oxidative stress like catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPX), glutathione S-transferase (GST), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR). They defend plants against oxidative onslaught leading to the restoration and improvement of yield in crops. This chapter articulates the current understanding of these enzymes in addition to their status and role at a molecular level in several crops. In addition, a concise account of transgenic variants of crops with a better expression of these antioxidant enzymes conferring them improvised tolerance to various stresses is given.

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Stress · Antioxidant · Superoxide dismutase · Catalase · Peroxidase · Transgenic plants

3.1 Introduction

Plants, due to their immovable nature, are exposed to a plethora of factors that are hostile to their overall growth and development. Some of these factors include extremes of temperature, light, scarcity or excessive water, high salinity, heavy metals, gaseous pollutants, ultraviolet (UV) radiation, etc., which represent different forms of abiotic stresses and present a grave threat to the cultivation of crops and the ecosystems culminating in tremendous crop yield loss throughout the world (He et al. 2018; Tester and Bacic 2005). The global scenario is that about 90% of cultivated lands are impacted by one or multiple of these abiotic stresses leading to 50–70% of agricultural productivity losses in chief food crops (dos Reis et al. 2012; Mantri et al. 2012; Waqas et al. 2019). Plants are also prone to many biotic stress factors under natural conditions like insects, pathogens (virus, bacteria, fungi, and nematodes), weeds, and herbivore pests, which cause considerable constraints in global agricultural production (Chen et al. 2020; Kovalchuk, 2016). The different forms of stresses are frequently interconnected in action and either independently or in combination, they manifest as physiological, molecular, and biochemical alterations that undesirably exercise their influence on plant development, productivity, and eventually crop yield (Bita et al. 2013; Tester and Bacic 2005). For guaranteeing global food security, sustainable agriculture production has a key role to play, albeit, stress conditions considerably undermine such endeavors. The plants require indispensably a modification in their architectural plan, which includes plant physiology, metabolism, and biochemistry to facilitate survival against adverse stress conditions (Chen et al. 2020; Rajput et al. 2021).

One of the modes through which these stresses exert their deleterious impact on various crops is by causing oxidative onslaught in their cells (Akter and Rafiqul Islam 2017; Sun et al. 2020). Like many other organisms dealing with oxygen, certain biochemical processes in plants lead to a usual generation of ROS such as superoxide ion ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), and non-radical molecular species like singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), etc. (Das and Roychoudhury 2014; Sharma et al. 2012). Under typical physiological situations, ROS has a definite role to play in the regulation of cell growth, gravitropism, hormone signaling. and development of various tissues in plants (Choudhary et al. 2020; Kwak et al. 2006). This stable milieu is disturbed by abiotic and biotic stressful factors that lead to enriched production or outburst of ROS in plants climaxing in the form of disruption of cellular homeostasis (Tripathy and Oelmüller 2012). The consequent accumulation of ROS is undesirable and can cause a serious threat to cells by instigating peroxidation of lipids, protein oxidation, impairment to nucleic acid molecules, inhibition of key enzymes, and stimulation of programmed cell death (PCD) or cell necrosis (van Breusegem and Dat 2006; Xie et al. 2019). On the other hand, there is a sort of cross talk between ROS and and relevant signalling pathways that leads to activation of antioxidant defense against the same (Chen and Raji, 2020). The dilemma of whether ROS will behave as a detrimental entity or facilitate proper signaling in cells is reliant upon the subtle stability between the scale of ROS generation and scavenging, which keeps the levels in minimal or requisite intensity. Detoxification or scavenging of surplus ROS is attained by well-organized antioxidative machinery involving the low-molecular-mass nonenzymatic and enzymatic antioxidants, which, therefore, are crucial components of stress tolerance in plants (Ahmad et al. 2010). To accomplish effective signaling, ROS must be retained at minimal intensities in cells to thwart the chances of any oxidative damage. The capability to maintain a high antioxidant potential in plants to nullify the noxious levels of ROS has been associated with enhanced tolerance to biotic and abiotic stresses (Chapman et al. 2019; Nadarajah, 2020). The focus of the study in the past few decades by concerned researchers has been the study of gene expression of these antioxidant enzymes *vis-a-vis* different stresses in plants and understanding the adaptability which they provide to cope in harsh environments (Rai et al. 2013). Comprehension of the detoxification process has led to the development of transgenic lines in crops with improved levels of antioxidants to make them tolerant to different stresses, which they encounter in the field. Tolerance to various environmental stresses is known to be provided more efficaciously by the synchronized expression pattern of multiple antioxidant enzymes in comparison with single or double expression (Ghimire et al. 2015; Lee et al. 2007). The chapter summarizes different aspects of the antioxidant enzyme defense system in crop plants and their part in the management of abiotic and biotic stress in natural and experimental conditions.

3.2 ROS in Crop Plants: Cellular and Productivity Effects

ROS are oxygen-containing entities that are extremely unstable and reactive (Lobo et al. 2010). Apart from the utilization of oxygen in various biochemical processes, approximately 1–2% of it expended by the plants is averted to generate ROS in certain subcellular sites such as mitochondria, chloroplasts, peroxisomes, apoplast, endoplasmic reticulum, cell wall, etc. They are typically produced in the intracellular and extracellular environments throughout the usual metabolic processes involving electron transfer reactions (Karuppanapandian et al. 2011; Sharma et al. 2012; Stephenie et al. 2020). The certain factors that exert stress like drought, salinity, and very high temperature cause stomatal closure limiting CO_2 fixation, and thus electron transport chains in two photosystems act as key bases of ROS under such circumstances (Gill and Tuteja 2010; Sharma et al. 2012). Several metabolic pathways, which involve peroxisomes like photorespiration, fatty acid β-oxidation, nucleic acid and polyamine catabolism, ureide metabolism, etc., give rise to ROS. Under stressful conditions, such as low availability of water, when the stomata remain closed, the CO_2 to oxygen ratio declines considerably, which favors photorespiration, and a tremendous quantity of H_2O_2 is produced in the cells (Anjum et al. 2016; Sandalio et al. 2013). The mitochondrial electron transport chain (ETC) has an ample supply of electrons in complexes to reduce O_2 to form ROS such as superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). Under stressful conditions, inhibition and alteration of its module take place leading to excess reduction process of electron carrier molecules and, henceforth, creation of surplus ROS (Rhoads et al. 2006; Sharma et al. 2012). The generation of ROS is restricted not only to the inside of the cell but in numerous plant species biotic stress that can lead to a generation of superoxide anions at the cell membrane from the activity of an NADPH oxidase. In addition, the activity of certain enzymes such as amine oxidases, oxalate oxidases, peroxidases, etc. acts as prospective sources of hydrogen peroxide (H_2O_2) in the apoplastic regions (Bolwell et al. 2002).

While an exceedingly high level of ROS causing oxidative stress is harmful to the plants, a basal optimum or minimal requisite scale of ROS is a boon for cells as it is crucial for the appropriate growth and development of plants (Mhamdi and van Breusegem 2018). These molecular species are also mediators in various physiological processes spanning the life cycle of plants, from seed germination to vegetative growth and ultimately to reproductive development (Manna et al. 2019; Singh et al. 2016). In higher plants, ROS are known to have a key role in many aspects of differentiation, development, and redox balance, biological interactions with other organisms, stress response signaling, and also the death of cells (Mittler, 2017). Apart from these ROS, reactive nitrogen species (RNS) like nitric oxide ($NO^{\bullet}$) and nitric dioxide ($NO_2^{\bullet}$) in addition to non-radicals such as dinitrogen tetroxide (N_2O_4) and nitrous acid (HNO_2) also lead to oxidative injury and tissue damage apart from molecular signaling depending upon concentration (Kapoor et al. 2019). Nitric oxide in cells can mediate elicitation of stress tolerance like salinity and water involving important cross talk with other free radicals such as H_2O_2 (Qiao et al. 2014). Under severe biotic and abiotic stress situations, the degree of ROS production surpasses the quenching capability of the cellular defense system culminating in oxidative damage leading to disturbance in equilibrium (Gill and Tuteja 2010). Relentless environmental pressures in the case of plants will lead to the generation of superfluous ROS due to which imperative and detrimental physiological actions are exerted like peroxidation of lipids, oxidation of nucleic acid molecules, denaturation process of proteins, inhibition of the activity of different enzymes, and even activation of programmed cell death process (Das and Roychoudhury 2014; Petrov et al. 2015). Lipid peroxidation initiates a chain of reactions that subsequently exacerbates oxidative stress by generating lipid radicals, which cause significant damage to many important proteins and DNA. When the ROS levels rise tremendously during highly stressful situations and cross the threshold values, the circumstances turn out to be so detrimental that it is usually deliberated as the lone parameter to estimate lipid destruction (Das and Roychoudhury 2014). The two key targets of the ROS in membrane phospholipids are the double bonds among carbon atoms and the ester linkage between fatty acids and glycerol. The polyunsaturated fatty acids (PUFAs) like linoleic and linolenic acid that are considered as two key constituents of the plasma membrane are the hotspot targets by ROS destruction especially superoxide

(O_2•–) and hydroxyl (OH$^•$). The hydroxyl radical (OH$^•$) is the utmost detrimental molecular species that can elicit a recurring chain reaction leading to the peroxidation of other membranes PUFAs (Das and Roychoudhury 2014). The reactive products of lipid peroxidation that include members such as aldehydes, ketones, and hydroxyl acids are responsible for the alteration of proteins by causing the oxidation of certain key amino acid residues. The activity profile of the protein is considerably modified due to varied changes such as carbonylation, glutathionylation, nitrosylation, and the formation of disulfide bonds (Sharma et al. 2012). Excessive ROS can considerably impact in the form of oxidative impairments to nuclear, mitochondrial and chloroplast DNA, which ultimately can lead to the malfunction or inactive encoded proteins (Guo et al. 2013; Hahn and Zuryn. 2019). Deoxyribose oxidation, nucleotide removal, strand breakage, and a variety of other changes in nitrogenous bases are some manifestations that are brought about in DNA by the ROS onslaught. Apart from that, there may be some undesirable changes in DNA-protein cross-linkages (Wang et al. 2020). Significant DNA degradation and undesirable modification have been detected in plants that are exposed to higher salinity and heavy toxic metals (Sharma et al. 2012).

Crop plants are kept from realizing their full genetic potential in terms of growth, reproduction, and yield due to the presence of a suboptimal and undesirable environment. One of the significant productivity limiting factors throughout the world in crops is the occurrence of oxidative stress due to various environmental stresses (Nxele et al. 2017). The rate of plant growth, development, photosynthesis, respiration, and many biochemical processes is perturbed by membrane collapse by excess ROS accumulation. The structure and arrangement of several cell-building constituents such as carbohydrates, lipids, proteins, and nucleic acids are compromised by ROS during stress conditions (Raza et al. 2019). Because of damage to cellular structures, oxidative stress impairs cellular functions that negatively affect germination, plant growth, and yield. The oxidative stress directly exerts its effect on different processes such photosynthesis, transpiration, energy metabolism, transpiration, metabolism of lipids, carbohydrates, synthesis of proteins and nucleic acids, stomatal conductance, cell wall integrity, and pigment distribution, collectively upsetting plant growth and development (Sharma et al. 2017) (Fig. 3.1).

3.3 Antioxidant Enzyme Defense System in Plants

Redox homeostasis in plants in the scenario of stressful circumstances is maintained by a double armor strategy – the enzymatic partners comprising of the superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST), monodehydroascrobate reductase (MDHAR), dehydroascorbate reductase (DHAR), and the low-molecular-weight nonenzymatic components like ascorbic acid, reduced glutathione (GSH), carotenoids, α-tocopherol, proline, phenolics, flavonoids, glycine betaine, etc. (Almeselmani et al. 2006; Das and Roychoudhury 2014; Xie et al. 2019). The ubiquitous and widely prevalent nature of both these

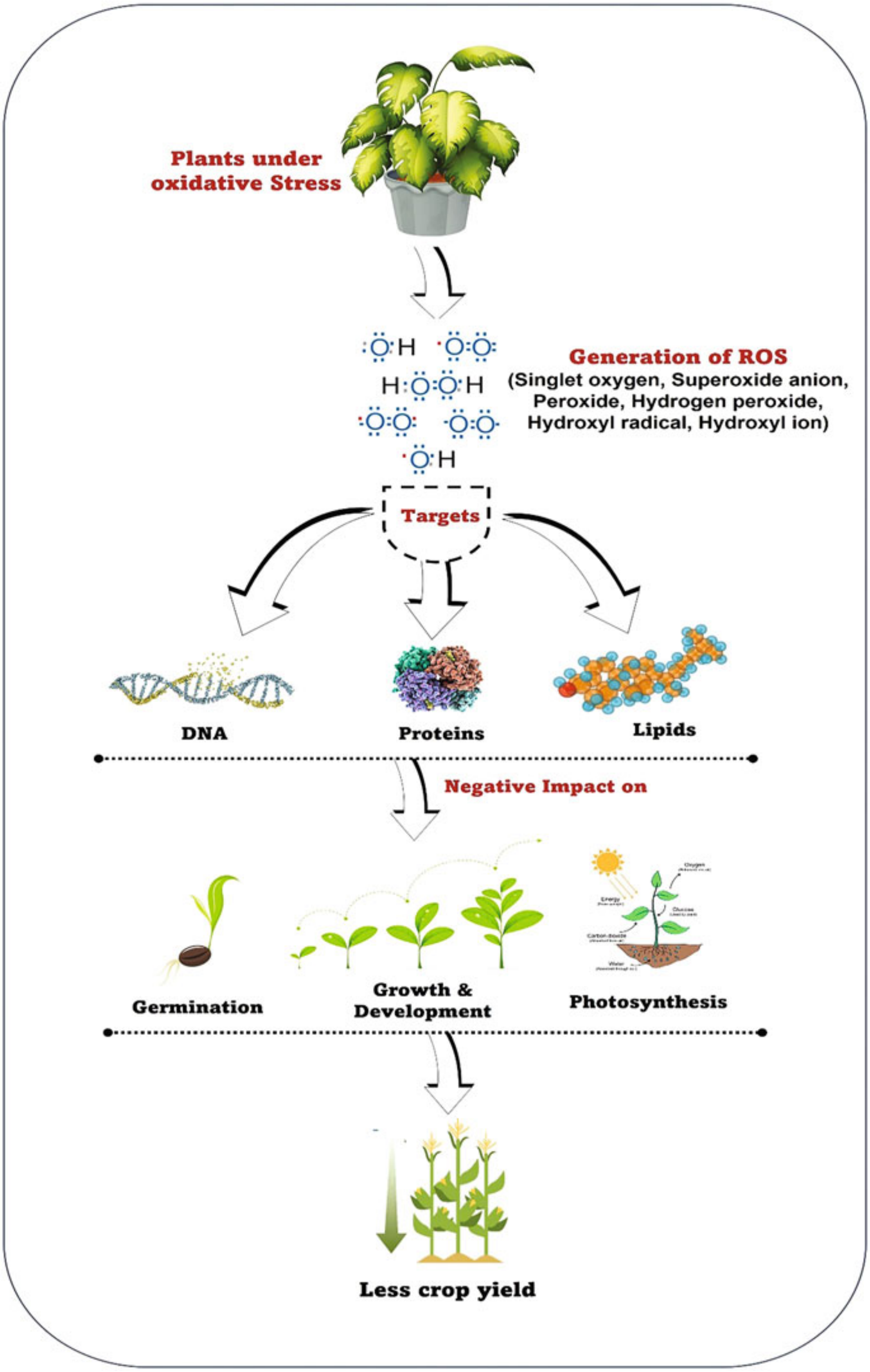

Fig. 3.1 Effect of stress on the different aspects of crop plants via oxidative onslaught and ultimately on their yield

defenses of antioxidant mechanism highlights the inevitability of detoxification of ROS for the survival of cells. The development of stress-tolerant crop plants is a well-directed and important step in this direction (Rajput et al. 2021). Understanding the role of the individual gene under the influence of different stress conditions can be useful in developing stress-tolerant plants. Transgenic strategies have been commonly brought into practice for the progression of plant health and therefore productivity under the varied situations of oxidative stress. Thus, transgenic plants can be engineered and tactfully designed to improve stress tolerance by enhancing the activities of antioxidant enzymes (Hasanuzzaman et al. 2020). A concise account of the enzymatic antioxidants and their role in ameliorating oxidative stress in various crops is presented.

3.3.1 Superoxide Dismutase (SOD, E. C. 1.15.1.1)

SODs are metalloprotein enzymes working with metals such as Cu, Fe, Zn, and Mn as cofactors, and the different isoforms are present in the chloroplast, mitochondria, cytosol, peroxisomes, and apoplast (Stephenie et al. 2020). SOD enzyme is well known to catalyze the conversion of superoxide radical ($O_2^{\bullet -}$) generated via various metabolic pathways into normal oxygen molecules and hydrogen peroxide, which comparatively is lesser harmful (Sharma et al. 2012). In aerobic organisms, the SOD enzyme is very forefront in conferring a defense against the toxicity exhibited by oxygen-mediated radicals. SODs establish the paramount line of defense to combat abiotic stress-induced enhancement of ROS and its reaction products (Gill et al. 2015; Saed-Moucheshi et al. 2021). In the case of plants, environmental adversities like the occurrence of drought, floods, very high or low temperature, the existence of toxic heavy metals, and macronutrient insufficiency often lead to the amplified generation of reduced oxygen reactive species, and in such scenarios, SOD is suggested to play a vital role in conferring plant stress tolerance. The SOD activity has been known to increase in plants when they have to face any of the abiotic or abiotic stress. An investigation with salt-tolerant and sensitive wheat seedlings treated with a 0.7 M NaCl as compared with control revealed that there was up to 1.5-fold enhancement in the MnSOD enzyme activity in the shoots of tolerant cultivars when compared with non-tolerant ones. Though all the cultivars displayed the diminished activity of MnSOD in root tissues, there was a considerable enhancement in Cu/ZnSOD activity (three to four fold) in tolerant cultivar roots, whereas it was diminished in the sensitive ones suggestive of a definitive role in salinity tolerance of these plants ((Inci) Eyidoğan et al. 2003). An increase in the gene expression of cytosolic Cu/ZnSOD was observed in chickpea (*Cicer arietinum* L.) in response to cold stress (4 °C treatment) wherein the increase in SOD activity in response to salt stress was attributed to post-transcriptional regulation (Hernández-Nistal et al. 2002). McKersie et al. 1993 showed that the progeny in transgenic Alfalfa (*Medicago sativa* L.) with surplus MnSOD isozyme exhibited rapid regrowth post freezing stress as compared with non-transgenes (McKersie et al. 1993). In one more recent study, the activity of SOD was found to be 1.38-fold greater in transgenic potato lines overexpressing StSOD1 in comparison with non-transgenic lines. In addition, the activity of POX and CAT was also found to get boosted in a transgenic line pointing toward a possible cross talk or interaction in the expression of antioxidant enzymes (Che et al. 2020; Rajput et al. 2021). The expression and activity profile of SOD can be regarded as an indirect selection criterion for the screening of drought-resistant varieties since it has been reported to correlate with oxidative stress tolerance in plants (Saed-Moucheshi et al. 2021).

3.3.2 Catalase (CAT, E.C.1.11.1.6)

The function of the catalase enzyme is the decomposition process of hydrogen peroxide (H_2O_2) to water and oxygen. Being the first antioxidant enzyme to be discovered, it is commonly found in almost all living organisms even certain anaerobes and consists of tetrameric iron porphyrin protein with subunits ranging from 54 to 59 kDa and molecular weight of 240 kDa, although exceptions are there (Du et al. 2008; Mhamdi et al. 2010). It is a powerful antioxidant enzyme that plays an indispensable part in signaling processes in an energetically feasible pattern in the cells, which are under any sort of environmental stress (Rajput et al. 2021). During stress in cells for energy and rapid generation of H_2O_2 through catabolic processes, the subsequent degradation of H_2O_2 by catalase in an energy-efficient fashion helps to keep the balance (Willekens, 1997). The catalase enzyme exists in all the main organelles where H_2O_2 is produced such as peroxisomes, mitochondria, chloroplast, and cytosol of plants. It functions as a single enzyme triggered by excessive H_2O_2 concentration or in collaboration with other antioxidant enzymes to mitigate the oxidative onslaught during different stresses in plants (Heinze and Gerhardt 2002). It has been well established by research that catalase exists in many forms encoded by corresponding genes and differentially expressed in the temporal, organelle, and stress-specific pattern (Rohman et al. 2020; Palma et al. 2020; I. Sharma and Ahmad 2014). The presence of multiple molecular forms of catalase isozymes such as CAT-1, CAT-2, and CAT-3 encoded, respectively, by structural genes Cat1, Cat2, and Cat3, respectively, are suggestive of its multipurpose role within the plant system (Matsumura et al. 2002; Rajput et al. 2021). Depending upon intensity, duration, and nature of stress, there can be an enhancement or diminution of the CAT activity (Sharma et al. 2012). An increase in catalase activity rate is supposed to be associated with resistance of drought in *Carthamus tinctorius* L. (safflower) (Zare 2011). A foliar pretreatment of H_2O_2 in maize seedlings causing an increase in the amount of catalase enzyme was shown to lessen the injurious effects of salinity stress on overall growth parameters and lipid peroxidation (Gondim et al. 2012). Transgenic rice plants expressing wheat CAT protein showed almost 2–5 times increase of activity in leaves as compared with non-transgenic ones, which conferred them with better capability to low-temperature stress of 4 ° C for eight days (Matsumura et al. 2002). Heat-sensitive rice mutants in rice have been demonstrated to amass ROS accompanied with diminished catalase activity under heat stress in contrast to

HTT-121 mutant described as heat tolerant, suggesting its key role in adapting to heat (Zafar et al. 2020). Combined overexpression of antioxidant enzymes Cu/Zn superoxide dismutase (MeCu/ZnSOD) and catalase (MeCAT1) in cassava, was shown to extend the shelf life of their storage roots by the maintenance of ROS homeostasis postharvest apart from a considerably improved tolerance capability to cold and drought stress in comparison to wild types (Xu et al. 2013).

3.3.3 Guaiacol Peroxidase (GPX, E.C. 1.11.1.7)

It is a heme-containing enzyme consisting of 40–50 kDa monomers that remove superfluous H_2O_2 in the stress conditions or usual metabolism and is ubiquitous in animals, plants, and microbes. It preferably causes oxidation of aromatic moiety containing electron donors, e.g., guaiacol and pyrogallol, at the expenditure of H_2O_2 (Das and Roychoudhury 2014; Erofeeva 2015). This enzyme has been deliberated as the important one in the elimination of H_2O_2. Apart from its isoenzymes being active in intracellular components such as cytosol, vacuole, and the cell wall, it also functions extracellularly as an efficient quencher of reactive intermediary forms of O_2 and peroxy radicals in stressful circumstances (Sharma et al. 2012). In addition to being involved in defense against abiotic and biotic stresses, it is also linked with several significant biosynthetic processes, such as lignification of the cell wall, wound healing, degradation of indoleacetic acid (IAA), and biosynthesis of ethylene (Anjum et al. 2016; Das and Roychoudhury 2014; Sharma et al. 2012). GPX activity is known to increase in plants under several forms of biotic and abiotic stresses and thus has a definite part in providing tolerance (Erofeeva 2015; Varga et al. 2012). Golfazane et al. 2017 demonstrated a greater level of GPX activity in the drought-tolerant genotype of canola in osmotic stress conditions, leading to the diminished buildup of H_2O_2 as compared with drought susceptible genotype (Golfazane et al. 2017). It was demonstrated that the increase of the GPX activity, at least in part, is responsible for the superior defense of salt-tolerant safflower plants from the salt-mediated oxidative onslaught in addition to its catalytic efficiency and stimulation of specific isoenzymes in comparison to salt-sensitive cultivar (Tayefi-Nasrabadi et al. 2011). In another study, a considerable enhancement in tolerance of pepper (*Capsicum annuum L.*) plants to chilling-induced oxidative damage as indicated by the reduction of foliar damage symptoms and levels of malondialdehyde and H_2O_2 was provided by exogenous supplement of abscisic acid (ABA) chiefly by improving GPX and SOD activities and corresponding gene expression (Guo et al. 2012).

3.3.4 Glutathione Reductase (GR, E.C.1.6.4.2)

GR enzyme is a flavoprotein oxidoreductase present in both prokaryotic and eukaryotic organisms, which utilize pools of NADPH for the reduction of oxidized form GSSG to GSH (reduced glutathione) and thus is helpful for the maintenance of higher GSH to GSSG ratio. Although most of the activity of GR isoforms is restricted to chloroplasts in the photosynthetic tissue, it is also located in minute amounts in cytosol, mitochondria, and peroxisomes (Das and Roychoudhury 2014; Sharma et al. 2012). GSH is converted to its oxidized form (GSSG) in the process of utilization to generate ascorbic acid from MDHA and DHA. It maintains the pool of GSH by contributing to the maintenance of the sulfhydryl (-SH) group, which acts as a substrate for glutathione S-transferases (Yousuf et al. 2012). The catalytic process of the enzyme is accomplished in two critical steps. The first step is the reduction of flavin domain taking place by reducer NADPH followed by oxidation and a redox-active disulfide bridge undergoes reduction in the process to produce a thiolate anion and a cysteine. In the second one, it is the reduction of GSSG that takes place by thiol-disulfide exchange reactions (Gill et al. 2013). The concomitant pool of GSH act as an antioxidant by reaction with detrimental ROS entities like H_2O_2, $^1O_2$, and $OH^\cdot$. The escalation of activity or overexpression of enzyme GR, therefore, leads to abiotic stress tolerance in many crops due to proficient ROS quenching tendency (Romero-Puertas et al. 2006). Antisense transgenic tomato (*Lycopersicon esculentum Mill.*) for chloroplast glutathione reductase gene under chilling conditions resulted in a greater buildup of $H_2O_{2,}$ excess electrolyte leakage, decrease in photosynthesis, and oxidizable P700 as compared with wild type plants emphasizing the importance of GR in alleviating the manifestations of the oxidative stress (Shu et al. 2011). Oxidative stress in the form of paraquat or H_2O_2 treatment has been observed to induce GR synthesis in a drought-resistant strain of maize (Pastori and Trippi 1992). Similarly, Koscy et al. (2002) observed that cold treatment induces a greater increase in GR activity in tolerant genotypes of wheat and maize as compared with sensitive ones. In the same study, osmotic stress and drought stress also lead to enhanced GR activity and total glutathione content especially to tolerant genotypes improving their adaptability to abiotic stresses (Kocsy et al. 2002). An upregulation of GR activity in wheat plants by the stimulation of distinctive isoforms was observed, which occurs as a defense mechanism against cadmium-generated oxidative stress in roots (Yannarelli et al. 2007). From time to time, there have been numerous endeavors for tapping the possibility of alteration in the expression of specific enzymes by genetic manipulation for the development of transgenic plants with upgraded levels of oxidative stress defense enzymes to augment stress tolerance. There have been reports of few transformed lines of tobacco plants with elevated levels of expression of GR accumulating greater concentrations of the GSH, which display augmented tolerance to herbicide paraquat (Creissen et al. 1994).

3.3.5 Ascorbate Peroxidase (APX, E.C.1.1.11.1)

As a scavenger of ROS in cells, APX belongs to the multi-copper oxidase family and functions as an essential constituent of the ascorbate-glutathione cycle. This enzyme is involved in catalyzing the reduction of H_2O_2 to H_2O by using two molecules of ascorbate as a reducing agent resulting in the production of two molecules of monodehydroascorbate (MDHA) in this process (Das and Roychoudhury 2014). It is considered a more useful enzyme in plants during stressful conditions for the management of oxidative stress because of its ubiquitous nature and better affinity for H_2O_2 as compared to CAT enzyme (Pignocchi et al. 2006; Sharma et al. 2012). Based on different amino acids, the family comprises many isoforms present in different locations such as cytosol, mitochondria, peroxisomes, and chloroplast encoded by discrete genes (Pignocchi et al. 2003). APX is highly significant in connection with alleviating the adverse effects of ROS in crop plants. By employing various techniques such as western blotting, enzyme activity assay, and biophoton emission, a strong correlation was found between APX amount and activity with increasing drought in soybeans (Kausar et al. 2012). Lead-contaminated soil was shown to stimulate APX activity in *Vicia faba* L., which plays a critical role in negating the effects of ROS in a condition of declining CAT activity (Wang et al. 2010). In another study, in *Prunus* hybrids after 70 days of water scarcity without irrigation, the activities of APX and other enzymes of the ascorbate-glutathione cycle were shown to increase with severe drought stress, whereas they showed a complete reversal in trend during the following rewatering phase more rapidly in shaded leaves (Sofo et al. 2005). Transformation of plum plants with genes encoding antioxidant enzymes including cytosolic ascorbate peroxidase (cytapx) has been demonstrated to augment the tolerance to salinity (Diaz-Vivancos et al. 2013). Overexpression of cytosolic ascorbate peroxidase (cAPX) gene derived from *Pisum sativum* L. in transgenic *Lycopersicon esculentum* L. (tomato) caused enhancement of 10 and 25-fold activity during salt and chilling stress minimizing the oxidative damage, which was visible in leaves (Wang et al. 2005).

3.3.6 Dehydroascorbate Reductase (DHAR, E.C. 1.8.5.1)

Dehydroascorbate reductase is an important ascorbate recycler enzyme belonging to the glutathione S-transferase (GST) superfamily that catalyzes the reduction of oxidized ascorbate (DHA) to ascorbate with reduced glutathione (GSH) acting as the reducing substrate. Thus, DHAR has the role to regenerate and maintain a pool of reduced forms of ascorbate within the cells for the subsequent detoxification of ROS. The oxidized glutathione (GSSG) by the action of glutathione reductase is again reduced to glutathione (GSH) with NADPH as an electron donor (Do et al. 2016). DHAR enzyme because of its role is copiously found in seeds, root and shoot distributed in both apoplast and symplast (Das and Roychoudhury 2014). DHA, a very short-lived molecule, has either chance of getting irreversibly hydrolyzed to 2, 3-diketogulonic acid or can be recycled to ascorbate by DHAR (Sharma et al. 2012). Metal toxicity, salinity, drought, and chilling act as the environmental stressing agents that trigger the DHAR activity in plants and confer them with the capability to scavenge ROS generated in such a milieu. An increase in ascorbate pool content has been reported due to overexpression of DHAR in tobacco and maize, which effectively enhances the ability to counter oxidative stress during stress conditions (Chang et al. 2017; de Tullio et al. 1998; Kwon et al. 2003). Overexpression of DHAR has been shown to enhance salt stress tolerance in rice plants (*Oryza sativa* L. japonica) by maintaining ASA pool leading to better growth and yield (Kim et al. 2014). Transgenic potato bearing the Arabidopsis cytosolic AtDHAR1 has been seen to possess enhanced DHAR activity and a level of reduced ascorbate. Therefore, they are reported to be more tolerant to herbicide, drought, and salt stresses in comparison with their wild varieties (Eltayeb et al. 2011). Improved ascorbate regeneration and accumulation via overexpression of DHAR in transgenic tomato have been known to provide tolerance to deleterious effects of salt stress resulting in better photosynthetic rate, germination rate, chlorophyll content, etc. (Li et al. 2012). Constant upregulation of cytosolic DHAR gene in *Lotus japonicus*, leading to improvement of apoplastic ascorbate pool, was reported to enhance the salt stress tolerance in it as compared to other legumes (Rubio et al. 2009).

3.3.7 Monodehydroascorbate Reductase (MDHAR, E.C. 1.6.5.4)

MDHAR is a Flavin adenine dinucleotide (FAD) enzyme that replenishes the ascorbate pool by catalyzing the process of its regeneration from the short-lived MDHA generated in APX-catalyzed H_2O_2 scavenging reaction utilizing NADPH as the electron donor. The activity of MDHAR is very common in plants since it has many isozymes which are located in several cell organelles like mitochondria, chloroplast, glyoxysomes, and peroxisomes in addition to the cytosol (Das and Roychoudhury 2014; Sharma et al. 2012). Numerous studies reveal an increase in activity of MDHAR in crop plants such as maize, tomato, wheat, etc. subjected to varied stresses (Feng et al. 2014; Hodges et al. 1997; Stevens et al. 2008). Ectopic expression of MDHAR gene from *Brassica rapa* L. in Arabidopsis was demonstrated to regulate other antioxidant genes like SOD, APX, GR, DHAR, etc., and the plants showed improved freezing stress tolerance with favorable redox status (Shin et al. 2013a, b). Likewise, in another study, transgenic Arabidopsis plants co-overexpressing *Br*MDHAR and *Br*DHAR (from *Brassica rapa* L.) consistently exhibited enhanced antioxidant capacity and redox status upon freezing stress relative to non-transgenic counterparts (Shin et al. 2013a, b). Overexpression of tomato (*Lycopersicon esculentum* Mill.) chloroplastic MDHAR in transgenic Arabidopsis has been reported to enhance its tolerance to temperature and methyl viologen-mediated oxidative onslaught resulting in the alleviation of photo inhibition of photosystems (F. Li et al. 2010). Overexpression of MDHAR gene from Arabidopsis in the case of tobacco plant confers improved tolerance resulting in the higher net rate of photosynthesis during salt, ozone, and polyethylene glycol (PEG) stresses (Eltayeb et al. 2007).

3.4 Antioxidant Enzyme Status in Crops Under Stress

Abiotic environmental stresses are known to initiate a cascade of physiological and molecular changes in plants, effectuating similar sort of responses in some cases.

3.4.1 Salinity

According to a reasonable estimate, about 50% of the total land under cultivation faces serious challenges due to an unprecedented rise in salinity. Besides its huge repercussions on plant growth and health, salinity poses a tremendous threat to world food security by dramatically downsizing the crop yield of agriculturally important plants. A global analysis of current trends and plausible future scenarios assume that crop yield loss due to salinity is likely to continue in the foreseeable time largely because the direct effects of salinity on crop yields are projected to either remain constant or increase in the future. Moreover, due to scarcity of freshwater, influence and incidence of salinity are thought to expand day in and day out (Habib et al. 2016). Among the plethora of biochemical changes, salinity-induced rise in reactive oxygen species (ROS) generation in plant species is most dramatic. In response to salinity, plants evolve a series of compensating mechanisms that are enough efficient to neutralize the negative consequences of salts. Additionally, it has been found that the activity profile of some antioxidant enzymes increases disproportionally in plants in extremely saline environments. As a case in point, it has been reasonably argued that the activities of CAT, APX, and GR rise considerably in both salt-tolerant KRL-19 and salt-sensitive WH-542 cultivars of wheat (*Triticum aestivum*) seedlings. However, the activities of these enzymes showed partial and full recovery upon desalinization in KRL-19 and WH-542, respectively (Mandhania et al. 2006). In yet another study, seedlings of barley subjected to NaCl (20 mM) for 0, 1, 2, and 5 days showed a considerable increase in the activities of SOD, CAT, APX, POX, and GR in the roots within 1 DAT. Interestingly, the elevated levels of these enzymes in different treatments were sustained by 5 DAT. Among all the enzymes, the activity of CAT revealed tremendously large increments. Among the antioxidant enzymes, CAT activity was increased the most drastically. Surprisingly, NaCl-stressed roots of barley exhibited a strong positive correlation between activities of SOD, CAT, APX, among others, and expression of constitutive as well as induced isoforms (Kim et al. 2005). Another interesting study on four different cultivars of potato showcased a significant rise in the production of CAT and POD during episodes of salt-induced stress. Moreover, salt-tolerant potato cultivars were found to be better protected against reactive oxygen species (ROS) outbursts due largely to the enhanced production of counteractive antioxidant enzymes (particularly SOD) in them (Rahnama and Ebrahimzadeh 2005). Another well-established *in vitro* investigation performed on rice (*Oryza sativa* L.) cultivar, BRS AG, showed that treatment of plants with 136 mM NaCl increases protection against salinity-induced oxidative stress via enhanced activities of antioxidant enzyme systems. A series of genes that encode different isoforms of antioxidant enzymes include OsSOD3-CU/Zn, OsSOD2-Cu/Zn, OsSOD-Cu/Zn, OsSOD4-Cu/Zn, OsSODCc1-Cu/Zn, OsSOD-Fe, and OsAPX1. Among all, OsCATB and OsGR2 revealed a proportionately large surge in their activities. Except for OsAPX6 that exhibited stability in its expression, all other target isoforms contribute to the escalated enzymatic activity in this species. Therefore, it is appropriately concluded that cultivar BRS AG of rice has profound defensive strategies against salt stress and its follow-up oxidative damages (Rossatto et al. 2017). Down the line of such investigations, it was observed that *Cuminum cyminum* L. plants treated with 50, 100, and 150 mM of NaCl significantly increase the activity SOD, APX, and CAT. As evidenced by the results of real-time quantitative reverse transcription-polymerase chain reaction, it was found that expression of iron-superoxide dismutase (Fe-SOD) and catalase (CAT) genes in this species increases considerably following 50, 100, 150, and 200 mM NaCl treatments. These changes were also effectuated in the expression profile of their respective mRNAs. In view of the above discussion, it can be precisely concluded that *C. cyminum*'s salinity-responsive antioxidant system enables it to survive in saline soils (Soleimani et al. 2017). A concise account of more examples wherein the introduction to salinity stress significantly altered the antioxidant enzyme activity in crops is given in Table 3.1.

3.4.2 Drought Stress

Drought condition is the manifestation of a disparity between the evapotranspiration flux and uptake of water from the soil, which represents one of the crucial stresses that plants encounter in numerous biogeographical regions. Plants experience drought stress during very little soil moisture, which is accompanied by low atmospheric humidity and high air temperature. In the vast majority of the world's agricultural fields, this stress factor alone is the most critical impediment to plant growth and crop productivity (Lamaoui et al. 2018). The reduction in transpirational water loss and thus drought stress, however, is alleviated by abscisic acid (ABA), an important plant hormone that facilitates the closure of stomata. The stomatal closure is critical in drought stress responses because it lowers the NADP+ regeneration and inhibits the CO_2 fixation via the Calvin cycle (Waterland et al. 2010). Because of the drawbacks of stomatal closure about photosynthesis, it must be done only when water conservation benefits outweigh the negative effects (Rajput et al. 2021). Through accelerated electron leakage to molecular oxygen, these unfavorable circumstances stimulate the production of ROS such as $O_2^{\bullet -}$, OH, and H_2O_2 radicals. Drought or water shortage stress induces the creation of a variety of ROS, including non-radical molecular forms like $^1O_2$ and H_2O_2 and highly reactive free radicals like alkoxy radicals ($RO^{\cdot}$), perhydroxy radical ($HO_2^{\cdot}$), $O_2^{\bullet -}$, and $^{\bullet}OH$ (Impa et al. 2012). SOD, APX, GPX, and chloroplastic APX activities were augmented in rice seedlings after drought stress of −0.5 to −2.0 MPa for 24 h to slow the generation of $O_2^{\bullet -}$ and progression and lipid peroxidation (Sharma and Dubey 2005). It was demonstrated that the severity and duration of water stress and fruit development stage influenced the changes in APX, CAT, SOD, GR, MDHAR, and DHAR activities and comparable transcript levels in tomato *Solanum lycopersicon* L.cv Micro-tom (Murshed et al. 2013). Regulations of plants' physio-biochemical responses to drought conditions could be used as markers for drought stress tolerance in the selection and breeding processes. In comparison with two other maize hybrids, drought-induced osmolyte buildup and robust antioxidant enzymatic defense systems averted serious damage in Dong Dan 80 hybrid (Anjum et al.

Table 3.1 Effects on antioxidant enzyme activity in crops under salinity stress

Crop plant material	Stress condition	Effect	Status of antioxidant enzymes
Wheat seedlings two cultivars Salt resistant Salt sensitive	50 and 100 mM NaCl	A decline in relative water content, cell membrane damage	Enhanced activities of CAT, APX, GPX (Mandhania et al. 2006)
Four rice varieties Salt tolerant (BRRI dhan 47, BINA dhan 8 and BINA dhan 10) Sensitive (BRRI dhan 28)	20, 40 and 60 mmol/L NaCl	Reduction in growth, root shoot biomass, chlorophyll content	CAT and APX increase in tolerant varieties, decreased GPX activity (Kibria et al. 2017)
CCRI-79 (salt tolerant) and Simian 3 (salt sensitive) Cotton cultivars	0, 80, 160, and 240 mM NaCl	Reduction in the dry weight of organs, a decline in net photosynthesis and stomatal conductance	Higher SOD, CAT, APX, and GR activity in CCRI-79 (salt-tolerant cultivar) (Zhang et al. 2014)
Maize plant Salt tolerant (USTB-297) Moderate salt-tolerant (USTB-109) Salt sensitive (USTB-265)	100 mM NaCl	Decrease in plant height	SOD activity is higher in USTB 297. Ct and APX activity are higher in USTB-109 and 265 (Cai et al. 2019)
Four potato cultivar seedlings—Agria, Kennebec (salt tolerant); Diamant, Ajax (salt-sensitive)	50 mM NaCl	50% decrease in shoot fresh mass in salt-sensitive cultivars	Enhanced activities of SOD, CAT, APX (Rahnama and Ebrahimzadeh 2005)
A. Amaranthus tricolor varieties, VA14 (salt-tolerant), and VA3 (moderately salt-sensitive)	50 mM and 100 mM salt	A decline in leaf dry weight per plant, specific leaf area, total biomass, and shoot dry weight, chlorophyll	Increase in activity of SOD, GPX, CAT (Sarker and Oba 2020)
Two barley varieties Afzal and EMB82-12	50,100, 200, 300, and 400 mM NaCl for 3 days	MDA content constant in Afzal, MDA enhancement in EMB82-12	Enhanced activity of CAT, APX, SOD, GPX in Afzal (Khosravine et al. 2008)
Two date palm cultivars (*Phoenix dactylifera* L.), "Umsila" (salt-tolerant), and "Zabad" (salt susceptible)	NaCl solution at 240 mM	Increase in H_2O_2 in both, MDA in Zabad	Enhanced activity of CAT, SOD, APX in leaf and root tissues of Usmila (al Kharusi et al. 2019)

2017). Soybean plants evolved antioxidative defense mechanisms, such as accelerated antioxidant enzyme activity and suitable soybean cultivars, were able to mitigate abiotic stresses under imbalanced WDC, particularly in crop rotations

(Iqbal et al. 2019). Melatonin treatment boosted the activities of key antioxidant enzymes like APX, SOD, CAT, and others, implying that it should be used on a wide level during drought conditions (Imran et al. 2021). Silicon greatly increased the lentil crop plants' ability to tolerate drought stress conditions by promoting antioxidant enzyme activity and diminishing reactive oxygen species production (Biju et al. 2017). In a study conducted, potato plants treated with the development rhizobacteria *Bacillus subtilis* HAS31 generated lesser ROS and MDA and had improved enzymatic activity of POD, SOD, and CAT, demonstrating that growth and yield were maintained under drought stress (Batool et al. 2020). In another recent study, selenium supplementation reduced the negative effects of drought by altering the physio-biochemical processes of plants, including an increase in production of APX, SOD, and CAT that eventually led to the enhanced yield and yield components of both crops, oilseed crops, *Brassica napus* L. (Canola) and *Camelina sativa* L. (Camelina) and under drought conditions (Ahmad et al. 2021). According to a recent study, drought stress triggered by PEG-6000 had a relatively less negative impact on photosynthesis in Bachar cultivar of faba beans (*Vicia faba*) than Giza 3 due to improved antioxidant enzyme operations (CAT, SOD, and APX) and elevated transcription levels of their respective genes (Abid et al. 2021).

3.4.3 Heavy Metal Toxicity Stress

Heavy metal intoxication of the environment, caused by human-made practices and/or natural events, is a common and severe challenge in the present era. The emergence of the industrial revolution has aggravated this issue to such undesirable limits that have necessitated more relevant scientific research in this direction and further course of action to lessen its impacts (Arif et al. 2019; Rai et al. 2019). Excessive accumulation of heavy metals reduces crop yields by inflicting direct or indirect damage to plant tissue and interfering with various biochemical and physiological processes in plants. They disrupt a variety of physiological processes in plants, including seed germination, accumulation and remobilization during germination, photosynthesis, and plant growth, to name a few (Ghosh and Sethy 2013). Heavy metal poisoning disrupts the redox balance in cells and reduces crop output by causing an outburst of reactive oxygen species. Plants respond to heavy metal stress in a variety of ways, but the majority of these involve cell detoxification via antioxidative scavenging mechanisms (Shahid et al. 2015; Yilmaz et al. 2017). Plants have restricted stress-avoidance mechanisms and require an adaptable way of coping with change. A popular attribute in the fight against stress factors is the synchronized function of antioxidant enzymes, which aids in the substantial reduction of cellular deterioration by curtailing reactive oxygen species (ROS). Understanding the interaction between oxidative stress and the role of antioxidant enzymes can lead to the development of certain plants that can withstand oxidative stress. These processes have proven to have enormous potential for remediating these pernicious metals via phytoremediation (Bhaduri and Fulekar 2012). As several researchers have pointed out, the severity of oxidative stress triggered by heavy metals varies by species and across genotypes, tissues, and /or developmental phases. Metal-susceptible plants, in general, exhibit severe symptoms when subjected to oxidative stress, whereas metal-resistant plants show only minimal or no oxidative damage (Juknys et al. 2012; Xie et al. 2019). Cadmium pollution leads to the production of ROS in *Oryza sativa* L. (rice) plants antioxidant stress and accumulation of peroxidation of membrane lipids. The cadmium exposure accelerated the activities of SOD, peroxidases, CAT, and other antioxidant enzymes in both leaves and roots, implying that rice seedlings cope with free radicals generated by Cd via coordinated, improved activities of the antioxidative enzymes engaged in detoxification (Yu et al. 2013). In yet another example, Cd and Pb interactive effects on rice enhanced generation of ROS (H_2O_2 and $O_2^{\bullet-}$), lipid peroxidation, protein carbonylation, etc. Despite increased antioxidative enzyme activity (GPX and SOD) in metal-treated seedlings relative to controls, CAT upregulated during the first week of metal exposure and then fell after two weeks (Srivastava et al. 2014). Recently, Thind et al. (2021) showed that the application of Si augmented the activities of various antioxidants, APX, SOD, POD, and CAT leading to the alleviation of parameters of oxidative stress induced by Cd uptake in root and shoot of two wheat cultivars (Thind et al. 2021). Antioxidant profiling of the tolerant (TPM-1) and sensitive (TM-4) cultivars of *Brassica juncea* after exposure to arsenate [As(V)] and arsenite [As (III) found that TPM-1 had a greater response to antioxidant enzymes and glutathione levels than TM-4. When compared with TM-4, these responses may have allowed TPM-1 to withstand higher As concentrations (Srivastava et al. 2010). It's likely that Al toxicity is mediated by oxidative stress and that the Al tolerant maize roots enhanced protection against Al-induced oxidative damage is due to, at least to some extent, their antioxidative system's greater activity (Giannakoula et al. 2010). GB may reduce Cd toxicity in cotton plants by minimizing Cd concentrations and regulating Cd-induced oxidative stress in various plant parts, potentially by improving the activity of the antioxidant enzymatic system (Farooq et al. 2016). Several fold enhancements of antioxidant enzymes APX, SOD, GR, and CAT were shown in *Pisum sativum* (pea) after treatment with $NiCl_2$ and $CdCl_2$ (El-Amier et al. 2019; Hasanuzzaman et al. 2020). Cr levels significantly increased SOD, APX, and GST activation, particularly in the leaves, and had a significant impact on the photosynthesis process in *Sorghum bicolor*. The antioxidative enzyme activity in the leaves was higher than in the root, implying that the leaves have a higher level of lipid peroxidation and hence a greater resistance to heavy metal stress (Yilmaz et al. 2017).

3.5 Conclusion

Reactive oxygen species (ROS) are irreversible by-products of regular cellular metabolism, which are produced by electron transport processes in various cellular components or organelles. The various biotic and abiotic stresses cause a buildup of ROS in cells leading to detrimental consequences; however, they mediate certain essential biological phenomena such as signaling at minimal concentrations. In crop plants, this causes a considerable loss in yield. The plant defense against ROS depends largely on an enzymatic component that constitutes several enzymes that help mitigate the ROS and bring the cells back to a state of homeostasis. There are well-established studies regarding the reaction mechanism of various antioxidant enzymes, which are engaged in the defense of plants against the ROS to withstand the stressful milieu. Advances in genomics, metabolomics, and proteomics have significantly contributed to a better understanding of biochemical mechanisms involved in cellular responses to oxidative stress. The transgenic method for overexpression of antioxidant genes individually or as cassettes can deliver tolerance in crop plants to multiple stresses and thus help to attain the goal to achieve sustainable global food security.

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Proteomic and Genomic Approaches for Antioxidant Enzyme-Mediated Defense Analyses in Higher Plants

4

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Abstract

Plants are sessile organisms and constantly encounter a number of environmental stresses, including salinity, water scarcity, life-threatening temperature extremes, toxic heavy metals, flooding/waterlogging (WL), etc., and biotic stresses like pathogen attack. All these stresses are further worsening due to drastic and harsh climate change. The aim of this chapter is to critically analyze the proteomic and genomic aspects and to comprehend the state-of-art knowledge regarding enzymatic antioxidant defense systems to cope with the various stresses faced by the plants. The practical solicitation of antioxidant enzymes in generating stress-tolerant transgenic plants is also discussed. The proteomic approaches for SOD, CAT, POX, GPX, GR, GST, APX, MDHAR, and DHAR enzymes and their recent molecular understanding are particularly discussed with reference to advanced techniques like LC-MS/MS, MALDI-TOF, and phylogenetic and conserved motif analysis. Genome-wide association studies (GWAS) including transcriptome, sequence tagged site, *CRISPR/Cas9*, and quantitative trait loci-based approaches regarding enzymatic antioxidants have been summarized with reference to stress conditions. The detailed study will help in providing

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comprehensive proteomic and genomic understandings about antioxidant enzyme-mediated defense in higher plants.

Keywords

Antioxidants · GWAS · LC-MS/MS · MALDI-TOF · Innate mechanism · QTLs

4.1 Introduction

Food security is a major concern to feed growing population, but perplexing environmental constrains are causing drastic reduction in overall crop yield (Shao et al. 2009). The higher plants, confronting environmental constraints (drought, salinity, light, and high and low temperature), are particularly associated with oxidative stress, resulting in the accumulation of excited oxygen species. These species regulate many cellular processes and are extensively known for duple role in plant physiology, for inducing oxidative stress and serving as signaling molecules in several developmental processes (Noctor et al. 2018). These noxious species are activated byproducts of oxygen, consisting of free radical or non-radical forms such as $O_2^{o-}/OH^o/OH_2^o$ and H_2O_2, respectively, sum up to cellular impairment, discomfort, and ageing mechanism. Plants have been equipped with two basic antioxidant defense processes, i.e., enzymatic and nonenzymatic systems to mitigate the oxidative stress. The nonenzymatic group comprised of low-molecular-weight molecules such as glutathione, carotenoids, phenolics, and vitamin (A, C, and E) while superoxide dismutase, peroxidase, and catalase formed enzymatic defense system to eliminate stress (Jaleel et al. 2009).

Stress tolerance mechanism is a multifaceted and multigenic trait, and progresses with traditional breeding procedures must be very challenging to feed growing population while molecular breeding techniques, based on genetic engineering, marker-assisted selection (MAS), and genome editing technologies, grasp great potential to empower farmers to cope with these concerns in a better way.

Post-translational medications (PTMs) of some proteins are significantly involved in the acclimatization of plants under environmental stresses. Growing confirmation has revealed that PTMs play critical roles in environmental stress regulation via regulating glycosylation, phosphorylation, and ubiquitination in plants. Therefore, it is essential for scientists to further discover key PTM-related genes for developing abiotic stress-tolerant crops (Wu et al. 2016).

Quantitative analysis of proteins is a strong approach to screen protein accumulation in plant samples. Proteomic analysis clearly defines the role of salt-responsive proteins in plants (Long et al. 2018). Proteomic study of soybean seedlings under salt stress revealed the upregulation of amino acid and carbohydrate, antioxidant, and protein metabolism (Ji et al. 2016). Rice genotypes exhibited an early upregulation of photosynthetic and antioxidant metabolism proteins under salt stress.

4.2 Mining of Proteomic Approaches for Enzymatic Antioxidant Response

Various morpho-physiological and biochemical attributes were considered as indirect indicators for biotic and abiotic stress tolerance (Teulat et al. 2003; Richards et al. 2002).

The general procedure for proteome analysis involved

- Sample preparation, including extraction and proteolytic digestion of the proteins.
- Peptide separation.
- MS analysis.
- Informatic data interpretation (Liu et al. 2019).

4.2.1 Proteomic Approaches

Proteomic techniques give most suitable way for the functional examination of translated genome sections. Crude proteins have been extracted and isolated by fractionation, which is the most promising method to attain better proteome exposure (Hashimoto and Komatsu 2007). More advancements and initiatives for fractionation technique need to be addressed to cope with the narrow proteome resolution (Hashimoto and Komatsu 2007).

4.2.1.1 LC-MS/MS

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is a highly precise technique enabling the detection of wide range of metabolites in biological samples (Sawada and Yokota Hirai 2013). The desirable peptide can be quantitively measured and identified by the assistance of this technique and the retaining time of respective peptide in any complex plant sample (Song et al. 2015). Selected reaction monitoring (SRM)/multiple reaction monitoring (MRM) approaches have been proved as foremost advancement tools to acquire reliable quantitative proteomic data (Lange et al. 2008). Song et al. (2015) using these techniques successfully identified aldo/keto reductase (AKR) and its isomers along with SOD, APXs, GR, and GSTs during ripening of strawberry fruit. All studied antioxidant of strawberry fruit significantly increased except cytosolic APX, CAT, 1-Cys, and 2-Cys PRX. Quantitative MRM and LC-MS/MS technique offers an organized and multi-focused enquiry of the enzymatic antioxidant in plant samples at each ripening stage. Several enzymes expressed differentially in *Solanum lycopersicum* under drought stress (Cheng et al. 2015). Quantitative and qualitative proteomic investigation revealed the dynamics of plant apoplast in adverse effect of drought in plants (Jaswanthi et al. 2019). Stress-related protein species particularly SOD and peroxidases were found to be upregulated, and 20 unique protein species were also recognized in drought-stressed proteome analysis (Zhou et al. 2013).

TMT labeling coupled with LC-MS/MS was used to check the stress resistance of wild and domesticated *Rhododendron chrysanthemum* at both cellular and

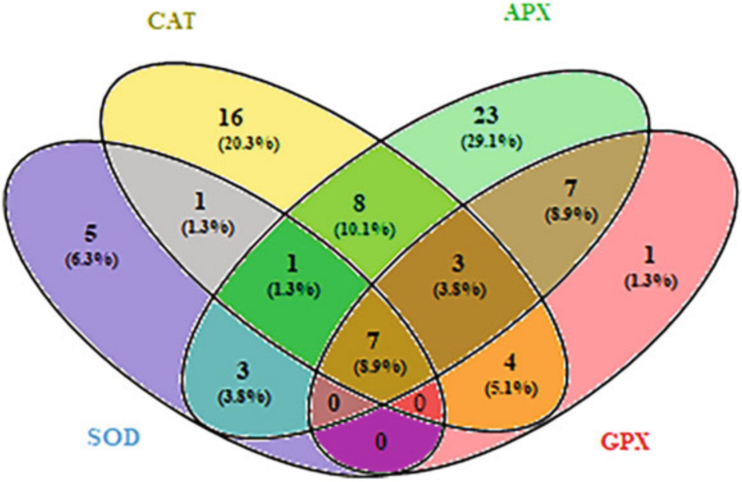

Fig. 4.1 Venn diagram for SOD, CAT, APX, GPX (number, percentage, and overlapping), and their interaction among four categories in *Rhododendron chrysanthemum* Pall

molecular level. Among 1395 identified proteins, 137 proteins were upregulated in the domesticated type of *R. chrysanthemum*. Significantly higher activity and expression levels were noted for SOD, CAT, APX, and GPX in this category. However, the enzymatic antioxidants interaction network analysis also expressed considerable role of these enzymes in plant's stress tolerance (Zhou et al. 2017) (Fig. 4.1).

4.2.1.2 MALDI-TOF-MS

Proteomic advancements using two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF MS) provide an appropriate method to detect differentially expressed proteins engaged in responses to numerous stresses (Agrawal et al. 2009). Thirty-four different proteins were detected in rice by MALDI-TOF mass spectrometry approach. The upregulation of L-ascorbate peroxidase, putative DHAR, and POD was observed in rice exposed to Cu and Cd stress (Lee et al. 2010; Rakwal et al. 1999). Song et al. (2013) recorded more pronounced expression of antioxidant proteins in the Cu-tolerant cultivar compared with Cu-sensitive cultivars. The upregulation of ascorbate peroxidase is the only prominent feature in the Cu-tolerant genotype. Transgenic tobacco plants (*NtGp*11) were compared with wild type for drought stress and identified 43 contrastingly expressed proteins by using 2-DE coupled to MALDI TOF-TOF MS/MS approach. Reduced glutathione (GSH) was found to have an essential role in mitigating osmotic stress by upregulating stress responsive genes. Further RT-PCR analysis had shown an increase in transcript levels of stress-resistance genes in NtGp11 compared with wild-type tobacco (Kumar et al. 2014).

4.2.1.3 Phylogenetic and Conserved Motif Analysis of Enzymatic Antioxidants

There are a total of 61 SOD protein enzymes in different plants. A phylogenetic tree of 61 proteins was predicted for the sake of evolutionary study, which categorized all 61 SOD proteins into four groups. First, second, third, and fourth groups were highlighted by red, blue, purple, and green colors, respectively. In the phylogenetic clade, SOD enzyme appeared to be in close relationship in different plant species in the gene bank.

According to motif analysis, almost all motifs occupy same locations except some genes like AFN42318.1 CuZn superoxide dismutase. C95A synthetic construct appeared on much different locations. *Raphanus sativus* have seven and *Eucalyptus grandis* have two new motif location.

According to Fig. 4.2, one ancestor is divided into three families in which one family is divided into 11 genes and their motifs are same except synthetic construct Cu/Zn of SOD. The second family is divided into 21 genes, and motifs are similar except one, i.e., *Raphanus sativus*. In the third family, almost all motifs are same and 28 genes involved in it.

According to Fig. 4.3, one ancestor is divided into seven families in which first, second, third, fourth, fifth, sixth, and seventh gene family belongs to 1, 10, 5, 9, 1, 5, and 9 genes, respectively. The motifs are different from gene to gene. Some motifs are same but mostly differ with respect to each other.

According to Fig. 4.4, one ancestor is divided into seven families in which the first family belongs to three genes or the second, sixth, and 13th family belong to four genes, and the fifth family belongs to Three genes. Third, fourth, seventh, eighth, ninth, 11th, or 12th family belongs to one gene. Tenth family contains ten genes, or 11th or 12th family belongs one gene. The motifs are different from gene to gene. Some motifs are same but mostly differ with respect to each other. Motif analysis shows that every motif has same location and same size except of some genes.

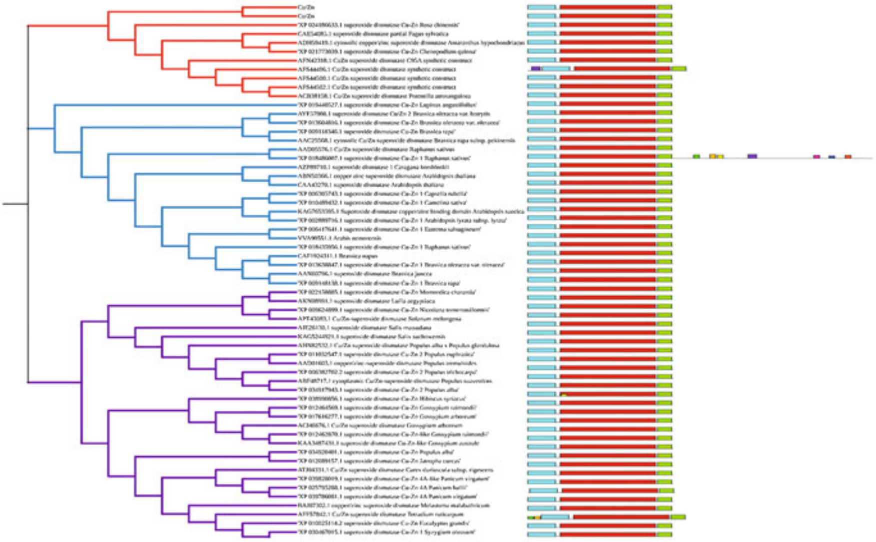

Fig. 4.2 Evolutionary analysis for SOD constructed by mega 7 and conserved motifs analysis by meme suite

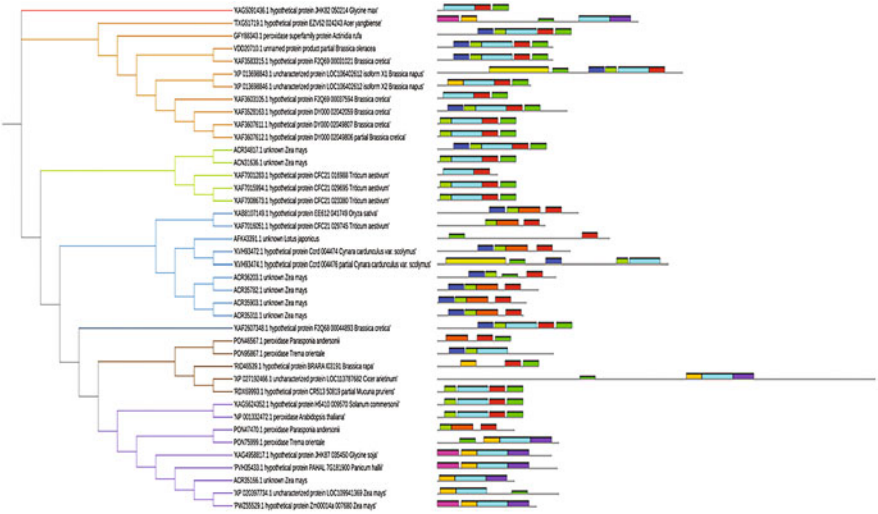

Fig. 4.3 Evolutionary analysis for POD constructed by mega 7 and conserved motif analysis by meme suite

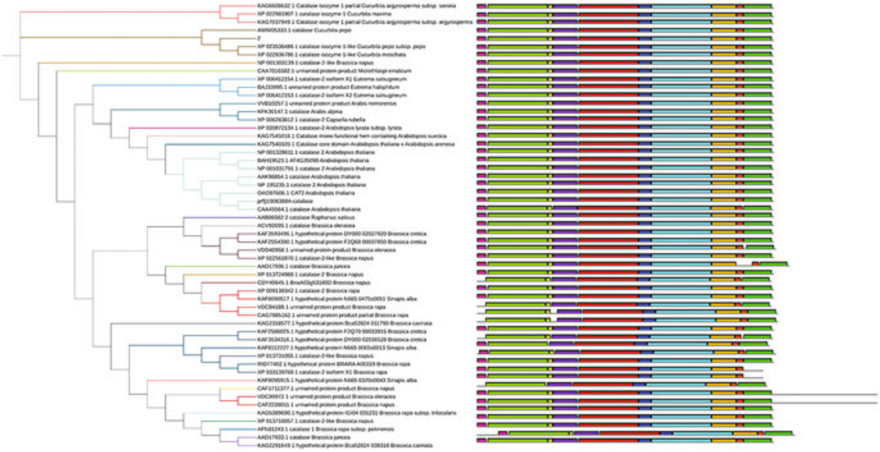

Fig. 4.4 Evolutionary analysis for CAT constructed by mega 7 and conserved motif analysis by meme suite

4.2.2 Genomic Approaches

Genome-wide association studies (GWAS) have endorsed significant contributions to enable the discovery of genomic variability by developing stress-tolerant crops. Marker-assisted selection (MAS) is closely linked to target gene for the indirect picking of trait of interest without phenotyping that trait. Huge advancement has been accomplished by MAS strategy for crop breeding under various ecological pressures where one or more genes participate in conferring stress tolerance.

4.2.2.1 Engineering of Stress-Specific Enzymatic Antioxidant Genes

For the last 10 years, transgenic approaches have been extensively applied to enhance plant defense system under hostile environmental conditions. Thus, genetically engineered plants can be a good source for cultivation with better activities of redox regulating antioxidant enzymes (Table 4.1).

Table 4.1 Engineering of enzymatic antioxidant genes in plants

Gene	Plant source	Transgenic plant	Results of overexpression	Character	Reference
MuWRKY3	*Macrotyloma uniflorum*	*Arachis hypogea*	↓MDA ↓H_2O_2 ↓$O_2^{\cdot -}$ ↑SOD ↑APX	Drought tolerance	Kiranmai et al. (2018)
MdATG18a	*Macrotyloma uniflorum*	*Malus domestica*	↑CAT ↑POD	Drought tolerance	Kiranmai et al. (2018)
DgNAC1	*Arabidopsis thaliana*	*Chrysanthemum*	↑SOD ↑CAT ↑POD	Salinity tolerance	Wang et al. (2017)
PaSOD	*Potentilla atrosanguinea*	*Solanum tuberosum*	↑SOD ↑APX	Sat tolerance	Shafi et al. (2017)
RaAPX	*Rheum australe*	*Solanum tuberosum*	↑SOD ↑APX	Sat tolerance	Shafi et al. (2017)
SbMYB15	*Salicornia brachiata*	*Nicotiana tabacum*	↑CAT ↑SOD ↑*MnSOD* ↑*CAT1*	Cd tolerance	Kumar et al. (2020)
CaGrx	*Cicer arietinum*	*Arabidopsis thaliana*	↑GRX ↑GR ↑GPX ↑GST ↑APX/CAT ↑SOD ↑MDHAR	AsIII Cr tolerance	Kumar et al. (2020)
AtDREB1A	*Arabidopsis thaliana*	*Solanum lycopersicum*	↑SOD ↑CAT	Chilling tolerance	Karkute et al. (2019)
DaAPX	*Dioscorea alata*	*Arabidopsis thaliana*	↑APX	Chilling and flood tolerance	Chen et al. (2019)
AtHDG11	*Arabidopsis thaliana*	*Nicotiana tabacum*	↑SOD	Chilling tolerant	Wu et al. (2021)
K2-NhaD	*Arabidopsis thaliana*	*Gossypium hirsutum* L.	↑SOD ↑CAT ↑POX	Salt tolerance	Guo et al. (2020)
IbCAT2	*Ipomea batatas*	*Escherichia coli* and *Saccharomyces cerevisiae*	↑CAT	Salt and drought tolerance	Yong et al. (2017)

Several genes have been identified for providing significant shielding effect against environmental stress in many plant species; however, still a number of genes are yet to be revealed in important crops. Genetic modification is extensively using biotechnology-based breeding methods (BBBMs) to lift up the plant's tolerance mechanism. Crucial tolerant genes have been isolated and successfully integrated into those plant species, which are at greater risk of environmental stresses.

4.3 Genome-Wide Analysis for Enzymatic Antioxidants

The emergence of genome engineering techniques greatly affected the progress of plant biology and agriculture as it allows very accurate genetic modification of subjective systems. Genome editing approaches are efficient way of modifying plant genome (Liu et al. 2017; Andersson et al. 2017).

4.3.1 Transcriptome-Based Approach for Enzymatic Antioxidant

Transcriptome study of SOD gene family exposed that variety of environmental stresses has regulatory impact on the expression of TaSOD genes. Different TaSOD genes were expressed differentially in retort to the same environmental stressor, which might be due to differential response of transcriptional regulators (Jiang et al. 2019). Broadly genes translating chlAPX isoenzyme are grouped into following categories.

- The first class consists of one gene but shows variation in post-transcriptional alternative splicing regulation for two isoenzymes, e.g., spinach, tobacco, pumpkin, and ice plant.
- Each gene of second group codify single isoenzymes, e.g., Arabidopsis, tomato, and rice.

The splicing of premature chlAPX mRNA has been extensively probed in *Spinacia oleracea* (Ishikawa and Shigeoka 2008) and found this phenomena fundamental in monitoring the expression pattern of stromal and thylakoid APX isoenzymes. Complementary DNA (cDNA) sequences revealed wide distribution of antioxidants throughout the plant kingdom. Redox regulating antioxidant enzymes are expressed by tiny gene groups in the plants (Passardi et al. 2007). *APX* cDNAs of cowpea leaves were constructed and mentioned as cytosolic, peroxisomal, and chloroplastic isozymes (D'Arcy-Lameta et al. 2006). Similarly, six *APX* isoforms of *Eucalyptus grandis* were putatively nominated as cytosolic, peroxisomal, and chloroplastic proteins (Teixeira et al. 2005). Same trend was shown in *Solanum lycopersicum* (Najami et al. 2008) and *Arabidopsis thaliana* (Chew et al. 2003). In *Oryza sativa*, SOD gene family contains eight members: two for each cytosolic, peroxisomal, chloroplastic, and mitochondrial (Pan and Yau 1991). The existence of organelle-specific peptides shows that transmembrane regions are present in the amino and carboxyl-terminal of newly formed polypeptide that specifies subcellular targeting of antioxidant and their isoenzyme (Teixeira et al. 2004).

4.3.1.1 Hybridization-Based Approach for Enzymatic Antioxidants

Natural hybridization is a recurrent method among vascular plants. Hybridization is referred to as an essential evolutionary power; it may lead to enhanced inter- and intraspecific genetic variation, participating in speciation process. Plant hybridization is also of great significance to truthfully identify hybrid individuals. Several DNA markers like RAPD (random amplified polymorphic DNA), AFLP (arbitrary fragment length polymorphism), RFLP (restriction fragment length polymorphism), SSR (simple sequence repeat), and SNP (single-nucleotide polymorphism) are used to identify the hybrids(López-Caamal and Tovar-Sánchez 2014). Avramova et al. (2017) had determined the difference in drought tolerance Egyptian, European, and South-African maize hybrids. The maize lines with varying geographic distribution displayed different molecular approaches to cope the stress. The European and South-African maize hybrids showed greater drought tolerance, correlated with improved activities of the enzymatic antioxidants in meristematic region of leaf. Such outcomes deliver evidence for a strong linkage between antioxidant regulation and hybrid response under limited water supply particularly in the leaf meristematic region.

4.3.1.2 Sequencing-Based Approach for Enzymatic Antioxidant

Expressed sequence tags (ESTs) are consisted of only exon part of transcribed DNA, so much smaller than the cDNAs. Assembly of overlapping EST sequences reveal polymorphism. However, homologous genes may cause disassembly of sequences, especially in polyploidy plant species such as in hexaploid wheat (Rudd 2003). This approach is also helpful particularly for crops having larger genome size, where complete genome sequence is not yet available (Ergen and Budak 2009).

Reem et al. (2020) had isolated and characterized ESTs of *Phoenix dactylifera* L. (date palm) tangled in response to saline stress by DD-PCR technique. The DD-PCR results exposed the presence of 17 (7 ESTs from Bertamoda and 10 ESTs from Malkabi) upregulated genes in salt-treated plants compared with nonstress plants. These fragments varied in length from 212 to 1361 bp (Abd El-Maksoud et al. 2020). Plants produced many antioxidant enzymes, efficiently scavenge free radicals. Genes related to ROS scavenging mechanism are deposited at the database. Among the 8835 nonredundant set, CAT3 (Contig5024), GSTF3 (Contig5012), and GST1 (Contig4958) consist of 53, 37, and 15 EST, respectively. The reference gene and their ESTs are deposited in their respective database for salt tolerant plant. qRT-PCR study revealed the countenance of CAT3 and GSTF3 were substantially increased under saline stress in *Arabidopsis pumila* (Huang et al. 2017).

4.3.1.3 CRISPR/Cas9-Mediated Response for Enzymatic Antioxidants

Desirable genome editing via CRISPR-Cas9 is a widely accepted genetic manipulation method in different living systems. The editing of gene/genome is widely in practice for easy handling and versatility in comparison with many formerly adopted genome editing techniques. Scientists have formed some modal mechanism with several plant species to adopt this editing system and introducing new prospects to go through laborious and time-consuming transgenic practices for important commercial crops (Montecillo et al. 2020; Andersson et al. 2017). Mariette Andersson et al. (2017) have also described the transitory solicitation of *CRISPR/Cas9*-mediated genome excision process in the protoplasts of tetraploid potato (*Solanum tuberosum*).

Non-expressor pathogenesis-related gene (NPR1) participated as a primary defense regulator. The regulation redox active antioxidant enzymes are relatively associated with *Solanum lycopersicum* glutathione-S-transferase (SlGST) activity. Further, the loss of SlNPR1 activity potentially hampers antioxidant gene expression under water deficit conditions (Li et al. 2019).

4.4 Quantitative Trait Loci (QTLs)-Based Approach for Enzymatic Antioxidant

QTLs seems to more stable with passing years under varying environmental stresses (Stevens et al. 2007). Scientists have been utilizing the latest available data and other potential competitive techniques to understand complete antioxidant mechanisms in plants, with particular focus to genotoxicity, transgenerational alterations, and QTLs (Gürbüz Çolak et al. 2020). Tomato size and its composition are a continuously varying trait, not only controlled by more than one gene but also influenced by the environment. Different molecular markers allow the partitioning of quantitative traits into discrete quantitative trait loci (QTL), which are helpful in designing genomic map (Saliba-Colombani et al. 2001).

Introgression line (IL) of *S. lycopersicum* M82, *S. pennellii* LA716, and *S. pennellii* was assessed for growth and antioxidant capacity with and without salt stress. The data was classified on the basis of quantitative trait loci (QTL) for monitoring the antioxidant accumulation under stress. The distribution of QTL in the IL population for these attributes may be helpful for breeding salt-tolerant tomato cultivars having higher antioxidant levels (Frary et al. 2010). Through evaluating the salt tolerance of 285 ILs at the seedling stage, a total of ten quantitative trait loci (QTLs) related to salt tolerance were identified on chromosomes 1, 5, 7, and 9–12, with individual QTLs explaining 2–8% of phenotypic variance (Wang et al. 2017).

4.5 Conclusion

Redox regulating enzymatic antioxidant (SOD, CAT, APX, and POX) scavenges ROS and is significant components of the plant's immune system. Genetic, proteomic, and computational biology studies have provided significant insights into their regulatory mechanism in diverse plant stress and hormone responses, in development, as well as in the evolution of the many gene families. Proteins interacting with their relevant transcription factors have been identified along with target genes for the regulation of antioxidant-mediated defense response in higher plants. Some advanced proteomic techniques like liquid chromatography–tandem mass spectrometry and conserved motif analysis using meme suite significantly helped in analyzing antioxidant enzymes under stress. QTLs are helpful in the development of plants with higher antioxidant potential under stress. However, to validate these results, comprehensive field trails are required at larger scale with different plant species under changing climatic conditions. Apart from the significant scientific study of antioxidant-mediated response in plants, more detailed study is lacking with reference to *CRISPR-Cas9* for enzymatic antioxidant response under stress. GWAS can be helpful in genomic variation in plants to mitigate adverse climatic conditions.

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5

Genetic Engineering Applications in Inducing Stress Tolerance in Plants Through Antioxidants

Deepu Pandita

Abstract

Reactive oxygen species (ROS) is a necessary evil for the adaptation of plants in an oxygen-containing environment. ROS is mainly produced by electron transport system of chloroplasts (photosynthesis), mitochondria (during respiration), peroxisomes, and plant cell membrane. ROS in low concentrations is critical in some important plant metabolic pathways. It is estimated that beyond all the O_2 absorbed, approximately 1% of it is diverted to generate ROS. Higher levels of ROS concentration can damage plant cells, chlorophyll, lipids, proteins, carbohydrates, and nucleic acids by oxidative damage and can cause disruption of transport of ions, loss of enzyme activity, protein cross-linking, DNA damage, and programmed cell death (PCD) pathway activation and death of cells. In order to evade the oxidative damage, chloroplasts, mitochondria, and peroxisomes in plants provide complex antioxidative defense against ROS by numerous enzymatic and nonenzymatic antioxidant components. Abiotic stress tolerance and stress-induced oxidative protection/tolerance has been accomplished in several crop plants by generation of transgenics through detoxification strategies of overexpression of a battery of enzymatic and nonenzymatic antioxidants that scavenge oxygen radicals.

Keywords

Antioxidants · Genetic engineering · Stress tolerance · Reactive oxygen species (ROS) · Oxidative stress

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5.1 Introduction

An inevitable consequence of adaptation of life to an oxygen-comprising environment was the nonstop production of reactive oxygen species (ROS) as the metabolism derivative in a biological system. ROS is mainly produced by electron transport chain system of chloroplasts (photosynthesis), mitochondria (during respiration), peroxisomes, and plant cell membrane or as a derivative of numerous cellular metabolic pathways. ROS originates from molecular oxygen and accumulate in plant tissues. In normal plant growth scenarios, production rate of ROS is low in different cell compartments. Exogenous abiotic environmental stresses for instance, chilling, drought, extreme heat, salinity, toxicity of heavy metals, pollutants, deficiency of nutrients, and UV can interrupt the cellular homeostasis, cell defense, and intra- and extracellular cell signaling and can induce the production and concentration of biochemically active reactive oxygen species (ROS) molecule through oxygen metabolism in that plant (Zurbriggen et al. 2009; Pandita 2021). But higher levels of ROS concentration can damage plant cells, chlorophyll, lipids, proteins, carbohydrates, and nucleic acids by oxidative damage and can cause disruption of transport of ions, loss of enzyme activity, protein cross-linking, DNA damage, and programmed cell death (PCD) pathway activation and death of cells. Hence, plants have evolved several complex mechanisms to maintain the ROS concentrations under balance by stringent reconnaissance (Ali and Alqurainy 2006; Ahmad et al. 2011a, b; Ozgur et al. 2013; Sharma et al. 2012). The ROS consists of both free radical (alkoxy radical (RO), superoxide radical ($O2^{\cdot -}$), hydroperoxyl radical ($HO_2^{\cdot}$), hydroxyl radical ($^{\cdot}OH$), and peroxy radical (ROdrogen peroxide (H_2O_2)). The free radical and nonradical forms are plant cytotoxic (Dismukes et al. 2001; Pandita 2021).

Toxic and destructive ROS production in plant cell alters metabolic functions of cell organelles like chloroplast and mitochondria. Their increased production is destructive and causes oxidative damage to organellar membranes and other vital biomolecules. Reactive oxygen species or reactive oxygen intermediate (ROI) are partially a reduced form of atmospheric oxygen (O_2). Their production is due to the excitation of oxygen (O_2) to form singlet oxygen ($^1O_2$) or transfer of one or two or three electrons to O_2 to form superoxide radical (O_2^-) or hydroxyl radical (HO^-). ROS acts as signal molecule for the activation of stress-response and defense mechanism in plant body. Oxygen is vital for the cell; however, under stress conditions, it undergoes a series of reactions to form reactive oxygen species and jeopardize survival of cells (Ahmad et al. 2010a, b, 2011a, b; Ahmad and Umar, 2011; Choudhury and Panda 2013). ROS and antioxidant balance completely scavanges ROS. This leads to the loss of ROS, which is a significant second messenger in intracellular signaling cascade. Disturbance of this balance leads to oxidative stress (Dalton et al. 1999; Tuteja, 2007; Khan and Singh, 2008; Tuteja, 2010; Ahmad et al. 2010a, b, 2011a, b; Ahmad and Umar, 2011). To avoid the oxidative damage, chloroplasts, mitochondria, and peroxisomes in plants provide complex antioxidative defense against ROS by producing numerous enzymatic and nonenzymatic antioxidant components (Sharma and Dubey, 2007; Zurbriggen et al.

2009; Gill and Tuteja, 2010; Saed-Moucheshi et al. 2014). Plants accumulate compatible solutes as well under abiotic stresses (Ashraf and Fooland 2007).

5.2 Reactive Oxygen Species (ROS) and Oxidative Stress

Molecular reduction of O_2 results in the production of ROS containing O_2-, H_2O_2, OH, and $^1O_2$ (Asada, 1999; Ahmad et al. 2008, 2010a, b, 2011a, b; Ahmad and Prasad 2012a, b; Ahmad et al. 2013). ROS free radicals comprise of oxygen with at least one unpaired electron. Unpaired electrons pair with other electrons for a stable conformation. These free radical forms swiftly react with other molecules and generate supplementary free radicals (Foyer and Halliwell, 1976).

Abiotic stresses increase generation and assemblage of very reactive reactive oxygen species. This sequentially leads to oxidative stress. Compounds responsible for tolerance, e.g., heat shock proteins (HSP) in temperature stress, also activate oxidative bursts (Panchuk et al. 2002; Lee et al. 2000). Advancement of ROS detoxification is emblematic of the reaction to abiotic and biotic stresses (Munné-Bosch et al. 2004). Disruption in cellular homeostasis reduces molecular oxygen and changes it into ROS by high energy electrons (Rizhsky et al. 2002a, b; Mittler, 2002). In living conditions, $^1O_2$, H_2O_2, $O_2{}^{\cdot}-$, and $^{\cdot}OH$ reactive oxygen species damage all organic components (Snider et al. 2008). ROS generates at low levels in chloroplasts, mitochondria apoplasm, and peroxisomes and endoplasmatic reticulum (Polle 2001; Ashraf 2009; Ahmad and Umar 2011; Ahmad and Prasad 2012a, b; Ahmad et al. 2013). Under normal conditions of growth, ROS production increases during environmental stress conditions (Laloi et al. 2004; Ahmad and Umar 2011; Ahmad and Prasad 2012a, b; Zare and Pakniyat 2012; Ahmad et al. 2013; Rasool et al. 2013) and reacts directly with numerous metabolites for instance, lipids (peroxidation), proteins (degradation, inactivation) and nucleic acids (DNA/RNA disruption) pigments, and other vital cellular molecules causing cell membrane injury and cell death (Apel and Hirt 2004; Bencze and Veisz 2005; Gao et al. 2008; Ashraf 2009; Mittler et al. 2011).

Abiotic or biotic stresses produce ROS by NADPH oxidases (Pei et al. 2000). Plants have three main pathways for ROS generation:

1. Electron transport chain (ETC) in photosynthetic chloroplasts and powerhouses of mitochondria.
2. A number of peroxidases and oxidase processes (glycolate oxidase, NADH oxidase, NADPH oxidase, amine oxidase, xanthine oxidase, and lipoxygenase).
3. Photosensitizer molecules holding chlorophyll (Blokhina et al. 2003).

Inadequate CO_2 fixation along with reduced ETC is the principal goal of ROS generation in chloroplasts. During photorespiration, glycolate oxidation into glyoxylic acid produces H_2O_2 in plant peroxisomes (Mittler et al. 2004). Stress disturbs functions of mitochondria, resulting in accumulation of ROS and in turn oxidative damage to lipids of membranes (Vacca et al. 2004; Suzuki and Mittler

2006). Electrons from NADH produced by enzymes of Krebs cycle reduce O_2 to reactive oxygen intermediates (ROI) by ETC (Davidson and Schiestl 2001). This process suppresses ROI production during stress conditions and induces several defense genes, e.g., scavenging enzymes of ROI (Mittler 2002). The ROS signaling molecule has roles in growth, development, biotic and abiotic stress, and the programmed cell death of plants. ROS overproduction causes oxidative stress. In oxidative stress, capability of cellular defense system to eliminate ROS molecules is lesser than ROS generation. The balance between the production of ROS and the consumption of ROS shifts toward higher generation of ROS (Ahmad et al. 2008, 2010a, b, 2011a, b; Ahmad and Umar 2011). The swift ROS production known as oxidative burst is an indispensable process. Respiratory burst oxidase homolog (Rboh) genes, which encode NADPH oxidases, are key producers of signal transduction-associated ROS (Miller et al. 2009). Certain signaling pathways exponentially enhance the ROS amount to battle against infection and stress in plants. This exponentially increased ROS is labeled as the oxidative burst. The positive power of ROS in low concentrations is decisive in certain vital plant pathways. The estimations state that beyond all the O_2 absorbed, approximately 1% of O_2 is sidetracked to ROS production (Tuteja 2010; Sharma et al. 2012). The localized and temporal ROS production and ROS scavenging are probably very critical in cellular and intracellular transduction of ROS signals (Panchuk et al. 2002). Therefore, two slightly opposite functions of ROS highlight the necessity to regulate steady state level of ROS in plant cells. This provides an extra potent approach for improving crop tolerance to various stresses (Suzuki and Mittler 2006). ROS-scavenging mechanisms protect plants (Yabuta et al. 2002; Yoshimura et al. 2004).

5.3 Scavenging of Reactive Oxygen Species (ROS)

Stress factors induce oxidative stress and antioxidative enzyme expression in bacteria, yeast, and plants (Morgan et al. 1986; Davidson et al. 1996; Jaleel et al. 2007; Esfandiari et al. 2007). ROS is a by-product of stress in plants, which damages cellular organelles (Xiaozhong and Huang 2000; Polle 2001). For protection of cellular and subcellular systems from oxidative damage, plant's responses have developed various ROS scavengers consisting of functionally interlocked protective detoxification systems of enzymatic and nonenzymatic antioxidants for neutralizing cytotoxic effects of ROS (Sairam and Tyagi 2004). The detoxification systems include protective molecules like isoprene, glycine betaine, α-tocopherol (vitamin E), and carotenoids and antioxidant metabolites, for instance, ascorbic acid and glutathione (Sakamoto and Murata 2001; Young et al. 2004; Markovska et al. 2009). Several plants under stressed condition synthesize and accumulate antioxidant enzymes as defensive mechanism. Antioxidant system consists of a battery of enzymes that scavenge oxygen radicals, for instance, dehydroascorbate reductase, ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), glutathione reductase, glutathione peroxidase (GPX), and peroxiredoxin (PrxR) (Kubo et al. 1999; Sairam et al. 2000; Shah et al. 2001; Iba 2002; Mittler et al. 2004).

Antioxidants in excess bind to ROI molecule and detoxify or scavenge it. Antioxidative activities of antioxidants upregulate under stress, but their activities are diverse in different species (Smirnoff 2005).

5.3.1 Nonenzymatic Antioxidants

The nonenzymatic antioxidants include glutathione (GSH), tocopherols, carotenoids, ascorbate (AsA), flavonoids, flavones, and anthocyanins (Fig. 5.1) (Schafer et al. 2002; Gupta et al. 2005; Ahmad et al. 2008, 2010a, b, 2011a, b; Ahmad and Umar 2011; Ahmad and Prasad 2012a, b; Ahmad et al. 2013; Rasool et al. 2013). These antioxidants function as redox buffers, which show ROS interaction and acts as metabolic interface that modulates proper induction of acclimation responses (Foyer and Noctor 2005). Ascorbate and glutathione are vital nonenzymatic antioxidants of ascorbate-glutathione cycle (Rasool et al. 2013; Ahmad et al. 2013).

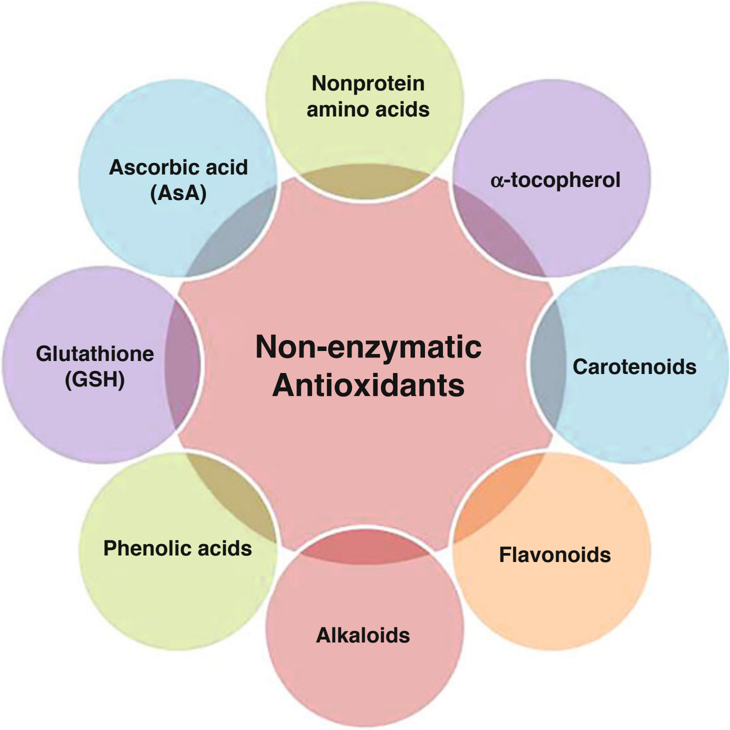

Fig. 5.1 Nonenzymatic antioxidants

5.3.1.1 Glutathione (GSH)

Glutathione (GSH) is tripeptide γ-glutamylcysteinylglycine (γ-glu-cys-gly) metabolite with significant role in plants. GSH appears in reduced form in tissues of plant. GSH is localized in cytosol, apoplast, chloroplasts, peroxisomes, mitochondria, vacuoles, endoplasmic reticulum, and scavenges peroxides (Noctor and Foyer 1998; Jimenez et al. 1998; Asada 1999; Gill and Tuteja 2010; Szarka et al. 2012; Ramírez et al. 2013). Inside plant cells, GSH exists in reduced form (GSH) and oxidized disulfide form (GSSG) (Shu et al. 2011).

GSH is the most important antioxidant and plays a vital function in antioxidative defense system and pathogen tolerance and detoxifies ROS by the regeneration of ascorbic acid through ascorbate-glutathione cycle and protects plants from oxidative damage (Foyer and Halliwell 1976; Noctor and Foyer 1998; Ogawa 2005; Shiu and Lee 2005; Shan et al. 2011). GSH is a substrate of glutathione S-transferase (GST) and detoxifies dehydroascorbate reductase (DHAR) and xenobiotics (Mendoza-Cozatl and Moreno-Sanchez 2006). GSH conserves cellular redox equilibrium by combining with its GSSG under stressful or normal conditions (Wang et al. 2008).

5.3.1.2 Ascorbate (Ascorbic Acid)

Ascorbate is a water soluble metabolite, ubiquitous antioxidant, and enzyme cofactor molecule, with localization in different organelles of cell, such as apoplast, chloroplast, cytosol, mitochondria, and peroxisome, has roles in photosynthesis, photo protection, growth of cell wall, and development, and fights against stress, biosynthesizes ethylene, gibberellins, hydroxyproline and anthocyanins (Wolucka and Van Montagu 2003; Mellidou et al. 2012). It plays central role in the removal of H_2O_2 and singlet oxygen (Noctor and Foyer 1998; Asada 1999). Ascorbic acid frequently occurs in reduced state (ascorbic acid/vitamin C) in plant leaves and chloroplasts under normal conditions and two oxidized ascorbate forms of mono-ascorbic acid and dehydroascorbic acid. The ratio of reduced and oxidized ascorbate forms is a principal factor that influences plant tolerance to oxidative stress (Conklin et al. 2000; Zechmann 2011; Cruz-Rus et al. 2012). Concentration of ascorbic acid in cells increases to millimolar range (Smirnoff 2005).

Ascorbate is the most powerful compound for detoxification of reactive oxygen species due to its capability to provide electrons in various nonenzymatic or enzymatic reactions. Ascorbic acid directly quenches O_2^-, $^1O_2$, and hydroxyl radicals, reduces H_2O_2 via glutathione-ascorbate cycle, and takes part in redox signaling, gene expression modulation, and enzymatic activity regulation (Foyer et al. 1997; Zechmann 2011). Ascorbate on reaction with reactive oxygen species produces monodehydroascorbate (MDHA), which disproportionates into dehydroascorbate (DHA) and ascorbate (Smirnoff et al. 2001). Ascorbic acid recovers tocopherols from tocopheroxyl radical and offers protection of membrane. Consequently, increased ascorbic acid levels in plants balance detrimental influences of oxidative stress (Smirnoff 2005).

5.3.1.3 Proline

Proline accumulates safeguard cellular redox potential and protects plants as ROS detoxification molecule. It maintains integrity of cell membranes and stabilizes antioxidant enzymes. Proline over accumulation has toxic effect on plant cells (Rizhsky et al. 2004a, b).

5.3.1.4 Tocopherols

Tocopherols (α-tocopherol) are lipid soluble metabolites with antioxidant and nonantioxidant potential, present in all plant parts produced by photosynthetic organisms with localization in biological membranes (Munné-Bosch 2005; Holländer-Czytko et al. 2005; Maeda and DellaPenna 2007; Ashraf 2009; Quadrana et al. 2013). Tocopherols detoxify lipid radicals and ROS (Holländer-Czytko et al. 2005). Plant tocopherols have four isomers (α-, β-, γ-, and δ-) (Ashraf 2009). It removes. OH radicals, superoxide radical, and singlet oxygen (Asada and Takahashi 1987). Leaves store most abundant form of α-tocopherol, and seeds store γ-tocopherol. The β-tocopherols and δ-tocopherols are less copious in plants. The α-tocopherol is biosynthesized from γ-tocopherol in green chloroplasts by γ-tocopherol methyl transferase (γ-TMT, VTE4) (DellaPenna 2005; Lichtenthaler 2007; Szymanska and Kruk 2008; Gill and Tuteja 2010; Szarka et al. 2012; Velasco et al. 2013). The α-tocopherol changes during stress into two phases. In initial phase, α-tocopherol content enhances under stress and reduces ROS levels. In second phase, under severe stress, α-tocopherol degradation is more than biosynthesis. Stress-tolerant plants face first phase, and stress sensitive plants have second phase. In photosynthetic biological organisms, fluctuations in tocopherol content were confirmed under drought, salinity, heavy metal, or high radiation stresses (Collakova and DellaPenna 2003; Ledford et al. 2004; Munné-Bosch 2005; Tounekti et al. 2011a, b).

5.3.1.5 Carotenoids

Carotenoids are lipophilic isoprenoids derived from organic pigment antenna molecules, localized in plant chloroplasts with multiple functions in plant metabolism and tolerance to oxidative stresses (Taiz and Zeiger 2006; Fraser et al. 2007). It scavenges singlet oxygen and protects chlorophyll from photoxidation (Demmig-Adams and Adams III 1992; Neubauer and Yamamoto 1992). The chlorophyll to carotenoid ratio is the indicator of seed tolerance to stress (Smolikova et al. 2011).

5.3.1.6 Flavonoid

Flavonoid phenolic pigments found floral parts; pollens and leaves of plants have antioxidant properties against various oxidizing compounds. Innumerable flavonoids act as potential inhibitors of lipoxygenase enzyme. Lipoxygenase enzyme transforms polyunsaturated fatty acids to oxygen comprising derivatives (Nijveldt et al. 2001).

5.3.2 Enzymatic Antioxidants

Enzymatic antioxidants take part in ROS detoxification in abiotic stress (Jiang and Huang 2001; Vacca et al. 2004). Peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) and GR, monodehydroascorbate reductase (MDHAR or MDAR), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR or DAR), and glutathione reductase (GR) scavenge superoxide and H_2O_2 and MDAR and GR regenerate ascorbate (Fig. 5.2) (Mittler 2002; Nagesh-Babu and Devaraj 2008; Ahmad et al. 2008, 2010a, b, 2011a, b; Ceylan et al. 2013). Accumulation of ROS, for instance, H_2O_2 under various environmental stresses, increased CAT and POX activities.

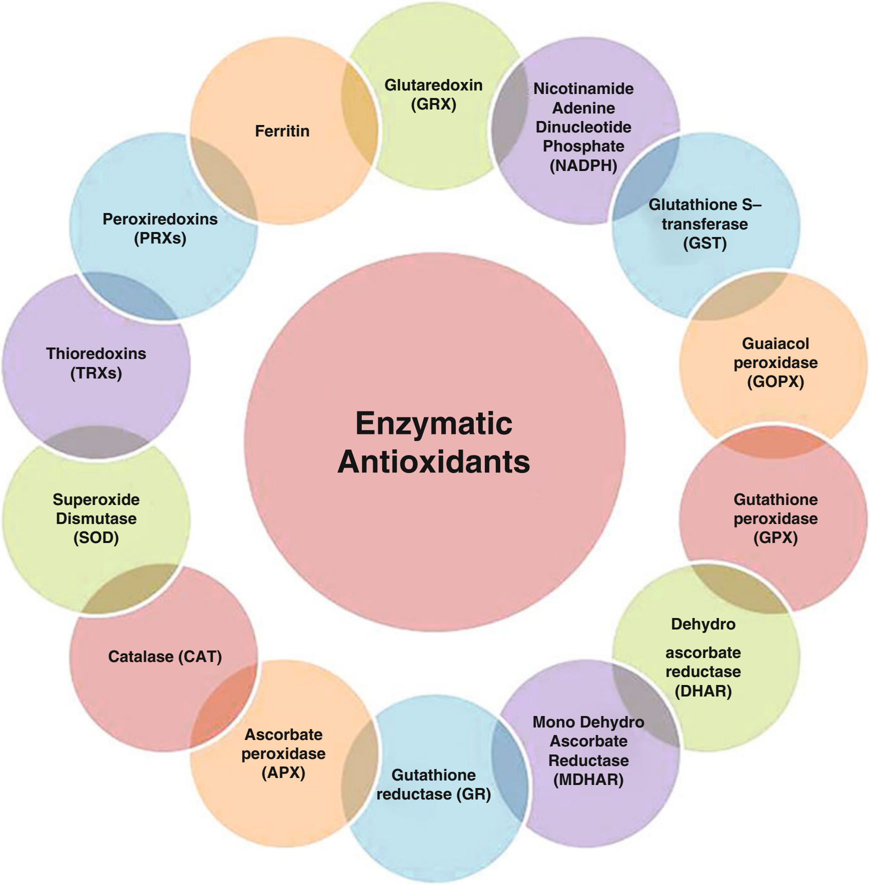

Fig. 5.2 Enzymatic antioxidants

5.3.2.1 Superoxide Dismutase (SOD)

The main and universal multimeric metalloprotein enzymatic antioxidant defense system in plants is SOD localized in chloroplast, cytsol, mitochondria, peroxisome, apoplast (Gür et al. 2010). SOD catalyzes dismutation/scavenging of two superoxide radicals O_2- into molecular oxygen and hydrogen peroxide (H_2O_2) (Abedi and Pakniyat 2010; Zare and Pakniyat 2012) and thus reduces hydroxyl radical (OH) formation, which is very toxic (Bowler 1992; Scandalios 1993). SOD isoforms, localized within cell apoplast, are differentially expressed in plants (Wingsle et al. 1999). According to SOD metal cofactor existing at their active sites, plants contain different types like manganese (Mn) (MnSOD), copper/zinc (Cu-Zn) (CuZnSOD), iron (Fe) (FeSOD), and nickel (Ni-SOD) in chloroplasts and mitochondria (Alscher et al. 2002; Halliwell 2006; Abedi and Pakniyat 2010; Zare and Pakniyat 2012).

Bacteria also contain these four SODs (Halliwell and Gutteridge 2006). After SOD changes superoxide radical to H_2O_2, then either CAT, APX, or POD reduce H_2O_2 to H_2O and O_2 in cytoplasm and cell organelles (Howarth 2005). Stress increases SOD enzyme activity for the detoxification of superoxide radicals (Abedi and Pakniyat 2010; Zare and Pakniyat 2012).

5.3.2.2 Catalase

Catalases (CATs) are tetrameric iron porphyrins. In plants, CATs generate inside peroxisomes and glyoxisomes. CAT catalyzes redox reaction in which H_2O_2 changes into oxygen and water. CAT specifically detoxifies H_2O_2. It also reacts with various organic hydroperoxides, for instance, methyl hydrogen peroxide (MeOOH) (Mittler 2002; Ali and Alqurainy 2006).

5.3.2.3 Ascorbate Peroxidase

Peroxidases remove the scavenging of H_2O_2 through oxidization of various cosubstrates. Various peroxidases are not specific and use various cosubstrates (Mano et al. 2001). Ascorbate peroxidase (APX) is a vital antioxidant enzyme having different isoforms in chloroplasts, mitochondria, cytosol, peroxisome, apoplast, and microsomes. These ascorbate isoforms detoxify H_2O_2 (Asada and Takahashi 1987; Asada 1999; Rasool et al. 2013; Ahmad et al. 2013). APX converts toxic H_2O_2 on reaction with ascorbate substrate to monodehydroascorbate (MDHA) (Payton et al. 2001; Blokhina et al. 2003). In plant cells, four classes of APX are chloroplast thylakoid bound (tAPX), glyoxisome membrane (gmAPX), chloroplast stromal soluble (sAPX), and cytosolic (cAPX). The ascorbic acid antioxidant plays a positive role for plant defense against oxidative stress. Ascorbic acid recovers by MDAR, DHAR, and GR, oxidized by APX. The stress increased APX gene expression in chloroplasts of tobacco plants and in turn increases resistance to salt stress and drought (Rizhsky et al. 2002b; Abedi and Pakniyat 2010; Zare and Pakniyat 2012). APX increases during stress and recovery from stress in tolerant genotypes approves that glutathione-ascorbate cycle is extra proficient in tolerants and provides tolerance to seedlings (Pang and Wang 2010). Plant glutathione peroxidases (GPXs) have Cys instead of seleno-Cys at their active sites. This decreases their catalytic action. Actually, some enzymes favor thioredoxin to GSH (Rodriguez-Milla et al. 2003).

5.3.2.4 Monodehydroascorbate Reductase (MDHAR)

Chloroplast, mitochondria, and cytosol cause the reduction of MDHA to ascorbate (Jimenez et al. 1997).

5.3.2.5 Dehydroascorbate Reductase (DHAR)

DHAR localized in chloroplast, mitochondria, and peroxisomes plays an important function in defense system and regenerates ascorbate from dehydroascorbate (Asada 1994).

5.3.2.6 Glutathione Reductase (GR)

GR is another enzyme localized primarily in chloroplasts or in low amounts in cell cytosol and mitochondria, which inhibits oxidation of enzymes and cell membranes from HTS and protects plants (Creissen et al. 1994; Almeselmani et al. 2006). It helps in reducing the glutathione (Edwards et al. 1990; Creissen et al. 1994).

Peroxiredoxins are homodimers with significant H_2O_2-scavenging systems (Rhee et al. 2005) and are 2-Cys peroxiredoxins, atypical 2-Cys peroxiredoxins, and 1-Cys peroxiredoxins (Georgiou and Masip 2003).

5.4 Genetic Engineering-Based Stress Tolerance in Plants Through Antioxidants

Most types of abiotic stress factors, for instance, high light, water deficiency, salinity, flooding, and temperature extremes, interrupt the metabolic balance of plant cells, resulting in highly stress-induced overproduction of ROS, which causes cellular damages and damage to lipids, nucleic acids, proteins, and carbohydrates (Kanofsky and Sima 1991; Mittler 2002; Mano 2002; Mittler et al. 2004; Foyer and Noctor 2005; Rodriguez and Redman 2005; Ahmad et al. 2009, 2010; Sharma et al. 2012; Suzuki et al. 2012a). Abiotic stress tolerance and stress-induced oxidative protection/tolerance have been accomplished in several crop plants by the generation of transgenics through detoxification strategies of overexpression of glutathione peroxidase, ascorbate peroxidase, superoxide dismutase, and glutathione reductase and other enzymatic and nonenzymatic antioxidants (Roxas et al. 1997; Zhu et al. 1999; Lee et al. 2007a, b; Zaefyzadeh et al. 2009; Kosová et al. 2011). The low molecular nonenzymatic antioxidants, for instance, carotenoids, ascorbate, tocopherols, and glutathione, play key role to survive with oxidative stress (Xie et al. 2008; Liu et al. 2009; Ahmad et al. 2010; Gill and Tuteja 2010; Miller et al. 2010; Shu et al. 2011; Mostafa and Ibrahim 2012).

A number of forms of nuclear-encoded SOD have been cloned in plants. FeSOD genes (FSD1, FSD2, and FSD3), Cu/ZnSOD genes (CSD1, CSD2, and CSD3), and MnSOD gene (MSD1) with an amino terminal targeting sequence occur in *Arabidopsis thaliana* (Scandalias 1990; Kliebenstein et al. 1999). CAT isozymes such as CAT1 and CAT2 are restricted to peroxisomes and cytosol and CAT3 to mitochondria in *Helianthus annuus* and *Brassica* (Polidoros and Scandalios 1999; Azpilicueta et al. 2007). CAT isozymes show temporal and spatial regulation and respond differentially to light (Scandalias 1990; Willekens et al. 1994). CAT1 is associated with genes of β-oxidation of fatty acids and superoxide dismutase. CAT3 is a senescence-associated gene (SEN2), whereas CAT1 displays mainly higher expression in male flower parts. H_2O_2 accumulation dramatically reduces in the transgenic cassava plants with upregulated CAT expression (Xu et al. 2013). The plants with knocked down catalase show correlation between the availability of intracellular H_2O_2 and GSH-GSSG ratios. The improved availability of H_2O_2 declines the status of GSH pools (Queval et al. 2009; Mhamdi et al. 2010). Catalase-deficient barley, tobacco, and *Arabidopsis* plants show distressed glutathione status because of the higher load on catalase-independent pathways for metabolizing H_2O_2 at intracellular levels (Smith et al. 1984; Rizhsky et al. 2002a; Queval et al. 2007). In tobacco and barley, Cat1 deficiency of Cat1-deficient plants produced white necrotic lesions on plant leaves after exposure to higher light intensities due to reduced ability to eliminate exogenous H_2O_2 and disturbs other defense system components (Kendall et al. 1983; Willekens et al. 1997). APX, CAT, and GPX central players remove H_2O_2 in plants. CAT deficiency induces APX mitAOX, MDA reductase, and GPX to compensate for CAT suppression (Mittler 2002). Decline in peroxisomal catalase activity increases sensitivity to ozone, and H_2O_2 induced cell death in *Arabidopsis thaliana*. Deleterious effects of salinity reduce by pretreatment with H_2O_2 and raised catalase activity in maize (Gondim et al. 2012). Modifications in grade of thiol–disulfide balance catalase deficiency and create an oxidized cellular redox in mutants (Queval et al. 2009). High light induces photorespiration and APX and GPX accumulation in catalase-deficient *Nicotiana tabacum* (Willekens et al. 1997). GPX induction in Cat2 deficient mutants acts as a well-defined alternate pathway to manage decomposition of H_2O_2 in catalase deficiency *Arabidopsis* (Queval et al. 2007; Mhamdi et al. 2010). Overexpression of BjCAT3 gene in *Brassica juncea* increased CAT activity by twofolds, which boosted Cd tolerance (Guan et al. 2009). Transgenic rice overexpressing CAT gene (katE) from *Escherichia coli* was salinity tolerant (Nagamiya et al. 2007; Moriwaki et al. 2008). ABA-induced expression of CAT1 is mediated by MAPK cascade-mediated ABA signaling in *Arabidopsis*. Therefore, close-fitting connection of H_2O_2 and ABA signaling due to CAT1 may be part of H_2O_2 signaling (Xing et al. 2008; Michelet et al. 2013). GST and CAT1 gene co-expression increased CAT and SOD activity in transgenic rice seedlings on exposure to both salinity and paraquat, whereas increased GST activity in transgenics took place only in plants stressed with paraquat (Zhao and Zhang 2006). Site-specific modifications in the activity of antioxidant enzymes have been reported. CAT stimulates in root and shoot; APX in endosperm and GR and POD are upregulated in shoots of drought-tolerant wheat genotypes (Devi et al. 2012).

The modification of DHAR expression is imperative for genetic engineering plants with property of stress tolerance (Amako and Ushimaru 2009). MDHAR overexpression reduces damaging consequences of ecological stresses (Eltayeb et al.

2007). In transgenics, overexpression of DHAR tolerates Al stress, tolerates salinity in *Arabidopsis*, and results in water deficiency and tolerance to ozone toxicity in tobacco plants by maintaining high ASC pool (Yin et al. 2010; Chen and Gallie 2005; Ushimaru et al. 2006; Eltayeb et al. 2006). DHAR overexpressing plants show enhanced growth of roots and lesser content of H_2O_2, less peroxidation of lipids, and levels of oxidative damage to DNA in Al stress (Yin et al. 2010). Transgenic tobacco overexpresses AtMDAR1 and showed 2.1-fold higher MDHAR activity and 2.2-fold higher level of reduced AsA and enhanced tolerance to O3, salinity, and drought stress (Eltayeb et al. 2007). Tobacco transformants express DHAR, GR, and GST genes and display changed antioxidant metabolism and better resistance to salt and chilling stress (Martret et al. 2011). Tomato fruits having enhanced MDAR activity showed improved chilling tolerance (Stevens et al. 2008). Salt and osmotic stress-tolerant transgenic tobacco plants show MDAR overexpression (Eltayeb et al. 2007).

APX family has four different isoforms, i.e., chloroplast stromal soluble form (sAPX), thylakoid (tAPX), glyoxisome membrane form (gmAPX), and cytosolic form (cAPX) (Noctor and Foyer 1998). In response to water deficit, salt, or ozone, APX expression increases in plants (Noctor and Foyer 1998; Yoshimura et al. 2000). In chloroplasts of tobacco, overexpression of APX enhances tolerance of plants to salinity and water deficiency (Badawi et al. 2004a, b). The ascorbate-glutathione cycle enzymes, for instance, APX and GR, were upregulated under high temperature in lily plants. These enzymes play an indispensable role in the alleviation of H_2O_2 accumulation. At transcriptional level, APX upregulation and generation of novel thermostable APX isoforms in *Arabidopsis thaliana* alleviate H_2O_2 accumulation (Panchuk et al. 2002).

Heat-tolerant genotype of cabbage exhibited higher GSH content. Due to increase in expression of APX, DHAR, and GR enzymes in apple leaves on exposure to heat stress, content of AsA, total glutathione, GSH, and GR also increase (Ma et al. 2008). APX and GR stop the oxidation of enzymes and cell membranes and henceforth play an imperative part in the protection of plants from heat (Almeselmani et al. 2006). The pAPX (peroxisomal type ascorbate peroxidase) scavenges H_2O_2 in peroxisomes (Shi et al. 2001). A gene pAPX (HvAPX1) from *Hordeum vulgare* was transferred to transgenic *Arabidopsis thaliana* plant against oxidative stress (Shi et al. 2001). Both CAT and APX enzymes detoxify H_2O_2 into H_2O and O_2. In oxidative stress conditions, CAT activity is declined in peroxisomes (Shi et al. 2001). In normal conditions, total APX enzyme activity does not fluctuate. Overexpression of HvAPX1 transgenic against salt tolerance only affects single APX isoenzyme instead of improving total APX enzyme activity (Tsugane et al. 1999). The cytosolic APX mRNA increases under ozone stress and causes detoxification of ROS (Kubo et al. 1995; Örvar et al. 1997). Antisense version of cytosolic APX reduced tolerance to ozone in tobacco plants (Örvar et al. 1997). OsAPXa overexpressing rice plants exhibited improved APX activity and lower levels of H_2O_2 and MDA in cold stress (Sato et al. 2011). Two different signals were likely generated in thylakoid ascorbate peroxidase (tylapx) and cytosolic ascorbate peroxidase 1 (apx1) gene knockout double mutants. The absence of chloroplastic H_2O_2 removing enzymes enhances heat tolerance in plants (Miller et al. 2007). The thylakoid-bound APX genes (LetAPX) from tomato on overexpression in tobacco lead to enhanced salinity tolerance (Sun et al. 2009).

Heavy metals cause oxidative stress because of better ROS production (Dietzk et al. 1999). SbMYB15 transgenic tobacco displayed heavy metal tolerance. SbMYB15 overexpression improved CAT and SOD activities, MnSOD (twofold), and $CdCl_2$ (three- or ninefold), and CAT1 (62-fold) (Sapara et al. 2019). Overexpression of CaGrx from chickpea increased heavy metal tolerance into *A. thaliana*. Transgenics exhibited enhanced activities of GRX, GST, GR, GPX, CAT, APX, SOD, and MDHAR in AsIII and Cr stress (Kumar et al. 2020). The gene expression for GSH biosynthetic enzymes was enhanced in Cd-tolerant transgenic *Brassica juncea* (Zhu et al. 1999). Yeast cadmium factor (YCF1) is a glutathione-S-conjugate transporter. In yeast, it transports Cd21 GSH in a vacuolar membrane protein (YCF1) and confers tolerance to Cd21 (Decottignies and Goffeau 1997; Morgan et al. 2013). GS or g-ECS overexpression in *B. juncea* showed improved stress resistance to various HMs (Cd, Zn, As, and Pb) because of higher capacity of GSH and PC biosynthesis (Reisinger et al. 2008). Aluminum (Al^{+3}) toxicity inhibits root growth (Tabaldi et al. 2009). Tolerant lines showed Al^{+3} tolerances because of improved GST activity (Darkó et al. 2004). Ascorbate-glutathione enzymes increased in Al^{+3}-stressed rice seedlings to cope with oxidative stress (Sharma and Dubey 2007). MDHAR or DHAR transgenic tobacco plants tolerate Al^{+3} stresses by regeneration of reduced AsA (Yin et al. 2010). Higher proline synthesizing transgenic plants showed better resistance to several abiotic stresses and oxidative damage. Transgenic green microalga *Chlamydomonas reinhardtii* overexpressing P5CS show tolerance to toxic cadmium heavy metal stress by accumulation of above twofold free proline content and GSH redox state (Siripornadulsil et al. 2002).

GR overexpression increased tolerance to plant oxidative stress because of increased antioxidant capacity. Transgenic plants produced by manipulation of various antioxidant enzymes of ascorbate-glutathione cycle show tolerance to salinity (Ashraf 2009; Kocsy et al. 2001). The cold tolerant coffea sp. shows higher level of Cu/Zn-SOD, APX, ascorbate, and tocopherol molecules for controlling oxidative stress produced during chilling (Fortunato et al. 2010). Acerola PMM transgenic tobacco has threefold more ascorbate content (Badejo et al. 2009b). In *Arabidopsis thaliana*, mutations in genes of D-Man/L-Gal pathway decreased AsA content (Dowdle et al. 2007; Linster et al. 2007). Overexpression of these genes improved AsA levels (Dowdle et al. 2007; Linster et al. 2008). *Arabidopsis* ERF (AtERF98) gene regulates AsA synthesis through the activation of synthesis associated genes (Zhang et al. 2012). The ascorbate content enhanced twofolds in transgenic tobacco transformed with GME and GGP than individual gene. The expression of acerola was 5- to 700-fold higher than in *Arabidopsis* (Badejo et al. 2009a). In *Solanum lycopersicon*, overexpression of GDP-Man-3, 5-epimerase gene (SlGME) increases AsA content and tolerance to salinity and cold (Zhang et al. 2011). Overexpressed GDP-D-mannose pyrophosphorylase (GMPase) gene of acerola shows a two- to threefold rise in ascorbate content in *Solanum lycopersicon* (Badejo et al. 2008). The GMPase levels change due to the change in AsA content on the basis of the presence or absence of light (Wang et al. 2011, 2013). GMPase gene shows overexpression in transgenics in normal conditions and heat stress. The activities of GMPase, DHAR, MDHAR, and APX and contents of DHA and AsA increase. Thus, potato and *Solanum lycopersicon* tolerate temperature stress because of overexpression of GMPase, which in turn increase AsA levels (Li et al. 2011a, b). During stress, expression of AsA induces and increases AsA biosynthesis in *Solanum lycopersicon* (Ioannidi et al. 2009). There is an association between the rise in content of AsA and expression of GDP-L-galactose phosphorylase (GGP) and GDP-D-mannose, 3′, 5′-epimerase (GME) in kiwifruit (Bulley et al. 2009).

Overexpression of either Gly I or Gly II glyoxalase system enzymes increases tolerance to abiotic stress in plants (Singla-Pareek et al. 2003, 2006, 2008; Lin et al. 2010; Wu et al. 2012; Viveros et al. 2013). GalUR overexpression in transgenic potato plants shows better tolerance to salinity and salinity-induced oxidative stress, accumulation of AsA, restricted increase in MG levels, higher GSH/GSSG ratio, and increased activities of APX, DHAR, GR, GST, GPX, and Gly I and Gly II. Increased ROS, MG detoxification, changes in GSH, and AsA redox state enhanced salinity tolerance in transgenics (Upadhyaya et al. 2011). The induction of plant-specific Tau class GSTU genes by various abiotic stresses provides improved protection of plants to oxidative damage (Jha et al. 2011). The effect of heat on glutathione and ascorbic acid levels was reported (Kocsy et al. 2002, 2004; Kumar et al. 2012; Dai et al. 2012). The transgenic tomato showed higher GPX activity and more resilience to abiotic stress but more susceptibility to biotic stress (Herbette et al. 2011). Tobacco GST with GPX overexpression in Nicotiana *tabacum* L. improved seedling growth in heat and salinity stress (Roxas et al. 2000). The proline biosynthesis suppression in transgenic plants with inducible gene coding for P5CR in the antisense direction improved drought sensitivity (de Ronde et al. 2000, 2001). Salt hypersensitive *Arabidopsis* mutant (p5cs1) had reduced root growth, proline content, and NADP1 to NADPH ratio and enhanced ROS production, severe chlorophyll destruction, elevated H_2O_2, and level of lipid peroxidation under severe oxidative stress (Szekely et al. 2008; Sharma et al. 2011). *Nicotiana tabacum* transformed with P5CS gene exhibited an increase in activities of APX, CAT, and proline accumulation and salinity tolerance (Razavizadeh and Ehsanpour 2009). “Swingle” citrumelo rootstocks were transformed with P5CSF129A gene encoding for proline biosynthesis (De campos et al. 2011). APX, CAT, SOD, and GR exhibited differential regulation in leaves of Swingle citrumelo transgenics with high endogenous accumulation of proline under drought stress and normal conditions (De Carvalho et al. 2013). P5CS gene overexpression in both sense and antisense directions was done in transgenic soybean plants. Antisense transgenics showed severe H_2O_2 and lipid hydrogen peroxide levels, higher GSH pool, and ROS injury. The sense transgenics showed slightest injury due to lower accumulation of H_2O_2 and higher proline and AsA pool in drought or combined heat and water deficiency stress (Kocsy et al. 2005). Transgenic sugarcane plants overexpress P5CS gene with 2.5-fold higher proline content but no osmotic adjustment under drought (Molinari et al. 2007). A rice transgenic overexpressing P5CSF129A gene showed higher salinity tolerance and reduced lipid peroxidation (Kumar et al. 2010). Salinity leads to excessive ROS generation and oxidative stress in plants produced as a derivative of aerobic metabolism (Chaparzadeh et al. 2004; Parida and Das 2005; Noreen et al. 2009; Ashraf 2009; Wang et al. 2012a, b).

OsOAT gene transgenic rice showed higher GSH pool, enhanced activities of GPX and POD, ROS scavenging activity, proline accumulation, and better water deficiency and osmotic stress tolerance (You et al. 2012). Transgenic *Solanum tuberosum* overexpressing l-gulono-c-lactone oxidase (GLOase) gene boosted basal levels of AsA content and showed improved persistence under various abiotic stresses (Hemavathi et al. 2010). The a-tocopherol overexpression increases plant tolerance to oxidative stress. Tocopherol cyclase (VTE1) catalyzes second last step of tocopherol biosynthesis. VTE1 overexpressing transgenic tobacco exhibited declined lipid peroxidation, electrolyte leakage, and H_2O_2 content in water deficiency conditions (Liu et al. 2008).

Synchronized overexpression of Cu/Znsod and apx or only apx in transgenic tobacco plants lessened the damage of drought stress (Faize et al. 2011). SOD (SOD-OX) overexpression in leaves enhanced tolerance to both acute and longer-term high temperatures (Artlip et al. 2009). In *Ipomoea batatas*, expression of Cu/ZnSOD and APX improved water deficiency resistance and the recovery rate from water deficiency stress (Lu et al. 2010). Expression of CuZnSOD, APX, and DHAR in transgenic tobacco plants was extra effective than single or double expression with improved tolerance to various stresses (Lee et al. 2007a, b).

Overexpression of MuWRKY3 gene from horse gram into groundnut plants leads to lower contents of MDA, H_2O_2, and $O_2{\cdot}-$ and improved SOD (three- to fivefolds) and APX (three- to sevenfolds) activities and increased water deficiency tolerance (Kiranmai et al. 2018). MdATG18a overexpression in apple increased the activities of CAT and POD and improved tolerance to drought stress by high frequency of autophagy and restriction of oxidative damage (Sun et al. 2018). Overexpression of chrysanthemum DgNAC1 gene lowered the accumulation of MDA, H_2O_2, and O2• − and significantly improved SOD, CAT, and POD activities and salinity tolerance in transgenics (Wang et al. 2017). PaSOD (*Potentilla atrosanguinea*) and RaAPX (*Rheum australe*) were overexpressed in potato dual transgenic. Transgenic potato increased activities of SOD and APX positive regulators to enhance tolerance to salinity by ROS regulation and lignin biosynthesis signaling (Shafi et al. 2017). DaAPX overexpression and supplementation of transgenic plants with H_2O_2 improved APX activity and improved flooding and cold tolerance (Chen et al. 2019). AtDREB1A overexpression in tomato plants increased activities of SOD and CAT and superior chilling stress tolerance (Karkute et al. 2019). Overexpression of StSOD1 gene improved SOD, POD, and CAT activities and improved cold tolerance in transgenic plants (Che et al. 2020). Chrysanthemum CmSOS1 overexpression enhanced SOD and CAT in transgenic plants (Wang et al. 2019). A summary of transgenic plants with increased antioxidant defense system activities under various stresses is documented in Table 5.1.

Table 5.1 Transgenic plants tolerant to oxidative stress

Mutant/ transgenic plant	Gene	Stress response	Reference/s
Arabidopsis thaliana	CaMT	Enzyme activities of APX, POD, GPX, and GRX enhance in transgenic plants	Dubey et al. (2019)
Arabidopsis thaliana	MpDGK2	Enzyme activities of CAT, APX, and POD enhance in transgenic plant.	Tan and Wang (2020)
Arabidopsis thaliana	OsSultr1;1	Enzyme activity is enhanced, i.e., GSH with As (III) toxicity	Kumar et al. (2019)
Arabidopsis thaliana	BnERF2.4	Enzyme activities of SOD, POD, and CAT enhanced in under waterlogging	Lv et al. (2016)
Arabidopsis thaliana	MaRAP2-4	Enzyme activities of CAT, GPX, and SOD enhance in transgenic plants under waterlogging	Phukan et al. (2018)
Arabidopsis thaliana	DaAPX	Enzyme activity of APX enhanced in transgenic plants	Chen et al. (2019)
Arabidopsis thaliana	HvERF2.11	Enzyme activities of SOD, POD, CAT, alcohol dehydrogenases by 2.1-, 2.3-, and 1.9-fold enhanced in three transgenic lines under waterlogging	Luan et al. (2020)
Arabidopsis thaliana	apx1/apx2	APX2 knockout lines produced more seeds under prolonged heat stress	Suzuki et al. (2012b)
Arabidopsis thaliana	HSF3/APX2	Enhanced ROS accumulation in plants	Panchuk et al. (2002)
Arabidopsis thaliana	CAM3	Thermotolerance reduced in CAM3 mutants and rescued/ enhanced in CAM3 overexpression	Zhang et al. (2009)
Arabidopsis thaliana	CAM3	AtCaM3 is involved in NO signal transduction as a downstream factor and is dependent on increased HS transcription factor DNA-binding activity and HSP accumulation	Xuan et al. (2010)

(continued)

Table 5.1 (continued)

Mutant/ transgenic plant	Gene	Stress response	Reference/s
Arabidopsis thaliana	Apx1/Apx2	H_2O_2 takes part in heat stress signaling pathway and in HSF activation during the early phase of heat stress	Volkov et al. (2006)
Arabidopsis thaliana	Mammalian L-gulono-lactone oxidase	Four- to sevenfold increase in ascorbate	Jain and Nessler (2000)
Arabidopsis thaliana	DHAR	Increased Salinity tolerance	Ushimaru et al. (2006); Chen and Gallie (2005)
Arabidopsis thaliana	GPX-2	Salinity, heavy metal, drought, cold, oxidative, and MV	Gaber et al. (2006)
Arabidopsis thaliana	APX2 and APX 3	Heat tolerance	Chiang et al. (2015)
Arabidopsis thaliana	MDHAR+ DHAR	Freezing oxidative tolerance	Shin et al. (2013)
Arabidopsis thaliana	AtERF98	Activation of ascorbic acid biosynthesis	Zhang et al. (2012)
Arabidopsis thaliana	VvWRKY30	POD, CAT, and SOD activities enhance in transgenic plants	Zhu et al. (2019)
Arachis hypogaea	MuWRKY3	Enzyme activities of SOD enhanced by 3–5 and APX by three- to sevenfolds in transgenic plants	Kiranmai et al. (2018)
Brassica campestris	Cu-Zn SOD + CAT	Salinity and SO2	Tseng et al. (2007)
Brassica juncea	GS	Heavy metal tolerance	Reisinger et al. (2008)
Chrysanthemum	DgNAC1	Enzyme activities of SOD by twofolds, CAT by twofolds, and POD by threefolds enhanced in transgenic plants	Wang et al. (2017)
Chrysanthemum morifolium	CmSOS1	SOD and CAT activities enhanced in transgenic plants	Wang et al. (2019)
E. coli	GR	Heat and MV	Achary et al. (2015)
Festuca arundinacea	Mn SOD +APX	Multiple abiotic stresses	Lee et al. (2007a, b)
Glycine max	GmMYB84	Enzyme activities of SOD, POD, and CAT enhanced significantly in transgenic plants	Zhang et al. (2020)

(continued)

Table 5.1 (continued)

Mutant/ transgenic plant	Gene	Stress response	Reference/s
Gossypium hirsutum	GR	Cold and photooxidative tolerance	Kornyeyev et al. (2003)
Lycopersicon esculentum	cAPX	Drought, heat, cold, and UV light	Wang et al. (2005a)
Malus domestica	MdATG18a	Enzyme activities of CAT and POD enhanced by 1.57–2.05-fold in transgenic plants	Sun et al. (2018)
Malus domestica	MdATG18a	Enzyme activities of SOD, POD, CAT, AsA, and GSH enhanced in transgenic plants, and decrease in ratio of GSH/GSSG under extreme temperature	Huo et al. (2020)
Manihot esculenta	APX and Cu-Zn SOD	MV and cold	Xu et al. (2014)
Nicotiana tabacum	ZmSO	Increased activity of GSH in transgenic plants	Xia et al. (2018)
Nicotiana tabacum	SbMYB15	Enzyme activities of CAT and SOD enhanced in transgenic plants and increase the expression of MnSOD, CdCl2 and CAT1	Sapara et al. (2019)
Nicotiana tabacum	TaFBA1	Enzyme activities of SOD, POD, and APX enhanced and CAT activity decreased in transgenic plants under heat stress	Li et al. (2018)
Nicotiana tabacum	Cu-Zn SOD	Salinity and drought	Badawi et al. (2004c)
Nicotiana tabacum	katE	Salinity	Al-Taweel et al. (2007)
Nicotiana tabacum	AtMDHAR1	Salinity, ozone and drought	Eltayeb et al. (2007)
Nicotiana tabacum	swpa4	Salinity, osmotic and oxidative	Kim et al. (2008)
Nicotiana tabacum	DHAR	Salinity and drought; ascorbic acid biosynthesis; aluminum	Eltayeb et al. (2007), Zhou et al. (2015b), Yin et al. (2010)

(continued)

Table 5.1 (continued)

Mutant/ transgenic plant	Gene	Stress response	Reference/s
Nicotiana tabacum	GPX	Salinity, cold and oxidative stress tolerance	Yoshimura et al. (2004)
Nicotiana tabacum	VTE1	Drought tolerance	Liu et al. (2008)
Nicotiana tabacum		Drought and ozone tolerance	Ushimaru et al. (2006)
Nicotiana tabacum	APX and Cu-Zn SOD	Methyl viologen and oxidative damage; salinity tolerance	Kwon et al. (2002), Negi et al. (2015)
Nicotiana tabacum	Cu-Zn SOD	MV and cold tolerance; oxidative and salinity tolerance; drought tolerance	Gupta et al. (1993), Jing et al. (2015), Negi et al. (2015)
Nicotiana tabacum	APX, Cu-Zn SOD and DHAR	Salinity and paraquat tolerance	Xu et al. (2014)
Nicotiana tabacum	EsSPDS1	Drought tolerance	Zhou et al. (2015a)
Nicotiana tabacum	CAT3	Heavy metal tolerance	Gichner (2004)
Nicotiana tabacum	SoCYP85A1	Enhanced activity of POD by 1.3- to 1.5-folds and SOD by 1.36- to 1.39-fold	Duan et al. (2017)
Nicotiana tabacum	GDP-L-galactose guanylyl transferase and GDP-mannose3’,5′-epimerase	Sevenfold	Bulley et al. (2009)
Nicotiana tabacum	Arabidopsis cytosolic DHAR	Tolerance to water deficiency and ozone stresses	Eltayeb et al. (2006)
Nicotiana tabacum	sHSPs	H_2O_2 is required for activation of sHSP synthesis	Konigshofer et al. (2008)
Nicotiana tabacum	BADH	Overexpression increased thermotolerance induced by accumulation of glycine betaine and repair of PSII from heat-enhanced photo inhibition in transgenic plants	Yang et al. (2007)
Nicotiana tabacum	MDHAR	MDHAR overexpression increased tolerance to salt and osmotic stresses	Eltayeb et al. (2007)

(continued)

Table 5.1 (continued)

Mutant/ transgenic plant	Gene	Stress response	Reference/s
Nicotiana tabacum	Human DHAR gene	Tolerance to cold and salinity stress	Kwon et al. (2003)
Nicotiana tabacum	Wheat DHAR gene	Tolerance to ozone	Chen and Gallie (2005)
Oryza sativa	Cu-Zn SOD	Salinity, drought, and oxidative tolerance	Prashanth et al. (2008)
Oryza sativa	P5CS	Drought and salinity tolerance in transgenic plants	Su and Wu (2004)
Oryza sativa	MDHAR	Salinity tolerance in transgenic plants	Sultana et al. (2012)
Oryza sativa	Cytosolic GR	Photooxidative stress tolerance	Kouril et al. (2003)
Oryza sativa	MnSOD	Salinity tolerance	Tanaka et al. (1999)
Oryza sativa	CAM1-1 CaM1-1	Isoform interpret a Ca21 signature regulating transcription of nucleoplasmic small HSP gene during heat stress	Wu and Jinn (2012)
Petunia hybrida	P5CS	Drought tolerance	Yamada (2005)
Populus davidiana X *Populus bolleana*	MnSOD	Salinity tolerance	Wang et al. (2005b)
Prunus domestica cv. Claudia Verde	SOD +APX	Salinity tolerance	Diaz-Vivancos et al. (2013)
Solanum lycopersicum	DREB1A/CBF3	Drought stress tolerance	Rai et al. (2013)
Solanum lycopersicum	AtDREB1A	Enzyme activities of SOD and CAT enhanced in transgenic plants	Karkute et al. (2019)
Solanum lycopersicum	CodA	Overexpression of choline oxidase shows higher levels MT-sHSP, HSP70, and HSC70 during heat stress in transgenic plants	Li et al. (2011c)
Solanum lycopersicum	GDP-L- galactose	Several fold	Bulley et al. (2012)

(continued)

Table 5.1 (continued)

Mutant/ transgenic plant	Gene	Stress response	Reference/s
Solanum lycopersicum	cAPX	cAPX overexpression enhanced resistance to heat in transgenic plants	Wang et al. (2006)
Solanum lycopersicum	Increased MDHAR	Chilling tolerance	Stevens et al. (2008)
Solanum tuberosum	GDP-L- galactose	Several fold	Bulley et al. (2012)
Solanum tuberosum	StSOD1	Enzyme activities of SOD enhanced by 1.38-folds, POD by 1.24-folds, and CAT by 1.37-folds in transgenic plants	Che et al. (2020)
Solanum tuberosum	GLOase	Accumulation of vitamin C with enhanced abiotic stress	Hemavathi et al. (2010)
Solanum tuberosum	APX and Cu-Zn SOD	Heat, oxidative stress and MV	Tang et al. (2006)
Solanum tuberosum	GLOase	L-ascorbic acid accumulation and tolerance to salinity and MV	Hemavathi et al. (2010)
Solanum tuberosum	P5CS	Accumulation of proline in response to salinity	Hmida-Sayari et al. (2005)
Solanum tuberosum	PaSOD, RaAPX	SOD enhanced by two- to sixfolds in PaSOD and one- to threefolds in double transgenic plants (DTP); APX enhanced by 5- to 11-fold in APX and four- to eightfold in DTP	Shafi et al. (2017)
Strawberry fruits	D-galacturonic acid	Two- to threefolds	Agius et al. (2003)
Triticum aestivum	MnSOD	Oxidative and photooxidative tolerance	Melchiorre et al. (2009)
Triticum aestivum	P5CS	Drought tolerance	Vendruscolo et al. (2007)

5.5 Conclusions

Stress from their neighboring environment affects plants in a complex modus. The schedule and localization of ROS production and scavenging during stress play strategic roles. Identification and characterization of synchronization of signaling events between ROS and oxidative stress will enable the generation of stress resilient crop plants. The findings stated above evidently validate pivotal functions of enzymatic and nonenzymatic antioxidants in inducing oxidative stress tolerance.

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Kinase-Mediated Signaling Cascades in Plant Abiotic Stress Physiology

6

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Abstract

Increasing human intervention in the environment due to population explosion, urbanization, industrialization, and overutilization of natural resources has resulted in changes in the overall ecosystem of the earth. Such changes lead to the pollution of the environment and alteration in natural cycles of water and temperature. All of these result in stressful environment, which is harmful for both plant and animal life. Plants in particular are highly susceptible to abiotic stresses because of their fixed nature. They have developed several signaling mechanisms, which help in the identification and reception of signals through the sensors. The signals, according to the type of stress, generate the responses as a preventive measure through signaling cascades. In the present chapter, an attempt

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has been made to understand various signaling pathways especially kinase signaling, which occur in plant systems in response to a particular type of abiotic stress.

Keywords

Abiotic stresses · Environmental pollutants · Kinase signaling · Signaling cascades

6.1 Introduction

Due to constant changes in the environment, plants have to face several stresses, which provide hindrance in their growth and development. These conditions are further hazardous to the plants as they are sessile in nature and unable to escape. Excessive water, salinity, and extreme temperature are the key environmental conditions that affect the plant development and control vegetation distributed geographically (Zhu 2016). Environmental pollutants, chemical pesticides, herbicides, and fertilizers resulting in excessive heavy metals in the soils and waters thus show strong adverse effects on plants. Hence, a large effect is seen on productivity of crops, and it is estimated that an average of 50% loss in yield is due to these abiotic stresses (Tuteja and Sopory 2008).

In order to survive in adverse conditions, plants need to adapt to these changing environmental factors. In order to do so, the stress signals are perceived by the plants, which are further transferred and ultimately result in a stress response. These responses include modifications in growth, development, and metabolism (Conde et al. 2011). Such changes in plant morphology and physiology are a result of changes in gene expression due to the stress signals. Altered expression of genes further lead to changes in many metabolic functions, and such responses occur both at cellular level and at the level of the entire plant in a synergistic manner (Tuteja and Sopory 2008). The primary signals such as hyperosmotic stress by drought and osmotic and ionic stress as a result of salinity lead to more complex secondary effects that include overproduction of reactive oxygen species leading to oxidative burst, loss of membrane integrity, and damage to vital biomolecules (Zhu 2016). The stress signal is primarily perceived by cell membrane that harbors signal receptors. With this, the downstream signaling cascade starts which aids in transferring the signals to the stress responsive genes (Tuteja and Sopory 2008). The responses to primary and secondary signals enhance the adaptation and hence tolerance of plants to the environmental stresses. The present chapter reviews the responses of plants to several stresses through signaling mechanisms mainly involving kinase group.

6.2 Plant Signaling Under Different Stressor

In response to varied environmental cues, the plants show modulation in gene expression, physiology, and metabolism, which affirms that plants are capable of sensing alterations in environmental signals (Zhu 2016). A very few putative sensors have been identified till recent times due to the functional repetitions or duplication in genes encoding sensor proteins. In case of plants, under abiotic stress, signal transduction pathways are conserved formed mainly by excessive amount of proteins such as receptors, G-proteins, kinases, phosphatases, transcription factors, channels, and transporters (Pandey et al. 2016). In case of eukaryotes, protein kinase occupies a large group of functional genes. In plants, protein kinases are grouped on the bases of functional diversity into different categories such as (1) RLKs (receptor like protein kinases), which perform similar function as RTKs (receptor of try kinase) present in animal system; (2) MAPKs (mitogen-activated protein kinases); (3) CDPKS (calmodulin domain-like protein kinases); (4) CDKs (cyclin-dependent kinases); and other kinases like SNF1/AMPK,PDK1 (pyruvate dihydrogenase kinase), HKs (histidine kinases), DGKs (diacylglycerol kinase), etc. (Chakradhar et al. 2019). Moreover, calcium (Ca) ions are one of the most imperative secondary messengers involve in signal transduction under stress and their levels are usually elevated under stress. These calcium sensors detect Ca^{2+} signals and transduce them downstream by binding to plethora of targets (Tuteja and Sopory 2008). These sensors identify and transduce signals provided by calcium signatures (Tuteja and Mahajan 2007), to target phoporylation networks resulting in alteration in gene expression. CaBP is an important protein triggered and buffered. This protein is activated in response to binding with Ca^{2+} and further reacts with wide array of target proteins in signaling cascades. On the other hand, buffer proteins bind to Ca^{2+} and sequester them. Few CaBP-triggered type proteins are calmodulin (CaM), CaM-binding proteins, and Ca^{2+}-dependent protein phosphatase and kinase (Reddy 2001).

Plants have been reported to have unique, putative sensors Ca^{2+}, which include 50 families of calmodulin-like proteins in *Arabidopsis* and they are termed CMLs. These calmodulin receptors contain more than 148 amino acids residues (Reddy, 2001; Luan et al. 2002). Furthermore, Vanderbeld and Snedden (2007) suggested that CMLs play pivotal role in sensing Ca^{2+}-regulated development and stress responsive cascades. In plants, CaM, i.e., CaMBP has been reported to activate small nuclear NTPases, NAD-kinases, Ca^{2+} ATPases, heat shock proteins, and few transcription factors. A hyperosmotic sensor has been identified in *Arabidopsis* plants, i.e., OSCA1 (reduced hyperosmolality-induced calcium increase 1) (Yuan et al. 2014). The cytosolic Ca ion levels are enhanced in response to plethora of osmotic agent, i.e., chilling, heavy metal, and elevated concentration of abscisic acid (ABA) in plants that was observed by employing calcium or aequorin receptors. In comparison with wild *Arabidopsis* plants, the *osca*1 loss of function mutants revealed a decline in calcium spike in response to osmotic stress induced by elevation in mannitols and sorbitol (Yuan et al. 2014). OSCA1 is a sensor that encodes plasma membrane proteins, which aid in the activation of hyperosmolality and calcium-gated channels. These plants didn't show any phenotypic alterations in response to drought and salt stress; hence, significance of OSCA1 under stressful environment still needs to be evaluated.

Another calcium sensor, i.e., SOS3 (salt overly sensitive 3) have been revealed to transducer signals downstream that is activated and interact with SOS2 protein kinases. This complex formed, i.e., SOS3-SOS2 further activates Na+/H+ antiporter activity of SOS1. More recently, SOS4 and SOS5 have also been identified. The SOS4 sensor encodes pyridoxal-5-phosphate (PLP), and SOS5 is an adhesion protein involved in normal expansion of cells (Mahajan et al. 2008). A large family of cyclic nucleotide-gated channels (CNGCs) has also been identified in plants along with families of glutamate receptor-like sensor (GLR) channels. Both the channels are significantly involved in the generation of cytosolic Ca^{2+}signals under stressful conditions (Swarbreck et al. 2013).

A few mechano-sensitive channels, viz., K2P, TRP, MscS-like Piezo, DEG/ENaC, etc., are non-plant systems (Hedrich 2012). In animals, TRP channels are identified as calcium channels, which sense alterations in membrane permeability in response to change in osmotic status (Árnadóttir and Chalfie 2010). The plants don't contain TRP sensors and DEG/ENaC genes, but the presence of family of MscS-like proteins (MSL) and Piezo homologs has been reported (Hedrich 2012). MSL8 is one of the most important MSL isolated from *Arabidopsis* pollens and were found to overexpress under hypoosmotic pressure during hydration-induced membrane tension (Hamilton et al. 2015). COLD1, another significant putative stress sensor, has been recently identified by Ma et al. (2015) in rice plants in response to chilling stress. Furthermore, they revealed that they were also found in *Nipponbare*, a subspecies of rice exposed to chilling stress of around 0–15 °C. COLD1 is a transmembrane protein identified in endoplasmic reticulum and plasma membrane and is found to interact with RGA1 (an alpha subunit of sole heterotrimeric G proteins) in plants. They further speculated that COLD1 might be involved in the modulation of calcium signaling under cold stress, although enhanced cold tolerance was not confirmed.

Hexokinases 1 (HXK1) has also been recognized as an evolutionary conserved glucose sensor, which regulates nutrient and hormonal signaling in plants exposed to varied environmental cues (Cho et al. 2006). More recent reports suggest that an unknown HXK1 nuclear complex has been revealed to modulate the expression of specific photosynthetic genes without the involvement of glucose metabolism but required the presence of VHA-B1 and RPT5B (metabolic enzymes) (Chen 2007). The fluidity of the cellular phospholipid membranes might get altered as a result of exposure to cold and heat stress (Sangwan et al. 2002). The alteration in the fluidity is sensed by membrane proteins, viz., membrane channels, plethora of receptors like kinase (RLKs), and transporters. A few molecular chaperones have also been reported to modulate signals under temperature stress. These chaperones bind to misfolded proteins formed due to heat denaturation of proteins. These misfolded proteins bind with chaperones release specific heat stress transcription factors resulting in the activation of heat-responsive genes (Scharf et al. 2012). Another imperative sensor, viz., H_2A2-containing nucleosomes, has been found to be thermosensor in plants and in yeast (Kumar and Wigge 2010). They further suggested that these nucleosomes wrap the DNA more tightly than H_2A containing nucleosomes. The expression of heat shock proteins (HSPs) and alternative histones such as H_2A2 nucleosomes encoding genes were elevated, which made DNA more available for transcription.

6.3 Kinase Signaling Under Temperature Stress

Exposure of plants to various environmental stresses especially range of temperatures has been long evident and attained a center stage in this arena of research. Plant metabolism and transciptomes are greatly affected by temperature stress. Both cold and heat stresses along with other abiotic factors are responsible for disrupting the water equilibrium of the cell, the effect of which is manifested as change in turgor pressure (Novakovic et al. 2018). The changes are evident in the case of low temperature environment, which cause dehydration. Behind the remarkable ability of plants to adapt to various stresses is an advanced and effective mechanism to restore ion and cell homeostasis. Among the plant armory to look after homeostasis are proficient stress detecting and signaling components, plant cell detoxification frameworks, perfect solute and osmoprotectant aggregation, and a fundamental revamp of solute transport and compartmentation (Conde et al. 2011). Remodeling of the cell wall of plant has been recognized through various proteomics (Kong et al. 2010; Komatsu et al. 2013) and more recently through metabolomics studies (Jorge et al. 2016), as key structural component in combating various abiotic stresses and making plant acclimatized to variety of stresses. Plant hormone signaling and cell wall integrity sensing have been characterized as fundamental mechanisms in acclimatization of plant toward various stresses.

The response of plants toward stress is a coordinated action of hormones at physiological level and genes encoding proteins; modifiers such as methylation, glycosylation etc.; transcription factors; and adaptors at genetic level (Tuteja 2007). The coordinated action of all these factors will relay signal that switch on the activity of various stress responsive genes (Tuteja and Sopory 2008). Elevated temperature has been found to effectuate heat shock protein (HSP) expression responsible for averting protein denaturation, thus maintaining homeostasis (Scharf et al. 2012), while freezing temperatures reportedly affect metabolism by inhibiting enzymatic action and restructuring of gene expression (Chinnusamy et al. 2007). The involvement of MAPK activation during heat stress- and cold stress-induced calcium signaling with the MAPK cascade has also been reported (Yang et al. 2010). Some of the common responses of plant toward both cold and heat stress is the participation of ROS and NO signaling along with proteosomal degradation and post-translation modifications such as SUMOylation (Chinnusamy et al. 2007). A tabulated review of various TFs responsible for triggering variety of mechanisms in making plants acclimatized toward cold stress is presented Table 6.1.

Cold and heat stresses both affect the metabolism of plants, and network of signaling pathways is activated to deal with the same. Cold stress is sensed by

Table 6.1 Summary of key factors/genes/TFs responsible for plant's cold acclimatization and signaling

S. no.	Transcription factors (TFs)/ genes	Effect of TFs	Reference
1.	AP2-domain proteins CBFs	Expression of COR genes	Chinnusamy et al. (2007)
2.	SUMO E3 ligase SIZ1	SUMOylation of ICE1	
3.	Ubiquitin E3 ligase HOS1	Polyubiquitination and subsequent proteasomal degradation	
4.	ICE1/2	Exercise the expression of CBFs and a transcriptional inducer of CBFs (CBF1–CBF3), ZAT12, NAC072 HOS9 in *Arabidopsis*	Benedict et al. (2006)
5.	SnRK2.6/OST1	Phosphorylation of ICE1 thereby activating CBF-COR gene expression cascade and enhancing freezing tolerance	Ding et al. (2015)
6.	MAP2K, MKK2	Controls COR gene expression and thereby increasing plant tolerance to freezing	Teige et al. (2004)
7.	MAPKs	Calcium influx and membrane fluidity cause activation of MAPKs	Sangwan et al. (2002)
8.	CPKs, CIPKs, and CRLK1	Regulation of COR gene expression and the MAPK cascade	Zhao et al. (2017)
9.	COLD1 (membrane protein)	Cold stress sensor and generates Ca^{2+} signal	
10.	CAMTAs	Activation of cold responsive genes	
11.	DEAR1, DREB, and EAR (ethylene response factor-associated amphiphilic repression motif protein1)	TF regulates cross talk between various signaling pathways	Kalia et al. (2017)
12.	DREB and AREB	Transactivation of ABA mediated rd29A promoter-GUS fusion gene in cold stress	Narusaka et al. (2003)
13.	Z15 (receptor like protein kinase identified from rice)	The expression of z15 by moderate to low temperature confirms its involvement in tolerance of early freezing stress in rice	Feng et al. (2019)
14.	OST1 a protein kinase	Regulates cold tolerance by phosphorylating the substrate BTF3 and BTF3L which in turn stabilizes CBFs under cold stress	Ding et al. (2018)

(continued)

Table 6.1 (continued)

S. no.	Transcription factors (TFs)/ genes	Effect of TFs	Reference
15.	GCN2 kinase	In *Arabidopsis* the activation of GCN2 kinase is found to activate by cold stress in a light dependent manner	Lokdarshi et al. (2020)

CBFs (C-repeat/dehydration-responsive element binding factors); SUMO E3 ligase SIZ1 (a SIZ-mediated small ubiquitin-like modifier (SUMO), ubiquitin E3 ligase; HOS1 (high expression of osmotically responsive gene 1); ICE1/2 (inducer of CBF Expression 1/2); SnRK2.6/OST1 (SNF1-related kinase 2.6/open Stomata1); MAP2K (mitogen-activated protein kinase); MKK2 (MAP kinase kinase2); MAPKs (mitogen-activated protein kinases); CPKs (Ca^{2+}-dependent protein kinases); CIPKs (CBL-interacting protein kinases); CRLK1 (calcium/calmodulin–regulated receptor-like kinase); CAMTAs (calmodulin-binding transcription activators); DREB (dehydration-responsive element-binding factors); AREB (ABA-responsive element binding factor)

COLD1, membrane protein which leads to CPKs and CBL-CIPK-induced cytosolic Ca^{2+}, which further activates MAP kinase cascade. This triggers the phosphorylation of TFs such as ICE1/2 and CAMTAs, responsible for the activation of cold responsive genes like OST1, followed by activation of ICE1. Similarly, heat stress acclimatization involves activation of MAPKs, which brings about change in membrane fluidity and governs HSP accumulation and thermotolerance (Chinnusamy et al. 2007). The signaling network for both heat and cold stress is displayed in the Fig. 6.1.

Among various abiotic stress factors, heat stress is one of the serious threats to the plants. In order to address the threats, variety of heat responsive mechanisms such as stomatal closure, hyper accumulation of HSPs, and increase ROS scavenging enzyme activity have been developed by plants (Mathur et al. 2014). In contrast to animals, plants are sessile and can’t escape troublesome temperature conditions. To endure, plants must most likely envision forthcoming harmful conditions early enough to trigger expression of genes and accumulate HSPs (Table 6.2).

6.4 Kinase Signaling Under Light Stress

The natural light environment of plants is constantly altered at high speed (Dietz 2015). In order to combat drastic variation in light intensity, the plants have evolved varied sensing and response generation mechanism. Most signals produced in response to light stress are mostly generated in chloroplast. The metabolic activity of chloroplast is coordinated to optimize the metabolism to accuse minimum damage (Spetea et al. 2014). Rapid photochemical modulation and molecular alterations are involved in acclimatization of plants to light stress (Dietz 2015). Chloroplast is metabolically multifunctional organelles that have a pivotal role in light sensing and signaling in response to varied environmental cues (Trotta et al. 2014). Varied short-term and long-term light stress results in plethora of alterations in ultrastructural and phenomenal variations such as (i) stacking of grana is enhanced in chloroplast and (ii) chlorophyll molecule per photosystem (PSII) is changed (Malkin and Fork 1981). Generally, the number of chlorophyll molecules per PSII is between 220 and 480 in the sun raised species and 630 and 940 in shade species.

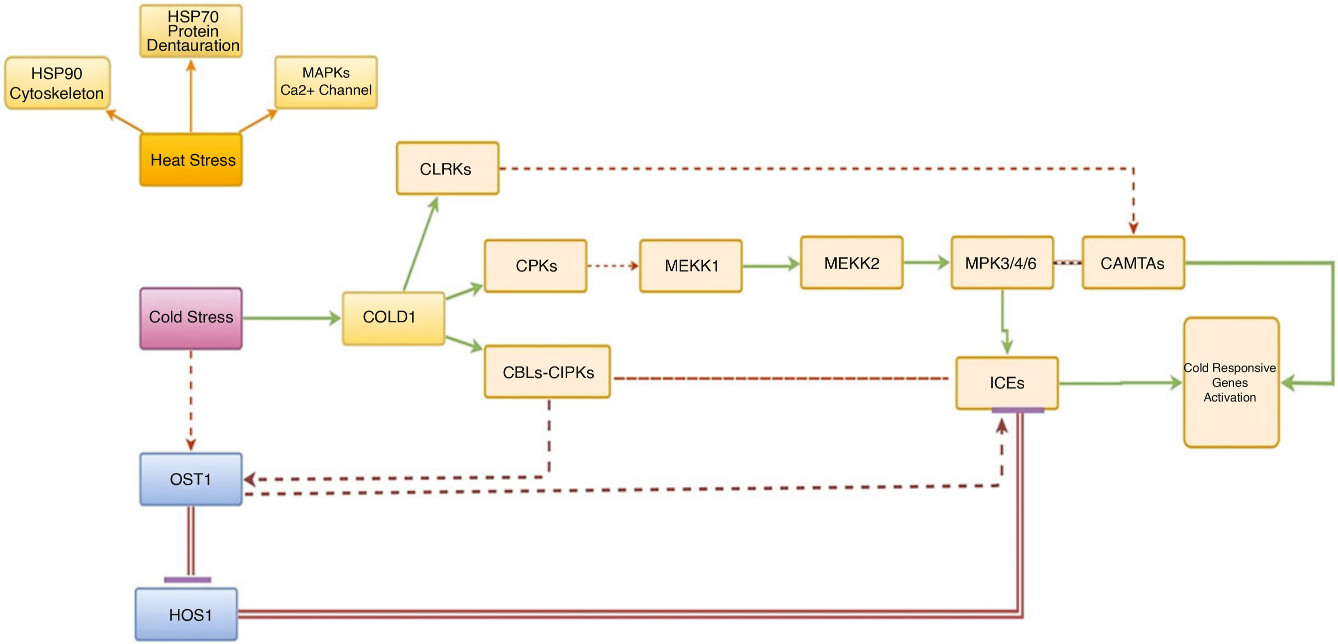

Fig. 6.1 Signaling pathway illustrating the key components of the plant temperature signaling leading to heat tolerance *via* heat-shock transcription factors (HSFs) and heat-shock proteins (HSPs) and cold tolerance *via* various transcription factors. (Solid arrows indicate activation, dotted lines denote regulation, and bars denote inhibition)

Table 6.2 Summary of key factors/genes/TFs responsible for plant's thermotolerance and signaling

S. no.	Transcription factors (TFs)/ genes	Mechanistic effect	Reference
1.	HSP90	Responsible for activation of transcription of HSPs and thus make plants thermotolerant	Yamada et al. (2007)
2.	MAPKs	Modulation of HSP gene expression which is responsible for membrane fluidity and ion signaling, thus making plant thermotolerant	Sangwan et al. (2002)
3.	HSP70	HSP70 along with HSP90 binds to misfolded proteins and thus release HSFs to activate heat stress cascade	Scharf et al. (2012)
4.	Hsp110/SSE (subfamily of HSP70)	The molecular chaperones assist in transcriptional activation, thus making plant heat tolerant	Wang et al. (2004)

Hsp110/SSE, HSP70 (heat shock protein 70), HSP90 (heat shock protein 90)

These variation results in acclimatization of plants to low light exposed leaves by increasing the number of chlorophyll molecules and lowered number of reaction centers. These low light exposed leaves activate the photosynthetic electron transport chain (PET) (Ruban et al. 2007). Change in the PET is included among the most spontaneous reaction to alterations in light intensity. Therefore, the electron produced from the PET are significant for modulation of short- and long-term acclimation response (Fey et al. 2005; Dietz 2015). Unanticipated exposure of plants to high intensity of light may result in light stress of varied levels, which might be dependent to PET over-reduction and inhibition of PSII (Driever and Baker 2011).

Along with these acclimation approaches, the modulation levels of photosynthetic components (Puthiyaveetil et al. 2012), antioxidative defense, and thylakoid ultrastructure (Pfannschmidt 2010) play a pivotal role. The changes in the light intensity results in alteration in ratios of linear to cyclic electron transport chain (Shikanai 2014). The excessively reduced energy is regained by enhancement in reduction state of chloroplast and elevation in activity of mitochondrial alternative oxidase (AOX) and plastid terminals oxidase (PTOX) enzymes, respectively (McDonald et al. 2011; Ivanov et al. 2012). Enhanced light intensity also results in activation of Calvin cycle through: (i) alkalization of stroma, (ii) increment in Mg^{2+} concentration, (ii) enhanced enzyme activity, viz., fructose 1,6-bisphosphate, and (iv) thiol-dependent activation of γ-subunit of ATP synthase (Nikkanen and Rintamäki, 2014).

6.4.1 Signaling in Response to Light Stress

Plants have a capability to employ various individual components of photosynthetic network in stimulation of light-dependent photosynthetic phenomena (Sierla et al. 2013). One of the most imperative environmental cues that exacerbate the production of ROS is high light, which in response results in generation of plethora of secondary metabolites (Zhu 2016). Although enhancement in ROS content might be hazardous for photosynthetic activity of plants (Karpinski et al. 2003), its accumulation results in the activation of signaling responses against plethora of stresses, specifically pathogens and light (Karpinski et al. 2003; Trotta et al. 2014). In plants, phospholipases are involved in various signaling events like seed germination and light-mediated processes (Tuteja and Sopory 2008). The protein kinases of C type, i.e., PKC, have also been shown to have role in light-induced stress responses (Chandok and Sopory 1998). In order to understand the molecular response of *Arabidopsis thaliana* mutant to light stress, Li et al. (2007) showed alteration in expression of high sugar responsive 8 (*hsr*8), which led to elevation in sugar responsive growth and gene expression. They further suggested that light grown-*hsr*8 plants had increased levels of starch and anthocyanin, whereas the content of chlorophyll was reduced. Although under dark conditions, *hsr*8 plants revealed sugar-hyper sensitivity, hypocotyls elongation, and development. Moreover, the findings further suggested the activation of various signaling cascades resulting in alteration in cell wall via PRL 1, which alters the expression of specific genes and subsequently the sugar metabolism (Li et al. 2007).

Qiu et al. (2007) revealed that light along with other metabolic signals triggers SUS (sucrose synthase) protein degradation in maize plants in response to etiolation. They further suggested that SUS degradation is prerequisite for the synthesis of other imperative proteins needed for autotropic metabolism. Furthermore, in the recent years, the involvement of various plant growth regulators (PGRs) including auxins, cytokinins, brassinosteroids, and ethylene have been reported to have significant role in light stress amelioration (Park et al. 2007; Jackson, 2007). Plants exposed to high light and heat conditions (Loreto et al. 2006) might have the involvement of volatile organic compound, which are components of plant-insect interactions that induced signaling. Two nuclear-encoding proteins, i.e., EXECUTER (EX1) and EX2, are located in the thylakoid membrane of the chloroplast, in response to enhanced singlet oxygen species generation, which triggers a signaling cascade (Wagner et al. 2004). They further showed that *Arabidopsis* plants (fluorescent mutant, *flu*) produce a shiver of singlet oxygen species at the dark to light transition stage. The enhanced level of singlet oxygen triggers deleterious alterations in the nuclear gene expression leading to chlorosis and cell apoptosis in wild type and not mutant type plants. The singlet oxygen has also been reported to stimulate signaling cascades independent of EX1 and EX2; the β-carotene's nonenzymatic oxidative breakdown by-products play a vital role in singlet oxygen-induced signaling (Ramel et al. 2012).

High light stress may lead to elevation in content of plastid metabolites, i.e., methyl erythritol cyclodiphosphate (MEcPP), one of the precursors of isoprenoid synthesis. The nuclear-gene encoding plastid proteins are activated by MEcPP-induced retrograde signals (Xiao et al. 2012). Furthermore, another metabolite phoshonucleotide 3′-sadenosine 5′-phosphate (PAP) is located in the plastids and is significant for stress signaling. PAP levels have been shown to enhance the following high light and drought stress (Estavillo et al. 2011); SAL1/FRY1, a bifunctional phophatase dephosphorylates inositol phosphate and PAP. The dysfunctioning of SAL1/FRY1 results in enhanced accumulation of PAP metabolites. Moreover, PAP has been shown to inhibit 5′ and 3′ exoribonuclease activity that results in enhanced expression of high light responsive gene expression (Estavillo et al. 2011; Gigolashvili et al. 2012). High light stress stimulates systemic response, causing enhanced systemic acquired acclimation (SAA). SAA participates in long-distance hydraulic electric signaling, calcium, and ROS networking (Choi et al. 2014). These stress conditions stimulate calcium and ROS signaling, which move with the speed of 1000 μm per second and were identified in various transgenic plants expressing calcium-susceptible fluorescent plants (Choi et al. 2014). They also result in transcriptional alterations in response to stress in the distal organelles and tissues (Miller et al. 2009). Figure 6.1 demonstrates light stress sensing and signaling in plants.

Vital participation of chloroplast envelope in cell signaling under light stress has been reported (Sun et al. 2011; Furumoto et al. 2011). For example, the *re* mutant of *Arabidopsis* reticulation plant leaves was found to be deficient in transmembrane proteins of the chloroplast membrane, which resulted in reticulated coloration and subsequent accumulation of ROS (Pérez-Pérez et al. 2013). The *re* mutant also showed enhanced cell death in response to ROS signaling in response to exposure to ozone fumigation at moderate environment (Overmeyer et al. 2008). Along with chloroplast and nucleus, recently mitochondrion is now considered important antegrade and retrograde controller of signaling networks (Schwarzländer and Finkemeier 2013). Few other factors, which play a major role in retrograde signaling, are changes in redox status linked with: (i) NADPH and thioredoxin (Bräutigam et al. 2009); (ii) ABA in the thylakoid membrane (Galvez-Valdivieso et al. 2009); (iii) sugars and chlorophyll anabolites as well as catabolites (Pružinská et al. 2003); (iv)superoxide anion and H_2O_2 (op den Camp et al. 2003); (v) glutathione, ascorbic acid, and lipid peroxides (Müller-Moulé et al. 2004); and (vi) phytohormones, viz., SA, ethylene, and JA (Mateo et al. 2006).

Tetrapyrrole, catabolites, and anabolites function as signaling molecules under stressed conditions and serve as an operational control under slight alterations in metabolism. Mutants with disturbed tetrapyrrole metabolism frequently show spontaneous cell death and are light dependent (Schlicke et al. 2014). It was reported by Pružinská et al. (2003) that *ACD1* gene (accelerated cell death 1) of *Arabidopsis* plants is a homolog to *LLS1* gene, i.e., lethal leaf spot 1 of maize plants. *LLS1* gene encodes the phaeophoride, a oxygenase enzyme activity. The maize mutant *lls1* accumulated enhanced levels of phaeophoride along with the formation of lesions in a light-dependent manner. Similarly, in tobacco plants, reduced plastic ferrochelate activity enhanced the expression of FeChl antisense RNA, which led to decline in chlorophyll content of leaves and consequently resulted in the formation of necrotic lesions in a light-dependent manner (Papenbrock et al. 2001). Moreover another light-dependent input is laid by cryptochrome and phytochromes, which have been reported to enhance the expression of various photosynthetic genes, specifically ribulose-1,5-bisphoaphate carboxylase (Berry et al. 2013).

It could be therefore concluded that varied light intensities, which exert plethora of effects on plants via photosynthetic processes and other metabolism, are dependent upon the duration and quantity of light stress (Kangasjärvi et al. 2005). An in-depth understanding of complex molecular interplay between environmental light signaling components is highly recommended, and efforts should be made to enhance the stress acclimation of plants for sustainable production and usage (Fig. 6.2).

6.5 Kinase Signaling in Response to Water Stress

Water stress has pernicious impact on plant's growth and development (McDowell 2011). It limits photosynthetic capacity of plant, and if condition prolongs, yield is significantly reduced (Osakabe et al. 2014). Plants have evolved mechanisms such as osmotic adjustment and antioxidant defense systems, which empower it to survive in such hostile environment (Khaleghi et al. 2019). Plants have also made remarkable alterations in regular signaling cascades to adjust their consumption of resources, adding more to its survival index (Osakabe et al. 2014). Elucidations of these networks will prove to be pivotal in improving stress tolerance in plants. In various signaling pathways, membrane-bound receptors play vital role in transmitting information to target molecule. RLK is cell wall-associated kinases (WAKs) that are involved in the perception of turgor pressure. Receptor-like kinase (RLK) family is involved in osmotic stress signaling in various plant species. These RLKs vary on the basis of different extracellular domains, which receive environmental stimuli (Christmann et al. 2013). It transmits information regarding the osmotic conditions outside the cell (Osakabe et al. 2013). AHK-1, acts as osmosensor, an *Arabidopsis* histidine kinase confined to plasma membrane. Drought tolerance was found to be increased in *Arabidopsis*. Its mutant had decreased ABA sensitivity, indicating that AHK1 plays a vital role as osmosensor under osmotic-stress signaling (Tran et al. 2007). Another very important component of plant's signal perception and its transduction is mediated by MAPK cascade. Many MAPK cascades were reported to play a pivotal role in abiotic stress signaling in plants (Sinha et al. 2011). It is noteworthy that several reports have been proposed that validates a direct association between MAPK signaling and water stress, enlisted as follows (Table 6.3).

6.6 Kinase Signaling in Response to Ionic Stress

Soil salinization limits the growth and productivity of crops and globally causes serious risk to agricultural practices (Kumar et al. 2008). Extreme level of salts in the plants leads to imbalance of ionic homeostasis, and, thus, plant cells generate the signal of ionic stress. These ionic signals are professed by the receptor or the salinity sensor, which are present on the plasma membrane of the cells, further it is controlled by the collective efforts of ion pumps, salt overly sensitive (SOS) pathways, and also by their downstream interacting partners, hence cause the removal of surplus ions from the cells (Shabala et al. 2015).

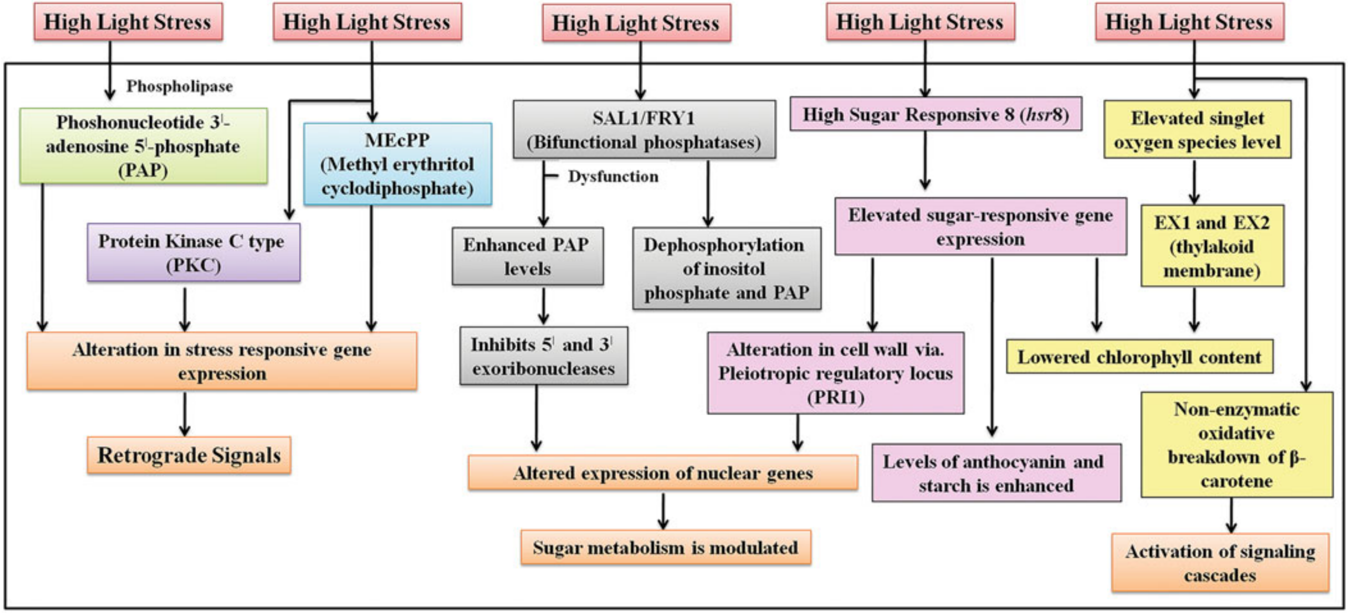

Fig. 6.2 Light stress sensing and signaling in plants

Table 6.3 List of MAPKs involved in stress signaling in different plants

S. no.	Plant	Stress	Unit of MAPK cascade involved	Reference
1.	*Arabidopsis*	Dehydration	AtMPK1, AtMPK4,6	Ichimura et al. (2002)
2.	Maize	Osmotic stress	ZmMPK7	Zong et al. (2009)
3.	Alfalfa	Drought	P44MKK4	Jonak et al. (1996)
4.	*Salicornia brachiata*	Dehydration	SbMAPKK	Agarwal et al. (2010)
5.	Tobacco	Osmotic stress	SIPK	Samuel et al. (2000)
6.	Rice	Drought	MAPKK1	Kumar et al. (2008)
7.	Alfalfa	Drought	p44MKK4	Jonak et al. (1996)
8.	Rice	Drought	OsMSRMK2, OsMAPK5	Agrawal et al. (2002)
9.	Rice	Dehydration stress	DSM1	Ning et al. (2010)
10.	Malus	Drought	MaMAPK	Peng et al. (2006)

6.6.1 Sodium (Na^+) Ions

Na^+ ions interfere with the functioning of the cells when get deposited in cells and tissues, hence proved detrimental. Na^+ deteriorate the functioning of the plant by damaging the cell wall, by imbalancing the ratio of Na^+ and K^+ ions inside the cell, and also by causing the adverse effects to the hydration shell of the molecules (Shabala et al. 2015). With the help of SOS pathway, a calcium-dependent protein kinase pathway, plants tolerate the Na^+ ions and regulate the signaling (Zhu 2002). In this pathway, an EF-hand calcium-binding protein SOS3 (CBL4) recognizes calcium signal of cytosole. SOS3 then turns on SOS2 (CIPK24), which is a CBL-interacting protein kinase (Halfter et al. 2000). Consequently, SOS1 is activated by the phosphorylation of SOS2. SOS1 is a Na+/H+ antiporter present at plasma membrane (Zhu 2016). Inactivation of SOS genes may lead to enhancement in the sensitivity of mutants exposed to salinity. SOS1 mutant exhibit most sensitivity in the presence of salinity, whereas sos2 are intermediate in this context and sos3 mutant proved to be least sensitive (Zhu 2000). SOS1 and high-affinity potassium transporter 1 (HKT1) have antagonistic functions.

In plants, HKT protein family is considered to be crucial to resist the salt stress. This family activated the parenchymatous cells and vascular system of *Arabidopsis*, which acts as a Na + importer (Rus et al. 2004). This protein family helps in the unloading of Na^+ from the roots through the xylem, so as to balance the level of salts. In the rice plants, OsHKT1;1 is present in the vascular system and acts as Na^+ transporter during salinity conditions, that further leads to increased Na^+ omission from the plant cell (Horie et al. 2005). Thus, HKT1 regulates the translocation of Na^+

from roots to shoots mainly in monocots. On the other hand, OsHKT1;4 gene more resourcefully helps in the removal of Na^+ from leaf of the japonica rice species, which are tolerant to salinity stress (Cotsaftis et al. 2012).

6.6.2 Calcium Ions

Calcium plays significant role as a crucial messenger in the adjustment and various other developmental activities of plants. Calcium-dependent pathway is helpful especially against the salinity stress conditions (Luan et al. 2002). For abiotic stress tolerance, it acts as a second most essential messenger (Sanders et al. 2002). Interaction of CBL4 (SOS3) (a Ca^{2+} sensor protein) with the CIPK24 (SOS2), a protein kinase at the plasma membrane and Na^+/H^+ exchanger pathway (SOS1), leads to the removal of Na^+ from the cytoplasm (Zhu 2016). Signaling process of Ca^{2+} is activated by Ca^{2+} sensor and respective target proteins.

Signal is transmitted through a downstream pathway by Ca^{2+}, where it links with the CBL, a protein sensor, and also interacts with protein kinases CIPKs (Lin et al. 2005). CBL family protein is an inimitable category of calcium sensors, which decodes calcium transients by balancing protein kinases (CIPKs). CBL10 mutant uptake low salt concentration signifies its distinctive characteristic in comparison with wild type (Kim et al. 2007). Different CBLs like CBL4 (SOS3) and the respective interacting kinase CIPK24 (SOS2), along with SOS1, contribute in efflux of Na^+ ions from the cell cytoplasm (Zhu 2016).

6.6.3 Magnesium Ions

For normal growth and development of plants, Mg^{2+} is required, but at the same time, their higher doses may prove toxic to the cells (Niu et al. 2018). Exposure of plants to high Mg^{2+} concentration is balanced by CBL2 and CBL3 proteins, which help in retaining the low Mg level; hence, these proteins also prove their role as a sequestration of Mg^{2+} from vacuoles (Tang et al. 2015). Four actively overlapping factors for the downstreaming of CBL2/3 such as CIPK3, CIPK23, CIPK26, and CIPK9 assist Mg^{2+} homeostasis in signaling pathway. This activity is regulated by CBL-CIPK pathway, which also contributes in detoxification mechanism (Gao et al. 2015).

6.6.4 Nitrate Ions

Nitrate is taken by the plants as a source of nitrogen (Crawford 1995), which involves high-affinity system specifically in low nitrate situation and low-affinity system during availability of enough nitrate (Krouk et al. 2010). Nitrate is translocated in plants with the help of three transporter families: nitrate transporter 1 (NRT1), nitrate transporter 2 (NRT2), and chloride channel (CLC). During the low nitrate concentration, AtCBL19-AtCIPK23 compounds are accountable for NRT1.1 phosphorylation that contributes in high binding affinity and transport capacity, in context with the more uptake of nitrate (Ho et al. 2009). AtCIPK8 is responsible for reacting against high nitrate by perceiving and activating the low affinity nitrate reaction, while AtCIPK8 helps in nitrate-regulating root growth for long duration (Ho et al. 2009).

6.6.5 Potassium (K^+) Ions

Potassium (K^+) ions are responsible for the growth and development in plants. Various aspects of crop yield and abiotic stress tolerance is influenced by K^+ (Ahmad et al. 2016). Hence for the homeostasis of K^+ in plants, K^+ transporters and channels are required to maintain across the plasma membrane. To tolerate the high salinity stress, Na^+/K^+ ratio is balanced in the cytosole (Adams and Shin 2014).

In *Arabidopsis,* AKT1 acts as key K^+ transporter as it arbitrates growth by uptake of K^+ by the roots through the help of external K^+ application and thus endorse K^+ affinity even in the low-K^+ concentration range (Xu et al. 2006). At less concentration of $K^{+,}$ akt1 mutants, cbl1/cbl9 and cipk23 cause retarded growth and chlorotic leaves. Similarly, AKT2 also acts as another K^+ transporter, which contributes in transporting K^+ across the plasma membrane (Thoday-Kennedy et al. 2015). CBL4-CIPK6 complex regulates plasma membrane focusing of *Arabidopsis* K+ channel AKT2 (Fig. 6.3) (Held et al. 2011). Further (de)phosphorylation pathway controls efficiently switch from influx to efflux. H + -ATPases are key constituents in the initial sensing during the lack of K^+ (Palmgren 2001). For K^+ possession and

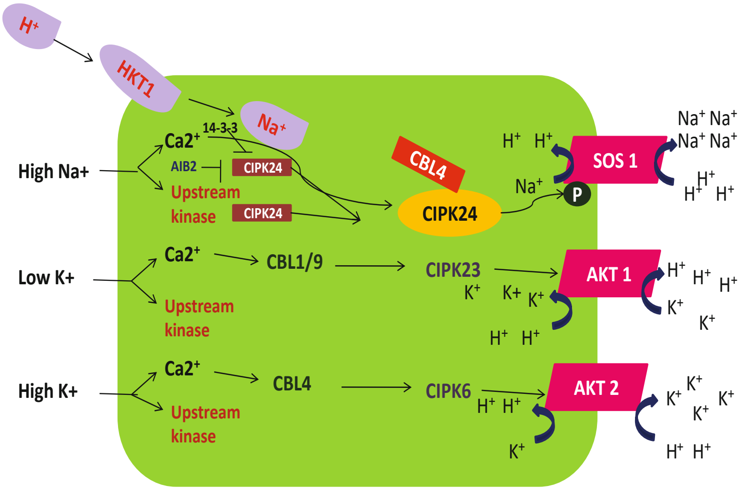

Fig. 6.3 High sodium, low potassium, and high potassium ion stress (AKT1: *Arabidopsis transporter 1, AKT2: Arabidopsis transporter 2,* HKT1: high-affinity potassium transporter 1, SOS1: salt overly sensitive. Activation is indicated by arrows and inhibition by bars)

allocation in rice plant, OsHAK1, OsHAK2, and OsHAK5, as K^+ transporters, contributes significantly (Chen et al. 2015). OsHAK1 helps in improving tolerance to salt and drought stress, when expressed in higher doses (Chen et al. 2017).

6.6.6 Phosphorus

Phosphorus (P), a component of nucleic acids and membrane phospholipids, acts as a key nutrient for the growth and development of plants and forms approximately 0.2% of their dry weight (Schachtman and Reid 1998). Plants uptake orthophosphate ion (Pi) from the soil, but reaction of inorganic and organic phosphates leads to deficiency of Pi (Bieleski 1973). There is involvement of CBL-CIPK system during low Pi in *Brassica napus* (Chen et al. 2012). BnCIPK6 cooperate with *Arabidopsis* CBL1, CBL2, CBL3, and CBL9 where BnCBL1 and BnCIPK6 were upregulated during Pi deficit conditions at low Pi condition; growth and yield in *Arabidopsis* is stimulated by BnCBL1 or BnCIPK6 (Chen et al. 2012).

6.7 Signaling Under Heavy Metal Stress

Plants exhibit various kinds of stresses such as biotic and abiotic. Out of which, heavy metal stress appears to be one of the most destructions that cause abiotic stresses. Overdoses of heavy metals not only causes phytotoxicity in plant but also results in changing the morphological, biochemical, and physiological behavior of plants. However, these heavy metals become more dangerous and cause serious human health hazards especially when they get to enter into the food chain web (Chakraborty et al. 2015). However, plants have gradually developed signaling mechanisms for adapting adverse overdoses of heavy metal phytotoxicity (Golldack et al. 2014).

Plants' defense responses like metal chelation, vacuolar sequestration, heavy metal intakes by transporters, and magnification of antioxidative mechanisms are mainly a result of such kind of intricate signaling networks occurring inside the cell that ultimately transmits the extracellular stimulant into an intracellular reaction and thus finally result in the formation of various kinds of crucial signaling components involved mainly under heavy metal stress. Important kinds of signaling networks working inside the heavy metal stresses are calcium signaling, hormone signaling, MAPK (mitogen-activated protein kinase) signaling, and ROS (reactive oxygen species) signaling (Fig. 6.4).

Out of the several stress-induced signaling, the most predominant and complicated type of signaling is the mitogen-activated protein kinase (MAPK) signaling mainly composed of MAPKKKs (mitogen-activated protein kinase kinase kinases), MAPKKs (mitogen-activated protein kinase kinases), and MAPKs (mitogen-activated protein kinase) (Hamel et al. 2006). MAPKs are serine/threonine kinases consisting of three-tier phospho-relay signaling, which is evolutionary conserved inside the plant kingdom (Jonak et al. 2002). MAPKs are considered one of the most

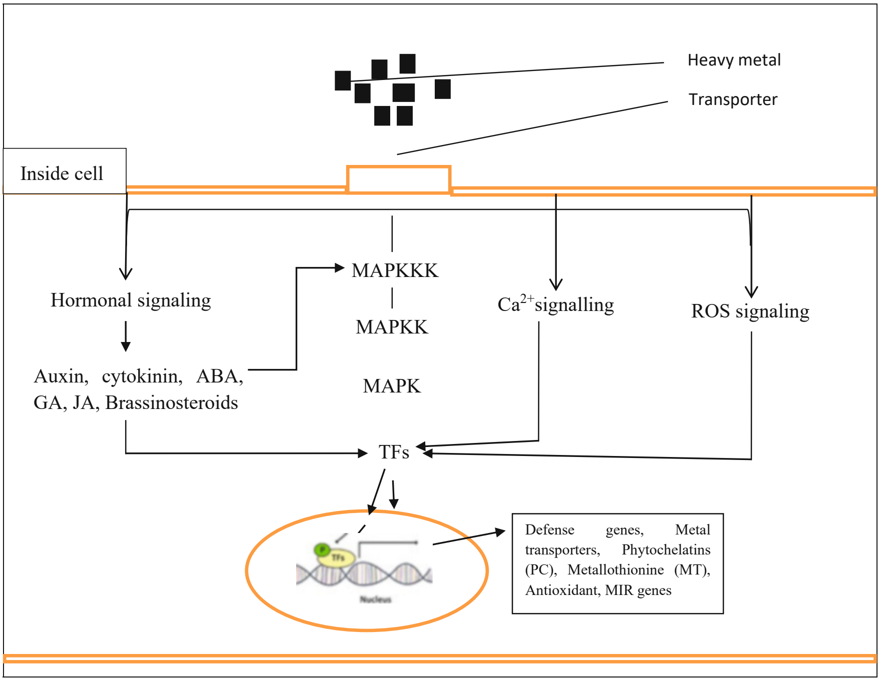

Fig. 6.4 This figure displays basic signaling molecules network working during metal stress. Heavy metals initiate signaling network that causes the activation of transcription factor (TFs) through phosphorylation (P), which regulates the expression of various stress-related genes

important signaling molecules for providing tolerance to biotic and abiotic stress (Rao et al. 2011). MAPKs are extremely conserved signaling components, which function mainly in response to distinct variety of stresses and also during many other developmental pathways (Sinha et al. 2011). MAPK cascade contains three-tier components. These are mainly MAPKKKs, MAPKKs, and MAPKs, which mainly helps in arbitrate phosphorylation reactions from receptor to target (Hamel et al. 2006). MAPKs are activated by specific metal-ligand and by ROS molecules, which were produced mainly during heavy metals stresses (Smeets et al. 2013). There are sufficient amount of reports showing the initiation of MAPK signaling in response to heavy metals such as Cd, Cu, and As (Smeets et al. 2013). However, depth investigation of a complete MAPK signaling network in response to specific heavy metal stress needs further investigation. However, in *Arabidopsis*, under $CdCl_2$ and $CuSO_4$, stress activation of MAPKs such as MPK3 and MPK6 was reported (Takahashi et al. 2011; Sethi et al. 2014).

Investigation of rice genomic profile displays participation of MAPK signaling cascade under Cr stress (Huang et al. 2014; Trinh et al. 2014). In wheat root apex, Al^{3+} exposure causes the activation of 48- kDa MAPK, which play a dominant role in transmitting Al-related and Al-resistant signals in wheat (Mossor-Pietraszewska

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The investigation in rice roots has shown that Zn stimulates quick activation of MBP by three MAPKs, which have approximate molecular weights of 34, 40, and 42 kDa (Lin et al. 2005). Pb stress in radish showed enhancement in four kinds of MAPKs such as MAPKKK7, MAPK6, MAPK18, and MAPK20 (Wang et al. 2013). These some aforementioned reports suggest the involvement of MAPKs in mediating metal stress; however, a detailed study of an entire MAPK cascade network working together for mitigating various kinds of heavy metal stress is required for the complete molecular level of understanding in order to investigate complete mechanistic pathway.

6.8 Conclusions and Perspectives

The plants have developed mechanisms to combat harsh environments through various signaling cascades that depend upon stress sensors for signal perception. The sensor sensitivity regulates the extent of defense mechanism that follows the stress. Research pertaining to identification of stress sensors, their action, and behavior in response to multiple stresses can be the goal of future studies as plants are exposed to multiple abiotic stresses simultaneously. Studies have also highlighted a plethora of compounds that are actively or indirectly involved in signaling mechanisms to strengthen the stress tolerance. Another aspect of study could be the identification of cross talk between these signaling molecules and their role in biotic and abiotic stresses. There is still a poor understanding about the roles of organelles such as chloroplast, mitochondria, peroxisomes, cell wall and cell membrane endoplasmic reticulum, etc. in stress. It becomes imperative to study the responses of organelles such as chloroplast, which is actively involved in light perception and along with mitochondria and peroxisomes is a major site of ROS production. Their perception to stress, behavior, and response to multiple stresses could be one of the major areas of work, which would be helpful in disclosing the pathways followed in these organelles. Overall, the multitude of mechanisms involved plant responses to environmental stresses is an important area of research, which would help in revealing vital information and increase our understanding toward plant behavior.

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Plant Peroxidases: Biomarkers of Environmental Stresses and Signaling in Plants

7

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Abstract

Plants faced several biotic and abiotic stresses during its life span. For maintaining the normal growth, plant produces the reactive oxygen species (ROS), which help in the tolerance of such stress. Fluctuation of the redox reaction in plants increases the production of ROS, which further adversely effects the plant physiological processes. Antioxidants governed and maintain the pathway as well as release of ROS. Till today, it became an interesting and challenging topic to understand the plant response to ROS. ROS is responsible for reversible and irreversible modifications of proteins, which act in various signaling pathways. Oxidative post-translational modifications (OX-PTM) cause structural modifications in target proteins and create oxidative damage. Initially, ROS were identified as a toxic by-product of aerobic metabolism. Now, it is clear that ROS play a key role in signal transductions of plants and controlled the process of growth and development. Biotic and abiotic environmental stimulus triggered the generation of ROS. The main site of ROS production in plants is chloroplast, peroxisome, and mitochondria. Apart from these cell walls, cell membrane, endoplasmic reticulum, and apoplast are also secondary site of ROS production. Degradation of biomolecules such as pigments, proteins, lipids, carbohydrates,

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and nucleic acid are the forms of cell damage, which ultimately cause plant cellular death. This chapter discusses the types, mechanism, and response of plant against these peroxides.

Keywords

Reactive oxygen species · Antioxidants · Stress responses · Oxidative post-translational modifications

7.1 Introduction

Peroxidase is heme-containing monomeric glycoproteins and a family of isoenzyme present in all plants. They utilize either H_2O_2 or O_2 to oxidize a wide diversity of molecules. These important enzymes are utilized in enzyme diagnostic assays, immunoassays, and industrial enzymatic reactions. In the molecular breeding of plants, peroxidase genes and their promoters can be used. To explore the physiological and molecular functions of peroxidase genes in plants, transgenic techniques have been utilizing (Jouili et al. 2011).

7.1.1 Plant Peroxidases

Guaiacol is a substrate that was used as the first colored reaction of biological material, as explained by Schönbein (1855). By semi-century later, to explain an enzyme extracted from roots of horseradish, horseradish peroxidase (HRP), the term peroxidase was used for the first time. In cell cultures of many plant species, bean (Arnison and Boll 1975), spinach (Sticher et al. 1981), tobacco (Pickering et al. 1973), and soybean (Griffing and Fowke 1985) peroxidases were observed, which are omnipresent in all living organisms (Hiraga et al. 2001).

7.1.1.1 Classes of Plant Peroxidases

Peroxidases are present in plants, animals, and microorganisms. Based on peroxidase catalytic properties and structure, they are divided into three super families (Welinder 1992, Table 7.1). In animals, fungi yeast, plants, and bacteria, the second peroxidase superfamily includes catalases (EC 1.11.1.6) (Hiraga et al. 2001). The plant peroxidase superfamily can be classified further into three classes on the basis of differences in primary structure (Welinder 1992, classes I, II, and III in Table 7.1). In plants, bacteria, and yeast such as microbial cytochrome c peroxidase, Class I plant peroxidases contain the intracellular enzyme such as (EC 1.11.1.5), ascorbate peroxidase (EC 1.11.1.11), and bacterial catalase-peroxidase (EC 1.11.1.6). Class II plant peroxidases are extracellular peroxidases from fungi, containing Mn^{2+}- dependent peroxidase (EC 1.11.1.13) and lignin peroxidase (EC 1.11.1.14). Class III plant peroxidases (EC 1.11.1.7), which were originally explained as peroxidases and which are the main concern of this article, are plant enzymes that are released outside

Table 7.1 Classification of peroxidases

Super family	Class	Member	EC number	Origin
Plant peroxidase		Glutathione peroxidase	1.11.1.9	Plant
		Catalase	1.11.1.6	Plant, fungus and yeast
	I	Cytochrome *c* peroxidase	1.11.1.5	Yeast and bacterium
		Catalase-peroxidase	1.11.1.6	Bacterium and fungus
		Ascorbate peroxidase	1.11.1.11	Plant
	II	Manganese-dependent peroxidase	1.11.1.13	Fungus
		Ligninase	1.11.1.14	Fungus
	III	Peroxidase	1.11.17	Plant

the cells or move into vacuoles. POX contains horseradish peroxidase, which is a commercially accessible enzyme that is often conjugated to an antibody for chromogenic identification (Hiraga et al. 2001).

These three classes of the plant peroxidase superfamily of enzymes are different in their catalytic properties and structures. Residues in their C-terminal of Class II peroxidases have an extra 40–60 amino acid in contrast to peroxidases in other classes (Welinder 1992). Groups of all classes of the plant peroxidase superfamily have ten simple α-helices. However, three specific helices are present in class III, but peroxidases enzymes of Classes I and II contain one fixed helix (Hiraga et al. 2001). Reductants (cytochrome c and ascorbic acid, respectively) represent strong specificity against Cytochrome c peroxidase and ascorbate Peroxidase. From small molecules to macromolecules, POXs oxidize many substances. However, there is low sequence similarity between the three classes, five independently positioned amino acids that are very significant for catalysis and structure as well as the helical folding of the whole polypeptide. They are strictly preserving among peroxidases in all three classes (Hiraga et al. 2001).

7.1.1.2 Functions

Plant peroxidases act as huge functional enzymes that could identify in plants, from shoot up to senescence. The different types of peroxidases and their origin are given in Table 7.1. The enzyme peroxidase and native ferric peroxidase are transferred into the compound I (comp) during the standard peroxidative cycle by catalyzing the reduction of H_2O_2. Another compound I and II catalyze in continue dehydrogenation reactions of a broad range of electron donor molecules such as phenolic compounds, auxin, or secondary metabolites lignin precursors. Widely speaking, ferriprotoporphyrin is the active part of peroxidases. Indeed, the ferrous heme (Fe IV=O) group contains compound I, which undergoes two continuous steps by AH2 to transform itself into a compound II (CompII). A native form of the enzyme included a ferric heme (Fe III). The reaction is shown to the generation of phenoxy radicals that combine spontaneously to form lignin polymers when the oxidized substrate is a phenolic compound (Chen and Schopfer 1999). If somehow, the phenolic substrate is restored by NADH or related reduced compounds. The resulting radicals (NAD) start a nonenzymatic oxidative cycle in which O_2 can decrease to O_2-, as O_2- can respond with another NADH molecule to give H_2O_2 and NAD. Peroxidases are known as NADH oxidase (Mäder 1980) and use NADH as the electron donor. They have been recommended to play a crucial role in the production of H_2O_2, which is required for lignification.

7.1.1.3 Subcellular Localization

The enzyme peroxidases Class III are commonly present in the apoplast and vacuoles (Andrews et al. 2002). They have excreted enzymes given by genes that encode a signal peptide. It is mediated by the entry of the developing peroxidase peptide into the endoplasmic reticulum. Therefore, they were present in the Golgi apparatus, the endoplasmic reticulum, and transport vesicles (Mäder 1980). The activity of the nuclei, mitochondria, and plasma membrane was identified by peroxidase. However, it seems that isoperoxidases with an acidic isoelectric point are present in cell walls (Passardi et al. 2004), while normal isoperoxidase is present either in the vacuole or in cell walls. Indeed, they have shown that cationic peroxidases could be discovered from the cell wall.

7.1.1.4 Multigene Family

The number of genes has increased widely from the appearance of the first Class III peroxidases, just before the advent of terrestrial plants, to the emergence of angiosperms (Passardi et al. 2004). The plant adaptation to terrestrial life can be connected with the multifunctional of peroxidases or characterized by the availability of oxygen at high proportions. Therefore, the evolution of a multigene family looks to be associated with the increasing difficulties of plant structure and the diverseness of their biotopes and pathogens (Hiraga et al. 2001).

7.2 Production, Scavenging, and Signaling of ROS

During entire life cycle of plants, there are many environmental conditions such as temperature, humidity, salinity, pathogen attack, herbivores attack, and mechanical stress, which are major challenges for them. A reserve signaling pathway is developed by plants which are nonparalleled in its complexity in living species for resistance of all this type of challenges. There are reprogramming of gene expression and metabolism in plants due to response of stress stimulus through signaling of hormones of plants, receptors of cell surface, photoreceptor, and plastids due to lights (Kami et al. 2010; Jaillais and Chory 2010; Vanstraelen and Benková 2012). A class of reactive forms of molecular oxygen plays a vital role in this signal integration and decision-making, collectively known as reactive oxygen species (Kangasjärvi et al. 2012). Due to many stimuli, either environmental or other cell organelles like peroxisomes mitochondria and chloroplast are generated ROS, and this is a hallmark of response against stress. The causes of generation of ROSs and

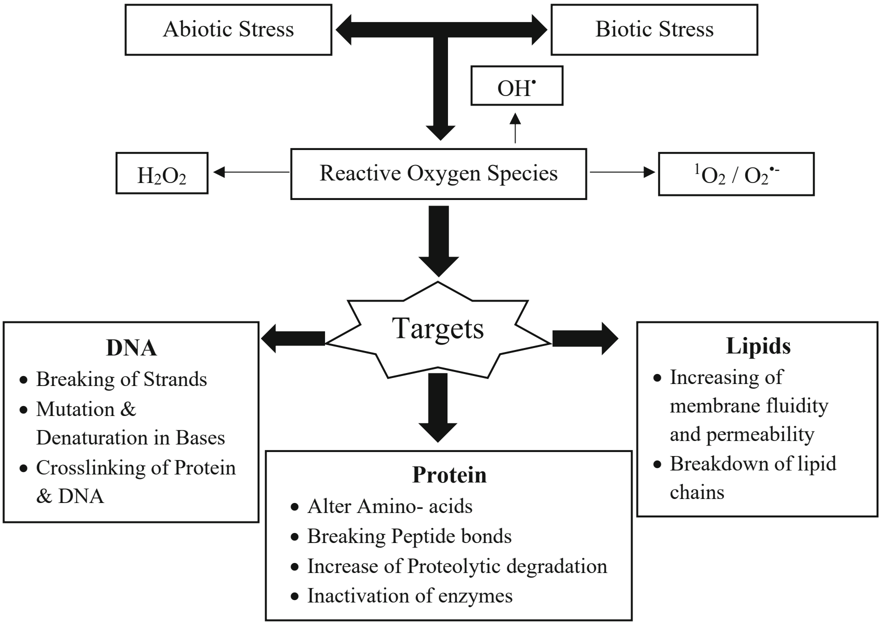

Fig. 7.1 Causes of ROS and their effect on plants

their ultimate effect are described in Fig. 7.1. The production of ROS mostly occurs in apoplast, peroxisome, chloroplast, and sometimes in endoplasmic reticulum, nucleus, and mitochondria (Shapiguzov et al. 2012).

7.2.1 Plastids (Chloroplasts) Responses to ROS

The ROS is being continuously generated in the chloroplasts as the energy is being transfer to O_2 due to partial reduction of oxygen. When cytochrome C oxidase interacts with O_2 generates water. Sometimes, $O^{\cdot -}{}_2$ is liberated due to the reaction of O_2 and discrete ETC constituents, and this is first produced ROS. After undergoing further reactions, superoxide radical ($O^{\cdot -}{}_2$) can also produce member of other ROS family. Singlet oxygen is produced by the reaction of O_2 and triplet state of chlorophyll in the antenna. It is an unusual member of ROS family, which is not produced by electron transfer to O_2 (Das and Roychoudhury 2014).

$^1O_2$ is generated by PSII via two ways (Das and Roychoudhury 2014). Firstly, when environmental stress disturbs the delicate balance between energy utilization and light harvesting, followed by triplet Chl (^{3}Chl*), which are formed and react with dioxygen ($^3O_2$), singlet oxygen ($^1O_2$) is liberated (Karuppanapandian et al. 2011). Secondly, due to over reduction of ETC, $^1O_2$ is produced by the light harvesting complex (LHC) at the PSII (Asada 2006). Due to accumulation of $^1O_2$ in the chloroplast, peroxidation of membrane lipid mainly PUFA (polyunsaturated fatty acid) takes place and damages the proteins of PSII at P680 reaction center. It can also cause the death of cells (Triantaphylides et al. 2008). It is important to control and scavenge the ROS in the chloroplast for survival of plants under stress conditions (Tseng et al. 2007).

7.2.2 Mitochondrial Responses to ROS

The mitochondria is the main production site of $O^{\cdot -}{}_2$ and H_2O_2 like injurious ROS (Navrot et al. 2007). Due to engaging in photorespiration, having rich environment of carbohydrate and O_2, plant mitochondria is different from animal mitochondria (Rhoads et al. 2006). Complex I and Complex III play lead role in the generation of ROS; hence, mitochondria is a crucial culprit because it stores energized electron to reduced O_2 and form ROS (Noctor et al. 2007). In Complex I (NADH dehydrogenase) at its flavoprotein region, O_2 directly decreases into $O^{\cdot -}{}_{2.}$ Due to shortage of NAD^+-linked substrates, a reverse electron flow occurs from Complex III to Complex I followed by the production of ROS that increased at Complex I (Das and Roychoudhury 2014). For the prevention of the oxidative stress in mitochondria, there are two types of vital enzymes named mitochondrial alternative oxidase (AOX) and mitochondrial SOD (Mn-SOD) (Das and Roychoudhury 2014). The main function of AOX is to maintaining the lower state of the UQ pool and reduced the production of ROS (Ho et al. 2008).

7.2.3 Peroxisomal Responses to ROS

Single-membrane-bound spherical micro-bodies, peroxisomes, and their integral oxidative metabolism are responsible for the responses against ROS. In matrix, hypoxanthine and xanthine are metabolized into uric acid by xanthine oxidase (E.C.1.17.3.2), and $O^{\cdot -}{}_2$ is liberated as a by-product. In peroxisomal membrane, NADPH-dependent electron transport chain having the component of Cyt b and NADH, which utilized O_2 as the electron acceptor and cytosolic $O^{\cdot -}{}_2$ is generated (Das and Roychoudhury 2014). There are three transmembrane proteins of peroxisomes, which cause the production of ROS, having molecular mass of 18 kDa, 29 kDa, and 132 kDa.

The electron donor of 18 and 132 kDa peroxisomal membrane polypeptide is NADH and NADPH that also act as electron donor for 29 kDa PMP to lower cytochrome c. During low water availability, stomata remain closed; in these stressful abnormal situations, the ratio of CO_2 to O_2 reduced and caused the occurrence of the increase of photorespiration followed by the formation of glycolate (Das and Roychoudhury 2014). With the help of glycolate oxidase, glycolate oxidized and gave rise to H_2O_2, and during photorespiration, it is a chief generator of H_2O_2 (Noctor et al. 2002). In peroxisomes, there are some other metabolic pathways that produced ROS such as β-oxidation of fatty acids and flavin oxidase pathway (Das and Roychoudhury 2014).

7.2.4 Apoplastic Responses to ROS

Apoplast is a notable site for H_2O_2 production due to the combination of abscisic acid(ABA) and stress signals during the time of stressful environmental (Hu et al. 2006). NADPH oxidase is expressed by *AtRbohD* and *AtRbohF* for the purpose of generation of apoplastic ROS, which is vital for stomatal closure via ABA induction (Kwak et al. 2003). In apoplast, there are some other enzymes that generate ROS like pH-dependent peroxidases, some polyamine oxidases, cell wall linked oxidases, etc., which are responsible for the production of H_2O_2 (Das and Roychoudhury 2014).

7.2.5 ROS Transport Through Cellular Membranes

The plasma membrane is surrounded with whole plant cell below the cell walls and plays a key role for the interaction with changeable environmental conditions and helped in the survival of the cells. The NADPH-dependent oxidases are remarkable due to the presence of different homologs in various adverse conditions and their gene expression, which are situated in the plasma membrane (Apel and Hirt 2004). NADPH oxidase transferred electron from cytosolic NADPH to O_2 and gives $O^{\cdot -}{}_2$ by the help of SOD (Das and Roychoudhury 2014).

7.2.6 Cell Walls Responses to ROS

The cell wall becomes active source of H_2O_2, $OH^{\cdot}$, $O^{\cdot -}{}_2$, and O_2 by hydroperoxidation of polyunsaturated fatty acids with the help of lipoxygenase (LOX) situated in cell walls during stressful conditions. Polyamines or diamines are utilized by diamine oxidase, which is located in the cell wall for the production of ROS (Das and Roychoudhury 2014). For reinforce of the cell wall with lignin during attack of pathogen, the lignin precursors are cross-linking with the help of H_2O_2-mediated pathways (Higuchi 2006).

7.2.7 Endoplasmic Reticulum Responses to ROS

The NADPH-mediated electron transport is situated in the ER including $CytP_{450}$, produced $O^{\cdot -}{}_2$ (Mittler 2002). A free radical intermediate (Cyt P_{450} R^{-}) are produced by the interaction of $CytP_{450}$ with RH, an organic substrate. Sometimes, this oxygenated complex decompose to Cyt P_{450}-Rh and liberated $O^{\cdot -}{}_2$ in the form of a by-product (Das and Roychoudhury 2014).

7.3 ROS-Sensing Mechanisms via Oxidative Post-Translational Modifications of Cysteine Residues

The oxidative post-translational modification (Ox-PTM) of Cys residues is a necessary mechanism that controls protein structure and functions. Cysteine (Cys) side chain's special properties allow various Ox-PTMs, which potentially results in diverse regulatory effects (Tripathy and Oelmüller 2012). The side chain of a Cys residue consists of a terminal thiol (−SH) functional group. At the core of the thiol, the sulfur atom is rich in electron, and its d-orbitals permit for the multiple oxidation states (Waszczak et al. 2015). The accessibility of different oxidation states permits the formation of a diverse range of Ox-PTMs containing sulfenylation (SOH), sulfhydration (SSH), S-nitrosylation, S-glutathionylation (SSG), disulfide bonds (RS-SR), sulfinic acid (SO_2H), and sulfonic acid (SO_3H).

Most Cys Ox-PTMs are stimulated by diffusible small molecules and are reversible. Via antioxidant defense system, they can decrease back to a free thiol (SH) or be transformed to other Ox-PTMs depending on the cell's redox-state (Waszczak et al. 2015). Many factors are involved in the reactivity of the individual Cys residue, its surrounding environment, and the composition of the local redox environment leads to the formation of a single Ox-PTM. A summary of the variety of different Ox-PTMs and the redox-chemistry is associated with their formation. Mainly, Cys Ox-PTMs are persuaded by reactive oxygen or nitrogen species molecules (ROS/RNS) that react with the free thiol on a Cys side chain (Waszczak et al. 2015).

Plants have evolved different strategies to keep ROS levels under a tight control that is governed by nonenzymatic and enzymatic ROS-producing and ROS-scavenging systems (Mittler et al. 2011).

Ascorbate (Asc) and glutathione (GSH) are the prime nonenzymatic cellular redox systems, with tocopherol and diverse alkaloid, carotenoid, and flavonoid metabolites often listed but consistently debated as physiologically relevant antioxidants (Hernández et al. 2009). Lower glutathione pool (high GSH/GSSG ratio) regulation is pivotal for cellular redox homeostasis, since GSH is used to regenerate oxidized ascorbate in the glutathione–ascorbate cycle (Del Río 2011). Asc and GSH work hand in hand with ascorbate peroxidases (Nakano and Asada 1981) and glutathione peroxidases (Mills 1957), respectively, which together with catalases, peroxiredoxins (Prxs), and superoxide dismutases establish the main enzymatic classes involved in ROS scavenging (Mittler et al. 2004). Glutathione is fully protonated at physiological pH because of its relatively high pK_a (Van Laer et al. 2013), and thereby its reactivity toward disulfides and ROS is rather limited (Waszczak et al. 2015).

The chemical properties of the sulfur atom (i.e., broad range of oxidation states) make Met and Cys residues the crucial sites of oxidation within proteins (Davies 2005). The −2 oxidation state of the sulfur atom is represented by the thiol group (R-SH) in Cys Ox-PTMs, which is the fully decreased form. Not all Cys residues in a protein are prone to ROS-mediated modifications, and the reactivity of distinct thiol-proteins toward ROS differs according to their physiological function and local redox environment (Waszczak et al. 2015). Between discrete Cys residues, the reactivity is strongly correlated with their pK_a, i.e., the potential to form the anionic form of the sulfur, called thiolate (R-S$^-$), which is much more reactive than the thiol.

The protonated thiol will be the dominant species, if the pK_a of the sulfur atom is higher than the pH of the solution. However, the majority of the thiols will be present as a thiolate (Cys prone to oxidation), if the pK_a is lower than the pH (Waszczak et al. 2015). The existence of dipoles or proximal charged residues as well as the hydrogen bonding between thiolates/thiols and neighboring residues can stabilize the cysteine thiolates (Harris and Turner 2002). Hydrogen bonding has a great credit on the pK_a of reactive Cys residues. Generally, the lower the pK_a is, the more hydrogen bonds a Cys-sulfur receives, and the more the thiolate form is stabilized (Roos et al. 2013).

The nucleophilicity of the Cys is also a vital factor in its reactivity; sometimes, a lower stabilization of the thiolate in Cys residues enhances its nucleophilicity, while a highly stabilized thiolate requires a great amount of energy to gain the transition state (Ferrer-Sueta et al. 2011). The steric accessibility of Cys residues within the three-dimensional structure of the protein is another important factor that controls the reactivity of Cys residues (Marino and Gladyshev 2010). The first step involves the reversible oxidation of reactive Cys residues to sulfinic acid (R-SOH) in ROS-dependent signaling. This modification is highly unstable and leads to further modifications, unless stabilized within its protein environment (Claiborne et al. 1993).

An extreme concentration of oxidant can result in further oxidation to sulfinic acid (R-SO$_2$H) and thereafter to irreversible sulfonic acid (Roos and Messens 2011). An ATP-dependent sulfiredoxin enzyme (Srx) catalyzed the reversion of the R-SO$_2$H modification that can reduce R-SO$_2$H to R-SOH in *Arabidopsis* (Rey et al. 2007). However, so far, R-SO$_2$H reduction is rather exceptional with the only two known substrates of *AtSrx*: mitochondrial *PrxIIF* (Iglesias-Baena et al. 2011) and the chloroplast 2-Cys Prx (Iglesias-Baena et al. 2010). On the other hand, R-SOH can react with free protein thiols to form intra- or intermolecular disulfide bonds (R-S-S-R/R-S-S-R′) or is modified by low-molecular-weight thiols (like GSH in plants), induced to Cys *S*- glutathionylation. Initially, *S*-glutathionylation events were regarded to serve as a protective mechanism on active-site Cys residues, preventing overoxidation and subsequent permanent protein damage (Waszczak et al. 2015). Only recently, the role of *S*-glutathionylation in redox signaling was recognized (Zaffagnini et al. 2012).

The reduction of deglutathionylation and disulfide bonds is controlled by thioredoxins (Trxs) and glutaredoxins (Grxs), respectively. Plants are equipped with a much more complex Trx/Grx network, compared with prokaryotes and animals. Fifty Grx/Grx-like and 44 Trx/Trx-like proteins are encoded by the *Arabidopsis* genome (Meyer et al. 2012). Trxs use multiple sources of reducing equivalents to perform the reduction of intra-/intermolecular disulfide bonds, depending on the subcellular localization (Waszczak et al. 2015). Light reactions reduce ferredoxin (Fdx) in chloroplasts, which in turn reduces ferredoxin–thioredoxin reductase (FTR), which eventually regenerates the Trx sulfhydryl groups (Schürmann and Buchanan 2008). Another origin of decreasing equivalents, common in the Trx and Grx systems, is NADPH, which after oxidation to NADP$^+$ is reduced by Fdx: NADP$^+$ reductase within the chloroplast stroma, also during the oxidative pentose phosphate pathway.

7.4 Intracellular Interactions Between Redox Signaling and Organelle ROS

Cytoplasmic NADPH is a core for redox signaling pathway for the detoxification of ROS. NADPH supplied electrons to ROS generating enzymes like NADPH oxidase and also maintained disulfide or thiol status. For the changes in the gene expression level in nucleus, all the signaling either from apoplast or cell organelles must have to pass through cytoplasm. Khandelwal et al. (2008) provided an example for the information of ROS where redox state of any cell is combined with other regulators. In last decades, the genetic approaches significantly contribute for understanding the mechanism of it. This is also helpful in the field of genetic engineering of crop plants (Tripathy and Oelmüller 2012).

7.4.1 Chloroplast-Mitochondrion Cross talk, Signaling, and PAP

Mitochondria are the end products of an endosymbiotic event, like chloroplasts, and have a portion of ancestral genome (Woodson and Chory 2008). It is a crucial signaling from mitochondria-to-nucleus in retrograde manner for coordination of the expression of nuclear genes encoding mitochondrial proteins with the expression of the mitochondrial genome (Rhoads 2011). The core function of chloroplast and mitochondria is to capture and utilize the energy in the metabolic exchanges. Apart from these functions, they are also coupled with cellular redox status (Bobik and Burch-Smith 2015). To regulate gene expression of mitochondria, the regulated translocation of proteins would mediate chloroplast signaling. Direct contact between mitochondria and chloroplasts would be made such translocation much easier, and by physical interaction, there may be direct communication (Bobik and Burch-Smith 2015). Mitochondria, peroxisomes, and chloroplasts have frequently been observed in close association in leaves, consistent with metabolic exchange among these organelles. Chloroplast-peroxisome association are established and followed by mitochondria recruited and formed triorganellar unit (Oikawa et al. 2015). These techniques are becoming less time-consuming and easier (McDonald 2014), and we can used these approaches for biology of plant cell (Bobik et al. 2014). This will be an energetic approach for interrogation of ultrastructure of plant cell body when coupled with fluorescence microscopy, as exemplified by recent work from Caplan et al. (2015).

7.4.2 During Stress, the Apoplastic and Organelle ROS Interactions

By the presence of plasmodesmata which is a specialized channel present in the cell wall between two adjacent cells, communication between plant cells is enhanced. Plasmodesmata are discovered 100 years ago, but the structure and regulation of plasmodesmata are not well understood till now (Bobik and Burch-Smith 2015). Recent advancement in plant biology and genetics helped very much to understanding their unknown fact and function. Plasmodesmata gives a way for the metabolite exchange and also water, ions, and product of photosynthesis; beside these also information coded by nucleic acids, proteins like transcription factor and hormones are essential for proper development of plants (Jackson 2015). Firstly, maize *sucrose export defective1* (Russin et al. 1996) mutant was reported (Bobik and Burch-Smith 2015). From sites of photosynthesis, because of callose accumulation at plasmodesmata of bundle sheath and vascular parenchyma locations, the export of photosynthate is decreased by *sxd* 1 mutants (Botha et al. 2000).

Photosynthesis is not inhibited by sxd1 mutants, while accumulation of starch and sugar occur in source cells (Provencher et al. 2001). The first clue is that chloroplast redox state may affect plasmodesmata provided by the *sxd1* mutant. However, mutants were un-differentiable from wild-type plants under optimal growth conditions; however they are much more sensitive to stress of photooxidative stage (Porfirova et al. 2002). With metabolism of tocopherol defects, cautiously examination of *Arabidopsis* mutants should take place for plasmodesmata-related changes in intercellular trafficking (Bobik and Burch-Smith 2015). Identification of the *gfp* arrested trafficking (*gat*) mutants can be done by a genetic screening with altered plasmodesmal function for *Arabidopsis thaliana* mutants (Benitez-Alfonso et al. 2009). The *gfp* is synthesized in the companion cells of the *Arabidopsis* mutant; in wild type, tissue phloem moves through plasmodesmata, while this is not seen in mutants, which means that intercellular trafficking is reduced by *gat* mutants. After about ten days of development become cease, the *gat1, 2, 4*, and *5* mutations are all seedling lethal (Bobik and Burch-Smith 2015). A thioredoxin-m3 (TRX-m3) is localized in plastid, and *gat1* roots is encoded by *GAT1* gene, which accumulate ROS in more amount than wild-type roots.

GAT1 overexpression leads to the reciprocal phenotype of increased intercellular transport. Hence, GAT1 probably functions in redox homeostasis like SXD1/VTE1, including the perturbation of plastid and chloroplasts redox state that leads to altered plasmodesmata. Also, in the *gat1* mutant, altered metabolic flux may change the redox state of TRX-m3 and eventually leads to plasmodesmata function (Benitez-Alfonso et al. 2009). A separate screen is managed by the lab of Zambryski for mutants of *Arabidopsis* with changing intercellular trafficking mediated by plasmodesmata (Burch-Smith and Zambryski 2012). Numerous *increased size exclusion limit (ise)* mutants were identified with the help of screening of the embryonically lethal mutant. *ISE1* and *ISE2* have been cloned and mapped (Kobayashi et al. 2007). Additionally, *ise1* and *ise2* embryos also consist of increased numbers of plasmodesmata with multiple branches to increased plasmodesmal trafficking (Burch-Smith and Zambryski 2010). Hence, due to defective chloroplasts, there are overlapping plasmodesmatal phenotypes of the *ise1* and *ise2* mutants (Bobik and Burch-Smith 2015).

7.4.3 ROS in Stomatal Closure and Plant Immunity

For the regulation of the closure of stomata, reactive oxygen species (ROS) acts as an important signal (Murata et al. 2015). Organisms, which are aerobic in nature, possess ROS ($H_2O_{2,}$ $HO^{\cdot}$, $O^{\cdot -}{}_2$ and $^1O_2$) as metabolites. Firstly for the regulation of stomatal closure, ROS is generated in apoplast of guard cells, and after this, sensing and signaling cause activation of anion channels (Sierla et al. 2016). NADPH oxidase of plasma membrane, which is also known as respiratory burst oxidase homologs [RBOHs], is considered ROS production in apoplast of plants and is known for stress-induced response developmental control (Sierla et al. 2013). NADPH oxidases are present all over and are evolutionarily conserved in nature (Sierla et al. 2016).

Molecules transported to intercellular space with the help of microcapillary, which are inserted to stroma nanoinfusion (Guzel Deger et al. 2015). Rapid stomatal closure is facilitated by nanoinfusion of flg22 and ABA (Guzel Deger et al. 2015). There is further a detailed study that is required for the role of RBOH generated ROS in the closure of stomata (Sierla et al. 2016). In the apoplast, amine oxidases and peroxidases helped in the production of ROS, aside from RBOHs (Sierla et al. 2016).

In guard cells, apoplastic signaling of ROS create complexity by both peroxidases and amine oxidases (Wang et al. 2012). Further studies are required for vital role of enzymes, their molecular identity, and functions, which generate ROS in stomatal movement (Sierla et al. 2016). Stomatal closure is done by accumulation of ROS in chloroplast, further ABA treatment, ozone, extracellular Ca^{2+} (Wang et al. 2012), and also by some other external stimulus. These discoveries demonstrate the chief role of ROS accumulation in chloroplast for stomatal movement (Sierla et al. 2013).

ROS accumulation gets in the guard cell vicinity of chloroplast by the help of ABA due to which in adjacent cells ROS signaling is increased (Zhang et al. 2001). An NADPH oxidase inhibitor diphenyleneiodonium is inhibiting the accumulation of ROS in chloroplast partially but not completely (Sierla et al. 2016). The late ROS peak was noticeably reduced in the double mutant of *atrbohD and atrbohF;* the late ROS peak is decreased, which demonstrates that the ROS derived by RBOH is engaged in initiating cytoplasmic or chloroplastic accumulation during treatment of O_3 (Joo et al. 2005; Vahisalu et al. 2010). These data depict a relation between the production of ROS by chloroplast and apoplast and describe a signaling in guard cell of chloroplast (Noctor et al. 2016).

7.5 ROS in Plant Development

Plants throughout their life cycle are subjected to different environmental stresses. In managing normal plant growth and improving their stress tolerance, reactive oxygen species (ROS) play crucial roles (Huang et al. 2019a). Having aerobic conditions for any organism, there is a chance to utilize oxygen as an electron acceptor and trapping their reacting quality for signaling and metabolism (Foyer and Noctor 2016). From seed germination to plant senescence, ROS are either produced or removed, due to which plants control their development to their adaptation in different environments (Huang et al. 2019a).

7.5.1 The Maintenance of Plant Vegetative Apical Meristems Engaged by ROS

ROS homeostasis shapes plant vegetative apex development indicated by emerging evidence. $O_2^{\cdot -}$ is required for cell divisions; it is mainly accumulated in the *Arabidopsis thaliana* in meristematic tissue of the root, and accumulation site of H_2O_2 is mainly the elongation zone, which is the confirmation of cell differentiation (Tsukagoshi et al. 2010). These two, i.e., meristematic zone and elongation zone, are called ROS microenvironment, and they are very crucial for distribution of transition zone. Cell of transition zone can be divided due to having gradient of ROS. Level of $O^{\cdot -}{}_2$ is decreased, and the level of H_2O_2 starts to increase the cells being elongating and stop dividing (Dunand et al. 2007). The balance of ROS is very essential for the transition zone. And this balance is provided by a transcription factor UPB1 (UPBEAT1). H_2O_2 itself also affects *the* expression of *UPB1*, and this system of regulation contained a feedback loop, which plays a role in both for ROS homeostasis and for root growth showed by further studies (Tsukagoshi et al. 2010). Additionally, distal stem cell (DSC) and the quiescent center (QC) are needed for root apical meristem (RAM) size maintenance (Huang et al. 2019b). The root stem cell niche (SCN) identity is affected by *Arabidopsis thaliana P-loop NTPase1* (*APP1)* via its control of local ROS homeostasis. Reduction in ROS levels accompanied disruption of *APP1*, which is an ultimate reason for increase in the rate of cell division at the point of quiescent center and root DSC differentiation (Yu et al. 2016). Plant root primary growth is regulated by ROS combined with hormones and some other signal molecules. ROS and auxin signaling acted antagonistically for the purpose of balancing root meristem growth in the RAM (Tognetti et al. 2017). For controlling the regulation of cellular ROS pathway, autophagy is a necessary mechanism and assisting the degradation of the oxidatively damaged peroxisomes is proposed by the findings. Brassinosteroids (BRs) also regulate root tip stem cell activity through ROS, which is shown in current studies (Huang et al. 2019b).

Due to binding of BR to its receptor kinase BRI1 (BRASSINOSTEROID INSENSITIVE1), the levels of H_2O_2 in cell are increased and the enhanced level of peroxide to altered the vital transcription factors in signaling of BR. The oxidative modification is responsible for increased transcriptional activity of BZR1; root meristem development is enhanced by increasing its interaction with ARF6 (AUXIN RESPONSE FACTOR6) and PIF4 (PHYTOCHROME INTERACTING FACTOR4) (Tian et al. 2018). There are limited details about the relationship between cytokinin and ROS in the apex growth regulations. Hormonal network and the ROS are interconnected and not to be considered as independent mechanism; they together activate physiological and stress adaptation response. In the *Arabidopsis* RAM, glutathione reductase (GR) plays a key role for the regulation of the levels of reduced glutathione in the *Arabidopsis* RAM (Huang et al. 2019a).

Oxidized glutathione enormous accumulation in *GR2* (*glutathione reductase2*) mutants leads to root apical cells entering the oxidized state and eventually results in abnormal growth. After applying glutathione exogenously, the normal phenotype is restored partially (Yu et al. 2013). For different *Arabidopsis* ecotype, a novel thioredoxin DCC1 has signify the shoot regeneration ability (Kka et al. 2018). Bust of *DCC1* activated the formation of mitochondrial ROS. Shoot regeneration further regulates by the process. Simultaneously, in the *DCC1*gene sequence, for the purpose of bud regeneration in different ecotype of *Arabidopsis*, there are about six different SNPs (single-nucleotide polymorphism) found, and the level of ROS is different in ecotypes harboring different SNPs (Zhang et al. 2018). ROS homeostasis acts crucially in various processes apical meristem maintenance, shoot initiation, etc. (Huang et al. 2019a).

7.5.2 Organ Morphogenesis Triggers by ROS in Plants

In all plant tissue, metabolically active ROS is found as a signaling component (Ishibashi et al. 2015). In rice, OsLEA5, the late embryogenesis protein, which is present, abundantly interacted with transcription factor ZFP and regulated APX *OsAPX1* gene expression for ABA-inhibited germination coregulation (Huang et al. 2018). A biosynthetic activity of phenylalanine of AROGENATE DEHYDRATASE3 (ADT3) plays a crucial role in cotyledon development and coordinating ROS homeostasis in etiolated seedlings of *Arabidopsis*. From heterotrophy to autotrophy, a crucial role is played by Phe at the time of the transition phase of seedlings by protecting the cells from damage of ROS (Para et al. 2016).

For the development of crown roots in case of rice, ROS also play an essential role. WOX11 is a transcription factor needed for crown root development, which is a WUSCHEL-related homeobox gene (Jiang et al. 2017). Under flooding conditions, ethylene is accumulated in crown roots of rice, and this is helped in the generation of ROS. With the help of other signals, ROS increased elongation of crown cells, and this leads to death of epidermal cells (Steffens et al. 2012). Salicylic acid prohibited the expression of genes related to the scavenging of ROS. In case of mutant *ABNORMAL INFLORESCENCE MERISTEM,* crown root synthesis is inhibited due to the decreased level of ROS, which helped in the synthesis of salicylic acid. Root development is again restart after using H_2O_2 externally (Xu et al. 2017).

7.6 Catalases of Plants Targeted on Nitric Oxide and Hydrogen Sulfide

The catalase system is the oldest known and first discovered antioxidant enzyme because it may be a main member of cell metabolism in maximum of the aerobic beings (Góth 2018). For example, it's been proved that human catalase displays 245 single-nucleotide polymorphisms, which are involved in diverse physiological and pathological situations, including hypertension, DM, insulin resistance, dyslipidemia, asthma, bone metabolism, or vitiligo (Kodydková et al. 2014). Besides these genetic factors, CAT activity could also be suffering from age, physical activity, and differences due to the season and certain chemical compounds (Palma et al. 2020). Additionally, catalase was found to manage lipid metabolism in liver without compromising the general oxidative damage of cells (Pérez-Estrada et al. 2019), and therefore, the modulation of its expression in cancer cells seems to be a technique to be potentiated for chemotherapy purposes (Palma et al. 2020).

7.7 Metabolomic-Guided Elucidation od Abiotic Stress Tolerance by Plants

Plants are unable to flee from unfavorable environmental conditions, e.g., biotic and abiotic stresses; thus, their responses are manifested through physiological and metabolomic changes (Maritim et al. 2015). Salt and water stresses are the main abiotic environmental conditions that reduce plant growth and end in significant yield losses (Llanes et al. 2018). Although plants have a good spectrum of mechanisms to adapt to adverse environmental conditions, the present understanding of mechanisms related to the power of plants to take care of their growth under abiotic stresses are poorly understood. All chemical species having molecular weight less than 1800 Da is known as metabolome, and their study is metabolomics (Hall 2018).

Therefore, the metabolites are the top products of cellular functions, and their levels are often considered because the plant responses to environmental or genetic manipulation (Llanes et al. 2018). In plants, metabolomic studies aim to spot and quantify the set of primary and secondary metabolites involved in biological processes. Plant primary metabolites are implicated within the normal plant growth, development, and reproduction, whereas the secondary metabolites are crucial to plant survival under unfavorable conditions by maintaining a fine-tuning with the environment (Llanes et al. 2018). Secondary metabolites vary from species to species, place to place, and even season to season, but essentially primary metabolites are highly conserved in their structures and abundances across the *Plantae* (Scossa et al. 2016).

The diversity of plant metabolites and their complicated regulatory mechanism highlights the necessity to investigate the biochemical nature of these compounds. Plant metabolome reported so far consists of roughly 30,000 endogenous metabolites that mainly comprise carbohydrates, amino acids, organic acids, and lipids (Llanes et al. 2018). Also, small molecules like plant hormones and signaling molecules are vital for plant growth and development. Plant metabolomic research depends largely on its methodologies and instrumentation to comprehensively identify, quantify, and localize every metabolite.

Thus, several strategies for the analysis of metabolites are being developed rapidly (Hegeman 2010): (1) metabolite profiling, identification and quantification of variety of predefined metabolites, which are related to a specific metabolic pathway(s); (2) metabolic fingerprinting, global screening of samples to discriminate among samples of different biological status or origin; (3) metabolite target analysis, qualitative and quantitative analysis of one or a couple of metabolites associated with a selected metabolic reaction; and (4) metabonomics, analysis of tissues and biological fluids for changes in endogenous metabolite contents resulting from disease or therapeutic treatments (Llanes et al. 2018).

Plant metabolism is notably perturbed under abiotic stress conditions. In the last years, metabolomics has been employed for the identification of putative metabolites responsible for phenotypes tolerant/sensitive to several environmental stressors. In general, the metabolic changes that are observed in plants subjected to worry may have different causes; thus, they differ in their significance and are expected to differently correlate with tolerance/sensitivity phenotypes. The main goal of studying metabolic changes during stress responses is to identify metabolites that allow the reestablishment of homeostasis and normal metabolic fluxes and to detect the accumulation of groups of compounds involved in mediating the strain tolerance (Llanes et al. 2018).

A set of primary metabolites (osmolytes and osmoprotectants) and secondary metabolites (defense metabolites) accumulate to strengthen plant stress tolerance. Among them, primary metabolites are the foremost important metabolites suffering from stress, usually as a result of impairment in CO_2 assimilation (Llanes et al. 2018). Although an increased accumulation of osmolytes by plants exposed to abiotic stresses has been reported, not all plant species synthesize all types of osmolytes; some species synthesize and accumulate very low quantities of a number of these compounds, whereas some others don’t do so in the least (Llanes et al. 2018).

7.8 Conclusions

Reactive oxygen species (ROS) can synthesize intracellular and extracellular locations. ROS can cause extensive damage to the integrity of the cell that causes death. To overcome such a situation, plants can be equipped with a wider range of defense measures including the morphological change in plants and metabolic and genetic level changes for the adaptation of nonfavorable environmental conditions. ROS has short half-life and high reactivity, which is very important for our understanding about the formation of ROS. Interaction between ROS and calcium signaling during multiple environmental stresses is still unanswered. Recent works reported many sources for the production and removal of ROS, different types of enzymes, and antioxidant molecules for the signaling to ROS. But still, there are many points, which are not disclosed about ROS, like how various ROS cause signaling in spite of having very short half-life and susceptible nature to many chemicals. Also, production of various ROS and their interaction with each other cannot be clearly understood. Further, we need some more work in order to understand complete mechanism and hope for the best.

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Molecular Mechanisms of Superoxide Dismutase (SODs)-Mediated Defense in Controlling Oxidative Stress in Plants

8

Raufa Batool, Muhammad Jawad Umer, Babar Hussain, Muhammad Anees, and Zhenying Wang

Abstract

Superoxide dismutases (SODs) are pervasive metalloenzymes that comprise the very first line of defense versus reactive oxygen species (ROS). It constitutes one of the most important enzymatic parts of detoxification of superoxide radicals that are produced in biological systems through catalyzing its dismutation to H_2O_2 and eventually to H_2O as well as O_2 depending on the catalase and peroxidase. In general, plant species includes several SOD isoforms varying in their active site metal ions, specifically Cu/Zn-SOD, Mn-SOD, and Fe-SOD. Numerous studies also stated that the tolerance levels of plants are positively associated with SOD activity at the same time as well as along with the number of SOD isoforms and founded the fact that "the greater the SOD activity, the greater the stress tolerance." Hence, the SOD isozyme profile of any plant could be used as a balanced

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marker used for stress tolerance in plants. Throughout this chapter, we have talked about the title role of SOD in abiotic and biotic stress tolerance, kinds of SODs, and the correlation of its activity and its isoforms along with stress tolerance level.

Keywords

Superoxide dismutase · H_2O_2 · ROS · Stress tolerance

8.1 Introduction

Reactive oxygen species (ROS) are produced as by-product in response to stresses both biotic and abiotic. ROS causes serious damages at DNA, protein, and lipid level leading to sever injuries in plants (Apel and Hirt 2004). Production of ROS leads to the activation of plant defense responses by modifying cell wall, phytoalexins, and proteins that are linked to pathogenesis (Segal and Wilson 2018). Moreover, hypersensitive response (HR) is also activated due to ROS production, and it is a defensive strategy to counter the injury or damage (Lamb and Dixon 1997). Plants respond to various stresses to scrub the reactive oxygen species by producing enzymatic and nonenzymatic molecules including catalases, peroxidases, and superoxide dismutases (SODs) (Broxton and Culotta 2016) (Fig. 8.1).

One of the most critical enzyme is superoxide dismutase which acts as a first line of cellular defense by actively participating in ROS homeostasis, thus converting superoxide anion ($O_2^{\cdot -}$) to H_2O_2 (Apel and Hirt 2004). SODs are further divided in three types on the basis of the metal cofactor they had, i.e., (1) manganese (Mn)-SOD located in mitochondria and the peroxisomes, (2) iron (Fe)-SOD (found in chloroplast), and (3) copper (Cu)/zinc (Zn)-SOD (located in cytosol, chloroplasts, and the peroxisomes) (Li et al. 2017).

Cu/Zn SOD having of two subunits, Cu and Zn, plays a crucial role in increasing the enzyme's activity and stability (Lin et al. 1995). Ratios of O_2^- to H_2O_2 can be altered by SODs (Chabory et al. 2010). H_2O_2 also has a potential to modulate the expressions of genes including TFs, anti-oxidative genes, and some genes linked to stress.

It has been proved that SODs are involved in maintaining the ROS homeostasis at intracellular levels. Overexpressing "MeCu/Zn SOD" and "MeCAT1" in *Manihot esculenta* resulted in an enhanced tolerance and ROS scavenging against various stresses including cold, drought, and oxidative stress (Xu et al. 2013). Similar studies have been reported in yeast and *Arabidopsis* by overexpressing "PutCu/Zn SOD" (Wu et al. 2016). In *Saussurea involucrata*, a gene, namely, "SiCSD," belonging to "Cu/Zn SOD," was overexpressed in tobacco, thus increasing tolerance to drought, cold, and other oxidative stresses (Li et al. 2017) (Fig. 8.2).

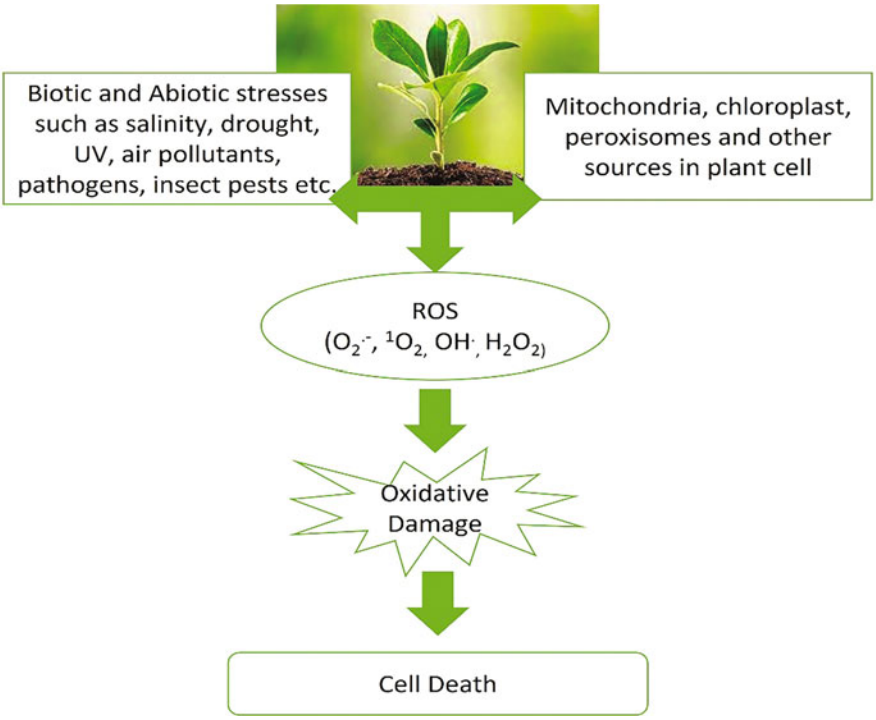

Fig. 8.1 The hypersensitive response (HR), an extreme measure to limit pathogen spread

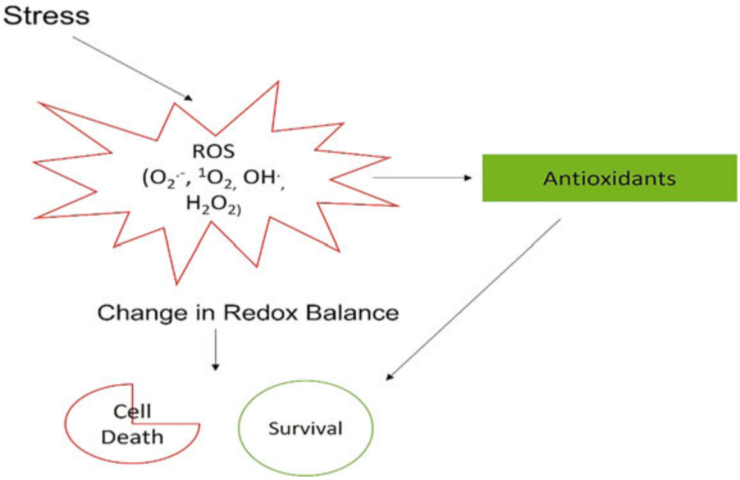

Fig. 8.2 Changes in redox balancing in response to stress

8.2 SOD-Mediated Mechanism in Plants Against Oxidative Stress

Life of aerobic organisms can be affected by the oxidative stress imposed by the oxygen reactions. Generally, these oxygen reactions occurred due to the presence of radicals of superoxide (SO) and peroxides (PO) which are nonreactive, but they can attach with other radicals and lead to destruction at cellular levels. Hydroxyl radicals are known as the top reactive radicals known so far and are produced from SO and HPO during Haber-Weiss reaction in the presence of metallic ions (Cadenas 1989). Hydroxyl radicals can cause DNA mutations, proteins denaturation, and peroxidation of lipids. Peroxidation phenomenon of in lipids is considered as an indication of oxidative stress (Gutteridge and Halliwell 1990). However, $O_{1/2}$ produced as a result of energy transfer to oxygen can be destructive (Knox and Dodge 1985) (Fig. 8.3).

According to Salin (1988), cellular reactions lead to the production of radicals of superoxide, along with H_2O_2 and oxygen in singlet form. It has been reported that superoxide radicals have a definite production area called as mitochondrial ETC. But in the chloroplasts, the production of singlet oxygen occurs during the process of energy transfer from the chlorophyll to the oxygen. Plants have developed certain strategies to cope with these free radicals and keep the damage to a minimum. Plants do this by not allowing the radicals to interact as it is understood that hydroxyl radicals are the most reactive ones if they combine with the source of their meaning. Mechanisms including both enzymatic and nonenzymatic sources are involved in the defense and elimination of free radicals to avoid damages at cellular levels. Bowler et al. (1992) had discovered SOD (EC 1.15.1.1), and when these SODs come in contact with the superoxide radicals, it leads to the production of H_2O_2. SOD is considered unique in a way that its activities determines the contents of O^{2-} and

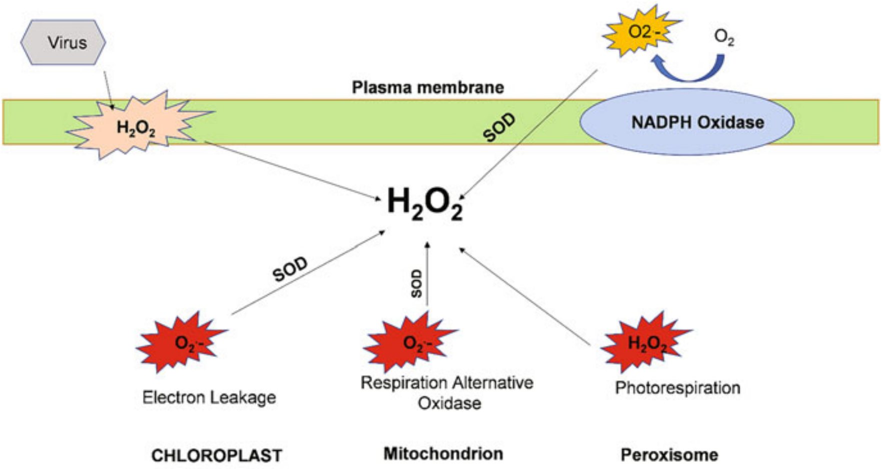

Fig. 8.3 Superoxide and hydrogen peroxide can react in a Haber-Weiss reaction to form hydroxyl radicals

H_2O_2, substrates of the Haber-Weiss reaction and, thus, considered central in defensive mechanisms.

8.3 Different Types of SODs and Their Localization

On the basis of metal cofactors such as Cu/Zn, Fe, and Mn presence, SODs have been categorized into three types, i.e., “Cu/ZnSOD,” “FeSOD,” and “MnSOD.” All these have different levels of sensitivities to the inhibitors such as KCN and H_2O_2 (Han et al. 2020). Of the three types, only MnSOD has resistance against KCN and H_2O_2. Cu/ZnSOD is sensitive to KCN and H_2O_2, whereas FeSOD has sensitivity against H_2O_2. FeSOD and MnSOD share the same structural similarity and are different from that of Cu/ZnSOD (Guleria et al. 2021). On the basis of phylogenetic studies, it has been proved that SODs with Mn and Fe metal cofactors evolved long ago even before the divergence of pro- and eukaryotes and are thus considered ancient as compared to Cu/ZnSOD which are found to evolve at the eukaryote lineage beginning. All the SODs are found to be located inside the nucleus, and they are transported to the designated locations via NH^2-terminal target sequences (Han et al. 2020). MnSOD are localized in mitochondria and Cu/ZnSOD in cytosol, and FeSOD are present in the stroma of the chloroplasts (Bafana et al. 2011) (Fig. 8.4).

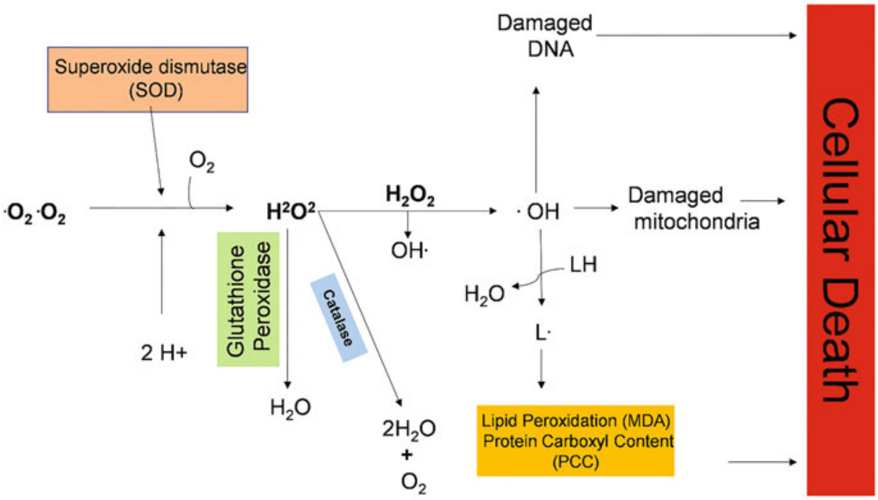

Fig. 8.4 Role of SOD in oxidative stress and antioxidant response

8.4 Response of SOD to Environmental Challenge-Induced Oxidative Stress

It has been well established that green leaves possess chloroplastic SOD in abundance, whereas cytoplasmic SODs and those in mitochondria are more in the germinating seedlings (Song et al. 2020). The activities of different enzymes are metal cofactor-dependent which means that variations do occur in the expressions in the presence or absence of metal cofactors. During normal growth, the SOD activities does not change much (Rahman 2007). Chloroplastic SODs become more abundant and active during the process of photosynthesis.

Under oxidative stress, plants become vulnerable, and phenotypic changes can be observed, and the activities of other reactive oxygen scavenging agents including catalases and glutathione reductases (GTHs) along with SODs decrease (Ighodaro and Akinloye 2018). Production of oxygen radicals results in the process of lipid breakdown by enzymes, namely, lipoxygenases in the effected plant tissue. Hence, the generation of H_2O_2 also promotes senescence as explained in rice plants by Bowler et al. (1992), thus supporting the concept that free radicals are involved in playing key roles in both senescence and the ageing (Song et al. 2020). Genes linked to SOD are sensitive and being regulated by the environmental influences possibly due to the formations of oxygen radicals (Fig. 8.5).

8.4.1 Photoinhibition

The production of hydrogen peroxide (H_2O_2) by illuminated chloroplasts was first studied by Mehler (1951). Subsequently it has been demonstrated that almost all of

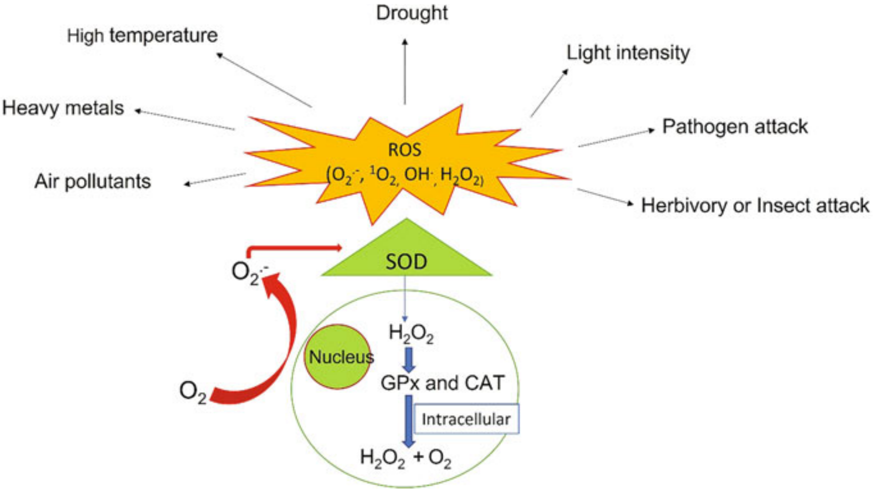

Fig. 8.5 Response of SOD in environmental stresses-induced oxidative damage

this hydrogen peroxide is originated from superoxide formed by the univalent transfer of electrons to oxygen from the electron acceptor of photosystem I and mainly from ferredoxin (Khorobrykh et al. 2020). Since ferredoxin generally passes its electrons to NADP (via ferrodoxinNADP reductase), the amount of superoxide made in this side reaction is directed by the amount of NADP available, which in turn rely on the activity of the Calvin cycle and the availability of CO_2. Hereafter, the extent of potential oxidant stress is reliant on the photosynthetic activity of the chloroplast. In general, high photosynthetic activity boosts the production of superoxide radicals and disorders the normal photosynthetic reactions. Such correlations are imitated in the behavior of chloroplast SOD. mRNA levels of the FeSOD of *Nicotiana plumbaginifolia* were not significantly affected by daily fluctuations of light and dark. Still, when plants were kept in the dark for three days before illumination, FeSOD mRNA levels increased dramatically in response to light (Triantaphylides et al. 2008). This initiation was not facilitated by phytochrome and could be reduced by adding 3-(3,4-dichlorophenyl)-I, 1′ -dimethylurea (DCMU), an herbicide that blocks electron transport in photosystem II, thereby blocking superoxide production from photosystem I. These results revealed that the chloroplastic SOD reacts not directly to light but boosts the superoxide formation arising from the inadequate transfer of electrons via the photosystems due to inadequate photosynthetic apparatus maintenance during the prolonged dark period. In addition to superoxide and hydrogen peroxide (and therefore the potential to form hydroxyl radicals), illuminated chloroplasts can produce singlet oxygen by transferring excitation energy from chlorophyll to oxygen. Carotenoids can enrich this problem because they can react with singlet oxygen at diffusion-limited rates and reduce the excited triplet states of chlorophyll that lead to singlet oxygen formation (Asada 2006).

During normal conditions, chloroplasts interactions normalized the occurrence of disorder and damage caused by photosynthetic energy transfer misuse. Thylakoid membranes are rich in antioxidants such as a-tocopherol and carotenoids, and available SOD, ascorbate peroxidase, provides an efficient enzymatic means for removing superoxide and H_2O_2. In addition, the increasing light intensity can upregulate the level of these antioxidants (Khorobrykh et al. 2020). However, because of the photosynthetic machinery’s continuous absorption of light energy, any distress of electron transport can lead to the donation of electrons to the wrong electron acceptor. This disruption caused the generation of reactive oxygen species. Such conditions only arose during the attack of herbicides, the herbicides that interfere with electron transport or CO_2 fixation and during conditions of photoinhibition, in which the absorbed light energy exceeds the capacity of the photosystems to direct it through photosynthetic electron transport. The conditions have possibly occurred in the presence of high light intensity and during high or low temperature (chilling or heat) accompany illumination. When plants are exposed to high light intensity sunlight, the photosynthetic capacity is disrupted, leading to the redirection of photon energy into processes that inhibit photosynthetic capacity. Maintained long enough, this condition leads to the destruction of photosynthetic pigments (commonly referred to as photooxidation). While this pigment bleaching is dependent upon oxygen and light and appears to be mediated to some extent by reactive oxyradicals, the reduced photosynthesis that precedes, it can occur largely in the absence of oxygen, hence questioning any involvement of active oxygen species (Foyer 2018). Under such conditions, photosystem II is the primary site of damage most likely because of the destruction of the 32-kDa QB polypeptide within the reaction center. Among other things, oxygen radicals have been implicated in this phenomenon (Halliwell and Gutteridge 2015). Photosystem I is usually less prone to damages, but its appearance is relayed upon electron passes from photosystem II and in the presence of oxygen. The results revealed that the most damage in photosystem I is mainly directed by the formation of superoxide from oxygen and or ferredoxin initiation in photosystem I. The iron-sulfur centers of photosystem I appear to be damage sites (Khorobrykh et al. 2020).

Further evidence for these oxygen-dependent and oxygen-independent events has been obtained from experiments with isolated spinach chloroplasts or thylakoids, which showed that adding SOD or catalase could only provide partial protection against photoinhibitory conditions (Wild et al. 1990). Nonetheless, a biotype of *Conyza bonariensis* possessing increase levels of chloroplastic SOD, glutathione reductase, as well as ascorbate peroxidase was reported to be resistant to photoinhibitory light. Injury resulting from the combination of light with cold temperatures appears to bear some similarity to that described above; but in addition, the peroxidation of membrane lipids is more noticeable. Indeed, the extent of this membrane damage may well govern chilling sensitivity because in *Anacystis nidulans*, blue-green alga genetic manipulation of fatty acid desaturation alone can result in alterations of chilling susceptibility (Wild et al. 1990). The effects of chilling are greatly directed by light, and reactive oxygen species have been concerned with the destruction of lipids and photosynthetic pigments that occurs (Wise and Naylor 1987). Consistent with the observations that oxygen radicals play some role in the cellular damage occurring due to photoinhibition, some reports document changes in SOD activity. In *A. nidulans*, the onset of death by photoinhibition was more apparent when cellular SOD activity had been decreased by prior incubation in an atmosphere of nitrogen (Abeliovich et al. 1974). In another blue-green alga, *Plectonema boryanum*, a mutant was isolated that was resistant to photooxidation (Steinitz et al. 1979). During exposure to photooxidative conditions, the SOD activity remained constant in the resistant mutant but dropped more than ten times in the sensitive parent strain. The maintenance of SOD activity was due to an increased synthesis of the thylakoid membrane-bound MnSOD and not to the soluble FeSOD. It was proposed that the increased synthesis of a hydrogen peroxide-insensitive SOD (the MnSOD) was important for maintaining high SOD activity during light-mediated stress because the H_2O_2 generated during photosynthesis may inactivate the sensitive FeSOD enzyme. In higher plants, the resistance against cold stress is considered to some extent; The ability to chilling-mediated photoinhibition may be due to the adaptation of the photosystems (Somersalo and Krause 1989). The process is dependable on the increased SOD activity and, in turn, increases in the levels of enzymes of the Halliwell-Asada pathway that acts as scavengers against H_2O_2 in chloroplasts cells (Schöner and Krause 1990). A new

SOD enzyme was identified in protein extracts from cold-hardened plants. It shows similar behaviors as a Cu/ZnSOD does in inhibitor tests with KCN and H_20_2, but its cellular location was not identified yet.

Furthermore, the high ratio of carotenoids as chlorophyll also boosts the means of scavenging reactive oxygen species. Research on chilling effects in chilling-sensitive tomato plants reveals that chloroplastic SOD was irreversibly inactivated, perhaps due to increased H_2O_2 concentration. This inactivation was proposed as the reason for the enhanced lipid peroxidation. Together with those obtained from lower photosynthetic organisms, these results suggest that a combination of chilling with light leads to increased H_20_2 formation that may eventually inactivate the chloroplastic SOD enzymes (Cu/ZnSOD and FeSOD).

Sunscald is a phenomenon related to photoinhibition caused by a combination of light and heat. It can severely affect the marketability of many kinds of fruits, flowers, and vegetables grown in warm climates. Oxygen radicals appear to be responsible for the damage done to photosystems and membranes (Steinberg and Rabinowitch 1991). The potency of some fruits, e.g., tomato, cucumber, and pepper fruit, to counter the effect of sunscald is correlated with the levels of carotenoids and with SOD but not peroxidase activity (Nahar et al. 2015). Furthermore, an artificial tolerance caused by experimentation in green tomatoes by way of monitored heat treatment parallels rises in SOD activity (Dias and Ortiz 2012). Whether or not these alterations in SOD activity have been due to alterations in chloroplastic, cytosolic, or mitochondrial SOD was not tested. Though *N. plumbaginifolia* leaves, which were exposed to heat shock, it was observed that there was an increase in the cytosolic Cu/ZnSOD mRNA levels, but not in the case of MnSOD and FeSOD. This production occurred freely of light (Tsang et al. 1991). Another situation was observed by exposing the plants to cold stress in combination to light. Here, the expressions of chloroplastic FeSOD mRNA were increased (Tsang et al. 1991). Still, if the plants were later sent back to optimum temperatures, MnSOD as well as cytosolic Cu/ZnSOD mRNA levels were detected. These various forms of SOD initiation facilitated by cold or heat shock in the light could therefore propose that the processes of photoinhibition are different in every case.

8.4.2 Paraquat and Other Herbicides

Any disruption in the photosynthetic activities results in the production of ROS, from photosystem I, ferredoxin, as well as excited chlorophyll. So, herbicides that have a direct impact on chloroplast activity may stimulate processes which generate destructive oxygen species. Herbicides which block photosynthetic electron transportation, like monuron, ioxynil, and atrazine, permit the excitation energy to be moved from chlorophyll to carotenoids, which is going to be damaged gradually as a result. When they are damaged, the light energy can be transferred to oxygen, producing singlet oxygen as well as other species that may be able to initiate lipid peroxidation (Aurand et al. 1977). Likewise, herbicides that are acting by limiting carotenoid synthesis, like “aminotriazole,” “metflurazone,” “fluridone,” "norflurazon," and "pyrichlor," remove a very important quencher of excitation energy, hence potentiating the creation of singlet oxygen along with other reactive oxygen species (Gill and Tuteja 2010).

Redox-active herbicides including acifluorfen, a diphenyl ether, may also be able to act through the creation of reactive oxygen. These compounds can accumulate photodynamic tetrapyrroles which can generate reactive oxygen species (Galvez-Valdivieso and Mullineaux 2010). Treating the cotyledons of cucumber with acifluorfen leads to a decrease in the contents of ascorbate as well as glutathione, along with enzymes in Halliwell-Asada pathway, catalase, and peroxidase, complemented by lipid peroxidation (Sade et al. 2011). On the other hand, though, (Schmidt and Kunert 1986) discovered that ascorbate, glutathione, as well as the glutathione reductase activity improved. Such differences might be due to the moment at which the events have been investigated. In the first case, the material could have been in the throes of death, and in the end, the plant might have been aggressively protecting itself against an herbicide. In a different report, protection from the damage caused by acifluorfen might be obtained through pre-treatment by a-tocopherol (Ensminger et al. 1985). Bipyridyl herbicides including paraquat as well as diquat enhance the oxidative stress directly through producing oxygen radicals. Of these two, paraquat has proved to be the most widely studied; both seem to have mediated the exact same effects. Also called methyl viologen (1,1-′-dimethyl-4,4′-bipyridinium chloride) paraquat which is a redox-active compound photoreduced by means of photosystem I subsequently reoxidized through the transfer of its electrons to oxygen, producing the superoxide anion (Bowler et al. 1992). This leads to the production of extremely damaging hydroxyl radicals as well as related species, thus causing the cellular damage, as paraquat can get electrons multiple sources specifically NADPH-dependent diaphorases so it is also damaging to non-photosynthetic organisms (Bowler et al. 1992) as well as from a NADPH cytochrome P-4S0 reductase present in the microsome. Such a mechanism can almost certainly happen in plants, too, though the elevated rates of electron transfer via the photosystems throughout illumination guarantee that photosystem I is the principal donor and that the consequences of paraquat under the light are significant than in the dark. Due to its indiscriminate toxicity, paraquat is currently prohibited as a herbicide in most nations. Nevertheless, the manner in which it generates superoxide radicals has resulted in its experimental use up for the study of oxygen toxicity in various organisms. In well-lit plants, paraquat triggers a rapid suppression of carbon dioxide uptake, subsequently lipid peroxidation, the termination of photosynthetic electron transport, and the collapse of chlorophyll (Varsamis 2008). SOD has been frequently correlated together with the mechanism of paraquat survival. In E. coli that contains both MnSOD as well as FeSOD, just the ex- enzyme is stimulated by paraquat (Hassan 1988). Paraquat actually induces for approximately 40 proteins in the E. coli, the vast majority of which have not yet been identified, involving antioxidant and the repair enzymes. Some of the following are positively regulated on the transcriptional levels by means of a gene product of the soxR locus (Greenberg et al. 1990). The significance of SOD in paraquat survival has been demonstrated by the isolation of SOD-deficient mutant which is hypersensitive to paraquat. Once the green alga *Chlorella sorokiniana* is cultivated in sublethal concentrations of paraquat, SOD activity boosts due to the synthesis of another new MnSOD isozyme. This stimulated MnSOD activity, all together with other protective enzymes, gives resistance to the higher doses of the herbicide. Likewise, treatment of *Spirodelu oligorrhiza* with benzyl viologen, which is a less reactive bipyridinium compound, increased SOD activity marginally, which might have been a factor in the plant's later resistance to paraquat (Jansen et al. 1996). *Dunaliella salina* reacts to paraquat by means of a more general initiation of SOD as well as catalase isozymes (Bowler et al. 1992).

Several cases have been studied on effects of paraquat on the endogenous SOD enzymes in illuminated plants. The treatment of *Phaseolus vulgaris* and Lemna plant leaves affects a general level of SOD activity (Radić et al. 2010; Taïbi et al. 2016). A study of the expression of tomato's cytosolic and chloroplastic Cu/ZnSODs showed that mRNAs for both were induced by paraquat. However, the former enzyme was the most affected strongly. In *N. plumbaginifolia*, chloroplastic, cytosolic, and mitochondrial SOD expression was analyzed at the mRNA level, and all three were strongly induced by paraquat, but in this case, the cytosolic Cu/ZnSOD was the least affected (Inzé and Van Montagu 1995). These differences are probably caused by the different light intensities used in each experiment because this can greatly affect the sites from which electrons are donated to paraquat. In this context, although (Azevedo et al. 1998) observed induction of all SODs in the light, paraquat treatment in darkness led only to induction of cytosolic Cu/ZnSOD expression. In maize, however, the chloroplastic Cui ZnSOD was induced in addition to the cytosolic Cu/ZnSOD by dark incubation with paraquat (Bowler et al. 1992), suggesting that there may also be differences between plant species. Following the intensive use of paraquat in certain world areas, several paraquat-resistant weeds evolved (Peterson et al. 2018). Biochemical analysis of these has allowed further insights into how plants protect themselves against oxidative stress. (Harper and Harvey 1978) analyzed SOD, catalase, and peroxidase activities in four paraquat-tolerant and eleven paraquat susceptible cultivars of perennial ryegrass (*Lolium perenne*). They found that constitutive activities of SOD, catalase, and sometimes peroxidase were higher in all the paraquat-tolerant lines than in the susceptible lines. All of the increased SOD activity was associated with the chloroplasts.

Similarly, a resistant biotype of *Conyza bonariensis* contained constitutively high levels of chloroplastic SOD, glutathione reductase, and ascorbate peroxidase (Shaaltiel et al. 1988). This variety was also reported to possess a mechanism of sequestration that prevented paraquat from entering the chloroplasts (Ye and Gressel 2000), but these results have been questioned. Genetic analysis of the variety indicates that the three Halliwell-Asada pathway enzymes co-segregate elevated activities, implying that one dominant nuclear gene is responsible for their control (Agarwal and Khimnani 2000). In contrast, the mechanism of paraquat resistance in a variety of barley grass (*Hordeum glaucum*) is not due to increased activities of these oxygen-detoxifying enzymes. Still, it may rather be related to uptake of the herbicide into the cell (Christopher 2018).

Paraquat-resistant plant varieties have also been artificially selected under experimental conditions. Paraquat-resistant tobacco was obtained by three successive screenings on paraquat-containing media; these contained constitutively sufficient SOD but not catalase or ascorbate peroxidase activities. All of the increased activity was due to Cu/ZnSOD, as shown in many inhibitor studies. Still, it could not be inhibited by antibodies against chloroplastic Cu/ZnSOD, suggesting that it was a cytosolic isoform. Thus, in callus material, which is not actively photosynthesizing, the main site of the oxidant stress generated by paraquat may be in the cytosol. Plants regenerated from such calli remained resistant to paraquat, even though the chloroplast is likely the chief site of superoxide formation in such material. Resistant tobacco plants were also successfully regenerated from paraquat-selected callus by (Miller and Hughes 1980). These contained high levels of catalase and peroxidase but not of SOD activities (Bowler et al. 1992). The selection of paraquat tolerant mutants of the fern *Ceratopteris richardii* led to the isolation of allelic mutants with recessive nuclear mutations. Biochemical studies could identify no differences in the levels of ascorbate, glutathione, SOD, catalase, peroxidase, glutathione reductase, dehydroascorbate reductase, and ascorbate peroxidase activities in the presence or absence of paraquat (Carroll et al. 1988); the uptake of paraquat was identical to that in the wild-type strain. A mutant with a mutation that enhances paraquat tolerance and is not linked to the other locus that has subsequently been isolated (Xi et al. 2012), but its effects on oxygen detoxifying enzymes have not yet been studied.

In summary, paraquat strongly influences the expression of SOD and other oxygen-detoxifying enzymes, as would be predicted from its mode of action. However, the importance of other mechanisms is not the precluded; for example, the resistance of one *E. coli* mutant was due to a decreased uptake of paraquat (Membrillo-Hernández et al. 1999), and *E. coli* cells deficient in spermidine biosynthesis have an increased sensitivity to paraquat (Minton et al. 1990). Studies with paraquat-resistant mutants, together with the realization that more than 40 proteins are induced by paraquat in *E. coli*, demonstrate that much remains to be learned about the basis of an organism's defense against oxidative stress, even when using paraquat, the simplest model system available.

8.4.3 Waterlogging and Drought

Waterlogging causes shortage of oxygen shortage on submerged parts of plant, and plants respond to it through alteration of protein synthesizing patterns. Several plants have the ability to survive this damaging period but immediately die upon re-exposure to air, indicating recovery phase is more susceptible to oxidative damage. A significant increase in SOD activity (up to 13-fold) was observed in resistant variety in comparison to sensitive variety during this anoxic phase (Kele and Ünyayar 2004). Mostly this increase is because of Cui ZnSOD. So, the elevated SOD activity was suggested to be most important in plant protection to air-exposure-induced oxidative stress. The induction of SOD prior to oxygenation can sometimes counteract the subsequent damage. Plant response against drought stress is a complex phenomenon which likely involve polyamines synthesis and a unique set of proteins whose function is not clearly known (Salah et al. 2019). Abscisic acid that regulates closing of stomatal guard is the first in response against water loss. Drought also reduces CO_2 availability for photosynthesis, which results in production of reactive oxygen species due to misdirection of electrons in photosystems. Therefore, mechanisms of reducing oxidative stress may perform secondary role in control of drought stress. In tomato, strong induction of cytosolic Cu/ZnSOD, while unaffected chloroplastic Cu/ZnSOD, was recorded against drought stress (Perl-Treves and Galun 1991). In drought-tolerant and drought-sensitive moss varieties, *Tortula ruralis* and *Cratoneuron filicinum*, respectively, the comparison between the drought-induced changes in SOD activity and catalase and lipid peroxidation was done (Pan et al. 2006) and revealed low lipid peroxidation level in drought-tolerant moss, along with increased levels of both enzymes and the opposite response was seen in the sensitive moss. (Apel and Hirt 2004) analyzed drought tolerant and in tolerant varieties of maize and found out that resistance is correlating with Cu/ZnSOD and glutathione reductase; however, increased levels of single enzyme apparently did not confer drought tolerance.

8.4.4 Pathogen Infection

The two types of plant-pathogen interactions were observed named as incompatible and compatible. Incompatible reactions (immune host, monotonous pathogen) are characterized by the appearance of hypersensitive response, a localized plant cell necrosis at the pathogen penetration site, which is responsible for preventing further spread of disease to other plant cells, whereas in compatible interaction (potential host, viral pathogen), the plant's cell death is not observed and pathogen can easily spread to other parts of the plant. Many biochemical events in the plant partake in a hypersensitive reaction, which involves the synthesis of ethylene and phytoalexin; reinforcement of cell walls with collagen, lignin, and related compounds; accumulation of glycoproteins high in cell wall-bound hydroxyproline; and synthesis of pathogenesis related (PR) proteins, including glucanases, chitinase, peroxidase, and proteinase. These observations indicate that superoxide, hydrogen peroxide, or both are intimately involved in determining the outcome of plant-pathogen interactions (Apel and Hirt 2004). Superoxide and/or hydrogen peroxide may be actively involved in killing pathogen. In tomato roots infected with *Meloidogyne incognita*, a considerable increase in SOD activity as compared to the roots of resistant varieties was observed (Maqsood et al. 2020). Similar response was noted in incompatible reactions of pea and potato to nematodes (Kaloshian et al. 2011). Incompatible reaction between *N. plumbaginifozia* and *Pseudomonas syringae* resulted in induction of mitochondrial MnSOD, but through this induction, the increase in oxidative stress due to increase mitochondrial activity occurred during infestation is clearly reflected. SOD might be involved more directly in large fungal groups *Cercospora*-induced defenses. These fungi produce cercosporin (1, 1 2-bis (2-hydroxypropyl)-2, 1 l-dimethoxy6,7-methylenedioxy −4 0.9-dihydroxyperylene-

3,1 O-quinone), a nonspecific phytotoxin sensitized by light to produce both singlet oxygen and superoxide (Segal and Wilson 2018). Cercosporin causes light-dependent peroxidation of plant membrane lipids, presumably mediated by toxic oxygen species. Several plant varieties were proved to be resistant to this toxin, with very little data available for their resistance mechanism. However, a paraquat-resistant tobacco variety with augmented SOD level but not catalase or peroxidase (Segal and Wilson 2018) is found resistant to cercosporin toxin.

8.5 Cross-Tolerance Phenomenon

Tolerance to specific ecological stresses can clearly arise from several potential processes, each one expected to involve multifaceted impacts, and a biotype lenient to a single condition may also be tolerant for others. SOD is one element that can decide this cross-tolerance phenomenon. *Chiarella*, a unicellular green alga, was the first organism in which this phenomenon was observed. Earlier growths of *C. ellipsaidea* in deadly concentration levels of paraquat, “an MnSOD activist,” can reduce the injury caused by the chilling facilitated photoinhibition (Clare et al. 1984); on the other hand, presence of sulfite the growths of *C. sorokiniana* boosts MnSOD content as well as gives resistance against the paraquat (Rabinowitch and Fridovich 1985). These phenomena have subsequently proven to be reported for numerous plants (Hickok et al. 1987; Jansen et al. 1990; Steinman and Ely 1990). Although the levels of cross-tolerance noted might not be enough in all circumstances of agrarian significance, it may well be of practical application because it is easier to experiment with leaf discs used for paraquat tolerance than to evaluate a complicated trait, for example, the drought tolerance. These findings also disclosed that several diverse stresses generate very much the same impacts at cellular levels, one element of that is an oxidative stress. The method of this trend can involve ethylene, well-known to be generated during numerous stress circumstances. Ethylene pretreating of mung beans, for instance, bestowed the protection from a subsequent experience to ozone, then hydrogen peroxide, and the paraquat (Mehler 1951). It is fascinating that ethylene stimulates plant MnSOD (Chai and Doke 1987).

8.5.1 SOD Regulatory Mechanism

SOD activity is induced by diverse stress conditions. At first glance, it is logical to assume that certain common components of these stresses are the chief mediators of SOD gene regulation. In *N. plumbaginifolia*, mitochondrial MnSOD responds to increased oxyradical formation in the mitochondria, while chloroplastic FeSOD responds to such an event occurring in the chloroplasts (Chai and Doke 1987; Tsang et al. 1991). Cytosolic Cu/ZnSOD probably responds to cytosol-localized reactions in a similar fashion. The effect of a particular stress on SOD gene expression is thus likely to be governed by the subcellular sites at which oxidative stress is generated. Because the genes encoding the SOD enzymes are clearly not coregulated, how may such responses be mediated? The ubiquity of superoxide and hydrogen peroxide suggests that they do not themselves direct the diverse profiles of SOD gene expression. The OxyR protein of *S. typhimurium*, a transcriptional regulator of hydrogen peroxide-inducible genes, activates these genes only when it has been oxidized. The soxR gene product of *E. coli* (Tsaneva and Weiss 1990) is probably regulated in the same way. Reactive oxygen compounds have also been implicated in the regulation of transcription in eukaryotic cells; a recent report presents evidence that the regulation of the transcription factor NF-KB by a wide range of diverse agents (such as TNF-a, calcium ionophores, interleukin-l, and phorbol esters) is mediated through reactive oxygen species (Schreck et al. 1991). How the observed complexity of SOD regulation in plants could be controlled simply by the oxidation/reduction state of a single transcription factor is not clear. However, specific regulation could be achieved if the signaling factor regulating each class of SOD were generated in specific compartments. To induce expression of the required SOD, this molecule would then need to transfer quickly from the chloroplast or the mitochondrion to the nucleus because all SODs are encoded there. The required specificity could be achieved if small molecular components specific to chloroplasts, mitochondria, or cytosol could be the primary sensors and signal transducers of compartment-specific stress. Lipid-derived molecules could serve in this role. Fatty acids specific for chloroplastic, mitochondrial, or plasma membranes could be cleaved by an oxidative event, leading to the release of a hydrophilic molecule that could diffuse to the nucleus and interact with particular transcription factors to activate the gene encoding the required SOD enzyme. Such a fatty acid derivative could meet the requirements for a signaling molecule: It would be small, specific, readily modified by reactive oxygen, and diffusible. Several examples of biologically active lipids from mammalian systems exist, including the prostaglandins, leukotrienes, and lipoxins, which originate from the oxidation of fatty acid derivatives initially cleaved from membrane lipids by phospholipases or lipoxygenases (Samuelsson et al. 1987). Genes that regulate SOD expression have not yet been isolated from any eukaryotic species. In plants, these may eventually be isolated by methods requiring promoter analysis, gel shift assays, DNase 1 foot printing, and the screening of expression libraries with DNA sequence motifs known to be recognized by the factor of interest. Alternatively, plant mutants may be used to isolate regulatory genes by genetic approaches. Although several plant mutants have been described that have mutations in regulatory genes controlling SOD expression, e.g., *Conyza bonariensis* (Shaaltiel et al. 1988) and *Lolium perenne* (Harper and Harvey 1978), the lack of good genetic maps in these species make them currently worthless for isolating the regulatory genes themselves. Soybean varieties have been described to have variant patterns of SOD activities when visualized on polyacrylamide gels, although the mutations are likely to be in structural genes encoding SOD and not in regulatory genes (Griffin and Palmer 1989). In maize, which has excellent genetic systems available, similar mutations were also found in some varieties (Baum and Scandalios 1982), but in addition, one strain was found that expressed reduced levels of the three Cu/ZnSODs but normal levels of MnSOD. It was proposed that a regulatory mutation may be responsible (18). The character was inherited as a recessive trait and was probably polygenic in nature. Some of these plant varieties may be of use in biochemical approaches to study SOD regulation. For example, by studying the proteins that bind to the promoters of different SOD genes using nuclear extracts derived from mutant and wild-type varieties, it may be possible to identify particular protein factors that are present in much greater amounts in one variety or that have altered expression patterns, e.g., constitutive in the mutant and inducible in the wild type. Such approaches may lead to the identification of the most interesting factors since their mutation was clearly shown a priori to affect SOD expression.

8.6 Genetic Alteration of SODs in Plants

The initiation of SOD in reply to the various ecological conditions discussed in the preceding section implies that it plays a significant role in a plant's defensive mechanism. It might be a central element, and in this case, its genetic modification could lead to stress-tolerant phenotypic characteristics. Instead, the information collected may merely illustrate the pervasiveness of oxidative stress in the plant processes. In this situation, changes in the SOD activities which are stemming from excessive production (or shortages) of SOD might like to disturb the typically augmented processes. Though likely to result in a clearer understanding of aerobic toxicity, such distress would likely be of not much use for agriculture. It has been proposed that a few of the stress-tolerant plant types evaluated with regard to SOD have bought tolerance by an increase in SOD activity only. This represents a total generalization of the biochemical level of these types of varieties, as well as the number of differences discovered with regard to the parental line is frequently a simple mirror image of how multiple parameters have been studied. Therefore, despite the fact that biochemical analysis of such mutants might suggest an important role of SOD, a genuine assessment of the consequences of altering SOD activities which are only in plants can only be acquired by genetic manipulation. To get the full picture, various SOD genes must be overexpressed, due to the fact that their enzyme products each have somewhat different properties. In specific, the deactivation of Cu/ZnSOD as well as FeSOD by means of H_20_2, their response to product as well as the distinct resistance of MnSOD in accordance with the H_20_2 may well be relevant to their particular effects. Enzyme activity must also be supplemented into one or more of the subcellular sections because various stress circumstances seem to affect them in various ways. Approaches that can result in decreased SOD activity, like the antisense (or ribozyme) technological development, are now viable and should be complementary to any overexpression experiments.

8.7 Cu/ZnSOD and MnSOD Overexpression

The very first report of the genetic modification of SOD in the plants termed a generation of tobacco as well as tomato plants which overproduced one chloroplastic Cu/ZnSOD originating from petunia (209). Tobacco plants expressing maximum levels have been studied for all their sensitivity to light-mediated paraquat injury sustained in leaf disc tests by quantifying $C0_2$ assimilations, photosystem II fluorescence, as well as chlorophyll bleaching. Under these circumstances, superoxide has the potential to be formed nearly exclusively inside the chloroplasts due to the high percentage of electron transfer via the photosystems. The genetically modified plants behaved no way differently from the plants that did not include the improved chloroplastic Cu/ZnSOD. Likewise, experiments to assess the effects of photoinhibitory circumstances (cold and elevated light) on genetically modified tomato plants showed no major difference between the plants which produced higher Cu/ZnSOD and the controlled plants. The conclusion was that the enhanced activities of SOD alone throughout the chloroplasts were not enough to give protection against oxygen toxicity because of the enhanced H_20_2 that this would create. The writers suggested that the genomic engineering of the chloroplastic H_20_2-detoxication system (in accordance with Halliwell-Asada pathway), as a supplement to SOD, might be required to manufacture a resistant phenotype. The impact of enhanced chloroplastic SOD activities on the activity of an endogenous pathway has not been tested. As could be forecast from the research with the other organisms, as well as from the biochemical attributes of SOD enzymes, excessive production of MnSOD generates different results. Inside our laboratory, we did the overproduction of *N. plumbaginifolia* stemmed MnSOD as well as targeted the enzyme either one to the mitochondria or to chloroplasts of the tobacco cells (Bowler et al. 1991). The leaf disc assays using paraquat were once again selected as the model system, and the light-mediated damage was evaluated by measuring the damage to membrane, photosystem II fluorescence, as well as the development of pheophytin, the derivative of chlorophyll created through the action of paraquat. Leaf discs taken from the plants which contained improved mitochondrial MnSOD performed very much the manner in which control material performed, as would have been predicted, because the key site of superoxide manufacturing is probable to be the chloroplasts during these experiments. Though, excess production of MnSOD into the chloroplasts has been granted protection from the paraquat toxicity which had been associated with the boosts in its activities. We assess that highest activity levels of MnSOD in genetically modified plants have been comparable to those of petunia Cu/ZnSOD in tobacco (Bowler et al. 1991), which does not grant protection to paraquat. Therefore, variations in activities of MnSOD and Cu/ZnSOD will not be able to describe the gap in these results; instead, we think that the varying H_2O_2 sensitivities of these two enzymes are in charge. We also studied the effect of paraquat upon leaf discs kept in full darkness. Subsequently, since photosynthetic electron transportation does not work in the dark, the comparative proportion of mitochondrially produced superoxide should increase. Still, electron supplies for paraquat do yet exist in the dark. These might be components of chlororespiratory electron transport route well-known to be fueled in the dark in the photosynthesis prokaryotes, as well as the green algae, due to the fact that recent evidence indicates it is as well present in the plant chloroplast (Garab et al. 1989). The findings of these dark experiments have been distinctly different to those of light experiments. Tiny increases of both chloroplastic and mitochondrial MnSOD have been harmful for the plant cells at the same time as larger productions presented resistance.

8.8 Overview of Stress Resistance Through Genetic Engineering of SOD

The findings discussed above have some bearing on the distinct impacts of SOD observed in bacterial and the animal systems, as well as to indicate a fine line between gain and damage resulting from alterations in the SOD activities.

We believe these results could be explained in accordance with Haber-Weiss reaction, a metal ion-catalyzed development of highly reactive hydroxyl radicals (OH·) from the relatively inert superoxide anion and hydrogen peroxide. Growth of SOD activity will alter the H_20_2 balance inside the cell, and this is going to either rise or decrease the probability of OH production. This will decide whether the genetically modified alterations in SOD activity have proved to be useful or harmful to the plant. Regrettably, this equilibrium between help and limitation might be so easily crisscrossed that there is no method to stress tolerance engineering through SOD alone can be successful.

It is obvious that the introgression of new genes linked to scavengers would results in an efficient defense by reducing the free radicals by maintaining the physiological balance. This might require, as (Shaaltiel and Gressel 1986) originally indicated, the exploitation of the entire oxidant stress defense method. For plant chloroplasts, this is going to be boosting the contents of SOD, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase—a real challenge for existing plant molecular biology. Ever-increasing the activities of all such enzymes in genetically modified plants would possibly be the best thing accomplished by manipulating the relevant regulatory processes which control their expressions. In *Conyza bonariensis*, at the very least, one nuclear localized gene seems accountable for increasing the activities of all mentioned enzymes (Shaaltiel et al. 1988), and therefore, its alterations may produce such a result. Though, there is much work to be made prior to the isolation of such genes. Still, this method is not without any difficulties due to the fact that the subsequent disturbances in oxidized as well as reduced ascorbate, glutathione, and NADP are more likely to produce further repercussions. One and only other methodology which is currently available to the molecular biologists is the overproduction of hydrogen peroxide-purifying enzyme along with SOD. Catalase as well as the peroxidase are two options, and catalase is probably the best, because it does not need any substrates used for catalysis. This self-adequacy would ensure against distress in additional cellular components. Such an approach might be best taken out by re-targeting catalase towards chloroplasts, cytoplasm, or mitochondria so that

H_20_2 can be removed at its location of formation. Likewise, targeting of the abovementioned enzymes to sites inside the cell at greatest risk from oxidative damage, e.g., membranes, might be a more detailed approach to reducing oxygen toxicity. As our knowledge of oxidative stress will improve considerably by attempting such approaches, it is possible that an optimized method for improving stress tolerance via the manipulation of a plant's defense system will be found, particularly as new methods become available for plant gene manipulation.

8.9 Conclusion

Superoxide dismutase, also known as first barrier to plant oxidative stress, partake the most fundamental role in scavenging mechanism of abiotic and biotic stress-induced reactive oxygen species. Foregoing discussion clearly indicates that plants having native or induced SOD activities induce more tolerance against different stresses. Numerous researchers have proved higher scavenging process of ROS in plants with higher level of SOD and its isoforms under stress. Plant SODs are classified in different types including Mn-SOD, Cu/Zn-SOD, and Fe-SOD, on the basis of isomers and active sites of metal ions, and they can be localized in different parts of the cell from which cloroplastic SODs were the most important ones. It has been proved by various findings that plant tolerance level and SOD activity with various isomers can be positively correlated. Therefore, plant SOD profiles can be utilized as a stable stress tolerance marker in almost all plant varieties.

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Glutathione in Higher Plants: Biosynthesis and Physiological Mechanisms During Heat and Drought-Induced Oxidative Stress

9

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Abstract

The growth and development of plants are affected by the adverse effect of environmental stresses including drought, salinity, high temperature, and toxic metal accumulation. Under environmental stresses, cell oxidative damage of plants generally occurs as a consequence of the overproduction of reactive oxygen species (ROS). While tolerant plants could survive against abiotic stress-induced oxidative stress by following various physiological mechanisms.

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Among various physiological processes, glutathione (GSH), a non-enzymatic antioxidant, is one of the key metabolites which plays a significant role in protecting the plant cells from oxidative stress. GSH directly or indirectly involves in detoxifying the ROS in plants' cells. Besides these roles, GSH also plays role in detoxification of methylglyoxal, formation of phytochelatins, interacts with plant hormones, other signaling molecules and its redox state triggers signal transduction, and also acts as a cofactor in several biochemical reactions. Therefore, GSH is measured as a versatile redox molecule and a perfect metabolite to have an involvement in plant growth and development, under both stress and normal conditions. The current chapter overviewed the earlier studies on the biosynthesis and physiological mechanisms of GSH during heat and drought-induced oxidative stress in plants.

Keywords

Plants · Abiotic stresses · Reactive oxygen species · Glutathione · Biosynthesis · Physiological mechanisms

9.1 Introduction

The growth and developmental period of plants are exposed to various environmental changes as a consequence of various environmental stresses such as heat, drought, etc. However, plants can regulate the cellular metabolism to cope up with environmental stresses. Environmental stresses like heat and drought-induced oxidative stress in plants are due to the excessive production of reactive oxygen species (ROS) in plants' cells or tissues that are stressed. At lower concentrations, ROS compounds play a role as a signal to activate the defense activity of plants against biotic and abiotic stresses. Therefore, the plant's cell exposed to environmental stress has to maintain the level of ROS under the threshold to prevent as well as to recover the plant's cell from oxidative stress due to the high concentration of ROS in the cell. To maintain the ROS under a threshold level when cells are in stressed conditions, plants make use of various enzymatic and non-enzymatic networks. However, concerning the plant genotypes, the epigenetic signature of plants, developmental stage of the plant, stress intensity, exposed cell/tissue types, and the efficiency of these networks, varies (de Pinto et al. 2015a, b; Centomani et al. 2015).

Among many other metabolites involved in these networks to modulate the level of ROS, glutathione (GSH), a non-enzymatic antioxidant, is one of the key metabolites playing roles in protecting plant cells from oxidative stress. GSH directly or indirectly involves in detoxifying the ROS in the plant cell (Foyer and Noctor 2005). Other than regulating the ROS level, GSH also plays role in detoxification of methylglyoxal (MG) (Hasanuzzaman et al. 2017), formation of phytochelatins (PCs) (Sharma and Dietz 2006), interacts with plant hormones, other signaling molecules and its redox state trigger signal transduction, and acts as a cofactor in several biochemical reactions (Foyer and Noctor 2005). These

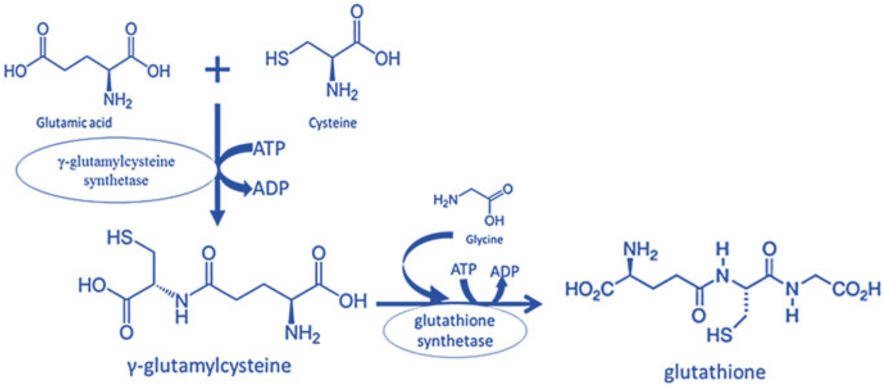

Fig. 9.1 A two-step reaction is involved in glutathione synthesis where γ-glutamylcysteine synthetase catalyzes the first reaction in plastid and glutathione synthetase catalyse the second reaction in plastid and cytosol at the expense of two molecules of ATP in each step

multiple functions of GSH and owing to its cysteine (Cys) moiety, GSH is considered as a versatile redox molecule and makes it a perfect metabolite to have an involvement in plant growth and development, under both stress and normal conditions.

Glutathione is a low molecular weight ubiquitous tripeptide (c-Glu-Cys-Gly, i.e., c-glutamyl-cysteinyl-glycine) that contains long hydrophilic groups and is found in all aerobic organisms (Gill et al. 2013). The molecular structure of GSH contains a peptide bond between the carboxyl group of the glutamate (Glu) side chain and the amine group of cysteine (Cys) and peptide bond between amine groups of cysteine (Cys) to a glycine. The Glu linkage of GSH leads to an increased reactivity with respect to its participation in the c-glutamyl cycle and protects GSH against attack by amino peptidases (Wonisch and Schaur 2001). It is found in the chloroplasts, cytosol, vacuoles, mitochondria, endoplasmic reticulum, apoplast, and the peroxisomes (Noctor and Foyer 1998). GSH synthesis generally takes place in the plastids (chloroplast), mitochondria, and cytosol (Zechmann and Muller 2010). However, the major processes which consist of a two-step reaction occur in the chloroplast where γ-glutamylcysteine synthetase (γECS; EC 6.3.2.2) is involved in the first step and glutathione synthetase (GSHS; EC 6.3.2.3.) in the second step at the expense of two molecules of ATP in each step (Fig. 9.1).

The γECS is found in plastids and GSHS is found both in the cytosol and plastids due to which plastids are considered as the site of GSH production in higher plants (Pasternak et al. 2008a, b). Wachter et al. (2005a, b) reported that the enzyme involved in the second step of GSH synthesis i.e., GSHS is encoded by a single copy nuclear gene in *Arabidopsis* with alternate transcription start sites leading to either targeted to plastid or cytosol. GSH is synthesized in its reduced form. In the plastids, GSH biosynthesis starts with the formation of an amide bond by a reaction between the c-carboxyl group of Glu and a-amino group of Cys to yield γ-glutamylcysteine by an enzyme γECS followed by the formation of an amide bond between the a-carboxyl group of the cysteine moiety in γ-glutamylcysteine and the a-amino group of glycine to form GSH by an enzyme GSHS (Galant et al. 2011). Six base pair deletion in the γ-ECS gene caused the reduction of GSH content making the *Arabidopsis* mutant sensitive to cadmium. The results suggest that the γECS is one of the main enzymes involved in GSH biosynthesis. After synthesis of γ-glutamylcysteine by the activity of γECS in the plastids, γ-glutamylcysteine can also be transported to the cytosol. The transported γ-glutamylcysteine could serve as the precursor for GSHS to synthesize GSH in the cytosol along with plastids as GSHS is found both in plastids and cytosol (Reichheld et al. 2009). Once synthesized in the cytosol, GSH can be imported or re-imported to plastids, mitochondria, and other organelles directly or in other forms to meet metabolic requirements (Mahmood et al. 2010). Once synthesized, glutathione in its reduced form becomes a substrate for several cellular reactions that results in oxidized glutathione. Dehydroascorbate reductase uses reduced glutathione as the substrate to yield ascorbate which in turn is used to detoxify the hydrogen peroxide. In this system, the pool of reduced glutathione is maintained by the activity of glutathione reductase as the balance between the oxidized glutathione and reduced forms of glutathione is a core component in maintaining cellular redox state (Meister 1995).

From the above discussion, it is confirmed that in plants under stress, GSH enhances plant tolerance against different biotic and abiotic stresses, including heat (high temperature) and drought (Hasanuzzaman and Fujita 2013). The current chapter is discussed on the biosynthesis and physiological mechanisms of Glutathione during heat and drought-induced oxidative stress in plants.

9.2 Glutathione and Its Role in Response to Heat and Drought-Induced Oxidative Stress in Plants

Plant growth and fitness are affected by adverse environmental factors such as drought, salinity, high temperature, and toxic metal accumulation. Plant growth and survival are dependent on their ability to modulate metabolism in response to environmental changes due to their sessile habits. Cell oxidative damage is caused by the overproduction of ROS in stress-exposed tissues in almost all adverse environmental conditions. The ability of a species/variety to detect changes in ROS concentration and cause the appropriate metabolic adjustments is often the determining factor in how it copes with environmental stress.

Plants have enzymatic and non-enzymatic networks that regulate the levels of reactive oxygen species. The effectiveness of these networks varies depending on the severity of the stress, the target tissue or cell compartment, the stage of development of the plant, and the genotype of the plant (De Gara et al. 2010; de Pinto et al. 2015a, b). It also depends on epigenetic signatures that alter the plant's ability to react to different stresses. Mendoza-Cózatl et al. (2008) found that glutathione is the most common source of organic sulfur transported in phloem. It is important in the interaction between plants and symbiotic nitrogen-fixing bacteria in the compartmentalization and neutralization of xenobiotics and heavy metals, and in the transport of secondary metabolites through the vacuole (Cheng et al. 2017; Noctor et al. 2012a, b). In Arabidopsis, GSH has also been proposed as the main donor of the reduced sulfur group for glucosinolate biosynthesis (Parisy et al. 2006). This non-exhaustive list of glutathione-related processes illustrates glutathione's function in various plant protection strategies. Glutathione is likely one of the most essential metabolites in the body's response to environmental stresses. In reality, glutathione and related enzymes appeared in aerobic organisms very early on, most likely alongside dioxygenic photosynthesis (Deponte 2013). Owing to its cysteine (Cys) moiety, the tripeptide GSH1 (γ-L-glutamyl-L-cysteinyl glycine) is a versatile redox molecule.

9.2.1 The Role of Glutathione During Drought Stress

One of the continuing effects of climate change is water shortage, which is likely to result in a significant reduction in crop productivity. Drought causes plants to close their stomata, which reduces endogenous water loss. The hormone abscisic acid is primarily responsible for drought-induced stomatal closure (ABA). When plants are deprived of water, ABA builds up in the xylem sap and travels to the guard cells, where it activates a signaling network, causing the guard cells to shrink and the stomata to close. Stomatal closure is caused by osmotic changes in guard cells as a result of ABA-induced ionic channel modulation. ROS generation in the apoplast and an increase in Ca^{2+} in the cytoplasm of guard cells are both parts of the ABA signaling network (Pei et al. 2000; Kwak et al. 2003). The role of GSH in ABA-induced stomatal closure has been addressed in several articles (Okuma et al. 2011; Akter et al. 2012, 2013). As part of its signaling cascade, ABA lowered GSH levels in cell guards, leading to stomatal closure according to Okuma et al. (2011). Indeed, a chemical therapy that lowers GSH levels in guard cells appears to improve ABA sensitivity in guard cells (Okuma et al. 2011; Akter et al. 2012). ABA-dependent stomata closure is similarly improved in Arabidopsis GSH-deficient mutant cad2-1, which lacks glutamylcysteine synthetase, the initial enzyme in GSH production (Okuma et al. 2011). Another reactive species implicated in the ABA-induced signaling pathway that promotes stomatal closure is nitric oxide (NO) (Bright et al. 2006). In fact, ABA-induced ROS generation causes guard cells to produce more NO. The negative feedback of ABA-induced stomata closure could potentially be due to NO (Wang et al. 2015). S-nitrosoglutathione (GSNO), storage of NO molecule in the cells that can operate as a trans-nitrosylating agent (Fig. 9.2; de Pinto et al. 2013; Locato et al. 2016), may play a role in this process.

9.2.2 The Role of Glutathione During Salinity

Another negative environmental characteristic that restricts the productivity of arable land is the excessive concentration of salts in the soil. High levels of NaCl in the soil are the main cause of salinity. Plant defense responses to salt stress are confounded

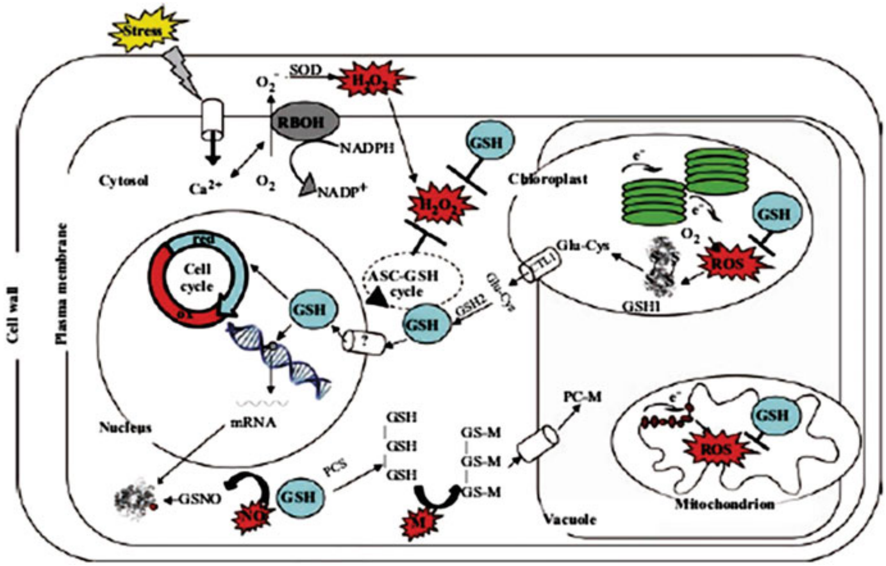

Fig. 9.2 The role of GSH in major protective defense mechanisms activated by plant cells against environmental stress

by the fact that an excess of salt in the soil causes both osmotic stress (a short-term effect) and ionic toxicity (a long-term effect) (long-term effect). Due to the high solute concentration in the soil, osmotic stress prevents the roots from absorbing water. Plants quickly respond to osmotic stress by closing their stomata, which reduces water loss. Plant tolerance to salt stress, on the other hand, may be attributed to their capacity to keep the K^+/Na^+ ratio within a physiological range (Munns and Tester 2008). As a result, because Na^+ competes with K^+ for intracellular transport, cellular K^+ content drops. Gupta and Huang (2014) found that low cellular K^+ levels had a negative impact on a variety of metabolic pathways. Because ROS are triggered by salt stress conditions, plant tolerance to salinity is also linked to its ability to reverse salinity-induced oxidative damage. Demidchik et al. (2010) discovered that salt stress causes a drop in cell K^+ levels, which is due to ROS activating guard cells outward rectifying potassium (GORK) channels. Brassica species that are salt resistant have a higher ability to retain K+ via lowering the sensitivity of the root K+ permeable channel to ROS (Chakraborty et al. 2016). The role of ROS in the signaling of salt-activated plant defense responses has also been observed (Ushimaru et al. 2006). Plants with ectopic expression of dehydroascorbate reductase (DHAR) have better salt tolerance. DHAR is a GST that regenerates the reduced form of ASC by temporary GSH conjugation as part of its catalytic action. As a result, ASC recycling is a component of the GSH metabolic network, which helps plants cope with oxidative stress caused by salt (Gallie 2013). GSH participates in the methylglyoxal (MG) detoxification route and is implicated in the defensive mechanisms triggered in response to salt stress. MG is a cytotoxic molecule that is produced in cells as a result of carbohydrate and amino acid catabolism. It also increases as a result of salt stress (Yadav et al. 2005).

9.2.3 The Role of Glutathione During Heavy Metals Stress

Increasing anthropogenic activities, mostly related to the metallurgical sector and agricultural practices such as mining, fertilizer use, and sewage sludge, have all released plentiful heavy metals in the soil over the last two centuries. Metal ion competition with necessary cations, which are routinely absorbed by plants, appears to be the main source of growth decrease induced by metal uptake. Although several metals, such as Fe, Mn, Cu, Zn, and Ni, are plant micronutrients, they become hazardous when their quantities exceed plant demands since they are cofactors of numerous enzymes (Anjum et al. 2015a). Nonessential metals, on the other hand, such as Cd, Pb, Hg, As, and Ag, are phytotoxic at low quantities and can poison people, as their presence in the diet has been linked to a variety of diseases (Järup 2003; Järup and Akesson 2009). Redox-active metals have more than one oxidation state and can participate in the Fenton reaction, which produces hydroxyl radicals (OH.). As OH is the most reactive ROS radical, it promotes cell oxidative damage, which can lead to metabolic dysfunction and cell death. Non-redox active metals, on the other hand, can boost ROS generation indirectly (Locato et al. 2017). Metals also compete with the cofactors of numerous ROS-scavenging enzymes, including SOD and APX, impairing ROS detoxification (Kliebenstein et al. 1998; Jespersen et al. 1997). Plants have evolved a multitude of ligands that chelate metals within cells to lower metal concentrations in the cytosol. Metal complexes are formed and translocated into the vacuole, where they are detoxified (Anjum et al. 2015b). Plants that are exposed to Cd produce GSH and PC by increasing the expression of genes that code for GSH biosynthetic enzymes. Arabidopsis GSH1 is activated in metal-induced oxidative conditions (Hicks et al. 2007a, b). Metal hyperaccumulating plants, which are metal-tolerant species that could be employed in phytoremediation because of their ability to absorb and endure high amounts of metals and so reduce metal pollution in the soil, have high GSH levels.

9.3 Biosynthesis of Glutathione During Heat and Drought-Induced Oxidative Stress in Plants

9.3.1 Molecular Basis of γ-Glutamyl Cysteine Synthetase (γ-ECS) and Glutathione (GSH)

In mammals and yeast, γECS also known as glutamate-cysteine ligase (GCL) catalyzes the rate-limiting step in GSH biosynthesis (Wild and Mulcahy 2000) which is similar for plants (Noctor et al. 1998). The γECS enzyme functions normally at less than its maximal rate, because the reaction catalyzed by γECS is feedback-inhibited by GSH as it is competitive with respect to glutamate. In feedback inhibition, the glutamyl moiety of GSH binds to the glutamate binding site of the enzyme (Meister 1995). In plants, the gene of γ-ECS was first isolated from *Arabidopsis thaliana* (May and Leaver 1994a, b). The expression analysis of *γ-ECS* genes showed that the transcripts accumulated when plants were exposed to adverse environments, including extreme temperatures and drought (Fang et al. 2016).

Oxidative stress caused by different environmental and cellular conditions increases GSH levels in plants (May and Leaver 1993). Several studies reported the increased expression of the genes encoding γ-ECS and GSHS and transcriptional regulation of the pathway under different environmental stress (Hasanuzzaman et al. 2017). However, the effects of different environmental stresses on GSH accumulation are not the same. Effects on the genes encoding the enzymes involved in GSH production in plants by different environmental stresses are different, like the expression of γ-ECS and GSHS and the levels of glutathione are increased in response to jasmonic acid and heavy metals (Xiang and Oliver 1998), however, when treated with GSH or H_2O_2, expression of those genes was unaffected (Schäfer et al. 1998). Similarly, the expression of γ-ECS in *Brassica juncea* (Indian mustard) and γ-ECS and GSHS in *Arabidopsis thaliana* was increased when treated with cadmium (Xiang and Oliver 1998). Generally, in plants, an increase in GSH is associated with the over-expression of γ-ECS, not GSHS, by increasing flux through the pathway. Similarly, a study by Meyer and Fricker (2002) also support the role of γ-ECS as a metabolic control point in the GSH synthesis pathway where it is found that the addition of cysteine, glycine, or glutamate does not enhance GSH synthesis. Interestingly, under the various oxidative stresses, both γ-ECS activity and cellular GSH level increased in *Arabidopsis* suspension cells, whereas transcription of γ-ECS was not upregulated (May et al. 1998). Similarly, *Arabidopsis* mutant phenotypes such as *rml1* (post-embryonic root development) (Cobbett et al. 1998), *cad2* (cadmium tolerance) (Vernoux et al. 2000) and *pad2* (plant disease resistance) (Parisy et al. 2007) are linked to γ-ECS gene.

Cloning and sequence analysis of γ-ECS from *Arabidopsis* (May and Leaver 1994a, b), *Medicago truncatula* (Frendo et al. 1999), *B. juncea* (Hothorn et al. 2006) and *Chorispora bungeana* (Wu et al. 2009) revealed that the plant γ-ECS enzyme is unrelated to the mammalian, yeast, or bacterial enzymes and indicated that this enzyme is grouped into different families categorized as non-plant eukaryotes, γ-proteobacteria, plants and α-proteobacteria (Copley and Dhillon 2002). However, the study based on crystallography analysis deciphered that the γ-ECS from *E. coli* (Hibi et al. 2004), plants (Hothorn et al. 2006) and yeast (Biterova and Barycki 2009) share a common 3D fold. The best-studied γ-ECS are those from non-plant eukaryotes. In mammals, γECS holoenzyme is a heterodimer that gets dissociated under non-denaturing conditions into 31,000 Da light or regulatory subunit and 73,000 Da heavy or catalytic subunits, respectively. The properties of a heavy subunit are the catalytic activity of γ-ECS and GSH feedback inhibition. Catalytic activity and GSH feedback inhibition are properties of the heavy subunit, but the kinetic properties of the heavy subunit can be significantly influenced by association with the light subunit (Huang et al. 1993a, b), however, the magnitude of influence of the light subunit on the catalytic properties of heavy subunit varies depending on the examined species or model system (Wild and Mulcahy 2000). The association of two subunits is mediated by the formation of intermolecular disulfide bonds (Fraser et al. 2002). The γ-ECS responds to the changes in the cellular redox environment to modulate the production of γ-glutamylcysteine, but this model doesn't extend to all the non-plant organisms. For example, γ-ECS from *Trypanosoma brucei* and heavy subunit of mammalian γ-ECS shares 45% amino acid similarity, however, *T. brucei* γ-ECS functions as a monomer (Lueder and Phillips 1996). Similarly, the γ-ECS of *E. coli* also functions as a monomer (Hibi et al. 2004).

In plants such as *Arabidopsis* (Hicks et al. 2007a, b), *Brassica juncea* (Hothorn et al. 2006; Gromes et al. 2008a, b), biochemical studies of γ-ECS revealed that γ-ECS enzyme is regulated by cellular redox environment through a process different from the heterodimeric enzymes. Kinetic analysis showed that a potential inhibitor of mammalian γ-ECS i.e., buthionine sulfoximine, which uses a random ter-reactant kinetic mechanism with a preferred order of binding for catalysis inactivated the *Arabidopsis* γ-ECS (Jez et al. 2004). In the yeast, magnesium ion (Mg^{2+}) of an active site of γ-ECS orients the glutamate γ-carboxylate in order to attack on the γ-phosphate of ATP, which is placed by an active site lysine and second Mg^{2+} and results into acyl-phosphate intermediate. The acyl-phosphate intermediate faces the nucleophilic attack by α-amino group of cysteine which is activated by the glutamate active site. The transition step involved in each reaction is stabilized by a conserved arginine that mediates peptide bond formation (Galant et al. 2011). In plants, the mechanistic analysis revealed altered the catalytic activity with more active oxidized protein than reduced protein caused by reversible disulfide bond formation (Jez et al. 2004). In yeast, it is reported that in the absence of GSH, the expression of genes encoding γ-ECS is induced by Met4, a transcription activator that is also involved in inducing the expression of genes related to sulphur assimilation (Wheeler et al. 2002). The promoter of γ-ECS gene contains a binding site for Yap1 transcription factor (Wu and Moye-Rowley 1994) which is also involved in transcriptional regulation of γ-ECS induced by oxidants such as H_2O_2 and heat shocks (Stephen and Jamieson 1997; Sugiyama et al. 2000). The depletion of GSH induces the Yap1 activity, thereby, increases the expression of Yap1 targeted genes. However, the expression of Met4 genes was found to be unaltered under the same conditions. It was found that activation of γ-ECS expression by Yap1 in absence of GSH is regulated by the Met4-dependent process with respect to the sulfur status of the cell (Wheeler et al. 2003).

In plants, GSH biosynthesis by γ-ECS under cellular redox environment influences a very simple post-translational control mechanism where the redox regulation of γ-ECS imparts a control switch for GSH production and GSH maintains the intracellular redox balance. The activity of γ-ECS is induced and the demand for GSH is increased under oxidizing conditions, and when the concentration of GSH increases, the cellular environment gains reduced potential, thereby decreases the activity of γ-ECS (Galant et al. 2011). To the intracellular oxidative signals, the γ-ECS enzymes provide the post-translational switch in GSH production which was confirmed through crystallographic and functional studies of γ-ECS (Galant et al. 2011). However, in plants regulated by a redox environment, γ-ECS functions as a homodimeric protein with two intermolecular disulfide bonds as opposed to heterodimeric (intramolecular) disulfide bonds in non-plant eukaryotic γ-ECS (Hicks et al. 2007a, b). The analysis of *B. juncea* γ-ECS showed that one disulfide bond (Cys178–Cys398) is at the link between monomers of the dimers and anchoring of a β-hairpin at the active site entrance and is mediated by a second disulfide bond (Cys341–Cys356) (Hothorn et al. 2006). Plants γ-ECS functions as a dimer in the oxidized state, whereas under reduced conditions, the disulfide bond of plants γ-ECS gets disrupted at Cys178–Cys398 which results in conversion of active dimer to less active monomeric form (Hothorn et al. 2006). When the disulfide bond (Cys178–Cys398) of γ-ECS in *B. juncea* (Hothorn et al. 2006) and disulfide bond (Cys186–Cys406) in *A. thaliana* (Hicks et al. 2007a, b) were inactivated through site-directed mutagenesis, the redox response of the γ-ECS were eliminated and γ-ECS was locked into its monomeric state.

The disulfide bond that is involved in the transition of the monomer/dimer state of γ-ECS is conserved all across the plant kingdom and also in α-proteobacteria (Gromes et al. 2008a, b). Various studies about the relationship between γ-ECS switch with respect to redox environment and disulphide bond formation for γ-ECS dimer organization in plants hinted response to redox environment and dimer formation could be related to the compartmentalization of GSH synthesis in the plastid (Gromes et al. 2008a, b). However, the compartmentalization of γ-ECS in sub-organellar positions pointed out different aspects of the regulation of GSH biosynthesis in plants under a cellular redox environment. The general mechanism is that γ-ECS is redox-regulated and under the oxidizing environment, the γ-ECS dimer is more active (Galant et al. 2011), however, in the chloroplast, stroma has reducing environment and lumen has the oxidizing environment, but γ-ECS activity is partitioned to the stroma which possesses a reducing environment and has enhanced the activity of γ-ECS (Rouhier et al. 2008; Meyer et al. 2009). The presence of both glutaredoxin system and thioredoxin system in the stroma of chloroplast suggest that one of the systems could be involved in maintaining the redox state of γ-ECS in the chloroplast, among which glutaredoxin may be important for modulating the activity of γ-ECS as suggested by physical properties of γ-ECS (Rouhier et al. 2008; Meyer et al. 2009). Similarly, for maintaining the reduced pool of less active γ-ECS, there is a potential link to the glutaredoxins system as suggested by the redox properties of γ-ECS (Galant et al. 2011). Heavy metal stress such as cadmium (Cd) tolerance in plants is imparted by the involvement of GSH dependent phytochelatin (PC) synthesis. A study found that the transcription of genes for PC synthesis, i.e., PCS1, and PCS2 along with γ-ECS, GSHS in *Arabidopsis* is positively regulated by a transcription factor ZAT6 under Cd stress. The γ-ECS could be a key target for ZAT6 as it is capable of specifically binding to the promoter of γ-ECS (Chen et al. 2016). The transient expression study of a transcription factor, WRKY12 showed that WRKY12 directly regulates the expression of both γECS and GSHS. It is found that under increased and decreased Cd accumulation by over-expression and loss of function of WRKY12 were due to repressing or expressing of the genes involved in the PC synthesis pathway. Further analysis showed that the transcription factor WRKY12 directly binds to the W-box of the γECS promoter (Han et al. 2019).

As discussed above, γ-ECS is involved in the first step and another enzyme i.e., GSHS is involved in the second step of GSH biosynthesis. GSHS is found both in the cytosol and plastids due to which plastids are considered the site of GSH production in higher plants (Pasternak et al. 2008a, b). A single gene encodes both the plastid-targeted and cytosol-targeted GSHS with alternate transcription start sites (Wachter et al. 2005a, b). There is no amino acid sequence homology of bacterial and eukaryotic GSHS. In bacteria, it functions as a tetramer (Yamaguchi et al. 1993), and it functions as a dimer in eukaryotes such as a mammal (De Jesus et al. 2014), plant (Yang et al. 2019a, b), and yeast (Sibirny 2019). GSHS of *Arabidopsis* shares around 40% amino sequence with humans (Rawlins et al. 1995), with yeast (Ullmann et al. 1996; Wang and Oliver 1996), GSHS of legumes with human (Moran et al. 2000 and Matamoros et al. 2003), maize, wheat, and soybean GSHS with human (Skipsey et al. 2005). Wang and Oliver (1997) reported an important activity related to glycine-rich loop in *Arabidopsis* GSHS which later was confirmed as an active site of the GSHS (Galant et al. 2009). Kinetic study of *Arabidopsis* GSHS showed that it uses a random ter-reactant process where binding of GSHS with either γ-glutamylcysteine or ATP is the first preference and addition of glycine to γ-glutamylcysteine for the formation of GSH is the last (Jez and Cahoon 2004). In the first preferred reaction, the GSHS mediates the transfer of γ-phosphate of ATP to γ-glutamylcysteine and a group of acidic residues arranges two Mg^{2+} to ATP which orients the γ-phosphate group in the active site and the reaction results in the formation of an electrophilic acyl-phosphate intermediate. The side chain guanidyl group of Arg454 of the electrophilic acyl-phosphate intermediate becomes the target side for glycine which interacts with the intermediates with its carboxylate moiety (Herrera et al. 2007). In many legumes, namely lentil, soybean, pea, alfalfa, bean, mungbean, and the thiol tripeptide homoglutathione (hGSH; γGlu-Cys-βAla) is formed from γ-Glutamylcysteine by hGSHS enzymes which has a higher affinity for β-alanine and low affinity for glycine which is generally present in tripeptide of glutathione. Similarly in maize, glycine peptide in the thiol tripeptide glutathione is replaced by glutamate when exposed to cadmium as it seems under cadmium stress that GSHS has a higher affinity towards glutamate than glycine peptide (Meuwly et al. 1995). Similarly in rice, GSHS is encoded by three different homolog genes designated as *OsGS1, OsGS2,* and *OsGS3*. The RNA interference-mediated knockdown of *OsGS2* significantly reduced the concentration of hydroxymethyl-GSH in rice plants, and in vitro enzyme assay revealed that the product of OsGS2 gene catalyzed the hydroxymethyl-GSH (h-GSH) in rice plants from γ-glutamylcysteine at the expense of ATP with higher affinity towards L-serine (tenfold) as compared to glycine as co-substrate (Yamazaki et al. 2019).

In many legumes, the thiol tripeptide homoglutathione (hGSH; γGlu-Cys-βAla) (Fig. 9.5b) can partially or fully replace the better-known thiol, glutathione. γ-Glu-Cys is formed from L-glutamate and L-cysteine by glutamate cysteine ligase (γ-Glutamylcysteine synthetase or γ-ECS) at the expense of ATP, and γ-Glu-Cys and β-alanine are condensed at the expense of ATP by a specific hGSH synthetase (hGSHS), an enzyme which has affinity for β-alanine and low affinity for glycine. Several studies reported the transcriptional regulation of the mechanism involved in the expression of the genes encoding γ-ECS, GSHS and GSH-related genes under different abiotic stresses. In wheat, under the heat treatment, the GSH, hGSH content and the activity of the two enzymes γ-ECS, GSHS of GSH synthesis was found to be increased (Kocsy et al. 2002). Similarly, the effect of heat stress on glutathione synthesis was studied in wheat genotypes consisting of chromosome substitution lines with different levels of freezing tolerance. In the substitution lines, genes in 5A chromosome of *Triticum aestivum* ssp. aestivum cv. The Chinese Spring responsible for moderate freezing tolerance was replaced with 5A chromosome of freezing tolerant genotype, i.e., *T. aestivum.* Ssp. aestivum cv. Cheyenne and susceptible genotype *T. aestivum*. Ssp. Spelta. The GSH and hGSH content, the ratio of reduced GSH or hGSH to oxidised GSH or hGSH forms and the activity of glutathione reductase (GR) were higher in freezing sensitive genotype, suggesting that the genes present in 5A chromosomes which are responsible for freezing sensitivity could be playing a regulatory role in GSH and hGSH synthesis during heat stress in wheat (Kocsy et al. 2004). Similarly in *Arabidopsis,* GSH was found to be modulating the expression of heat shock proteins via BZIP10 and MYB21 transcription factors (Kumar and Chattopadhyay 2018).

Foyer-Halliwell-Asada cycle or commonly known as the ascorbate–glutathione cycle helps in removing the H_2O_2, on one of the reactive oxygen species that imparts oxidative stress to the plants. This pathway comprises of AsA, GSH, four enzymes viz., ascorbate peroxidase, monodehydroascorbate, reductase, dehydroascorbate reductase, and glutathione reductase. Regulation of these pathways helps in mitigating the oxidative damage in plants under abiotic stresses (Hasanuzzaman et al. 2019). Under the heat stress in maize plants, AsA–GSH system plays a key role in maintaining the ROS homeostasis in cells by finely-tuning the redox metabolism, thereby minimizing the potentially toxic effects of ROS (Tiwari and Yadav 2020). The reduced GSH is oxidized into oxidized GSH (GSSG) while detoxifying the ROS especially H_2O_2 by glutathione peroxidases (such as Gpx1p, Gpx2p, and Hyr1p). The GSSG is converted back to reduced GSH by GR. GR, a flavoprotein oxidoreductase that acts as a substrate for GSH- S-transferases is involved in the reduction of glutathione disulfide (GSSG) to the sulphydryl form of GSH by employing NADPH as a reductant via the ascorbate–glutathione pathway, thereby plays a role in abiotic stress tolerance (Yousuf et al. 2012; Harshavardhan et al. 2017). Generally, under normal conditions, GSH/GSSG basal ratio is about 20:1 (Mhamdi et al. 2010a, b). However, when plants are exposed to oxidative stress, this ratio can go down significantly. Like heat stress, drought is considered as one of the severe environmental conditions that create oxidative stresses in plants. A study in mung bean (*Vigna radiata* L. cv. Binamoog-1) showed increased GSH, GSSG content, decreased GSH/GSSG ratio, and increased GSH-S-transferase activities in drought-tolerant genotypes (Nahar et al. 2015). Similarly, in *Amaranthus tricolor*, ascorbate-glutathione content, ascorbate-glutathione redox, and ascorbate-glutathione cycle enzymes activities were found to be increased in drought-tolerant genotypes (Sarker and Oba 2018). Lou et al. (2018) reported about the enhanced activities of the enzymes involved in the ASA-GSH cycle and enzymes responsible for maintaining GSH homeostasis in the cell under drought stress in winter wheat (*Triticum aestivum* L.).

9.3.2 Glutathione Biosynthetic Genes in Transgenic Plants During Abiotic Stress Tolerance

Many enzymatic and non-enzymatic pathways are involved in regulating the levels of ROS in the cellular environment. Among the non-enzymatic metabolites, GSH is one of the important metabolites involved to modulate the level of ROS and is a key metabolite playing the role of protecting plant cells from oxidative stress. It directly or indirectly takes part in the detoxification of ROS in the plant cell (Foyer and Noctor 2005). Apart from the ROS level modulation, GSH also plays role in detoxification of methylglyoxal (MG) (Hasanuzzaman et al. 2017), formation of phytochelatins (PCs) (Sharma and Dietz 2006), and interacts with hormones, signaling molecules and its redox state triggers signal transduction and act as a cofactor in different biochemical reactions (Foyer and Noctor 2005). These multiple functions of GSH and owing to its cysteine (Cys) moiety, GSH is a versatile redox molecule and makes it a perfect metabolite to have an involvement in plant growth and development, under both stress and normal conditions. Thus, it is now a fact that when plants are under stress, GSH enhances plant tolerance against different biotic and abiotic stresses, including heat (high temperature) and drought (Hasanuzzaman and Fujita 2013). The reduced GSH is oxidized into oxidized GSH (GSSG) while detoxifying the ROS especially H_2O_2 by glutathione peroxidases (such as Gpx1p, Gpx2p, and Hyr1p). The GSSG is converted back to reduced GSH by GR. GR, a flavoprotein oxidoreductase that acts as a substrate for GSH-S-transferases, is involved in the reduction of glutathione disulfide (GSSG) to the sulphydryl to form GSH by employing NADPH as a reductant via the ascorbate–glutathione pathway, thereby playing a role in abiotic stress tolerance (Yousuf et al. 2012; Harshavardhan et al. 2017). Considering the role of GSH described above, it is clear that the enhanced activities of GSH, GSH biosynthesis enzyme, GSH utilizing enzymes, and GSH regenerating enzymes play a crucial role in plants against abiotic stresses. The use of transgenic plants over-expressing the genes related to GSH biosynthesis and the genes associated with regulation of GSH level in the cell has clearly demonstrated the vitality of GSH in plants to protect it from oxidative stress caused by different abiotic stresses. Some of the experiments, where the transgenic approach have been used to understand the role of genes for GSH biosynthesis and genes associated with GSH modulation, are listed in Table 9.1.

9.3.3 Regulation of Glutathione Biosynthesis

Glutathione (GSH) is a tripeptide, γ-L-glutamyl-L-cysteinyl glycine, found in all living cells. It is a non-protein sulfur analogue of alcohol compounds. Its

Table 9.1 Transgenic plants over-expressing genes associated with GSH biosynthesis enzyme, GSH utilizing enzymes, and GSH regenerating enzymes imparting abiotic stress tolerance

Transgene/s	Source of transgene	Transgenic organism	Tolerance to	References
Glutathione reductase (GR) or Glutathione synthetase (GSHS)	*E. coli*	*Populus species*	Photo-inhibition	Foyer et al. (1995)
GSHS	*E. coli*	*B. Juncea* L	Cadmium	Zhu et al. (1999a)
γ-ECS	*E. coli*	*B. Juncea* L	Cadmium	Zhu et al. (1999b)
Serine acetyltransferase (*SAT*)	*E. coli*	*Solanum tuberosum*		Harms et al. (2000)
γ-ECS	*E. coli*	*P. species*	Herbicides	Gullner et al. (2001)
Arsenate reductase (ArsC) and γ-ECS	*E. coli*	*Arabidopsis thaliana*	Arsenic	Dhankher et al. (2002)
γ-ECS fused to chloroplast transit sequence of pea and GSHS under CaMV 35S promoter	*E. coli*	*B. juncea* L.	Organic pollutants	Flocco et al. (2004)
γ-ECS	*E. coli*	*Populus species*	zinc(2+)	Bittsánszky et al. (2005)
γ-ECS	*E. coli*	*.A thaliana*	Arsenic and mercury	Li et al. (2005)
SAT, Phytochelatin synthase (PCS) and γ-ECS	*E. coli* and *S. pombe*	*Nicotiana tabacum*	Cadmium	Wawrzyński et al. (2006)
γ-ECS)	*E. coli*	*A. thaliana*	Arsenic and mercury	Li et al. (2006a)
γγ-ECS and GSHS	*E. coli*	*A. thaliana*	Mercury	Li et al. (2006b)
Phytochelatin synthase (AsPCS1) and γ-ECS	*Allium sativum* and *S. cerevisiae*	*A. thaliana*	Cadmium and arsenic	Guo et al. (2008)
γ-ECS and GSHS	*E. coli*	*B. Juncea* L	Heavy metals	Reisinger et al. (2008)
Glutathione S-transferase (GST)	*Choristoneura fumiferana*	*A. thaliana*	Cold	Huang et al. (2009)
GST	*Glycine soja*	*N. tabacum*	Drought and salt	Ji et al. (2010)
γ-ECS	*Phragmites australis*	*Agrostis palustris*	Cadmium	Zhao et al. (2010)
γ-ECS	*E. coli*	*Populus species*	Heavy metals	Ivanova et al. (2011)
(γ-ECS	*E. coli*	*Populus deltoides*	Arsenic	LeBlanc et al. (2011)

(continued)

Table 9.1 (continued)

Transgene/s	Source of transgene	Transgenic organism	Tolerance to	References
γ-ECS	*B. juncea*	*Oryza sativa*	Salt	Bae et al. (2013)
γ-ECS under Rab21 strong promoter	*O. sativa*	*O. sativa*	Salt	Choe et al. (2013)
GST lambda class, (GSTL)	O. sativa	*A. thaliana*	Arsenic cold, osmotic stress and salt	Kumar et al. (2013)
GST	*Populus species*	*A. thaliana*	Salt and drought	Yang et al. (2019a)
Glutathione Peroxidase (GPX)	*Nelumbo nucifera*	*O. sativa*	Salt	Diao et al. (2014)
γ-ECS	*Lycopersicon esculentum* Mill	*N. tabacum*	Salt and drought	Kumar et al. (2014)
GST tau class (GSTUs)	*Citrus sinensis*	*N. tabacum*	Herbicide, salt and drought	Cicero et al. (2015)
GST tau class (GSTUs)	*Glycine max*	*N. tabacum*	Salt	Kissoudis et al. (2015)
γ-ECS	*Pyrus calleryana*	*E. coli*	Cadmium, salt, osmotic stresses.	Li et al. (2015)
GSHS under OsCc1 constitutive promoter	*O. sativa*	*O. sativa*	Abiotic stress	Park et al. (2017)
GSHS	*B. napus*	*N. tabacum*	Salt	Kuluev et al. (2018)
GF14b	*Triticum aestivum*	*N. tabacum*	Salt and drought	Zhang et al. (2018)
GST	*Trichoderma virens*	*N. tabacum*	Cadmium	Dixit et al. (2011)
GST	*Medicago sativa*	*N. tabacum*	saline-alkali stresses	Du et al. (2019)
GPX	*Rhodiola crenulata*	*Salvia miltiorrhiza*	Drought	Zhang et al. (2019)

concentration varies from tissue to tissue with a maximum in liver tissues (1–10 mM). GSH is found in the form of disulfide-oxidized (GSSG) (Kaplowitz et al. 1985). GSH is an active compound and accounts for>98% of the total GSH (Forman et al. 2009; Ballatori et al. 2009). In Eukaryotes, 80–85% GSH is found in the cytosol, 10–15% is found in the mitochondria, and trace amounts is found in the endoplasmic reticulum (Yuan and Kaplowitz 2009). GSH is a ubiquitous

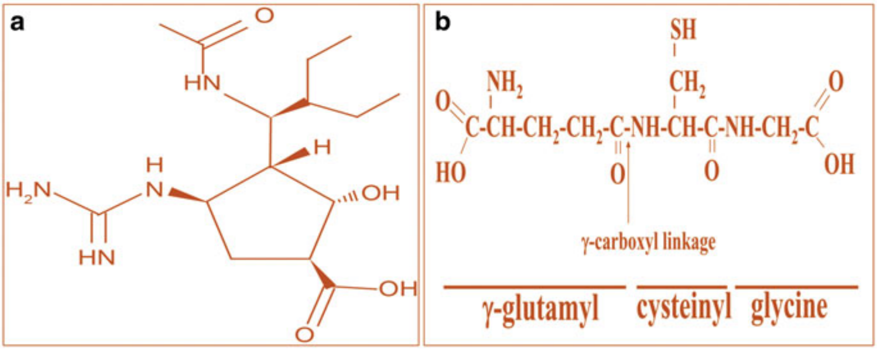

Fig. 9.3 (**a**) Chemical structure of glutathione; (**b**) Structure of γ-glutamylcysteinyl glycine, where the N-terminal glutamate and cysteine are linked by the γ-carboxyl group of glutamate

intracellular peptide with diverse functions and plays very important roles in redox signaling, detoxification of xenobiotics, cell cycle progression, acts as an antioxidant, apoptosis, and helps in storage of cysteine (Forman et al. 2009; Ballatori et al. 2009; Lu 2009; Pallardó et al. 2009; Liu and Pravia 2010). The structure of GSH is presented in Fig. 9.3.

The peptide bond linking glutamate and cysteine of GSH is thru the γ-carboxyl group of glutamate in place of the traditional α-carboxyl group. This uncommon association is a concern to hydrolysis via way of means of handiest one recognized enzyme, particularly γ-glutamyltranspeptidase (GGT), that is the handiest gift at the outside surfaces of sure mobileular types (Meister and Anderson 1983). As a consequence, GSH is immune to intracellular degradation and is the handiest metabolized extracellularly via way of means of organs with GGT (Fig. 9.3).

Plants without glutathione can't survive. Plants reply to environmental stresses via way of means of regulating metabolic pathways that feature to counteract ensuing cell damage. In plant, life uncovered to temperature extremes, heavy metals-infected soils, drought, and air pollutants. The era of ROS and changes within the side of the intracellular redox surroundings perturb cell physiology (Ogawa 2005). As a part of their reaction to those environmental stresses, plant life produces glutathione, which act as an antioxidant by way of means of quenching ROS species and is worried within side the ascorbate-glutathione cycle that gets rid of per-oxidases (Noctor and Foyer 1998; Rouhier et al. 2008). The primary GSH formation pathway is presented in Fig. 9.4, which is highlighted that Gsh1 and 2 genes play a significant role during abiotic stresses in plants. In the cytosol, the GSH is synthesized by two ATP dependent pathways (Mullineaux and Rausch 2005):

1. L-glutamate + L-cysteine + ATP → γ-glutamyl-L-cysteine + ADP + Pi,
2. γ-glutamyl-L-cysteine + L-glycine + ATP → GSH + ADP + Pi.

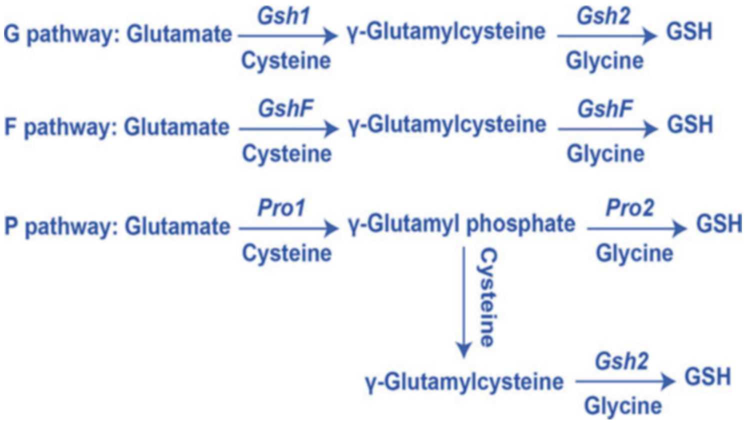

Fig. 9.4 Biosynthetic pathway of glutathione (GSH)

Factors Affecting the Regulation of Glutathione (GSH)

Following are the factors which affect the regulation of glutathione in different ways:
(A) Factors that determine cysteine availability.
This is the most important factor that affects the synthesis of glutathione.

- (a) Diet and amino acid uptake.
- (b) Methionine metabolism and the transsulfuration pathway.
- (c) Role of γ-glutamyltranspeptidase (GGT).
- (d) Concentration of ATP.
- (e) The activity of γ -ECS (associated with chloroplast).

The first step of GSH biosynthesis has taken into consideration the price restricting and catalyzed via way of means of glutamate-cysteine ligase (GCL, previously γ-glutamyl cysteine synthetase), which is famous as an absolute requirement for both Mg^{2+} or Mn^{2+}. GCL consists of a heavy or catalytic (GCLC, Mr. ~ 73,000) and a mild or modifier (GCLM, Mr. ~ 30,000) subunit, which can be encoded via way of means of unique genes in species as divergent as human beings and fruit flies (15–17) (Yan and Meister 1990; Huang et al. 1993a, b; Dalton et al. 2004). The second step in GSH synthesis is catalyzed through GSH synthase (GS). This enzyme has now no longer been studied as notably as GCL. GS purified from rat kidney has a Mr. of about 118,000 daltons, consisting of seemingly equal subunits (Oppenheimer et al. 1979).

(B) Regulation of GCL.

- (a) Regulation of GCL catalytic subunit (GCLC).
- (b) Transcriptional regulation of GCLC.
- (c) Post-transcriptional regulation of GCLC.
- (C) Regulation of GCL modifier subunit (GCLM).
- (D) Regulation of glutathione synthase (GS).

9.4 Antioxidative Function of Glutathione During Heat and Drought-Induced Oxidative Stress in Plants

Glutathione (gamma-glutamyl-cysteinyl-glycine or GSH), a cysteine-containing tripeptide, plays a crucial part to protect the lipids, nucleic acids and proteins from oxidative damage. With thiol-disulfide exchange, GSH controls the protein metabolism and activities in plants. In the case of oxidative stress, glutathione takes part as the main function of protection and detoxification as a cofactor of glutathione peroxidases and glutathione-S-transferases. The interaction between glutathione and components for the antioxidant defense system is synergistic. In recent times, glutathione (GSH) is being studied most to explore its antioxidative attributes. This is mainly because of endogenous synthesization of it in the plant body which creates a high concentration in plants. Many roles of it have been depicted though Fig. 9.5. GSH plays an important role during abiotic stress in plants. It coordinates with AsA turnover and is oxidized to GSSG (Hasanuzzaman et al. 2018). Moreover, some other thiol-dependent enzymes, GPX and GST, use GSH as cofactor, hence, converted to GSSG.

9.4.1 Redox Regulation Through Glutathione

Glutathione can remove the reactive oxygen species (ROS) directly or indirectly like many other metabolites, hence, it can work as an antioxidant. However, the signaling functions and antioxidant activity of the GSH are interdependent as both of these need GSTs and PRXs enzymes which can reduce hydrogen peroxide or other organic peroxides through thiol-mediated pathways (Noctor et al. 2012a, b). The unique antioxidative and signaling mechanism of GSH is believed to be its omnipresent allotment in plant cells and low redox potential.

9.4.2 Glutathione Redox State

Glutathione reductase (GR) activities have been observed in many parts of the plants viz., cytosol, peroxisomes, chloroplasts, and mitochondria (Stevens et al. 2000; Romero-Puertas et al. 2006). GR1 gene is majorly responsible for enzymatic activities in leaves (Mhamdi et al. 2010a, b). Mitochondria and plastid-related enzymes are regulated by GR2 gene (Chew et al. 2003). GSH can play an important role in affecting the role of ROS production and/or ROS removal under oxidative stress conditions (Gomez et al. 2004). Many previous studies reported a strong link between the high accumulation of hydrogen peroxide and changes in glutathione redox state (Chaouch et al. 2010). Under oxidative conditions, glutathione disulfide (GSSG, the oxidized form of GSH) accumulation may be explained as the net result of oxidation processes that overcome, even if only slightly, the capability of glutathione reduction (Noctor et al. 2013; Fig. 9.6).

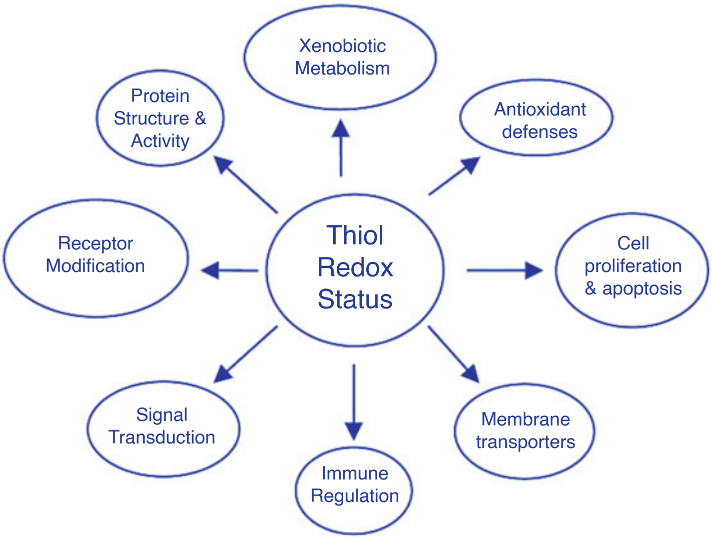

Fig. 9.5 Antioxidative roles of GSH

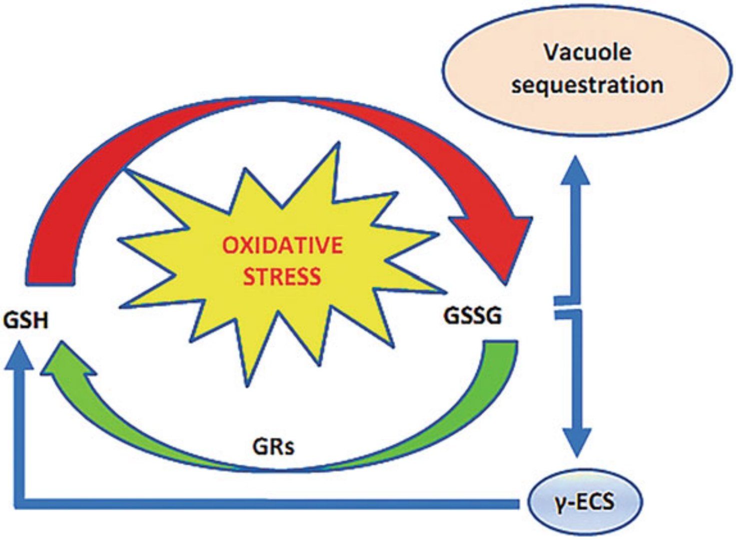

Fig. 9.6 Changes in glutathione content and redox state under oxidative stress

In cases of oxidative stress, GR1 gene activity is the most important (Mhamdi et al. 2010a, b). In stress conditions, production of GSSG does not decrease the GSH pool; rather, it has been found that the total GSH pool has been increased due to GSSG accumulation (Mhamdi et al. 2010a, b). Such phenomenon may be attributed to the new synthesis of GSH and compartmentalization of GSSG (Noctor et al.

2012a, b). Cysteine and GSH production are activated under oxidative stress as a result of increment in GSH biosynthesis (Gromes et al. 2008a, b; Queval et al. 2009). In addition, a considerable quantity of the GSSG generated by oxidative stress can be accumulated in the vacuole (Queval et al. 2011a, b) by ABCC transporters (Lu et al. 1998a, b).

9.5 Glutathione Transporters in Plants During Heat and Drought-Induced Oxidative Stress

Being the primary producers of organic sulfur, glutathione transporters are irreplaceable in the sulfur cycle of plants. Glutathione is one of the major representatives of reduced sulfur in plants and other organisms (Leustek et al. 2000; Noctor et al. 2002). It is a tripeptide (γ-glutamyl-cysteinyl Gly), an important metabolite for plant growth and development. Glutathione synthesized in the cytoplasm and chloroplasts of plant cells as a result of the activity of γ-glutamyl Cys synthetase and glutathione synthetase enzymes has multiple functions. These include transport and storage of reduced sulfur, control of sulfur assimilation, protection against biotic and abiotic stresses, participation in protein folding and cell cycle, antioxidant function against oxidative stress, biosynthesis of iron-sulfur proteins in mitochondria in the biosynthesis of iron-sulfur proteins, redox signaling, flowering, apoptosis in detoxification of metals, and xenobiotics (Rodriguez-Manzaneque et al. 2002; Kumar et al. 2011; Owens and Hartman 1986; Foyer et al. 2001).

9.5.1 Biosynthesis and Subcellular Distribution of Glutathione

The synthesis of glutathione in plants occurs mainly in the cytoplasm, partly in chloroplasts (Wachter et al. 2005a, b). Glutathione is synthesized in the cytoplasm from glutamate, cysteine, and glycine by two ATP-dependent enzymes - γ-glutamyl cysteine synthetase (GSH1) and glutathione synthetase (GSH2). These enzymes were found in the chloroplast and cytoplasm of Arabidopsis (Foyer et al. 2001). Although glutathione is mainly stored in the cytoplasm, it is required also in other cell organelles: mitochondria, nuclei, endoplasmic reticulum, and vacuoles. The amount of glutathione in cell organelles determined by various biochemical analysis methods amounted to 0.5-5 mM in chloroplasts, 1–3.52 mM in the cytoplasm, and about 0.73 mM in the vacuoles (Krueger et al. 2009; Foyer and Halliwell 1976; Noctor et al. 2002).

Glutathione is a form of reduced sulfur transported for long distances by phloem and xylem tubes (Herschbach et al. 2000). Glutathione transporters are located in the membranes of organelles and realize the export and import of glutathione. Accumulation of glutathione in the nucleus is an indication of its important role in the proliferation and cycle of the cell. Ions and other small molecules, including glutathione, penetrate into the nucleus through the pores (Bellomo et al. 1997). The location of ATP-dependent glutathione carriers in the nucleus was identified in studies (Ho and Guenthner 1994). The antiapoptotic factor, Bcl-2, was shown to be responsible for the formation of pores in the nucleus, and these pores provide the diffusion of glutathione into the nucleus (Voehringer et al. 1998; Markovic et al. 2007). Bcl-2 factor is also responsible for the transport of glutathione to the mitochondria.

The endoplasmic reticulum is considered to be a more oxidizing medium than the cytoplasm. Studies showed that both reduced and oxidized forms of glutathione are transported across the ER membrane at different rates (Banhegyi et al. 1999). Disorders in glutathione homeostasis can strongly affect the function of the endoplasmic reticulum. Glutathione transporters of the endoplasmic reticulum in plants have not yet been studied at the molecular level. Chloroplasts carry out their own glutathione biosynthesis. Chloroplasts also can uptake glutathione from the cytoplasm. According to previous genetic studies, selective blocking of glutathione biosynthesis in chloroplasts does not affect plant development. This means that chloroplasts meet their own need for glutathione (Zaman et al. 1995). Further biochemical research found the presence of high and low affinity transporter systems that carry glutathione to the chloroplasts. This transport does not depend on light or ATP but can be inhibited by glutathione disulfide and activated by hydrogen peroxide (Noctor et al. 2000; Pasternak et al. 2008a, b). The molecular identification of these transporters has not been performed yet. CRT-like (CLTs) transporters carry glutathione from chloroplasts to the cytoplasm.

9.5.2 Plant Glutathione Transporters

Glutathione transporters have been studied for many years, and the available data is mainly on the biochemical characteristics of glutathione transport (Griffith and Meister 1979; Iantomasi et al. 1997; Banhegyi et al. 1999). However, there is little information at the molecular level. The first successful identification of a glutathione transporter was in yeast (Bourbouloux et al. 2000). The first identified glutathione transporter was a multidrug resistance-associated protein (MRP) belonging to the ATP-Binding Cassette (ABC) transporter family. This transporter is called HGT1 and transports GSH, GSSG, and the glutathione-N-ethylmaleimide conjugate (GS-NEM). This transporter was shown to be involved in the removal of glutathione from the vacuolar membrane in yeasts (Rebbeor et al. 1993).

In Arabidopsis, the transport of glutathione to the nucleus was identified during cell proliferation (Vivancos et al. 2010). The existence of low and high affinity glutathione transport systems was established using biochemical analyses (Noctor et al. 2012a, b; Queval et al. 2011a, b). Absorption of oxidized glutathione by the isolated vacuole in barley occurs rapidly. In contrast, the absorption of reduced glutathione by the vacuole is very weak. The transport of glutathione in the vacuole depends on ATP and is carried out by special ATPases (Tommasini et al. 1993; Martinoia et al. 1993).

Chloroquine resistance transporters (CRT-like) have been identified to transport glutathione from chloroplasts to the cytosol in plants (Maughan et al. 2010). Three transporters- CLT 1, CLT 2, and CLT 3 belonging to the family of CRT-like transporters were studied in Arabidopsis. In Arabidopsis, CLT1 was first cloned with complementation for the severely glutathione-deficient glutathione1 mutant for BSO resistance. Expression of *AtCLT1* in *Xenopus oocytes* resulted in a 3-four-fold increase in intracellular glutathione levels. Expression of all three genes (*AtCLT1, AtCLT2, AtCLT3*) also led to the accumulation of glutathione in *Xenopus oocytes*. *AtCLT* genes located in plastids are responsible for the transport of glutathione from chloroplasts to the cytoplasm. Mutations in the *AtCLT1* and *AtCLT3* genes have led to a decrease in the amount of glutathione in the cytoplasm as well as an increase in the sensitivity of the organism to microbial infections (Maughan et al. 2010). The amount of glutathione in the roots of mutants also decreased compared to wild species (Maughan et al. 2010).

The OsCLT1 gene has been identified in an arsenate-sensitive (As) mutant of the rice plant (Murugaiyan et al. 2021). Encoding CRT-like transporters, this gene is located in the membrane of plastids. The molecular mass and protein length of OsGT1 are 86 kD and 766 amino acids, respectively. OsCLT1 plays an important role in glutathione homeostasis, probably by mediating the export of γ-EC and glutathione from plastids to the cytosol. The OsGT1 transporter is also capable of transporting GSH, GS conjugates, oxidized glutathione, and some peptides. These indicate that OsGT1 has a wide range of substrate specifications (Zhang et al. 2004). There are at least 3 homologs of *OsGT1* in the rice genome. Expression of *OsGT1* in yeast has also led to the assimilation of exogenous glutathione. The weak expression of OsGT1 in different parts of the rice plant grown under normal conditions indicates that it does not play a significant role in the transport of sulfur under normal conditions but OsGT1 is strongly expressed during biotic or abiotic stresses, especially during drought and heat stress. OsGT 1 stimulates the growth of the yeast *hgt1* mutant in a glutathione environment used as a source of sulfur. The OsGT1 transporter, expressed in the yeast strain, is responsible not only for the absorption of glutathione but also for amino acids and peptides.

The *AtOPT4* transporter gene found in Arabidopsis is defective in methionine (MET) synthesis and glutathione uptake (Zhang et al. 2016). Studies have revealed that the *ATOPT4* gene is expressed in the epidermal cells of onions and that the amount of glutathione in the leaves, roots, and stems of the mutant species does not differ significantly from the wild type. The low content of glutathione in *atopt2/atopt4* silique mutants suggests that they play a role in glutathione transport. The expression of the *AtOPT4* gene in the Rosette plant is responsible for the long distance transport of glutathione (Stacey et al. 2006). Thus, it realizes the transport of glutathione to various tissues of the plant. *AtOPT4* is both a low affinity and a high affinity oligopeptide transporter. Thus, *AtOPT4* encodes a low affinity plasma membrane glutathione transporter, which contributes to glutathione loading/unloading in siliques. In yeast strain, *AtOPT4* could also transport oligopeptides such as KLGL, GGFM, YGGFM, and IIGLM (Osawa et al. 2006).

AtOPT6 is an oligopeptide transporter similar to AtOPT4 (Cagnac et al. 2004; Pike et al. 2009). AtOPT6 in hgt1 yeast mutant can restore growth in a medium containing reduced glutathione as the sole sulfur source. AtOPT6 can also transport

Cd, Cd/GSH conjugate, and glutathione-*N*-ethylmaleimide conjugate (Cagnac et al. 2004). But in *Xenopus laevis oocytes,* AtOPT6 is a low affinity transporter, responsible only for the transport of glutathione (Pike et al. 2009). *AtOPT6* could also transport plant signaling peptides, including the amino acid conjugate jasmonate-isoleucine, the active form of the oxylipin signal jasmonic acid, and the sulfated pentapeptide hormone phytosulfokine. The expression of the *AtOPT6* gene is strongly induced by the herbicide primisulfuron and to a lesser extent by the effect of abscisic acid. However, Cd does not affect the induction of this gene expression (Cagnac et al. 2004). AtOPT6 is a transporter that allows glutathione to be transported to the phloem cell or various parts of the plant. AtOPT6 is located in the plasma membrane of specific cells and conducts a long distance transport of glutathione. The transport of peptides by AtOPT6 also promotes plant development as well as plant resistance to various pathogens and pests. In addition to the above, AtOPT6 also plays a role in plant signaling (Pike et al. 2009).

The BjGT1 gene from the mustard plant (*Brassica juncea*) was cloned and found to be homologous to the high affinity HGT1 glutathione transporter in *Saccharomyces cerevisiae* (Bogs et al. 2003). The molecular mass and protein length of BjGT1 are 74 kD and 661 amino acids, while for HGT 1, these parameters are 91 kD and 799 amino acids. *BjGT1* is highly expressed in leaves, very little in stems, not expressed in roots, and it is strongly induced by Cd in leaves and stems.

The *ZmGT1* transporter cloned from maize (Zea mays) is homologous to transporters of different plants. Expression of the *ZmGT1* gene is strongly induced by atrazine, and the level of transcripts increases 4-5 times. The strong induction of ZmGT1 by atrazine suggests that this transporter is involved in the detoxification of xenobiotics (Pang et al. 2010, 2012).

AtMRP1 and AtMRP2 transporters identified in Arabidopsis carry GS conjugates. AtMRP1 is structurally and functionally homologous to human multidrug resistance-associated protein (HmMRP1). It has been studied for its ability to generate GS conjugate pump activity in a transfected cell. In parallel, AtMRP2 transports the chlorophyll catabolite carrying substrate (*Bn*-NCC-1) in *Brassica napus* (Lu et al. 1998a, b). In plants, the expression profiles of these two genes differ. AtMRP2 is responsible for the transport of glutathionated herbicides and anthocyanins. AtMRP 2 has several times higher transport activity than AtMRP1 and also conducts Bn-NCC-1 transport (Lu et al. 1998a, b).

9.6 Conclusion

From the above discussion of the chapter, it may be concluded that plants are facing the adverse effect of environmental stresses and induced oxidative stress as a consequence of the excessive production of reactive oxygen species (ROS), although tolerant plants could survive against abiotic stresses by the production of various antioxidants. Among them, glutathione (GSH), a non-enzymatic antioxidant, play a significant role to protect plants from oxidative damages. It is involved in the detoxifying the ROS in plant cells through detoxification of methylglyoxal, formation of phytochelatins, interacts with plant hormones, other signaling molecules and its redox state triggers signal transduction. The GSH also acts as a cofactor in various plants' biochemical reactions and measures as an adaptable redox molecule that involve in plant development both under stress and normal conditions. The knowledge of the involvement of GSH during abiotic stresses will be helpful for the sustainability of crop production in the modern era of climate change.

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Role of Tocopherol in Conferring Abiotic Stress Tolerance in Plants

10

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Abstract

Abiotic stressors have a significant impact on plant growth, productivity, and survival, as well as posing a threat to world food security and supply. Various mechanisms linked with abiotic stress cause plant cells to create oxygen radicals and their derivatives, known as reactive oxygen species (ROS). Tocopherol production is thought to be one of the strategies that allow plants to adapt to their constantly changing environment. Tocopherols are lipophilic antioxidants that can be made by photosynthetic organisms. By scavenging lipid peroxyl radicals in thylakoid membranes, this antioxidant deactivates photosynthesis-derived reactive oxygen species (mainly $^1O_2$ and OH^-) and stops the spread of lipid peroxidation. Tocopherol levels vary based on the severity of the stress and the species' sensitivity to it. As a result of the changed expression of pathway-related genes, degradation, and recycling, changes in tocopherol levels are widely thought to contribute to plant stress tolerance, while reduced levels favor oxidative damage. Understanding how plants understand environmental signals and convey them to cellular machinery to trigger adaptive responses is critical for crop improvement initiatives aiming to produce stress-tolerant crop varieties. Tocopherols accumulate in plants and have strong connections with abiotic stress tolerance, according to a huge number of studies undertaken in the previous few decades. The potential roles and mechanisms of tocopherols, as well as their link

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to abiotic stress tolerance in plants, are discussed in this chapter. This chapter may aid readers in learning more about tocopherols and how they act in different environments, as well as gaining an understanding of how this knowledge can be used to promote abiotic stress tolerance in plants.

Keywords

α-Tocopherol · Abiotic stress tolerance · ROS · Vitamin E

10.1 Introduction

As a result of global climate change, abiotic stressors on plants have become more common and strong (Ghosh et al. 2021; Surabhi 2018) and can cause cellular and developmental processes in plants to be disrupted throughout their lives (Kerchev et al. 2020; Mohammadi et al. 2020). Plants produce a high amount of reactive oxygen species in response to numerous abiotic stimuli (such as drought, salt, waterlogging, ultraviolet radiation, temperature, and heavy metal) and induce lipid peroxidation (Arif et al. 2016; Singh et al. 2017). Plants have evolved intricate and well-organized strategies to cope with various abiotic stressors and protect themselves (Munns and Tester 2008). Tocopherol biosynthesis and accumulation are regarded as one of the essential host plant responses to oxidative stress caused by diverse abiotic stressors (Semchuk et al. 2009; Lushchak and Semchuk 2012). Only photosynthetic species, such as plants, algae, and most cyanobacteria, generate tocopherols, lipid-soluble antioxidants (Maeda and DellaPenna 2007; Quadrana et al. 2013). Tocopherol levels in plant tissue range from extremely low to extremely high in leaves and seeds (Munné-Bosch and Alegre 2002), and its makeup varies between species and within a species tissues (Badrhadad et al. 2013).

There are four types of tocopherols: alpha (α), beta (β), gamma (γ), and delta (δ). Tocopherol has proven to be a ubiquitous component of all higher plants when it comes to antioxidants (Kamal-Eldin and Appelqvist 1996; Mene-Saffrane and Pellaud 2017). The most prevalent tocopherol is α-tocopherol, which plays an important function in plants (Hirschberg 1999). Increased activity of antioxidative enzymes (Superoxide Dismutase, Peroxidase, and Catalase) and the concentration of non-enzymatic antioxidants with enhanced water relations are two ways α-tocopherol aids membrane protection (Ali et al. 2020). Normally, α-tocopherol accumulates in leaves and flowers, but seeds are high in γ-tocopherol (Szymańska and Kruk 2008; Velasco et al. 2013). Most plant species are deficient in β- and δ-tocopherols. γ-Tocopherol methyltransferase (*γ-TMT*, *VTE4*) in the envelope of plastids synthesizes alpha-tocopherol from γ-tocopherol in chloroplasts and stores it in plastoglobuli of the chloroplast stroma and thylakoid membranes (Gill and Tuteja 2010; Szarka et al. 2012). Their antioxidant function includes the prevention of membrane lipid peroxidation as well as the scavenging of reactive oxygen species and lipid peroxy radicals (Kruk et al. 2005; Kruk and Trebst 2008). PSII is protected by tocopherol against oxygen singlets (Krieger-Liszkay and Trebst 2006; Munné-Bosch 2005). Other roles of tocopherol in plant metabolism include sugar transfer from leaves to the phloem (Soltani et al. 2012). Tocopherols play an important function in redox homeostasis. Tocopherols, which are non-enzymatic antioxidant systems, are the most important preserved mechanism in plants that provides stress tolerance.

Tocopherol biosynthesis has been explored in-depth in a variety of agricultural and plant species (Hussain et al. 2013; Semchuk et al. 2009; Lushchak and Semchuk 2012). Tocopherol overproduction is caused by a variety of stress signaling mechanisms. Tocopherol expresses abiotic stress signals via engaging in a signal cascade, allowing for greater development and stress tolerance (Sattler et al. 2004). Many studies have shown that plants with higher levels of tocopherol have better abiotic stress tolerance (Semchuk et al. 2011; Munné-Bosch 2005). There is a link between endogenous tocopherol and abiotic stress tolerance in plants. Exogenous tocopherol supplementation has been linked to abiotic stress tolerance in numerous investigations (Ellouzi et al. 2013; Espinoza et al. 2013). Furthermore, numerous plants have been metabolically altered for induced tocopherol production, and such transgenic plants have shown improved survival and tolerance to diverse abiotic challenges (Abbasi et al. 2007; Hofius et al. 2004; Yusuf et al. 2010).

The current chapter gathers information related to biosynthesis and the prospective roles and mechanisms of tocopherols, as well as their association with abiotic stress tolerance in plants, based on the background issues and opportunities.

10.2 Types and Chemical Nature of Tocopherol

Tocopherol is a chemical compound that is related to vitamin E. Tocotrienols and tocopherols, both of which have a six-chromanol ring head and an isoprenoid side chain, make up the vitamin E family. Tocopherol is of four different forms that occur in alpha (α) containing three methyl groups, beta (β) containing two methyl group, gamma (γ) containing two methyl group, and delta (δ) containing one methyl group form (Fritsche et al. 2017; Shahidi and de Camargo 2016). The differences among α-, β-, γ-, and δ-tocopherol are only due to the position and number of the methyl (–CH_3) substitution(s) on the aromatic (chromanol) ring (Ajjawi and Shintani 2004). Thus, the vitamin E family comprises eight stereoisomers (tocopherols and tocotrienols), but only the RRR-form is found in nature. The most frequent kind of vitamin E discovered in tissues is γ-tocopherol, which has been the subject of more than 95% of investigations (Sen et al. 2007). γ-Tocopherol has been demonstrated to protect polyunsaturated fatty acids (PUFAs) against oxidation in oil-storing seeds like Arabidopsis, extending seed longevity. This suggests that γ-tocopherol plays a role in seed desiccation tolerance (Sattler et al. 2004). Tocopherols are exclusively generated in photosynthetic organisms such as algae and all higher plants, and they are ubiquitous elements of them (Hussain et al. 2013). Tocopherols' antioxidant properties are linked to the transfer of phenolic hydrogen to oxidative free radicals. Of different types of tocopherols, α-tocopherol is considered to be a potential antioxidant, whereas β- and γ-tocopherols have moderate potential as antioxidants

Table 10.1 Different types of tocopherols and their molecular formula

Types of tocopherol	Systematic names given by IUPAC (International Union of Pure and Applied Chemistry)	Molecular formula of tocopherols
Alpha (α) tocopherol	(2R)-2,5,7,8-tetramethyl-2-[(4R,8R)-(4,8,12-trimethyltridecyl)]-6-chromanol	$C_{29}H_{50}O_2$
Beta (β) tocopherol	(2R)-2,5,8-trimethyl-2-[(4R,8R)-(4,8,12-trimethyltridecyl)]-3,4 dihydrochromen-6-ol	$C_{28}H_{48}O_2$
Gamma (γ) tocopherol	(2R)-2,7,8-trimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-6-chromanol	$C_{28}H_{48}O_2$
Delta (δ) tocopherol	(2R)-2,8-dimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-6-chromanol	$C_{27}H_{46}O_2$

and δ-tocopherol has the lowest (Kapoor et al. 2015). Different types of tocopherols with their systematic names and molecular formula are shown in Table 10.1.

10.3 Biosynthesis of Tocopherol

The biosynthesis pathway of tocopherols in photosynthetic organisms was discovered in the mid-1980s by radio-trace research (Grusak and DellaPenna 1999). Tocopherol biosynthesis involves two metabolic pathways: the first produces the aromatic ring of the tocopherol molecule using homogentisic acid via the cytosolic shikimate pathway, and the second produces the tocopherol tail (phytyl diphosphate) via the plastid methylerythritol phosphate pathway. Tocopherols are formed when the aromatic ring and the phytyl diphosphate tail come together (Herrmann and Weaver 1999).

10.3.1 The Shikimate Pathway Leading to Homogentisate Formation

This metabolic pathway has been studied in microorganisms as well as higher plants. The shikimate pathway is involved in the synthesis of a variety of aromatic amino acids as well as precursors for a variety of secondary metabolites such as vitamins and pigments (Herrmann and Weaver 1999). Phosphoenolpyruvic acid, a glycolytic cycle molecule, and erythrose-4-phosphate, a transitional compound, are converted into chorismate in this process. The enzyme 5-enolpyruvylshikimate 3-phosphate synthase is responsible for the reversible production of 5-enolpyruvylshikimate 3-phosphate and phosphoenolpyruvate (Velíšek and Cejpek 2011) and inhibited by glyphosate (Lushchak and Semchuk 2012). Chorismate is a precursor for several metabolites, including vitamin K, tocopherols, and aromatic amino acids, and is formed as the final step in the shikimate pathway (Lushchak and Semchuk 2012). Chorismate is converted to prephenate by the chorismate mutase enzyme, which leads to two distinct processes that result in the synthesis of phenylalanine and tyrosine. HPP (*p*-hydroxyphenyl pyruvate) is a step in the production of tocopherols

(Lushchak and Semchuk 2012). The interaction of arogenate with tyrosine in plants results in the synthesis of HPP. To make p-hydroxyphenyl pyruvate and homogentisate, a precursor of tocochromanol, some fixed carbon is added to tyrosine (Rippert et al. 2004; Sterkel and Oliveira 2017).

10.3.2 Tocopherol Phytyl Tail Synthesis

The action of 1-deoxy-d-xylulose 5-phosphate (DOXP) synthase condenses glyceraldehyde-3-phosphate and pyruvate into 1-deoxy-d-xylulose-5-phosphate in the first stage of the methylerythritol pathway (Lushchak and Semchuk 2012). DOXP is transformed into isopentyl diphosphate by the catalysis of five enzymes in a row (IPP). Four molecules of IPP are bonded together to generate C-20 geranylgeranyl diphosphate (GGDP) (Munné-Bosch and Alegre 2002). GG reductase converts geranylgeranyl diphosphate to phytyl diphosphate during multistep processes in chloroplast membranes. Chlorophyllase degrades chlorophyll during leaf senescence, resulting in the production of free phytyl. This could be a forerunner of tocopherols (Valentin et al. 2005).

10.3.3 Compression of Phytyl Diphosphate and Homogentisate

2-Methyl-6-phytyl 1,4-benzoquinone is produced via the reaction of homogentisic acid with phytyl diphosphate, which is catalyzed by homogentisate phytyl transferase (HPT) and methyl-phytyl benzoquinol (MPBQ). It's a crucial step in the production of tocopherols. MPBQ methyltransferase catalyzes the formation of 2,3-dimethyl-6 phytyl-1,4-benzoquinone (DMPBQ) from 2-methyl-6 phytyl-1,4-benzoquinone (MPBQ). Tocopherol cyclase, which produces γ-tocopherol, catalyzes the DMPBQ. α-Tocopherol is formed when α-tocopherol is methylated by the enzyme γ-TMT (γ-tocopherol methyltransferase) (Sadiq et al. 2019).

10.4 Tocopherol Acts as an Antioxidant Molecule

Tocopherol is a lipid-soluble, nonenzymatic antioxidant. It (mostly α-tocopherol) considerably reduces ROS production (mostly $^1O_2$ and $OH^{\cdot}$) in collaboration with other antioxidants (e.g., ascorbic acid). Tocopherol biosynthesis increased in stressful situations, offering better protection against oxidative stress by lowering ROS generation (Shao et al. 2008; Semchuk et al. 2009; Lushchak and Semchuk 2012).

Tocopherol is abundant in the thylakoid membrane of chloroplasts, which is also a major source of reactive oxygen species (ROS). Because both ROS formation and tocopherol manufacturing take place in the same place, it's likely that the presence of α-tocopherol in chloroplast membranes is linked to tocopherol's ability to scavenge ROS such as $^1O_2$ and $LOO^{\cdot}$ and protect the photosynthetic apparatus from oxidative stress (Munné-Bosch 2007; Lushchak and Semchuk 2012). In addition to generating tocopherol, the enzymes MPBQ and DMPBQ displayed antioxidant activity by donating two electrons (Liebler and Burr 2000). Tocopherol's main antioxidant role is to keep the membrane structure of polyunsaturated fatty acyl chain reactions stable (Sattler et al. 2003). Tocopherols work in tandem with other antioxidants like glutathione and ascorbate to keep plastid redox balance in stressed plants, Munné-Bosch (2005) discovered. In Arabidopsis thaliana plants, degradation of *α*-tocopherol and glutathione had a deleterious impact on chloroplast integrity and functionality (Drobot 2013). Antioxidant levels increased by a factor of ten in *Arabidopsis* plants exposed to strong light (Kanwischer et al. 2005). In *Phillyrea angustifolia* plants exposed to water shortage, powerful coordination of *α*-tocopherol and salicylic acid has been discovered (Szarka et al. 2012).

10.5 Tocopherol Contribution Towards Abiotic Stress Tolerance

Tocopherol is an antioxidant that aids in the resistance of plants to abiotic stress. Tocopherol-induced stress protection is influenced by plant species, stress intensity, and physiological condition (Kapoor et al. 2015; Li et al. 2008; Munné-Bosch 2005). Stress tolerant plants have higher levels of tocopherol, whereas sensitive plants have lower levels of tocopherol under stressful conditions, resulting in oxidative damage, according to various researches (Smirnoff 1993; Munné-Bosch 2005; Munné-Bosch and Alegre 2002). Munné-Bosch (2005) argues that the levels of α-tocopherol in stressed plants are controlled in two stages. In the first phase, *α*-tocopherol is produced to scavenge ROS, resulting in increased protection by preventing oxidative damage. Net tocopherol loss occurs in the second phase as a result of extreme stress produced by tocopherol degradation exceeding its synthesis. If a *α*-tocopherol shortage is not corrected through rapid synthesis or exogenous tocopherol supplementation, lipid peroxidation increases, and cell death occurs. Abiotic stress-tolerant species go through the first degradation phase, while stress-sensitive species go through the second (Munné-Bosch 2005; Das and Roychoudhury 2014). Under such unfavorable conditions, plants combat oxidative stress by producing low and high-mass antioxidants (Lushchak 2011). Figure 10.1 depicts how tocopherols scavenge reactive oxygen species (ROS) and achieve abiotic stress tolerance.

10.6 Tocopherol's Mode of Action in Changing Environment

10.6.1 Drought/Water Deficit Stress

Drought affects a lot of crops all around the world (Ghosh et al. 2015, 2018; Mishra et al. 2021). Many plant species, such as *Arabidopsis* and tobacco (Liu et al. 2008), sage balm (*Salvia officinalis*), and lemon balm (*Melissa officinalis*) (Munné-Bosch and Alegre 2003), create a large amount of *α*-tocopherol when they are exposed to a lack of water. The intrinsic levels of tocopherols in most species are thought to be insufficient to adequately counteract the negative effects of drought stress (Bose

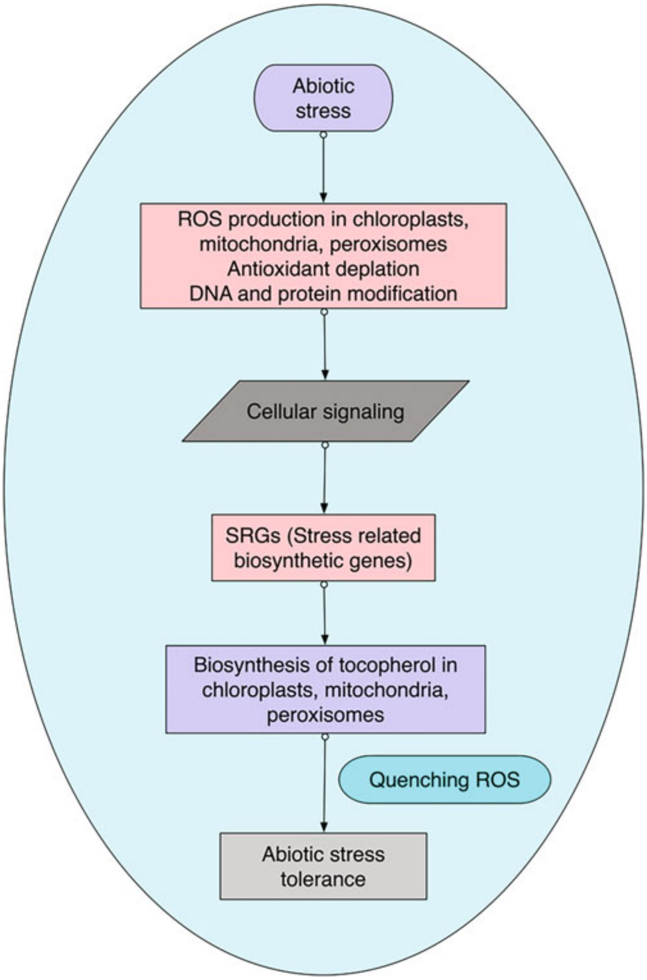

Fig. 10.1 The schematic illustration shows the role of tocopherols in the physiological process of plant adaptation that regulates tolerance to abiotic stress. Upon insight of abiotic stress signal, the associated signaling pathway is triggered, resulting in the activation of stress-responsive genes related to biosynthesis and accumulation of tocopherol, which protects and renders plants tolerant to abiotic stresses by quenching ROS

et al. 2014). As a result, some researchers have advocated for the exogenous delivery of tocopherols to drought-stressed plants, either as a foliar spray or as a seed pre-treatment (Jie et al. 2008; Mekki et al. 2015). In an experiment, the influence of seed priming with varying levels (50 and 100 mg L^{-1}) of α-tocopherol was investigated in carrot plants under water deficit conditions, and it is suggested that seed priming with 100 mg L^{-1} α-tocopherol was effective and proved beneficial in improving plant growth characteristics, osmoprotectants, and the oxidative defense system of carrot plants in such environment (Hameed et al. 2021). Foliar-applied

α-tocopherol improved maize cultivar growth, which was linked to improvements in photosynthetic pigment, water relations, antioxidative mechanism, and better nutrient acquisition in root and shoot, as well as tocopherol contents and a reduction in lipid peroxidation, resulting in maize cultivars with increased drought tolerance (Ali et al. 2020). Ali et al. (2019) reported that exogenously administered α-tocopherol could help wheat cope with the negative effects of heat and water stress. It's possible that the exogenous application of α-tocopherol improves internal levels because of its role in plant metabolic regulation (Jamil et al. 2015; Noman et al. 2018). Under stressed and non-stressed conditions, foliar sprays of α-tocopherol significantly increased the plant's endogenous levels and led to significant growth enhancement. Under the impact of α-tocopherol foliar application, increases in plant biomass production are favorably associated with improvements in plant water relations and the manufacture of biosynthetic pigments such as chlorophyll and carotenoids. The increase in plant water status could be attributed to α-tocopherol's effect on the H-ATPase system, which plays a role in cellular osmotic adjustment as an essential component of cellular membranes. Because of its involvement in cellular osmotic adjustment, α-tocopherol plays an important role in preserving cellular water relations under stressful conditions (Ali et al. 2020). Foliar application of α-tocopherol dramatically enhanced the leaf water content of water-stressed plants, confirming its drought-protective effect. This could be owing to its role in cellular turgor potential management, as well as its role in cellular osmotic adjustment by improving osmolyte production, resulting in improved growth by establishing an environment conducive to enhanced growth (Um et al. 2020).

10.6.2 Salinity

Salt stress is one of the major restrictions to agricultural productivity, impacting at least 20% of the world's arable land (Rizwan et al. 2015; Kumar et al. 2020). Salinity causes osmotic and ionic (high Na^+/K^+ ratio) stressors in lants, resulting in water deficits, phytotoxicity, and nutritional discrepancies (Safdar et al. 2019; Van Zelm et al. 2020). It has been discovered that using 100 mg L^{-1} tocopherol as a commercial formulation will help soybean plants grow and produce more when they are irrigated with moderately saline water (EC < 6.25 dS m^{-1}) (Mostafa et al. 2015). Exogenous administration of α-tocopherol reduced the negative effects of salt stress in flax cultivars, according to another study (Sh 2014). Seed soaking with the α-tocopherol enhanced shoot and root dry weight, 100 achene weight and total achene weight plant^{-1}, catalase and peroxidase activity, total phenolic content, ascorbic acid, and α-tocopherol pre-sowing seed treatment reduced lipid peroxidation in sunflowers (*Helianthus annuus* L.) (Lalarukh and Shahbaz 2020). α-Tocopherol seed treatment reduced the toxicity of salt (NaCl) stress by enhancing antioxidant activity, particularly catalase and peroxidase, which may be responsible for lowering reactive oxygen species levels. Catalases and peroxidases, which are found mostly in peroxisomes and apoplasts, detoxify H_2O_2 by converting it to oxygen and water (Mittler 2002; Fagerstedt et al. 2010) and provide phenols to protect the plant from oxidative damage by removing reactive oxygen species (ROS) (Zabalza et al. 2007). Catalase and peroxidase activity in faba beans and cotton (Orabi and Abdelhamid 2016; Hussien et al. 2015), as well as catalase and peroxidase activity in wheat and eggplant (Farouk 2011; Shaheen et al. 2013), have all been found to play an important role in protecting plants from oxidative damage.

10.6.3 Flooding/Waterlogged Condition

Plants' regular growth, physiological functioning, and productivity are severely hampered by flooding (Bailey-Serres et al. 2010; Feng et al. 2020). Waterlogging causes hypoxic conditions around the root system of plants, resulting in the production of ethanol and CO_2, affecting a variety of factors such as water and nutrient uptake, carbohydrate mobilization, ROS metabolism, production of superoxide radicals, hydrogen peroxide, and hydroxyl radical, as well as membrane lipid peroxidation, resulting in mechanical damage to the water-stressed part of the plant (Arbona et al. 2017). Sadiq et al. (2016) found that a foliar spray of α-tocopherol boosted plant growth and antioxidant enzyme activity, resulting in increased stress tolerance.

Under anoxia, the content of tocopherols in wheat and rice roots differed, as the tocopherol content of wheat roots increased rapidly at the start of anoxia but decreased over the next two days, whereas the tocopherol content of rice roots increased slightly only at the end of the 7-day incubation both under anoxia and aeration (Chirkova et al. 1998). According to Lin et al. (2004), short-term flooding stress studies revealed no significant changes in the level of α-tocopherol in the roots of many tomato and eggplant cultivars. Instead, incubation of the rhizomes of *Iris germanica* and *Iris pseudacorus* under anoxia led to significant decreases in both α- and β-tocopherol contents only under prolonged anoxia incubations, i.e., 45 and 12 days for the rhizomes of *Iris pseudacorus* and *Iris germanica*, respectively.

There are only a few investigations on the tocopherol pool's reactions to plant oxygen deprivation stress (Chirkova et al. 1998; Paradiso et al. 2016; Lin et al. 2004). Nonetheless, as the amount of tocopherols has been demonstrated to rise in wheat roots under low oxygen stress, the data show a wide range of reactions in the tocopherol pool of the species tested (Chirkova et al. 1998) and in *Arabidopsis* suspension cell cultures (Paradiso et al. 2016), but a significant decrease in the tocopherol content was observed both in the rhizomes of *Iris pseudacorus* and *Iris germanica* and in shoots of wheat seedlings (Chirkova et al. 1998). Furthermore, no significant changes in the content of tocopherols in tomato and eggplant cultivar roots were identified during flooding stress trials (Lin et al. 2004) and in roots of rice seedlings under anoxia (Chirkova et al. 1998). The diversity of reactions may be influenced by factors such as the plant species or organ utilized in the experiment, but the experimental setup may also have an impact. The duration of anoxia tests in most investigations was short, lasting only a few hours (Paradiso et al. 2016) or three to seven days (Lin et al. 2004; Chirkova et al. 1998), while the anoxic incubations of *Iris germanica* and *Iris pseudacorus* continued up to 12 days and 45 days, respectively. Instantaneous flooding might apparently increase α-tocopherol content in tuberous roots of sweet potato (*Ipomoea batatas* L.) (Eguchi et al. 2015).

10.6.4 Heavy Metals

Toxic metals can change biochemical and physiological processes, such as oxidative stress (Sharma et al. 2020; Lee et al. 2019). In various investigations, tocopherol levels were found to be higher when subjects were subjected to metal stress (Artetxe et al. 2002; Gajewska and Skłodowska 2007; Ruciska-Sobkowiak and Pukacki 2006; Yusuf et al. 2010). Cu and Cd ions increased tocopherol levels in *Arabidopsis thaliana* by six- and fivefold, respectively, when the plant was exposed to them (Collin et al. 2008). Vitamin E-deficient mutant showed sensitivity to Cd^{2+} and Cu^{2+} (75 μM), whereas the wild type displayed better growth traits (Collin et al. 2008). The buildup of tocopherol increased in carrot (*Daucus carota*) plants exposed to Cd stress (36 mM) for 2, 4, 7, and 14 days, according to a study (di Toppi et al. 2012). In wheat seedlings, 50- and 100-mM nickel (Ni) for 9 d resulted in a 38% and 60% increase in tocopherol content in shoots (Gajewska and Skłodowska 2007). Increases of α-tocopherol also have been observed in the lead (Pb)-treated lupin (Ruciska-Sobkowiak and Pukacki 2006).

10.6.5 Ultraviolet (UV) Radiation

Plants respond abnormally to UV-B radiation in a variety of ways, which harms plant development, physiology, and yield while also causing oxidative stress (Bais et al. 2019; Bornman et al. 2019; Mpoloka 2008). Tocopherol is thought to be a possible UV-B stress protector (Munné-Bosch and Alegre 2002). Endogenous α-tocopherol from plastids reduced ROS, lipid peroxidation, and H_2O_2, preserving membrane integrity under UV-B radiation in a variety of plant species (Sharma et al. 2012). The thylakoid membrane's α-tocopherol scavenges ROS and lipid alkyl and peroxyl radicals, preserving membrane structure and function (Hess 2017). UV-B exposure caused MDA production in *Spinacia oleracea* leaves (DeLong and Steffen 1998), but this was countered by an increase in endogenous α-tocopherol levels, which reduced lipid peroxidation and negated the oxidative stress effects. Furthermore, α-tocopherol is said to protect the membranous antioxidant system (DeLong and Steffen 1998). Incorporating α-tocopherol into phosphatidylcholine liposomes alleviated UV-B-induced oxidative damage (Pelle et al. 1990). As a result, α-tocopherol appears to have the capacity to mitigate the negative effects of UV radiation in some plant species under certain conditions.

10.6.6 Extreme Temperature

High temperature stress has a variety of effects on plants at different stages of development, resulting in significant yield loss (Zhang et al. 2018; Ding et al. 2020). High temperature stress, on the other hand, has been linked to increased tocopherol production and accumulation (Mokrosnop 2014; Szarka et al. 2012). High tocopherol levels were induced in *Helianthus annuus* plants when they were exposed to a temperature of 35 °C during the reproductive stage, as well as an increase in seed oil amount (Dong et al. 2007). In a study with lettuce, Tang et al. (2011) discovered that high temperatures activated tocopherol cyclase, resulting in increased vitamin E production. Orabi et al. (2017) conducted a pot experiment on cucumber (*Cucumis sativus* L.) plants and discovered that by reducing hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and electrolyte leakage, plants improved their resistance to low temperature stress. In heat-stressed wheat seedlings, external treatment of α-tocopherol resulted in greater endogenous tocopherol levels, increased chlorophyll contents, photosynthetic rate, membrane integrity, and elevated CAT, POD, and SOD, but a significant reduction in H_2O_2 and MDA levels (Kumar et al. 2012).

Tocopherol protects plastid membranes from oxidative stress at low temperatures (Matringe et al. 2008). Low temperatures are another damaging extreme that has a negative impact on plants. This is stress for plants in tropical regions during the winter season, not just in countries with very low temperatures (Farooq et al. 2009). For many, economically important crops at various growth stages, the impacts of low temperatures, which cause significant harm to plants, have been researched for a long time. This stress is a source of concern for plant productivity and growth (Wang et al. 2009). In numerous plant species, the tocopherol inflections in response to temperature extremes, both high and low, have been examined, and they demonstrate tocopherol's beneficial involvement in extreme temperature stress resistance (Kanayama et al. 2013).

10.7 Bioengineering Approach: Development of Tocopherol-induced Stress-Tolerant Crops

Transgenic plant species are thought to be capable of producing considerable levels of tocopherols (Table 10.2). For example, Kumar et al. (2012) claimed that transgenic *Brassica juncea* plants were more drought-resistant than wild type plants due to higher α-tocopherol levels. Similarly, Cela et al. (2009) discovered a large level of α-tocopherol in a transgenic *Arabidopsis* drought-tolerant line. In another study, Espinoza et al. (2013) discovered the *VTE2* gene, which produces α-tocopherol in response to water stress and is thought to protect photosynthetic machinery from photooxidative stress. The early initiation of drought stress in *Cistus clusii* triggered the homogentisate phytyl transferase gene, which increased the α-tocopherol level by 62% (Munné-Bosch et al. 2009). Liu et al. (2008) found that transgenic tobacco plants exposed to drought stress had greater levels of total tocopherol due to

Table 10.2 Overexpression of tocopherol biosynthetic genes in transgenic plants showing stress tolerance

Transgenic plant	Overexpressing gene and gene source	Gene product[a]	Protective effects[b]	References
Potato	*γ-TMT* and *HPT* genes from *Arabidopsis thaliana*	*γ-TMT and HPT*	‡	Upadhyaya et al. (2020)
Brassica juncea	*γ-TMT*	*γ-TMT*	†	Yusuf et al. (2010)
Brassica juncea	*γ-TMT*	*γ-TMT*	††	Kumar et al. (2012)
Oryza sativa L.	*OsVTE1*-OX	*VTE1*	†	Ouyang et al. (2011)
Tobacco	*VTE2*	*VTE2*	††	Espinoza et al. (2013)
Brassica juncea	*γ-TMT*	*γ-TMT*	+	Kumar et al. (2012)
Green alga	*VTE3*	*VTE3*	+	Luis et al. (2006)

[a] *γ-TMT* γ-tocopherol-methyltransferase, *HPT* homogentisate phytyltransferase

[b] † salt tolerance, †† Drought tolerance, ‡ Salt and heavy metal stress tolerance, + Heavy metal stress tolerance

overexpression of the induced *Arabidopsis VTE1* gene. Because of the elevated content of α-tocopherol in transgenic *Brassica juncea*, salt tolerance features were detected (Yusuf et al. 2010). Because of the increased content of α-tocopherol in transgenic potatoes, they were able to withstand salt and heavy metal stress (Upadhyaya et al. 2020). Ouyang et al. (2011) claimed that transgenic *Oryza sativa* L. has greater salt tolerance due to increased α-tocopherol levels.

10.8 Conclusion

Tocopherol is a well-known antioxidant that aids in the adaptation of plants to abiotic stress. Transgenic plants might help in reducing various abiotic stresses by enhancing the tocopherol levels. The physiological, biochemical, and molecular pathways by which it gives abiotic stress tolerance are poorly understood; thus, its overall impacts on plants should be researched. Exogenous tocopherol could boost growth and stress tolerance by increasing endogenous tocopherol levels. Tocopherol's signaling role may be more complicated; yet, as part of a complex signaling network involving ROS, antioxidants, and phytohormones, tocopherol is a good candidate for influencing cell signaling toward stress tolerance. Tocopherol is connected with other antioxidants to reduce ROS. Tocopherols' involvement in regulating several metabolic processes at the cellular and whole-plant levels requires a deliberate effort. It's also crucial to figure out whether extensive usage of tocopherols in the field is cost-effective, beneficial to growers, and environmentally friendly. Field trials are essential to see if results acquired in a controlled laboratory or greenhouse can be replicated in the field with a variety of edaphic and atmospheric variables. Some attempts have been made in recent years to tailor the genes that control tocopherol production under stressful settings. A thorough understanding of these mechanisms will contribute to the development of tolerance to abiotic stresses and the availability of tocopherol-rich diets for humans.

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Plant Glutathione Transferases and Their Role in the Mitigation of Abiotic Stresses

11

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Abstract

Abiotic stress in the environment is a major concern and a worldwide problem as plants have their own molecular responsive mechanism to deal with the abiotic stress. Thus, we need to explore those compounds which react against abiotic

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stress or stimulate other compound to compensate abiotic-induced damages. Plant glutathione transferases or glutathione-s transferases (GSTs) are multifunctional enzymes and consist of many classes. GSTs play an important role in alleviating oxidative stress from plants including H_2O_2 content, reactive oxygen species, and methylglyoxal, etc. In addition, GSTs being hydrophilic, highly reactive, and stable compound effectively scavenges ROS by inhibiting oxidation of thiol (–SH) group and creating redox buffer that aids in the stabilization of cellular redox state while heavy metals detoxify by binding with –SH group and decreased concentration of metals in cytosol. Thus, GST may be used in innovative agricultural and biotechnological tools to develop abiotic resistant transgenic lines. Therefore, the goal of this chapter is to emphasize the importance of GSTs in order to raise awareness about an environmentally safe compound (GST) that can help to alleviate abiotic stress.

Keywords

Plant glutathione · Classification · Tolerance mechanism · ROS and MG tolerance · Metal resistance

11.1 Introduction

Environmental stresses are the major factors that alter the plant growth, development, and productivity. Abiotic stresses, including salinity, drought, temperature, and heavy metal toxicity can hinder the plant metabolism causing severe detrimental effects, even plant death (Raza et al. 2021; Hussain et al. 2020). Conversely, plants have developed different evolutionary mechanisms to tackle such adversities by regulating their physiology and metabolisms (Fang et al. 2020). One of the earliest signaling in plant stress response is the production of reactive oxygen species (ROS) including super oxides (O_2^{-}), hydroxyl radicals (OH^{-}), and hydrogen peroxide (H_2O_2) (Hasanuzzaman et al. 2020). However, accumulation of ROS can damage the cells by oxidation of the biomolecules (Juan et al. 2021). Therefore, meticulous regulation of ROS signaling is the key for the plant stress response against various abiotic stresses. To realize this, plants use the antioxidant system consisting of both enzymatic and non-enzymatic members. These antioxidants protect the plants from

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the oxidative damage by ROS and also modulate the defense responses (Hussain et al. 2020). Among the enzymatic members, Glutathione S-transferases (GSTs) are a large family of enzymes having multifaceted functions in plants. More importantly, the GSTs are known for their crucial role in the phase II detoxification in plants (Hasan et al. 2021). The s-conjugation reaction between glutathione (GSH), an important non-enzymatic antioxidant, and the electrophilic moiety in the stress-induced substances is catalyzed by GST (Perperopoulou et al. 2018). GSTs have been reported to alleviate several abiotic stresses in plants, including salinity, drought, temperature, and xenobiotics in important plants such as rice, chickpea, cherry tomato, and radish (Kortheerakul et al. 2021; Kumar et al. 2021; Hunter et al. 2021; Lai et al. 2021; Kalita et al. 2020). Many reports showed that GSTs performed various functions in plants such as plant growth and metabolism. In addition, it can tolerate stresses such as cold and salt tolerance, water and nutrient deficiency, metals stress and pathogen infections (Skopelitou et al. 2015; Wang et al. 2019). Apart from stress response, GSTs have been reported to get involved in many key plant physiological processes, including growth and development, detoxification, phytohormone biosynthesis, secondary metabolite processing, nodule function, and even several non-catalytical processes (Kumar and Trivedi 2018). In this chapter, we have presented the comprehensive report on the plant GSTs in mitigating various stress effects. In addition, we have discussed the different types of GSTs and their roles in regulating plant growth and development. We have also presented a detailed conclusion and future prospective on the positive modulatory roles of GSTs under plant stress.

11.2 GSTs: Their Origins, Function, and Classification

The GST classification has developed over the years in tandem with the increasing availability of genetic data and the identification and characterization of new isoforms and classes. GSTs were first discovered in rats and then afterwards identified in mammals, insects, plants, fungi, and microbes. GSTs were previously split into three categories in mammals: cytosolic, mitochondrial, and membrane-associated GSTs, with the latter being further differentiated into microsomal GSTs and leukotriene C4 synthetases (Kraus 1980). Later on, the same three subfamilies were renamed soluble GSTs, kappa GSTs, and membrane-associated proteins in eicosanoids and glutathione metabolism (MAPEG) (Jakobsson et al. 1999). Mammalian GSTs were classified into the alpha, mu, pi, sigma, theta, and zeta classes based on immunological cross-reactivity and sequence similarity (Mannervik et al. 1985). Most non-mammalian GSTs were classified as heterogeneous thetas at the time (Buetler and Eaton 1992). The first classification system for plant GSTs was based on sequence similarity and the intron-exon organization of the genes. Following that, three and then four distinct types of plant GSTs were identified, including type I (herbicide-detoxifying GSTs), type II (GSTs similar to mammalian zeta GSTs), type III (primarily auxin-induced GSTs), and type IV (GSTs similar to classical mammalian theta enzymes) (Droog et al. 1995). Nevertheless, as more biochemically described plant GSTs were available in the late 1990s, it appeared that some plant GSTs clustered with specific mammalian GSTs, while others looked to be plant-specific. This, together with the publishing of the Arabidopsis thaliana genome, contributed to the development of a revised phylogenetic classification in plants based on the Greek-letter designations approach, which was broadly employed for non-plant GSTs (Dixon et al. 1998a, b). GSTs are identified by a two-character species code (At for A. thaliana), followed by the three letters "GST," a Greek or Latin letter identifying the class, and a number distinguishing members of the same class. As a result, "AtGSTF1" is assigned to isoform 1 of the Phi (F) class in A. thaliana. The Phi (replacing former Type I), Zeta (replacing former Type II), Tau (replacing former Type III), and Theta (replacing former Type IV) classes were introduced in plants, as well as two groups more distantly related to another known plant GSTs, the Lambda (L) and dehydroascorbate reductase (DHAR) classes (Dixon et al. 2002). The most recent phylogenetic study, which used well-annotated genomes of terrestrial plants (*A. thaliana, Hordeum vulgare, Oryza sativa, Physcomitrella patens, Pinus tabulaeformis, Populus trichocarpa,* and *Solanum lycopersicum*) and only proteins with the two regular N- and C-terminal domains (see below), identified 14 GST classes: phi (F) (Lallement et al. 2014). All kingdoms share some of these classes, such as Zeta and Theta, but Lambda, Tau, and DHAR are only found in plants. Although the phi class is commonly described as being unique to plants, comparable sequences have been found in fungi, bacteria, and protists (Morel et al. 2013). Despite its drawbacks, the primary sequence remains the most practical criterion for categorizing these proteins to date. GST's evolutionary history appears to be somewhat complicated, and numerous theories have been offered. Due to the presence of theta-class GSTs in bacteria, the initial evolutionary model hypothesized in the early 1990s that canonical (soluble) GSTs of plants, mammals, and fungi arose from this ancestral gene through duplications and divergent development (Pemble and Taylor 1992). This idea was later disproved by considering biochemical aspects such as the nature of the catalytic residue, as well as the oligomeric state of the proteins and their tridimensional structure when solved (Mashiyama et al. 2014). The structural data revealed that soluble GSTs' N-terminal domain adopted a TRX fold, implying that soluble GSTs' evolutionary history is linked to one of the TRX superfamily members. Soluble GSTs, according to this concept, originated from a TRX/GRX progenitor to which a C-terminal helical domain was added. The dimerization of some GSTs, the replacement of the original catalytic cysteine by a serine, and finally the replacement of this residue by tyrosine in many mammalian GST classes result in subsequent significant changes. Although these major phases are expected to remain true, the current model is still incomplete because it excludes newly discovered classes such as mPGES2, GHR, Metaxin, Hemerythrin, Iota, and Ure2p, to name a few.

11.3 Types of Plant Glutathione Transferases

Plant GSTs are categorized into six classes on the basis of gene sequencing, gene organization, and protein homogeneity; phi, zeta, tau, theta, lamda and dehydroascorbate reductase (DHAR) (Cummins et al. 2011a, b; Frova 2003). Meanwhile, GST iso-enzymes which contain the similar class share 40–60% resemblance in their core molecular arrangements, while enzymes which belong to dissimilar groups share less than 20% sequence identicalness (Armstrong 1997). The tau and phi group are found to be more plant-specific, as they are extra numerous as compare to others (Dixon et al. 2001). The function of the tau protein is to defend plant cells from variety of living and non-living stressors (Moons 2003; Thom et al. 2002). GSTs are abundant in the cytoplasmic matrix and classified into several groups according to their origins, catalytic amino-acid branches, sequence resemblance, as well as substrate selectivity (Kumar and Trivedi 2018; Labrou et al. 2015). Theta and zeta have limited xenobiotic activeness and they are also found in animals (Kumar and Trivedi 2018). Furthermore, phi, tau, zeta and theta have catalytic serine branch while lambda and DHAR strictly maintained cysteine groups in their active position motif (Lallement et al. 2014).

11.4 Plant GSTs' Essential Properties

Plant GSTs are a broad collection of multifunctional enzymes that catalyse a variety of processes involving the conjugation of glutathione (GSH; γ-Glu– Cys-Gly) into electrophilic compounds to generate more soluble derivatives that can be transferred to the vacuole and metabolized further (Labrou et al. 2015). Plant GSTs are divided into three superfamilies (cytosolic, mitochondrial, and microsomal) and three classes: tau (U), phi (F), theta (T), zeta (Z), lambda (L), γ-subunit of eukaryotic translation elongation factor 1B (EF1Bg), dehydroascorbate reductase (DHAR), metaxin, tetrachlorohydroquinon dehalogenase (TCHQD), Ure2p, microsomal prostaglandin E synthase type 2 (mPGES-2), hemerythrin (GSTH), iota (GSTI), and glutathionyl-hydroquinone reductases (GHRs) (Csiszár et al. 2016). GSTs make up a significant portion of total soluble proteins in plant cells; for example, they account for 2% of soluble protein in wheat seedlings (Pascal and Scalla 1999). In *Arabidopsis thaliana*, *Solanum lycopersicum*, *Oryza sativa*, and *Triticum aestivum*, for example, genome sequence data revealed numerous GST homologs arranged in complicated supergene families (Labrou et al. 2015); for example, there are 55, 81, 83, and 98 members, respectively (Gallé et al. 2009a, b). The tau and phi classes are the largest in plants and play critical roles in the remediation of environmental pollution caused by organic xenobiotics such as herbicides and industrial chemicals (Cicero et al. 2015a, b). In *Arabidopsis thaliana*, 42 of the 55 GSTs are characterized as tau and phi (Dixon and Edwards 2010). The presence of a conserved Ser residue at the catalytic region distinguishes biologically active tau and phi GSTs from other GST classes (Nianiou-Obeidat et al. 2017). Glutathione-dependent hydroperoxidase (GPOX) activity in fatty acid hydroperoxides and glutathione conjugation activity in lethal lipid peroxidation products are also seen in the tau and phi classes (Nianiou-Obeidat et al. 2017). Because these enzymes are primarily involved in xenobiotic metabolism, they have a high affinity for a wide range of harmful compounds, including xenobiotics and endogenous stress metabolites, such as lipid peroxides and reactive aldehydes, which may result in high abiotic stress tolerance (Gallé et al. 2009a, b). Some tau-class GSTs (*AtGSTU19* and *AtGSTU24*) appear to be important in safener-induced genome activation, according to thorough research. These genes' induction kinetics describe two types of xenobiotic response (XR), a quick (20 min) and a gradual (60 min) XR (Brazier-Hicks et al. 2018). The most recent findings reveal that a rapid XR is functionally linked to herbicide safening and that testing of oxylipid-inspired safeners with different electrophilic characteristics resulted in a separate rapid XR (Brazier-Hicks et al. 2018). Other GST subgroups have a variety of activities, including hormone signaling and peroxidase and isomerase activity (Gallé et al. 2009a, b). The discoveries about phi- and tau-class GSTs, as well as their proportions to other members of the GST superfamily, highlight their importance. Simultaneously, the complex regulation of GST activity is dependent on transcriptional and post-translational regulation, which is managed by several promoter elements and transcription factors, as well as phosphorylation and S-glutathionylation, which may be light-dependent (Dixon and Edwards 2010).

11.5 Plant GST Genes and Their Functions

In tau class, 28 genes were identified which are present inside cytosolic complex and nucleus while their functions are still unknown (Estévez and Hernández 2020). Thirteen genes detected in the phi class were found in cytosolic complex and chloroplast with pigment transportation operation (Estévez and Hernández 2020; Kitamura et al. 2004). Meanwhile, three genes related to theta group present in the peroximase and nucleus and take part in hydroperoxide reduction reaction (Dixon et al. 1998a, b; Estévez and Hernández 2020). Furthermore, two genes identified from zeta class are situated in cytosol and operate in tyrosine degradation (Dixon and Edwards 2006; Estévez and Hernández 2020). In addition, three genes related to lambda group and present in cytoplasm, chloroplast, and peroxisome and functions against salt stress (Estévez and Hernández 2020; Zhai et al. 2013). While four genes identified from DHAR occur in cytosolic complex, chloroplast and peroxisome are involved in ascorbate recycling (Estévez and Hernández 2020; Reumann et al. 2009). Moreover, various GST genes are recognized or discovered from miscellaneous plant varieties including angiosperms, gymnosperms, and non-vascular plants (Wang et al. 2019). For example, 55 GST genes were found in *Arabidopsis thaliana* (Sappl et al. 2009a, b) and 79 in *Oryza sativa* (Jain et al. 2010; Soranzo et al. 2004). Wang et al. (2019) determine 330 *TaGST* genes from the wheat genome and found that tandem and segment duplicates were involved in the extension of *TaGST* family while replicated genes might pass through wide sanctify selection. The gene-expressing making and *cis*-substances in promoter section of 330 *TaGST* genes implicit their functions in proliferation and advancement as well as tough conditions.

The analysis of 14 *TaGST* genes exhibited that they could combat to several abiotic tenses and hormones, particularly salt stress and abscisic acid (Wang et al. 2019). Moreover, Hasan et al. (2021) classify a total of 92 putative *MtGST* genes in *Medicago truncatula* that code for 120 proteins. All of these sets were divided into 12 classes according to phylogentic correlation and occurrence of structural conserved area/theme. Among these classes, seven were found to have segmental repetition. Transcriptional analysis indicated that *MtGSTU5*, *MtGSTU8*, *MtGSTU17*, *MtGSTU46*, and *MtGSTU48* upregulated significantly when exposed to many abiotic and biotic pressures. In addition, strains of *MtGSTU8*, *MtGSTU14*, *MtGSTU28*, *MtGSTU30*, *MtGSTU34*, *MtGSTU46*, and *MtGSTF8* were greatly upregulated when exposed under drought condition for 24 h and 48 h (Hasan et al. 2021). Moreover, the complete genome-wide sequence of the japonica and indica rice subspecies was performed (Goff et al. 2002; Yu et al. 2002). EST and genomic divisions of the Genbank/EMBL/DDBJ databank were screened through in silico, in which 59 putative GST genes and 2 pseudogenes were recognized (Soranzo et al. 2004). It was found that 40 from 59 rice alleles fitted into tau class of GSTs. Meanwhile, additional potential tau GST alleles (GenBank Accession No. AF309378) were found through homology explorations in the NCBI record (Soranzo et al. 2004). A gene *VvGSTF13* could increase salinity and drought stresses in Arabidopsis (Xu et al. 2018). The expression examination of *OsGSTL1*, *OsGSTL2*, and *OsGSTL3* showed that rice lambda class not only improve plant growth and development but also withstand against various biotic and abiotic stresses as well as metalloids, low temperature, drought, and salt stress (Kumar et al. 2013a, b). Genetically developed accession expressed *PpGST*, a zeta GST gene contained in fruitlet of *Pyrus pyrifolia* Nakai cv. Huobali, exhibited comparatively regular proliferation in drought, NaCl and metal treatments. Moreover, the T1 transgenic tobacco lines displayed remarkably decreased superoxide anion production rate as compared to wild type exposed in abiotic conditions. The results showed that upregulation of *PpGST* in tobacco increased the tolerance of T1 lines to oxidative destruction under abiotic stresses (Liu et al. 2013). Examination of Arabidopsis GST genes, the introduction of *AtGSTU19* and *AtGSTU24,* and higher GST functions were detected in the leaves and roots of 8-week-old plants when treated with 100 mM NaCl in a solution culture experiment (Horváth et al. 2015).

11.6 Response to Stress by Glutathione Transferases

Plants, such as all living creatures, are subjected to predictable as well as unpredictable changes in the environment. Owing to their sedentary habits, plant development and growth along with their survival depends on the plant's capacity to modulate the metabolic rate in order to offset these kinds of environmental changes. Nearly all adverse ecological conditions result in cell oxidative injury that is caused by excessive production of reactive oxygen species (ROS) during the stress-exposed tissues. Undeniably, exactly how a species/variety handles with any certain environmental stress is frequently because of its capability to sense alterations in the ROS concentrations and to activate the sufficient metabolic modifications (De Gara et al. 2003). It also is dependent upon the epigenetic signatures which alter the plant's ability to respond to specific stress conditions (Centomani et al. 2015). Glutathione is perhaps one of the most significant metabolites participating in the defense responses versus the environmental stresses. Indeed, glutathione as well as its associated enzymes developed very early on in aerobic organisms, possibly in conjunction with di-oxygenic photosynthesis (Deponte 2013). Because of its cysteine (Cys) molecule, the tripeptide GSH1 (γ-L-glutamyl-L-cysteinylglycine) is a flexible redox molecule. Nevertheless, its part extends beyond ROS scavenging as well as the redox homeostasis. Glutathione is the main form of organic sulfur carried in phloem. It plays a crucial role in an interaction between plants and mutually beneficial nitrogen-fixing bacteria, in compartmentalizing and neutralizing of xenobiotics and heavy metals, as well as in the vacuolar transportation of secondary metabolites (Cheng et al. 2017; Noctor et al. 2012). GSH has also been proposed as the key contributor of the diminished sulfur group meant for Glucosinolates biosynthesis in *Arabidopsis* (Parisy et al. 2007). This non-exhaustive list of procedures involving glutathione underlines its role to play in various plant defense tactics. Emphasis is being placed on the three biggest abiotic stresses—drought, salinity, and heavy metal contamination—due to the fact that their significance has been growing under climate change as well as a result of anthropic events.

11.7 Glutathione Role in Abiotic Stress

Various approach and evidence feature that GSTs substantially contribute to the adaption and tolerance to the abiotic environmental stresses like the drought, salinity, and the cold stress. Various GSTs, and in particular members of Tau and Phi classes, are differently expressed in response to abiotic stress indications (Csiszár et al. 2014; Lan et al. 2009). On numerous occasions, higher GST expression is associated with increased stress tolerance such as seen in tomato, wheat, as well as in barley (Gallé et al. 2009a, b; Rezaei et al. 2013; Sun et al. 2010). Abiotic stress tolerance facilitated by the chemical priming such as treating along with SA, corresponds with a spike in GST expressions (Csiszár et al. 2014; Horváth et al. 2015). Genetically modified overexpression of GSTs has provided the additional knowledge in their method of action that has contributed to the abiotic stress adjustment. Oxidative stress tolerance via GPOX activity, initiation of antioxidant equipment, and alterations in the redox state were observed following the overexpression of a GST in tobacco belonging to tau class that resulted in salt, as well as chilling stress tolerance in the tobacco seedlings (Roxas et al. 2000). Overexpression of tau-class GSTs from several different species, as, for example, the Glycine soja *GsGSTU13*, the *A. thaliana AtGSTU19*, as well as in the *Prosopis juliflora PjGSTU1*, have resulted in the improved stress tolerance (George et al. 2010; Jia et al. 2016). Interestingly, salt and oxidative stress tolerance by the overexpression of rice *OsGSTU4* was accompanied by pleiotropic effects such as reduced sensitivity to ABA and auxin, as well as upregulation of pathways related to defense responses, sulfate reduction, and phenylpropanoid and flavonoid biosynthesis pathways (Sharma et al. 2014). This is indicative of multilevel interactions that can merely be explained by the enzyme's catalytic functions. Such pleiotropic effects might be the cause of the increased tolerance to drought and salt stress of transgenic tobacco plants overexpressing a sweet orange *CsGSTU*, despite the lack of GPOX activity (Cicero et al. 2015a, b). Zeta-class GSTs have been identified, through different approaches, as significant contributors to abiotic stress tolerance despite the lack of significant GST/GPOX activity to standard GST substrates such as CDNB. In rice, the presence of a naturally occurring *OsGSTZ2* allelic variant with significantly lower isomerase activity correlated with reduced cold tolerance (Kim et al. 2011). Curiously, when the very same gene was over-expressed in rice, it bestowed cold tolerance that coincided with increased GST as well as the GPOX activity of rice leaf extracts (Takesawa et al. 2002). Likewise, over-expression of *Tamarix hispida* "ThGSTZ1" improved drought and the salt stress tolerance with the simultaneous increase of overall GST as well as in GPOX activity (Yang et al. 2014a, b). Possibly, GSTZ isomerase activity on the way to maleylacetoacetate, an electrophile that could potentially alkylate a wide variety of macromolecules (Blackburn et al. 2006), decreases an accumulation of oxidants under stress, ultimately facilitating the homeostasis in control of plant antioxidant machinery. The expression profiles of the rice GSTL class were thoroughly studied, exposing the tissue and developmental stage-controlled expressions. *OsGSTL2* presented the very highest expression levels, and the induction under abiotic stress (Kumar et al. 2013a). *OsGSTL2* over-expression offered increased tolerance to drought, salinity, and the cold stress (Kumar et al. 2013b). Functional evaluation of soybean *GmGSTL1*, which is upregulated in salt stress conditions, indicates that its protective action against salinity possibly comes from its interactions with antioxidant flavonoids—quercetin as well as kaempferol. *GmGSTL1* remained conjectured to serve as a catalyst for the reduction of oxidized flavonoids reestablishing their antioxidant function and thereby contributing to the oxidative stress tolerance (Chan and Lam 2014). So far, in studies referred to above, GST-protective roles against abiotic stress exists in direct connection with its protein abundance suggestive of the immediate role of its catalytic functions or protein interactions along with cellular metabolites. Nevertheless, this is in contrast to the remark that the silencing of *AtGSTU17* stemmed in increased drought and the salt stress tolerance (Chen et al. 2012). Plants displayed anatomical and physiological alterations like the smaller stomatal aperture which resulted in a lower transpiration rate and the increased growth of primary and lateral roots. Moreover, *AtGSTU17*-silenced plants demonstrated higher ABA and GSH contents (Chen et al. 2012). These findings offer substantial evidence for the signaling roles of plant GSTs that expand beyond their catalytic activities and could be further utilized to increase plant strength under abiotic stress.

Biotechnological manipulation of plant GSTs can open up new opportunities towards improving the reliability of crops versus the continuing climate change. Technological developments in ~omics and other technologies may provide substantial information on GST-mediated stress tolerance mechanisms. For instance, metabolomics analysis of GST over-expressing plants proposed that these plants reach an accustomed state as efficiently as possible (Kissoudis et al. 2015a, b). Such adjustments may be further lessened by stress-inducible expression. For example, co-engineering of a tau-class GST with a glutathione synthetase substantially increased herbicide tolerance in contrast to single transformants (Skipsey et al. 2005). Comparable methods with Lambda GSTs as well as the antioxidant secondary metabolite overproduction, e.g., flavonoids (Nakabayashi et al. 2014) co-engineering, can be far more effective in achieving robust stress tolerance. Fine adjustment GST enzymatic characteristics as well as substrate availability may materialize basic research towards biotechnology applications in agricultural crops.

11.8 Role of Plant Glutathione in Detoxification of Reactive Oxygen Species and Methylglyoxal

Plants constantly exposed to variety of stress through extreme temperatures, drought conditions, organic pollutants, heavy metals, and salt concentration leading to the generation of reactive oxygen species (ROS), which may alter many physiological mechanisms (Foyer and Noctor 2005b). Glutathione, being hydrophilic, highly reactive, and stable compound, effectively scavenges ROS by inhibiting oxidation of thiol (–SH) group and creating redox buffer that aids in the stabilization of cellular redox state (Meyer and Hell 2005). Since GSH occurs in reduced, as well as in oxidized forms (Mahmood et al. 2010), the cysteine thiol (–SH) group instantly donates an electron to unstable ROS. As a result, GSH becomes reactive and interacts with another GSH to produce GSSG, which directly assists in the detoxification of hydrogen peroxide (H_2O_2). In the next step, glutathione reductase (GR) acts on GSSG using nicotinamide adenine dinucleotide phosphate (NADPH) and regenerate GSH (Jozefczak et al. 2012). However, it was documented that increased stress reduces the capacity of GR to cope with the massive consumption of GSH causing reduction in free GSH contents (Cuypers et al. 2011).

Glutathione not only actively participates in ROS scavenging but also regulates antioxidant enzymes. Glutathione (GSH) and ascorbate (AsA) serve as redox buffers and play an active role in the AsA-GSH cycle in plant cells (Smeets et al. 2005). The oxidation and reduction of AsA and GSH enable ascorbate peroxidase (APX) to convert H_2O_2 to H_2O (Jozefczak et al. 2012). It was documented that AsA is maintained in reduced state in plant cells owing to the activity of dehydroascorbate reductase (DHAR), which utilizes GSH as an electron donor (Mittler et al. 2004; Foyer and Noctor 2005a). Therefore, GSH and AsA pools in reduced forms are necessary for optimum function of plant cell against stress; otherwise, APX is instantly inhibited and unable to respond with H_2O_2 and may result in the toxicity to the plants.

Methylglyoxal (MG, α-oxoaldehyde) is a cytotoxic highly reactive organic compound produced as byproduct in plants as a consequence of numerous abiotic stresses through multiple enzymatic and non-enzymatic processes. Methylglyoxal not only causes oxidative stress through production of free radicals (photo reduction of O_2 to $O_2^{\cdot -}$) (Saito et al. 2011), which exacerbates damage to plant cells, but it also disrupt antioxidant enzymes that are capable of scavenging ROS (Wang et al. 2009). During stress conditions, the production of MG increased by two- to sixfolds leading to disruption in various cellular functions (Yadav et al. 2005) and DNA damage (Hasanuzzaman et al. 2017b). Therefore, emphasis also needs to be paced on detoxification of MG while dealing with different types of abiotic stress (Hoque et al. 2008). It was reported in the literature that glyoxalase system, which is composed of lactoyl-glutathione lyase (Gly I) and hydroxyacyl-glutathione hydrolase (Gly II), detoxifies MG in a coordinated manner (two steps) utilizing GSH as cofactor (Hasanuzzaman et al. 2017a). In the first step, MG produces hemithioacetal through its reaction with GSH, which is subsequently transformed to S-D-lactoyl-glutathione (SLG) mediated by Gly I. In the next step, SLG transformed to D-lactate through hydrolysis mediated by Gly II, simultaneously producing GSH (Yadav et al. 2008).

11.9 Role of Plant Glutathione Transferase in Heavy Metals Stress

Heavy metals (HMs) are the main abiotic stress which can cause harmful effects in plants. HMs toxicity could lead to extreme accretion of reactive oxygen species (ROS) and methylglyxol (MG) resulting in lipids peroxidation, protein oxidation, enzymes inactivation, impairment of DNA, and/or affecting important components of plant cells (Hossain et al. 2012, 2021; Rai et al. 2019; Xu et al. 2020). However, higher plants contained well developed anti-oxidant defense mechanism and a glyoxalase scheme to capture ROS and MG. Furthermore, HMs that have access to a plant cell can be confiscated by amino acids, organic acids, GSH, and GSTs (Hossain et al. 2012, 2021; Kumar and Trivedi 2018). GSH plays a main role in controlling ROS and MG as well as their derivatives in plant cells, while GSH enzymes such as glutathione S-transferase act cumulatively and uniformly in order to efficiently protect the damage caused by ROS and MG; meanwhile GST also detoxify HMs through complexation, chelation, and vacuolar sequestration processes (Hossain et al. 2012). Furthermore, HMs' stress increases GSH concentration in plants; meanwhile a positive relationship was found between feedback induction and improved expression of genes encoding groups of GST and glutathione peroxides (GPX) gene families in metal stress (Shri et al. 2009). Many investigations have showed enhanced tolerance to a number of environmental stresses, such as heavy metals cadmium, and nickel in transgenic lines expressed GSTs in *Aspergillus nidulans*, Indian mustard (*Brasscia jucea*), tobacco, and poplar tree (*Populus trichocarpa)*, among others (Rai et al. 2003). Zhang and Liu (2011) reported that *pKHCG* transgenic alfalfa plants showed a significant resistant against the mixture of cadmium and trichloroethylene.

11.10 Role of Plant Glutathione Transferases in Salinity and Drought Tolerance

The climate change scenario is a real threat to agriculture specially to the crop plants as abiotic stresses have been attributed to up to 50% reduction in crop production worldwide (Hasanuzzaman et al. 2012). Among the abiotic stresses, drought and high salinity have significant impact on the crop yield around the globe. Solely, the increasing drought spells in different areas will affect the two-thirds of world population, and the salinity is expected to affect 50% of total cultivable lands by 2050 leading to food scarcity (Hasegawa 2013; Naumann et al. 2018). On the other hand, about 45 million hectares irrigated area of the word has already been considered under salt stress that affect overall crop yield in those areas (Munns and Tester 2008). Abiotic stresses like drought and salinity affects the plant growth, physiology, and biochemical process that are involved in different pathways. Drought causes the stomatal closure that disturb the gaseous exchange, thermal conductance, and photosynthetic efficiency of leaves leading to production of reactive oxygen species (ROS), while salinity leads to ionic toxicity and also impart physiological drought ultimately leading to reduction in production (Pinheiro and Chaves 2011; Seleiman et al. 2021).

In plants, the expression of glutathione transferases (GSTs) have been observed during different growth stages. GSTs have been found to be expressed under different biotic and abiotic stresses including drought and salt stress (Xu et al. 2015). A detailed study of GSTs on the Arabidopsis showed that the expression of these genes is induced by early stress and plays an important role in protection of plants against the oxidative and hormonal stresses (Sappl et al. 2009a, b). A mutant-based study showed that *AtGSTU17* gene plays a negative role towards the drought and salinity stress as an increased amount of ABA production was observed in the mutant plants. Moreover, the application of glutathione resulted in induction of high level of ABA and other phytohormones that play crucial role in different physiological processes like seed germination, stomatal conductance, and transpiration rates (Chen et al. 2012). Similarly, the exogenous application of GSH improved the salinity tolerance in *V. radiate* due to production of antioxidants, while the tobacco transgenic plants expressing *GmSTU4* gene showed high amount of metabolites like proline that is an osmo-protectant and have also been found to induce tolerance against high salinity and herbicide under the stress conditions (Kissoudis et al. 2015a, b). The genome wide in–silico and expression analysis of glutathione transferase genes in different crop species including cotton (Xu et al. 2017), tomato (Islam et al. 2017), pumpkin (Kayum et al. 2018), brassica (Wei et al. 2019), wheat (Hao et al. 2020), and melon (Wang et al. 2020) also reveal their role in conferring the abiotic stress in higher crop plants. These studies showed the importance of GSTs in regard to stress tolerance in plants and also encourage the further functional studies of individual genes (Fig. 11.1).

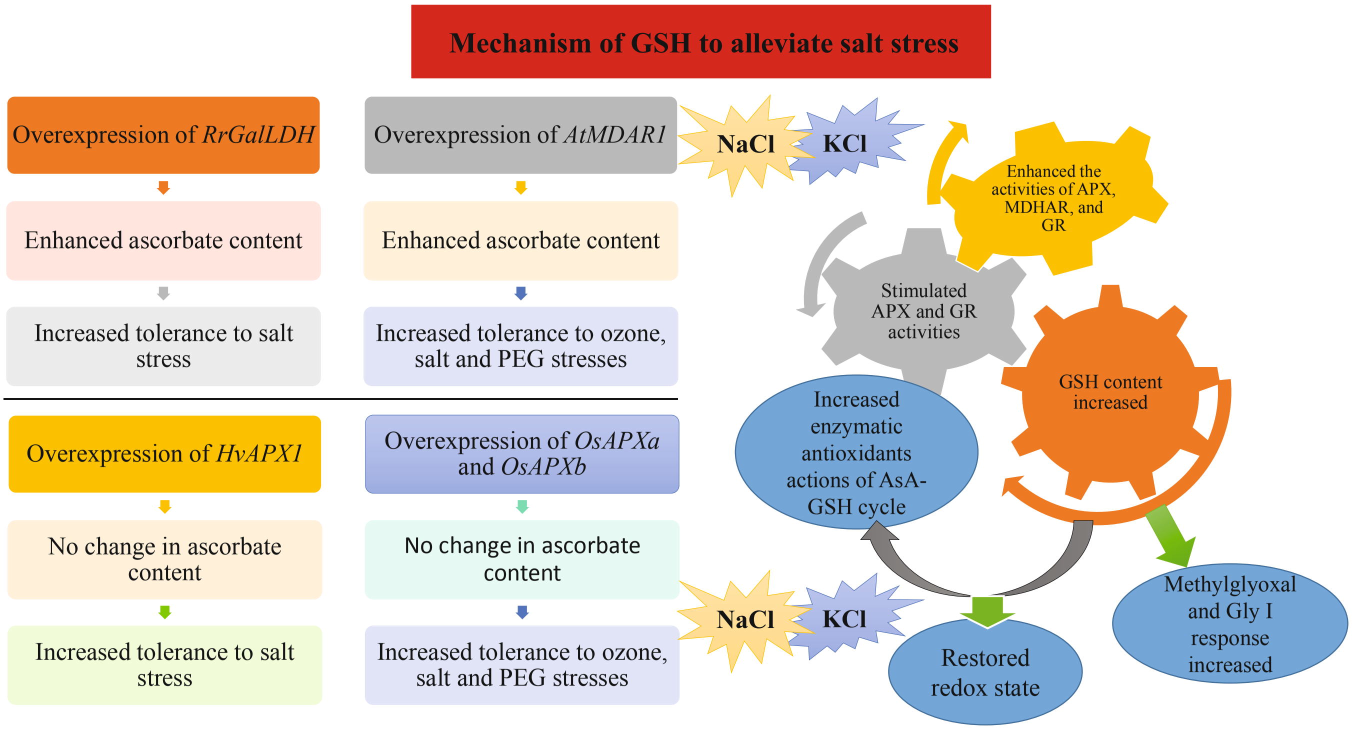

Fig. 11.1 Mechanism of GSH and its genes to alleviate salinity stress

11.11 Mechanisms of Glutathione Transferases

Glutathione (GSH, γ-glutamylcysteinylglycine) is a key tripeptide hydrophilic metabolite produced in a plant cell that plays an essential role in metabolic functions, signal transduction, protection against oxidative stress, defense against extreme temperatures, and electrophilic species detoxification (Schröder 2001; Hatzios et al. 2005; Asgher et al. 2017). The formation of GSH is governed by two adenosine triphosphate (ATP) dependent stages, initiating with the attachment of the carboxyl group of glutamate to the amine of cysteine through peptide bond (Hasanuzzaman et al. 2017a) catalyzed by γ-glutamylcysteinyl synthetase (GSH1) with subsequent inclusion of glycine mediated by glutathione synthetase (GSH2) (Gill et al. 2013). Glutathione exists in both reduced (GSH) and oxidized (GSSG) form, but it is primarily maintained in reduced status inside plant cell owing to the enzymatic action of glutathione reductase (GR) or glutathione peroxidase (GPX) (Mahmood et al. 2010). More than 90% of total GSH is located in cytosol, while the remainder is found in mitochondria or other organelles (Lu 2001). In addition, certain plant species produce homoglutathione (hGSH, γ-glutamylcysteinyl-β-alanine), which functions similarly to GSH (McGonigle et al. 1998). The details about the synthesis of glutathione and its metabolism are presented in Fig. 11.2.

The detoxification of metals and xenobiotics mainly depends on the contents of GSH and glutathione S-transferases (GSTs) activity in plants. Glutathione is non-enzymatic scavenger of oxidative stress that not only inhibits protein denaturation by safeguarding thiol (–SH) group, but also acts as precursor for GSTs enzymes (Hasanuzzaman et al. 2017a; Sidhu and Bali 2021). Glutathione S-transferases are multigene family of isozymes that play a range of functions in plant developmental processes such as stress tolerance, endogenous metabolism, and xenobiotic detoxification in phase II reactions (Schröder 2001; Noctor et al. 2002). At present, seven classes of GSTs were reported in the literature: phi (GSTF), tau (GSTU), zeta (GSTZ), theta (GSTT), lambda (GSTL), dehydroascorbate reductases (DHARs), and tetrachlorohydroquinone dehalogenase (Cummins et al. 2011a, b). Among them, GSTF and GSTU were the most widely reported class of GSTs that were involved in xenobiotics detoxification in various plant species (Cummins et al. 2011a, b). Although GSTs were reported in various plant species, still there is a poor understanding of their endogenous roles in cellular protection and as carrier proteins. The capacity of GSTs to withstand various types of biotic and abiotic stress is attributed not only to electrophilic species detoxification by glutathione and attenuation of cellular redox status (Foyer and Noctor 2005b) but also to the production of secondary metabolites and auxins, their restriction and transport within plant cell by the following equation (Lamoureux et al. 1994; Lamoureux and Rusness 1993).

$$\mathrm{X}-\mathrm{Z}+\mathrm{GSH}\rightarrow\mathrm{GS}-\mathrm{X}+\mathrm{H}-\mathrm{Z}$$

Xenobiotics contain functional groups such as alkyl, amide, amino, carboxyl, halogens, hydroxyl, and nitrile which are susceptible to enzymatic transformations. Metabolism of xenobiotics in plants involve phase I, phase II, and phase III

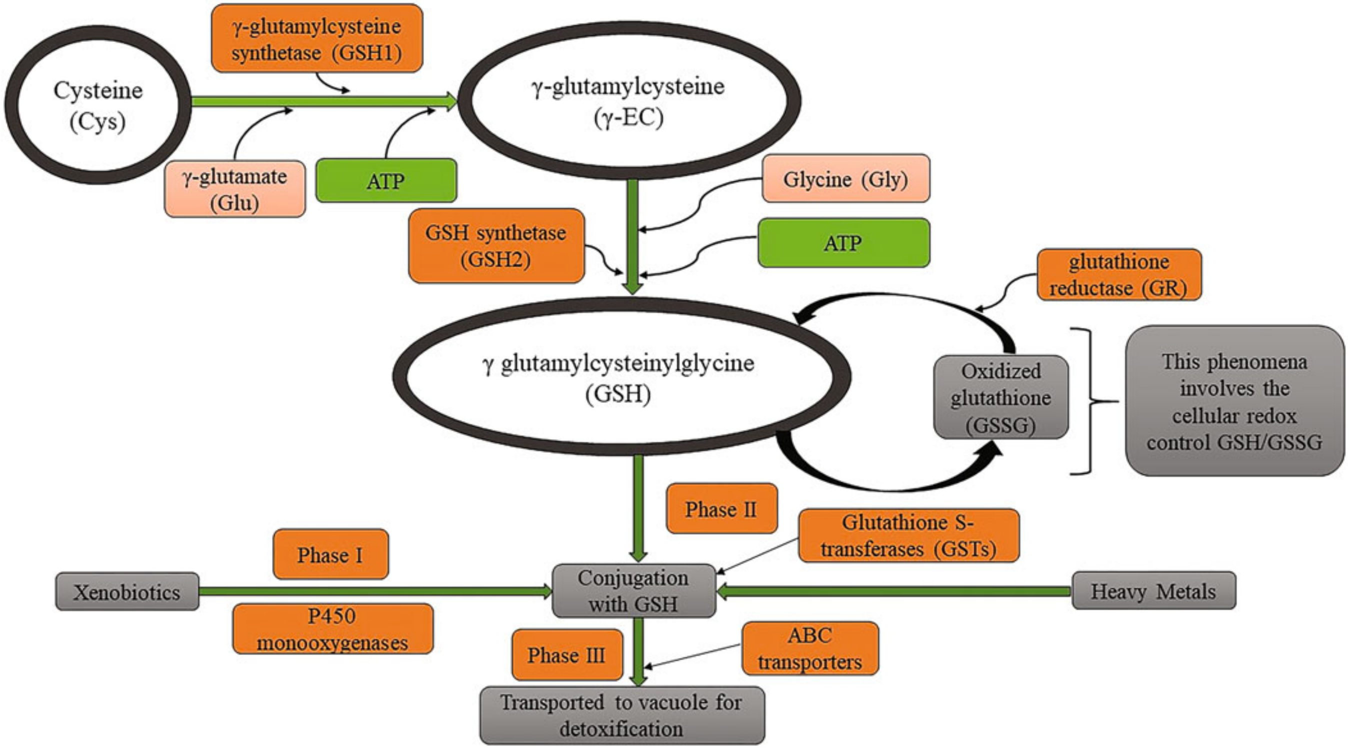

Fig. 11.2 Synthesis of glutathione (GSH) and schematic representation of heavy metals and xenobiotics detoxification pathway by GSH and glutathione S-transferases (GSTs). Phase I and Phase II occurs in cytosol, while Phase III in vacuole

reactions, which are analogous to human hepatic metabolism (Sandermann Jr 1994). Once xenobiotics enter plants, they undergo oxidative, reductive, or hydrolytic reactions (phase I) by P450 monooxygenases and peroxidases, which activate them to act as highly reactive intermediates for conjugation with glutathione mediated by a wide array of GSTs that make these compounds less toxic (phase II) (Wang et al. 2010) or peptidases degrade these glutathione conjugates back to cysteine (McGonigle et al. 1997). It was documented in the literature that electrophilic xenobiotics bind to GSH through the cleavage of halogens, nitro group or double bonds in exchange for cysteinyl sulfur present in GSH, which is mediated mostly by phi (GSTP) and Tau (GSTU) classes of GSTs. In the last step (phase III), xenobiotics conjugates are either transported to the vacuole or apoplast of plant cells via ATP-binding cassettes (ABC transporters), where they were further metabolized by hydrolytic reaction to CO_2 or deposited outside the cell (Edwards et al. 2011). These final compounds interact with gamma-glutamyl transpeptidases, carboxypeptidase, or dipeptidases enzymes in the vacuole, resulting in further breakdown (Schröder et al. 2008).

On the other hand, detoxification of heavy metals requires synthesis of chelators and storage of metal complexes in plant cell (Hernández et al. 2015). Heavy metals have a high affinity for the thiol (–SH) group of free cysteine, resulting in rapid oxidation (Jozefczak et al. 2012). As a result, reduced metal may initiate a Fenton reaction leading to the production of hydroxyl radicals ($HO^{\cdot}$), which are toxic for plants (Fahey and Sundquist 1991). Therefore, low levels of free cysteine (50 μM) within plant cell is necessary for protection against hydroxyl radicals ($HO^{\cdot}$), which may produce from Fenton reaction (Fahey and Sundquist 1991). It was reported that oxidation of thiol (–SH) is substantially decreased when free cysteine amino group attach with glutamate and glycine to form GSH using GSH1 and GSH2 (Hasanuzzaman et al. 2017a). These chemical reactions help in maintaining low concentration of cysteine and high concentration GSH inside plant cell by preventing the production of hydroxyl radicals ($HO^{\cdot}$) by Fenton reaction (Jozefczak et al. 2012). In addition, heavy metals bind to thiol (–SH) group of GSH (nucleophilic in nature) upon entering in plants using GSTs (Ahmad et al. 2010) and make non-toxic metal complexes and transport them to the vacuole of plant cells (Kumar et al. 2013a, b, c) via ATP-binding cassettes (ABC transporters), where they were further metabolized (Edwards et al. 2011). However, excessive consumption of GSH for heavy metals sequestration reduces its cytosolic concentration, therefore, balance in GSH/GSSG ratio is very critical for survival of plant (Yadav 2010).

11.12 Conclusion

Environmental stresses such as cold, salinity, drought, and heavy metals can damage plant growth and yield. Plant GST genes may be significantly used for bioremediation of combined environmental pollutants. For instance, by developing transgenic cultivars which are resistant to multi abiotic stresses, they can be used for future agricultural productivity. As a result of their versatile qualities, GSTs are an essential and fascinating area of research for functional characteristics. Many GST member genes need to be investigated to learn more about their roles in salt, drought and metal stress, as they have significant impact on salt and drought tolerance as well as heavy metal remediation.

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Role of Ascorbic Acid in Alleviating Abiotic Stress in Crop Plants

12

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Abstract

Environmental stresses cause significant damage to plant growth all over the world thereby significantly declining the yield. The damaging effects of stresses result from the alterations induced on key metabolic pathways including germination, root growth, water uptake, mineral assimilation, photosynthesis, protein synthesis, and respiration. Among the main causes of stresses induced growth damage is the excessive generation of toxic reactive oxygen species imparting several damaging effects like degradation of enzymes, proteins, nucleic acids, etc. Several responses are common to almost all stress factors however some are stress specific. The indigenously existing tolerance mechanisms are upregulated to alleviate the damaging effects of stresses. Among the key tolerance, metabolites ascorbic acid has a central role in stress mitigation by its antioxidant property and has been reported to alleviate the stress damage by strengthening the associated tolerance mechanisms. With this backdrop, present review focuses on summarizing the effects of stresses on plant growth and the role of ascorbic acid there in.

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Ascorbic acid · Environmental stresses · Metabolic pathways · Photosynthesis

12.1 Introduction

Among the abiotic environmental factors are drought, salinity, temperature extremes, metal(loid)s stresses, UV radiations, etc. All of these mentioned stress factors impart damaging effects in the crop plants and also alter their distribution (Ahmad et al. 2018; Abdel-Farid et al. 2020; Arif et al. 2020; Ahanger et al. 2021; Fig. 12.1). Adverse effects of stresses can be stress specific while some are common to all stresses like photo inhibition, reduced growth and yield. Stresses alter metabolism of plants reflecting in significant decline in growth and yield potential. Altered metabolism is linked directly with the reduced root growth, reduced absorption of mineral ions and water from soil, reduced protein synthesis, altered cellular homeostasis, and enzyme activity (Ahanger and Agarwal 2017a, b; El-Beltagi et al. 2020). One of the key contributors to stress-induced damage to plant growth and metabolism includes the increased generation of toxic reactive oxygen species (ROS, Fig. 12.1). Among the key ROS are included: hydrogen peroxide, superoxide, singlet oxygen, hydroxyl, nitric oxide, etc. Stress-induced increase in ROS triggers oxidation of proteins, lipids, nucleic acids, and fatty acids thereby hampering the functioning key cellular organelles including chloroplast, mitochondria, membranes, etc. (Laxa et al. 2019; Huang et al. 2019). ROS-mediated damage to membrane structural stability influences their function as well as other key organelles like

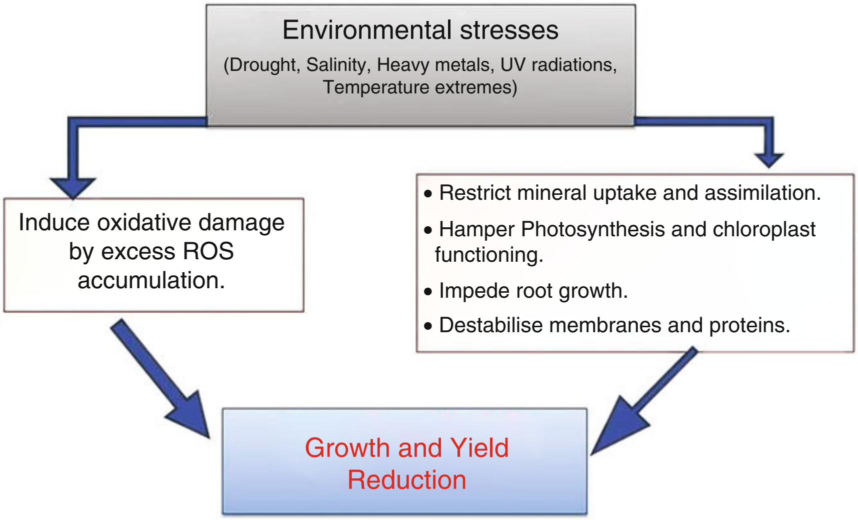

Fig. 12.1 Mechanistic illustration of stress effecting yield and growth of plants

chloroplast and mitochondria thereby affecting the processes operating within them (Dietz et al. 2016). There have been enough reports available confirming the damaging effects of stress-generated ROS on the photosynthetic attributes, electron transport, enzyme functioning, and overall growth performance of the plants (Huang et al. 2016; Ahmad et al. 2018). Plants have developed mechanisms to counteract the damaging effects of stresses so that the major functions of cellular metabolism can be protected. Among the key mechanisms are included: (a) antioxidant system, (b) osmolyte metabolism, (c) ion exclusion, (d) phyto-chelation, (e) secondary metabolite accumulation, etc. These tolerance mechanisms are precisely regulated at genetic and molecular levels. The strength of tolerance mechanism is obviously reflected at the cellular and whole plant level in terms of plant functioning and yield performance. All these mentioned, tolerance mechanisms are constituted of enzymatic and nonenzymatic components. Upregulation of antioxidant system (Singh et al. 2016; Ahanger et al. 2017), osmolyte accumulation (Sharma et al. 2019; Soliman et al. 2020), ion exclusion (Negrão et al. 2011), secondary metabolite accumulation (Yadav et al. 2021), and phyto-chelating agents (Otero and Tupas 2018) have been reported in plants in response to environmental stresses. These tolerance mechanisms protect plant metabolism from the damaging effects of stresses by mediating the quick elimination of toxic ROS maintaining their levels at optimal concentrations (Ahmad et al. 2010, 2021). Besides, having a role in ROS scavenging, other roles include the maintenance of tissue water potential through accumulation of compatible osmolytes leading to protection of structural and functional integrity of key cellular components (Burg and Ferraris 2008), chelation of toxic metal ions through accumulation of chelating agents/compounds (Angulo-Bejarano et al. 2021), and exclusion and compartmentation of toxic ions into less sensitive cellular spaces like vacuole or cytosol through upregulation of transport proteins and exchangers (Wu and Li 2019). Considerable research work has been done evaluating the physiological, biochemical and molecular mechanisms regulating the abiotic stress potential of crop plants. Among the antioxidants, ascorbic acid is having a key role in plant protection against stress. Ascorbic acid regulates redox homeostasis, cell division, cellular expansion, signaling and cell wall growth (Wang and Huang 2019; Chen et al. 2021). It has a vital role in alleviation of the stress-mediated damage to plant growth through its active role in ROS neutralization via enzymatic and non-enzymatic detoxification pathways (Ahanger et al. 2017). Besides this, it is believed to serve as cofactor for enzymes involved in photosynthesis, biosynthesis of phytohormone, secondary metabolites and anthocyanins (Smirnoff 2001). For example, the enzymes of xanthophyll cycle like violaxanthin de-epoxidase, ethylene biosynthesis like 1-aminocyclopropane-1-carboxylic acid oxidase, and the abscissic acid and gibberellic acid biosynthesis like 2-oxoaciddependent dioxygenases (Smirnoff and Wheeler 2000).

Ascorbic acid is commonly known as vitamin C. It is water soluble and one of the abundant and essential metabolites for plants and animals. For animals, it acts as an important dietary supplement for humans and primates because of their inability to synthesize it. Ascorbic acid concentration within a cell is determined by biosynthesis and degradation potential in addition of the redox pathways (Akram et al. 2017;

Bilska et al. 2019). There are evidences reporting the stress sensitivity of plants exhibiting low ascorbic acid synthesis reflecting in significant restrictions in their growth, development, and yield productivity. Moreover, several studies using transgenic approach including knockout, as well as overexpression have demonstrated significant role of ascorbic acid in increasing the tolerance of plants to various environmental stress (Medina et al. 2021). Improved tolerance to drought and extreme temperature (Bao et al. 2016), salinity (Xue et al. 2018), and heavy metals (Alamri et al. 2018) due to enhanced ascorbic acid accumulation/synthesis has been widely reported. Four pathways for synthesis of ascorbic acid have been identified which include (a) Smirnoff-Wheeler pathway (D-mannose/L-galactose pathway), (b) D-galacturonate pathway, (c) the L-gulose pathway, and (d) the myo-inositol pathway. It has been established that D-mannose/L-galactose pathway also known as Smirnoff-Wheeler pathway is the dominant one (Fenech et al. 2019). The genes and enzymes involved in the synthesis of ascorbic acid have been well characterized and through various studies their roles have been established in contributing/regulating the biosynthesis of ascorbic acid (Wang and Huang 2019). Manipulating the ascorbic acid biosynthesis pathway through transgenic approaches has been reported to influence growth performance and stress tolerance significantly (Fenech et al. 2019). The accumulation of ascorbic acid is fine regulated by orchestration of biosynthesis, recycling, degradation, and transport pathways (Broad et al. 2020). Biosynthetic pathway is connected between cytoplasm and mitochondria, while degradation occurs within the apoplast (Mazid et al. 2011). Present review summarizes the beneficial role of ascorbic acid in improving the tolerance potential of plants to stresses by highlighting the effect on key stresses.

12.2 Ascorbic Acid and Drought Stress

Drought is one of the key damaging stress factors resulting due to reduced water availability, and global climate change has further intensified the situation. Plants are very much sensitive to drought and damage varies with the intensity and duration of drought (Kapoor et al. 2020). Drought stress has been demonstrated to decline root growth, germination, biomass, and yield production by influencing the key metabolic pathways like photosynthesis, protein synthesis, mineral uptake, and assimilation, etc. (Queiroz et al. 2019; Kapoor et al. 2020). Water deficit or drought suppresses photosynthesis by directly affecting leaf area, stomatal movements, and evapotranspiration which ultimately induce osmotic stress and hence arrest of photosynthetic apparatus (Zare et al. 2011; Bhargava and Sawant 2013). Restricted access to carbon dioxide following the stomatal closure directly influences the activity of Rubisco, nitrate reductase, and other key enzymes. Besides, this drought considerably alters the accumulation of secondary metabolites (Begum et al. 2021; Ahanger et al. 2021) by affecting the enzymes involved in their metabolism. Drought mediated alteration in redox homeostasis has been reported by several workers (Kapoor et al. 2015; Shah et al. 2017) and hence alterations in metabolism and growth of plants has been reported. Drought stress significantly affects the antioxidant functioning and the accumulation of osmolytes thereby affecting the tolerance potential to considerable extent (Zhang et al. 2020a, b; Hou et al. 2021). In addition, the decline in uptake and assimilation of mineral elements is also an important damaging consequence of drought. Drought affects the functioning of transport proteins carrying uptake of mineral ions and the activity of enzymes involved in metabolism of minerals (García-Caparrós et al. 2019). Drought has been reported to affect the synthesis of ascorbic acid significantly and reports are contradictory. Reports available have demonstrated increase (Parveen et al. 2021) as well as decrease (Seminario et al. 2017) in content of ascorbic acid in plants under drought. Increased synthesis of ascorbic acid directly affects the drought tolerance in plants by influencing the activity of enzymes of key tolerance mechanisms like ascorbate-glutahione cycle. Alteration in the content of ascorbic acid is directly linked with the expression of genes involved in regulating the biosynthesis and catabolism of ascorbic acid (Seminario et al. 2017). The active role of ascorbic acid in mitigation of drought stress-induced growth decline has been confirmed through the studies exploiting its exogenous application effects on several tolerance mechanisms. For example, Shafiq et al. (2014) have demonstrated significant improvement in growth, mineral uptake, and photosynthesis in *Brassica napus* L. through modulation of antioxidant defense system and osmolyte accumulation following the exogenous application of ascorbic acid. Ascorbic acid improves the stomatal and non-stomatal attributes of photosynthesis by maintaining the redox homeostasis thereby protecting major cellular functioning like enzyme functioning, membrane structure, and stress signaling (Venkatesh and Park 2014). Upregulation of key enzymes like ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase has been reported due to exogenous application of ascorbic acid resulting in maintenance of redox homeostasis and hence drought tolerance (Singh and Bhardwaj 2016). Under drought stress, protection of membrane functioning due to exogenous application of ascorbic acid has been attributed to upregulation of activity of antioxidant enzymes (Penella et al. 2017). Arabidopsis thaliana transgenic plants over-expressing the AtOxR gene accumulated greater ascorbic acid and escaped the drought by declining the generation of hydrogen peroxide (Bu et al. 2016). Zhu et al. (2014) have demonstrated that increased expression of stress inducible EsWAX1 from *Eutrema salsugineum* imparts drought tolerance in Arabidopsis by promoting ascorbic acid synthesis by significantly enhancing the expression of gene VTC1, GLDH and MIOX4 involved in its synthesis. Ascorbic acid can also crosstalk with key phytohormones like ABA to promote signaling for better alleviation of drought as has been reported by Zhang et al. (2020a, b). In another study, Bao et al. (2016) have demonstrated that co-expression of 9-*cis*-epoxycarotenoid dioxygenase (SgNCED1) and yeast D-arabinono-1, 4-lactone oxidase (ALO) genes improve drought and chilling tolerance by improving ascorbic acid content in *Nicotiana tabacum* and *Stylosanthes guianensis*.

Drought stress results in oxidative damage to plants by increasing the accumulation of toxic reactive oxygen species including hydrogen peroxide, superoxide, etc. (Sharma and Dubey 2005). Drought-mediated oxidative affects drastically damages the structural and functional integrity of key macromolecules and ultimately altering the major cellular functioning and hence yield productivity (Sehgal et al. 2017). Growth and photosynthetic inhibition in drought stressed plants is often a culminated effect of the reduced water availability and the subsequent decline in water use efficiency (Talbi et al. 2020). Drought severely affects the net photosynthesis (Pn), light- and CO_2-saturated net photosynthesis (Pmax), stomatal conductance, the maximal rate of Rubisco carboxylation (Vcmax), and ribulose bisphosphate regeneration (J(max)) along with leaf carbon and nitrogen concentration in *Deschampsia flexuosa* (Albert et al. 2011). Moreover, drought-mediated decline in photosynthesis results from the direct influence on the soil moisture and field capacity reflected as reduced photosynthesis, light use, and relative growth rate (Xu et al. 2009). Exogenous application of ascorbic acid has been reported to significantly improve the photosynthesis by modulating the water relations, membrane structure, antioxidant system, and accumulation of secondary metabolites and osmolytes (Cevik and Unyayar 2015; Farooq et al. 2020). Exogenous application of ascorbic acid alleviated the drought stress-induced damage in safflower by eliminating ROS through upregulation of the antioxidant defense system, osmolyte accumulation, and maintenance of redox components (Farooq et al. 2020). In another study, Khazaei et al. (2020) have demonstrated better performance and tolerance to drought in *Capsicum annuum* through significant enhancement in water content, antioxidant functioning, and membrane stability due to exogenous application of ascorbic acid. However, the exact mechanisms are largely unknown; therefore, molecular and genetic studies are required to unravel the exact mechanisms. Besides, this exogenous ascorbic acid improved the accumulation of phenol peroxidases and total phenol content thereby strengthening the antioxidant system for quick neutralization of toxic ROS so that growth is least affected (Farooq et al. 2020). In wheat, Malik et al. (2015) have reported significant enhancement in growth and membrane functioning through modulation of antioxidant enzymes, proline accumulation, and tissue water content maintenance after treatment of ascorbic acid either through foliar, priming, or rooting medium. Increased growth and yield productivity under drought in ascorbic acid-treated wheat plants has been direct effect on leaf area index, tissue water content, protein oxidation, and lipid peroxidation (Kotb and Elhamahmy 2013). Ascorbic acid application improves protein, carbohydrate content, and water use efficiency in chickpea under drought stress (Farjam et al. 2015). Application of ascorbic acid reverses the influence of drought on the ABA, polyamine, and proline accumulation, and reduces the accumulation of ROS with concomitant enhancement in the leaf water status and stomatal conductance (Terzi et al. 2015). Drought tolerance in ascorbic acid-treated chickpea plants results from the significant enhancement in the activities of key enzymatic antioxidants and the accumulation of non-enzymatic antioxidants as well as osmolyte accumulation imparting a visible positive effect on the photosynthesis, growth, and yield (El-Beltagi et al. 2020). Improved drought tolerance in rice was demonstrated due to the upregulation of antioxidant system and osmolyte accumulation, and among the antioxidant enzymes, the increased activity of ascorbate peroxidase contributed more to hydrogen peroxide elimination as compared to catalase or peroxidase which was related with the greater accumulation of ascorbic acid in tolerant cultivars (Wang et al. 2019). Ascorbic acid improves water stress tolerance in common bean by enhancing pigments content, activity of carbonic anhydrase and antioxidant enzymes, accumulation of secondary metabolites like phenols, flavonoids, and tannins, thereby resulting in reduced oxidative damage and increased free radical scavenging activity (Gaafar et al. 2020). Research unraveling the molecular mechanisms is extensively needed to understand ascorbic acid-mediated growth regulation under drought stress.

12.3 Ascorbic Acid and Salinity Stress

Salinity is another key environmental factor affecting the normal growth and development in plants thereby restricting the yield potential of crop plants. Salinity stress mainly arises due to excess accumulation toxic ions like sodium, chloride, etc. in the soil solution (Isayenkov and Maathuis 2019). Among the key reasons for salinity include the usage of bad waters or saline waters for irrigation, excessive use of chemical fertilizers, and other improper management practices. The key damaging consequences of salinity stress include osmotic and ionic stress resulting in oxidative effects on plants (Isayenkov and Maathuis 2019). Excess salinity hampers germination, root growth, access to mineral ions, enzyme activity, and photoinhibition (Khan et al. 2021). In response to salinity, stress plants trigger the tolerance mechanisms for alleviating the damage to extent, and in this context, plants can be classified as halophytes or glycophytes (Pan et al. 2020). Halophytes have special mechanisms to tolerate excess salinity as compared to glycophytes which are sensitive to external salinity. Halophyte including *Atriplex*, *Aeluropus*, *Suaeda*, *Cakile*, *Mesembryanthemum, Salicornia*, *Thellungiella*, etc. are the potential candidates for the identification and characterization of salt-responsive genes, and among the genes are included: those coding for antiporters like *SOS, NHX,* HKT, ion channels like aquaporins, Cl^-, Ca^{2+}, etc., antioxidant like *SOD*, *CAT*, *APX*, *BADH*, etc. and other novel genes including *SDR1*, *SRP*, etc. (Mishra and Tanna 2017). There has been continuous effort from researchers all over globe to exploit these genes for improvement of salinity tolerance in glycophytes. In addition, the efficient sequestration and exclusion of toxic ions has a key role in salinity tolerance in plants (Isayenkov and Maathuis 2019). In addition, salinity stress alters the metabolite profile and triggers transcriptional modulation regulating the key pathways of metabolism including photosynthesis, signaling, and antioxidant system (Qin et al. 2021; Wang et al. 2021). Plants over-expressing the salinity stress responsive genes have been reported to exhibit reduced oxidative damage through modulation in the activity of antioxidant enzymes (SOD and CAT), accumulation of amino acid (proline and valine) and organic acids (glyceric acid, phosphoenolpyruvic acid and ascorbic acid), and secondary metabolites (Wang et al. 2021). Besides the modulations in the antioxidant machinery, osmolyte accumulation, and ion compartmentalization, salinity stress triggers significant alteration in the phytohormones metabolism and hence their altered accumulation (Arif et al. 2020). It has been reported that significant enhancement in the synthesis of ABA as increase in ethylene has also been reported (Nazar et al. 2014). Salinity-mediated modulation in polyamine metabolizing enzymes has been reported (Ahanger et al. 2019) resulting in significant modulation in their accumulation (Roychoudhury et al. 2011); hence, growth modulations are evident. Salinity altered salicylic acid in glycophyte *Solanum lycopersicum* and the wild-related halophyte *Solanum chilense* (Gharbi et al. 2016). On the other hand, phytohormones have been shown to improve salinity stress tolerance significantly, but only the optimal concentration can be beneficial. There have been considerable reports demonstrating the alteration in the content of ascorbic acid in plants exposed to salinity stress. For example, salinity exposed *Solanum lycopersicum* (Ahmad et al. 2018) and cherry tomato (Abdelgawad et al. 2019) exhibited decline in ascorbic acid content, whereas maize (AbdElgawad et al. 2016), *Limonium stocksii* (Hameed et al. 2015), wheat (Ahanger et al. 2017), and *Hordeum vulgare* L (Hassan et al. 2021) exhibited increase. Reports showing the essential role of ascorbic acid in protection of plant growth and yield performance are available, and it has been shown that exogenous application of ascorbic acid can be an active strategy to mitigate the salinity-induced damage. Recently, Hassan et al. (2021) have demonstrated a significant beneficial role of exogenously applied ascorbic acid in improving the salinity stress tolerance in *Hordeum vulgare* through modulation in the ion uptake, antioxidant metabolism, osmolyte accumulation, and expression of stress responsive genes. These modulations by ascorbic acid treatment alleviated the decline in photosynthesis by mediating reduced accumulation of toxic ROS. Pretreatment of *Vicia faba* with ascorbic acid improves salinity by increasing the synthesis of carbohydrates and proteins reflecting in growth improvement (Mohsen et al. 2013). Mittal et al. (2018) have reported increased protein, phenol content, and total antioxidant activity in *Brassica rapa* pretreated with ascorbic acid. Ascorbic acid improves nitrate reductase activity and antioxidant enzyme activity in salinity stressed canola with concomitant increase in fatty acid content (Bybordi 2012). Increased growth and biomass accumulation in *Limonium stocksii* due to ascorbic acid application has been attributed to increased activities of superoxide dismutase, catalase, ascorbate peroxide, and glutathione reductase and the accumulation of proline and total soluble sugars resulting in maintenance of lesser ROS levels and higher tissue osmolarity (Hameed et al. 2015). Saccharum spp. exposed to salinity stress were shown to exhibit better salinity tolerance due to the upregulation of superoxide dismutase and peroxidase activity and the accumulation of proline due to ascorbic acid treatment, thereby resulting in increased growth and biomass production (Ejaz et al. 2012). Singh and Sengar (2019) have demonstrated significant growth enhancement and salinity tolerance in in-vitro grown *Saccharum officinarum* due to ascorbic acid-induced enhancement in the activity of antioxidant enzymes (catalase and peroxidase). Exogenous application of ascorbic acid potentially reduces the ROS accumulation, and the endogenously existing factors can significantly contribute to maintenance of ascorbic acid content; for example, increased expression of ABI4 gene has been reported to lessen ascorbic acid content by affecting the *VTC2* expression and hence increasing salt sensitivity (Kakan et al. 2021), and such regulatory interaction has also been confirmed by others as well (Luo et al. 2021).

The involvement of transcription factors in ascorbic acid content and the stress tolerance has been reported. Ethylene responsive factor (ERF98) imparts salinity tolerance by increasing ascorbic acid content through activation of the genes regulating the D-mannose/L-galactose pathway and the myo-inositol pathway gene MIOX4, as well as of AsA turnover genes (Zhang et al. 2012). Exogenous ascorbic acid protects photosynthesis and growth under salinity stress by upregulating antioxidant enzyme activity and endogenous ascorbic acid content (Ekmekçi and Karaman 2012). Applied ascorbic acid alleviates the detrimental effects of salinity by maintaining higher K/Na ratio and proline accumulation thereby contributing to maintenance of tissue water content with concomitant reduction in electrolyte leakage (Aliniaeifard et al. 2016). Ascorbic acid application can potentially mitigate the damaging effects of salinity through improved physiological and biochemical attributes contributing to enhanced yield attributes (Ishaq et al. 2021a, b). Under salinity stress, ascorbic acid application enhances germination, fresh and dry weight, and reduces the oxidative damage by upregulating antioxidants system with concomitant increase in amylase and protease activities which was not consistent with tie duration (Chen et al. 2021). Genetic and functional analysis studies of GDP-D-mannose pyrophosphorylase in *Glycine max* have revealed its key role in plant development and stress tolerance, and higher transcript levels were found in were found in leaf than other tissues, besides its over-expression in *Arabidopsis* and soybean increased salinity stress tolerance via increased ascorbic acid synthesis resulting in significant decline in superoxide anion accumulation and hence lipid peroxidation (Xue et al. 2018). It should be mentioned here that the modulations in gene expression, biochemistry, and physiology of plants due to exogenous application of ascorbic acid are largely unknown therefore further studies are required to unravel the more detailed mechanisms.

12.4 Ascorbic Acid and Flooding Stress

Soil flooding is another factor that significantly affects the growth and plant functioning. Flooding creates complex stress which is also known as submergence or logging. Evapotranspiration and precipitation directly determines the distribution pattern of plants all over the world (Panda and Barik 2021). Besides this, the rainfall and level of groundwater table has considerable impact on maintenance of aquatic ecosystems during dry periods and also affecting the physiological characteristics of plants to altered water availability (Katerova et al. 2021). Floods are responsible for significant damage to crop yield of major crops like rice, maize, sorghum, sugarcane, etc. (Panda and Barik 2021). Flooding can be considered as waterlogging if water is superficial, and when entire plant is covered by water, it is considered as submergence (Panda and Barik 2021). Continuous submergence restricts the oxygen movement thereby leading to hypoxia. Flooding affects germination, growth, and yield significantly (Xiao et al. 2020). Reduced growth and yield under flooding can be attributed to greater accumulation of ROS and hence triggering the cell damage (Katerova et al. 2021). Synthesis of ATP and its management, metabolism of starch, ROS production and scavenging, element uptake and assimilation, and redox homeostasis are affected by flooding stress (Zhou et al. 2020). Changes in photosynthesis, respiration, architecture, and the phytohormones signaling are triggered due to flooding (Zhou et al. 2020). Plants tend to modulate the tolerance mechanisms for protecting the major cellular pathways like photosynthesis from logging-induced damage (Katerova et al. 2021).

The alteration in the synthesis of endogenous ascorbic acid and its role in alleviation of flooding-induced growth restriction is less researched area. Exogenous application of ascorbic acid to *Vigna angularis* has been reported to reduce the logging-induced decline in growth by declining lipid peroxidation and elevating levels of jasmonic acid (Ullah et al. 2017). Potential to delay the apparition of oxidative damage under flooding stress has been reported to show correlation with the antioxidant functioning, i.e., the activity of antioxidant enzymes and the accumulation of non-enzymatic antioxidants including ascorbic acid (Arbona et al. 2008). It should be mentioned here that there are very rare reports on discussing the role of ascorbic acid in flooding tolerance and the influence of flooding on the endogenous ascorbic concentration of ascorbic acid.

12.5 Ascorbic Acid and UV Stress

Ultraviolet radiations severely influence the normal plant metabolism and growth restricting the yield potential of crop plants (Valenta et al. 2020). UV radiations are harmful to living organisms causing damage to key macromolecules like membranes, proteins, lipids, and DNA. Due to damage to the ozone, the UV radiations enter directly to the atmosphere (Hollosy 2002). Plants utilize sun light for photosynthesis and cannot avoid the exposure to UV radiations due to their sessile nature (Hollosy 2002). Reduced growth due to UV exposures is related to decline in chlorophyll and protein content (Salama et al. 2011). Growth decline under UV exposure results from the declined photosynthesis. The sites of UV radiation damage within the photosynthetic apparatus include oxygen-evolving complex, D1/D2 reaction center, and the donor and acceptor sides of PSII. In addition to this, the light harvesting complex II is inactivated, and expression of genes coding for PSII proteins is also affected (Khudyakova et al. 2019). The UV-B radiation stress has been reported to affect primarily the Mn cluster of water oxidation complex, whereas other sites including D1, D2, cytochrome, and quinone molecules are the subsequent targets. Besides this decline in Rubisco, chlorophyll, stomatal conductance, photosynthesis, maxima quantum efficiency, effective efficiency of PSII, and formation of necrotic spots on leaves altering their morphology are common effects (Mariz-Ponte et al. 2021). The photosynthetic damage due to UV radiations is also attributed to increased ROS accumulation (Liu et al. 2018). Radiation stress declines germination and growth significantly as it has been reported in *Triticum aestivum, Helianthus annuus, Glycine max, and Pinus maximartinezii* reflecting as altered length of radicle and plumule (Pournavab et al. 2019). Exposure to UV radiations reduces photosynthesis, ammonium uptake, and nitrated reductase activity in *Gracilaria lemaneiformis* (Xu and Gao 2012) while UV accumulation of absorbing compounds increased and the accumulation of these compounds have significant correlation with NADP malic enzyme (Xu and Gao 2012). Exposure to UV radiations reduces reproduction but enhances the accumulation of phenolics (Valle et al. 2020). The influence of UV radiations shows rather varied effects on the activity of nitrate reductase and can improve its activity under nutrient deficient conditions (Krywult et al. 2013), and this is believed to be due to the presence of specific loci that are responsive to radiations (Morrison et al. 2010). Treatment of UV-B radiation decreases the oxygen evolution rate, sugar content, and nitrate reductase activity but showed no effect on the chloroplast ultrastructure and plant weight (Quaggiottia et al. 2004). Cotton treatment of UV-B or UV-A hampers the functioning of antioxidant system by declining the synthesis of ascorbic acid, thereby negatively affecting the growth (Dehariya et al. 2011). However, Li et al. (2017) have observed contrasting response to UV-C exposure in terms of ascorbic acid accumulation in subtropical fruits including litchi, longan, and rambutan, with litchi exhibiting increased ascorbic acid content while longan and rambutan exhibiting decline, thereby having the similar impact on the radical scavenging activity measured as DPPH and ABTS activity. Therefore, it is evident that the plants show contrasting response to UV radiations, therefore knowing the exact mechanisms involved need to be unraveled. Research studies discussing the role of ascorbic acid treatment in mitigation of UV stress-induced damage in plants are not available.

12.6 Heavy Metals and Ascorbic Acid

Heavy metal(loid)s are the nonessential metal ions that have been reported to influence the plant growth negatively. Heavy metals include the metals that have a density greater than 5 g/cm^3. Nearly 53 heavy metals have been identified, and among these, seventeen (17) are available for living cells based on their solubility and are essential for organisms as well as the ecosystem (Anjum et al. 2014). At optimal concentrations, the metal ions including Fe, Mn, and Mo are essential micronutrients whereas others including Ni, Cu, Zn, V, Co, Cr, and W are trace elements, however, at greater concentrations, they prove toxic for plant growth (Angulo-Bejarano et al. 2021). Metals including Cd, As, Pb, Hg, Ag, U, etc. have been reported to interrupt the normal growth and metabolism of plants through their toxic and damaging effects (Angulo-Bejarano et al. 2021). In present times, heavy metal pollution is increasing all over the world and is considered as one of the key factors for loss of agricultural productivity (Anjum et al. 2014). Heavy metals are mainly released from the industries as well as the bad agricultural activities and vehicular activities. The presence of heavy metals in the soil solution influences plant growth, energy synthesis mechanisms, and trigger senescence (Zu et al. 2016). Heavy metals adversely affect root growth, absorption, transport, and assimilation of key essential mineral ions and enzyme functioning, thereby hampering the normal metabolism and hence significant decline in growth and reproduction follows

(Ahmad et al. 2010, 2021). Reduction in chlorophyll synthesis, stomatal and non-stomatal attributes of photosynthesis, Rubisco synthesis, and the alteration of the redox components are evident effects of heavy metal stress (Ahanger et al. 2020a). Among the other common effects of heavy metals are included: reduced growth and yield production, yellowing of leaves, hampered absorption, and assimilation of mineral elements leading to reduction in photosynthesis and excessive ROS generation, causing loss of membrane structure and function and oxidation of proteins and lipids (Hameed et al. 2016). Heavy metals induce ROS generation by stimulating the activity of NADPH oxidases, replacing cations from binding sites of enzymes leading to enzyme activity inhibition by binding with the thiol (-SH) groups (Shahid et al. 2014). The toxicity, response, and tolerance to heavy metals vary with the plant species and the cultivars (Ahmad et al. 2021). Like other stresses, heavy metal tolerance is also a complex event that is precisely regulated at molecular, biochemical, and physiological levels (Ahmad et al. 2010). Therefore, coordination among the key complex physiological and biochemical processes regulated by the modulations in the gene expression and alterations in the metabolome is key to elicit a proper stress signal and hence the tolerance (Ahanger et al. 2020b). Heavy metals plant performance through their impact at different structural and functional levels. Reduction in germination, shoot and root elongation under heavy metal stress can be majority due to impaired cellular division. Reduced germination, shoot, and root growth in terms of length and weight due to heavy metals has been reported by several workers (Yao et al. 2021). Root becomes stubby, stunted, and brittle with apices swollen and damaged. Heavy metals reduce the cell wall elasticity and therefore get easily affected by the mechanical stress (Gall et al. 2015). The interaction of heavy metals with the plant constituents intensifies the effects of metals. Ahmad et al. (2018) have demonstrated significant decline in growth parameters, photosynthesis, enzyme activity, and mineral uptake resulting in oxidative damage to membranes due to mercury stress. Mercury stress impedes nitrogen and phosphorous mobilization in spinach (Gothberg et al. 2004). Mercury restricts growth, photosynthesis, osmolyte accumulation, antioxidant metabolism, and functioning of glyoxylase system thereby inducing oxidative stress (Ahmad et al. 2021). Per et al. (2016) have observed significant decline in growth and phytocystatin activity in mustard cultivars due to cadmium stress. Arsenic stress imparts damaging effects on the growth and metabolism of plants by altering the key metabolic pathways including mineral uptake and assimilation, photosynthesis, respiration, enzyme functioning, and metabolite synthesis (Ahmad et al. 2010). Further the oxidative damage resulting from excess accumulation of ROS in addition of the translocation factor of particular heavy metal as well as the shoot and root tolerance index determine the toxicity of heavy metals in plants (Ahmad et al. 2010). Among the key determinants of phytoremediation potential and hence the heavy metal tolerance are bioconcentration factor, bioaccumulation coefficient, and translocation factor (Amin et al. 2018).

Modulations in the endogenous concentration of ascorbic acid due to heavy metals have been reported to affect the tolerance potential of plants significantly. The increased heavy metal sensitivity in ascorbic acid-deficient plants is notably due to alterations in the redox homeostasis triggering hindrances in normal metabolism (Bielen et al. 2013). As an active component of ascorbate-glutathione cycle, ascorbic acid regulates growth directly or indirectly by scavenging ROS, and the entire redox system together with enzymes like ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase efficiently protect plants against heavy metal stress-mediated damage (Ahmad et al. 2021). The ascorbate-glutathione cycle functioning has been considered as one of the key contributors for the maintenance of ascorbic acid content besides the identified biosynthetic pathways (Ishikawa and Shigeoka 2008). In lead-stressed wheat, Alamri et al. (2018) have reported significant alleviation of oxidative damage, nitrogen, and sulphur assimilation through upregulated antioxidant metabolism and mineral ion uptake due to exogenous ascorbic acid treatment. Further they have attributed ascorbic acid-mediated growth enhancement to increased chlorophyll synthesis, Rubisco activity, RWC, and N, P, K, Mg, and Ca. Exogenous ascorbic acid alleviated copper stress-induced growth decline in wheat by modulating the protein, carbohydrate, proline, cellulose, lignin, and the content of lipids and phospholipids (Al-Hakimi and Hamada 2011). Increased accumulation of ascorbic acid is due to trehalose application contributed to copper stress tolerance by maintenance of redox homeostasis (Mostofa et al. 2015). Research reports have shown that plants exhibiting enhanced expression of ascorbic biosynthesis show greater stress tolerance. Bu et al. (2016) have reported that over-expression of AtOxR improves metal stress tolerance in Arabidopsis through increased accumulation of ascorbic acid. However, the influence of ascorbic acid on the metal ion transporters is largely unknown, and further research in this direction can provide further understanding about the actual mechanisms.

12.7 Ascorbic Acid and Temperature Stress

Temperature extremes are also key environmental factors influencing growth and development of plants and their productivity. High as well as low temperatures impart decline in germination, cell proliferation, enzyme functioning, photosynthesis, and mineral assimilation (Huang et al. 2016). Global climate change intensifies the damaging effects by imparting sudden changes in variations in temperature regimes. Alterations in optimal temperatures results in considerable damage in productivity (Zhang et al. 2020a, b). By affecting the metabolic pathways like respiration and photosynthesis, temperature stress shortens normal life cycle and hence restricts the plant productivity (Barnabás et al. 2008). Early obvious alterations induced by temperature fluctuations include hampered chloroplast protein stability and the enzyme activity (Zhang et al. 2020a, b) and reduced import of small subunit of Rubisco into plastids with significant change in electron transport and chlorophyll fluorescence (Hu et al. 2020). Changes in cellular differentiation, elongation, and proliferation under temperature stress result due to the damage to cell membranes and microtubule organization (Koutalianou et al. 2016). Among the key primary targets of high temperature stress include the chloroplast stroma and thylakoid membranes, thereby inducing significant decline in carbon flux (Wise et al. 2004). Photosynthetic enzymes including those of C_3 or C_4 cycle are very much sensitive to temperature stresses (Song et al. 2014). Proteomic studies have shown considerable changes in photosynthetic and carbon metabolism proteins induced by low temperature stress, and acclimation is related to energy dissipation in photosystems in sensitive plants, while as an electron sink, the enhanced photosynthetic carbon, assimilation can be a key factor for imparting tolerance in tolerant plants. Besides the alterations of the thylakoid membranes, the accumulation of starch granules can be also seen during cold stress (Calzadilla et al. 2019). Low temperature induced decline in photosynthesis, stomatal conductance, and relative water content and contribute maximally to growth decline in *Brassica oleracea* genotypes (Rodríguez et al. 2015). Temperature stress-mediated increase in ROS triggers chlorophyll decline and photoinhibition, thereby declining the carbon fixation rate. Besides this, decline in phenolic contents under temperature stress declines the antioxidant potential which can also negatively influence the growth (Soengas et al. 2018). Like other stresses, temperature extremes also induce the increased accumulation of toxic ROS, thereby imparting significant damage to membranes, proteins, lipids, nucleic acids, etc., hence leading to altered metabolism and plant performance (Munjal 2019). Excess ROS generated due to temperature stress induce oxidative damage to membranes affecting their fluidity and ion transport properties besides influencing the enzyme functioning, protein synthesis, and also affects the protein cross linking (Munjal 2019). ROS production under heat stress can integrate the signaling mechanisms for triggering downstream mechanisms for improving the temperature stress tolerance (Medina et al. 2021).

Tolerance to temperature extremes also depends on the efficient working of pathways responsible for synthesis of key molecules having significant role in the alleviation of damaging effects of extreme temperatures (Ljubej et al. 2021). The upregulation of antioxidant system, accumulation of proline, sugars, free amino acids, phenols, flavonoids, and the expression of temperature responsive genes significantly determine the tolerance potential of plant to temperature fluctuations (Leuendorf et al. 2020; Ljubej et al. 2021). Priming with ascorbic acid has been reported to affectively ameliorate the chilling-induced oxidative stress and growth decline in tomato through modulation in the accumulation of proline, endogenous ascorbic acid content, mineral uptake, and the expression of catalase HSP70, HSP80, and HSP90, reflecting in increased antioxidant potential and reduced oxidative stress capacity and total oxidative capacity. Temperature stress (low or high) declines the content of ascorbic acid (Elkelish et al. 2020). Zhang et al. (2018) have shown that L-galactono-1, 4-lactone dehydrogenase (GLDH) over-expressing and mutant plants exhibiting increased and deficient ascorbic acid synthesis have increased and decreased photosynthesis respectively which was attributed to ascorbic acid-mediated modulations in the chlorophyll, soluble sugars, Rubisco subunits, and ROS production. Ascorbic acid has a key role in thermal energy dissipation in plants to protect photosynthesis (Bilska et al. 2019). Temperature stress-induced alterations in the endogenous ascorbic acid content are related to the effect on the expression level of genes controlling its synthesis and recycling (Xiang et al. 2020). Foliar application of ascorbic acid to strawberry alleviated the temperature stress-induced growth decline by enhancing chlorophyll content, relative water content, cell turgidity, endogenous ascorbic acid, and the peroxides isozyme levels (Ergin et al. 2014). Transgenic plants exhibiting increased expression of dehydroascorbate reductase showed greater tolerance to low temperature by maintaining greater ascorbic acid content, and it has been consolidated that transcriptional regulation mediates the stress tolerance. Cold tolerance in plants reflected as reduced oxidative damage due to upregulated functioning of ascorbate-glutathione cycle results from the maintenance of higher concentrations of redox components (Xing et al. 2019). Reports discussing the role of exogenous ascorbic acid application on modulation of tolerance mechanisms against temperature variations are scanty; therefore, it is imperative to focus on these areas to further understand the mechanisms and underpin the key crosstalk mechanisms. However, research studies have shown that overexpression of enzyme components or other intermediate components contributing to ascorbic acid synthesis or maintenance of its cellular concentration contribute to temperature tolerance. Overexpression of ascorbate peroxides enhances tolerance to heat and chilling in tomato (Wang et al. 2005, 2006). Overexpression of GDP-mannose pyrophosphorylase in tobacco enhances the synthesis of ascorbic acid imparting a significant positive effect on the antioxidant enzyme functioning, thereby preventing ROS accumulation and hence the oxidative effects (Wang et al. 2011). Increasing expression of ascorbate peroxidase imparts chilling tolerance to rice (Sato et al. 2011) and has been reported to protect the photoinhibition in transgenic tobacco by improving PSII activity by lessening ROS accumulation and lipid peroxidation under chilling and high temperature stress (Sun et al. 2010). The protective role of ascorbic acid against heat stress-induced decline in photosynthesis has been ascribed to its role as alternative PSII electron donor (Tóth et al. 2011). Therefore, it can be concluded that ascorbic acid through its active involvement in other key protective mechanisms assists in preventing the damage to photosynthetic apparatus and plant metabolism significantly.

12.8 Conclusion and Future Prospects

Abiotic stresses prove to be damaging factors for the growth and development of major crop plants. Growth and metabolic alterations posed due to stress factors results from the oxidative damage, impeded mineral assimilation and photosynthesis, and increased programmed cell death. Molecular mechanisms controlling the physiological and biochemical pathways at a whole-plant level are triggered in response to stress to cope up with the stress-induced damage. Modulation of the tolerance mechanisms for alleviating the stress effect have been reported widely due to ascorbic acid treatment (Fig. 12.2). Such beneficial effects have been confirmed through transgenic research. Identification of key target genes for improving the endogenous ascorbic acid levels for improved stress tolerance needs further research. In addition to this, the downstream signaling targets of ascorbic acid-medicated stress tolerance also need further investigation.

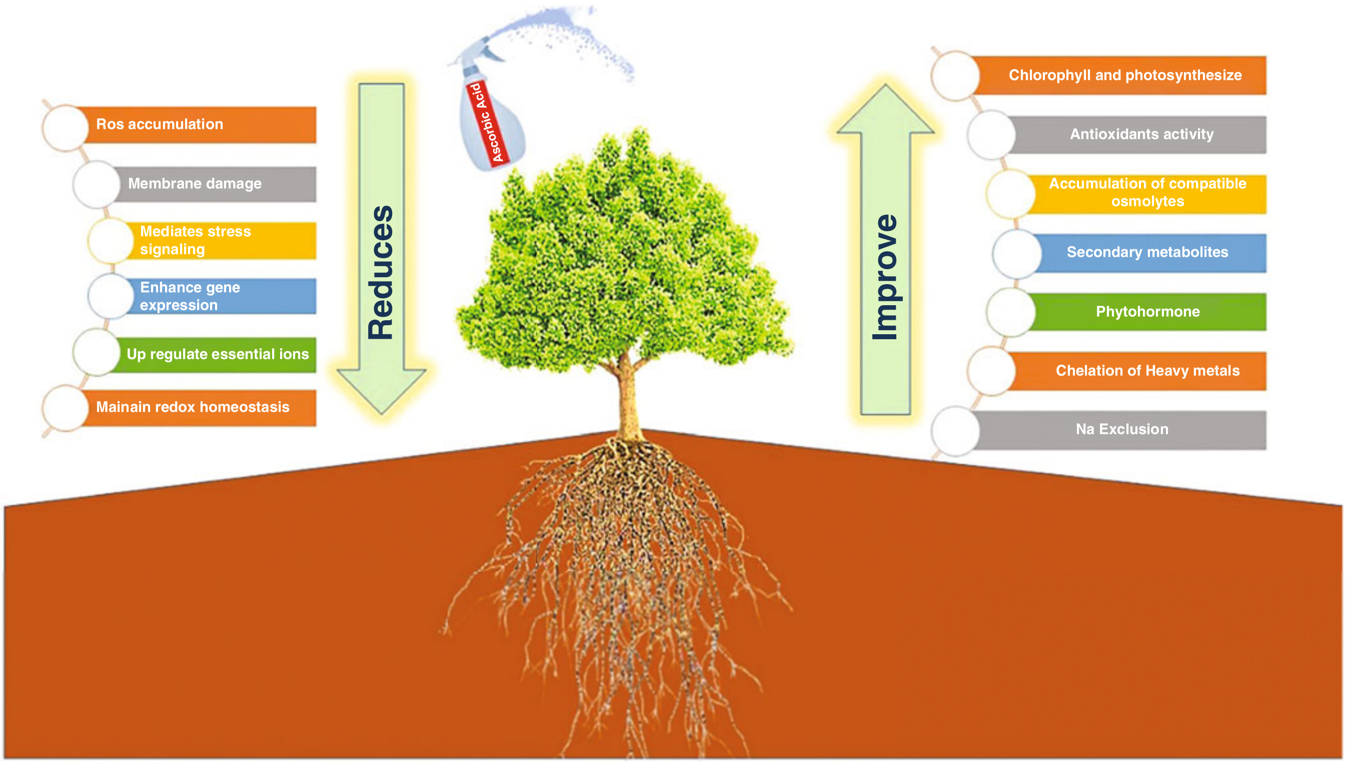

Fig. 12.2 Role of ascorbic acid in overcoming stress in plants

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CRISPR/Cas-Mediated Genome Editing Technologies in Plants for Stress Resilience

13

Deepu Pandita

Abstract

Climate change can affect agriculture through various abiotic (temperature: low/high, salinity, heavy metals, water submergence, and water deficiency) and biotic (bacterial, viral, fungal) stress factors. Climate change leads to above 50% of worldwide losses in the yield of major crops per year. Nutrition demand is going to nearly double by the 2050. Recent genome editing approach of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) system is a leading, powerful, versatile, ground-breaking, and smart plant genome editing tool with greater efficiency for designing of stress resilient crops. CRISPR/Cas can cause activation and interference of genes related to stress regulatory networks for advanced tolerance to stress scenarios.

Keywords

CRISPR/Cas system · Abiotic and biotic stress · Stress resilient crops · Genetic engineering · Genome editing

13.1 Introduction

Climate change is affecting agriculture in inestimable ways and threatens food security by causing more than 50% of worldwide losses in the yields of major crops every year. Food demand at worldwide levels is going to nearly double by the 2050s (Tilman et al. 2011). In anticipation of Gregory Mendel's breakthrough discoveries, man-mediated natural selection of plants was a time-consuming and labor-intensive process and achieved plant reproduction goals causing damage to

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gene pool diversity including genes related to abiotic (drought, salinity, cold, heat, submergence, etc.) and biotic (pathogens) stress tolerance and resistance (Somssich 2019). Time (6–7 years) consuming due to intensive backcrossing and backbreaking conventional crop breeding (Fig. 13.1) can improve crops, but it is not prolific in climate resilience due to complex inheritance and high genotype × environmental interactions and transfers accessory genes along with Gene of Interest (GOI) into transgenic plant (Bhat et al. 2016). The genetically engineered plants can be divided into three generations. The first generation of genetically engineered plants was targeted towards traits of tolerance/resistance towards abiotic stress or herbicides. The second generation of genetically engineered plants highlighted more on marketable profits, for instance, shelf life or nutritive value. Third generation of genetically engineered plants include functional foods boosted with pharmacological products (Fernandez-Cornejo et al. 2014). Genetic engineering via RNA interference (RNAi) and omics approaches of transgenic breeding are suitable strategies to improve stress tolerance due to direct relationship of genotype with the phenotype studies, transfers only gene of interest (GOI) into transgenic plant (Fig. 13.1) and to avoid decline in crop yields caused by climate change during the twenty-first century (Tester and Langridge 2010; Verma and Deepti 2016; Hyun 2020). Use of genetically modified (GM) crops and transgenic breeding is limited because of public concerns about their potential benefits, risks and safety, and efficacy of genetically modified organisms (GMOs) (Fernandez-Cornejo et al. 2014; Prado et al. 2014; Raman 2017). Recent genome editing technique of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) system has attracted attention as a potent genome editing tool that can efficiently generate hereditary mutations by targeting genes in a site-specific manner (Fig. 13.1) and produce high yielding climate-resilient crops and to understand the molecular mechanisms of stress tolerance in crop plants (Hyun 2020). CRISPR/Cas system finds use in both the induction (CRISPR activation) and repression (CRISPR interference) of genes. Consequently, CRISPR/Cas can activate tolerance (T) genes besides suppressing sensitivity (S) genes (Zafar et al. 2020a). CRISPRa-system-based OsNCEB3 overexpression in *Oryza sativa* enhanced accumulation of abscisic acid and tolerance to salt and water deficiency stress (Huang et al. 2018). The types, modes of action, and biological mechanisms of various CRISPR/Cas systems have been elucidated in our several chapters (Pandita et al. 2021; Pandita 2021a, b, c).

13.2 CRISPR/Cas-Mediated Genome Editing Technologies in Plants for Abiotic Stress Resilience

Plants are domineering source of food, vegetables, fruits, and medicines for human consumption (Pandita 2020a, b; Pandita et al. 2020; Pandita and Pandita 2020, 2021; Zehra et al. 2020). Heat, salinity, heavy metals, and water deficiency abiotic stresses (Fig. 13.2) stop growth of plants and source overwhelming yield damages (Pandita 2019; Pandita and Wani 2019). Plant sensitivity genes (Se genes) boost damaging consequences of abiotic stresses. Genome editing approach of CRISPR/Cas system

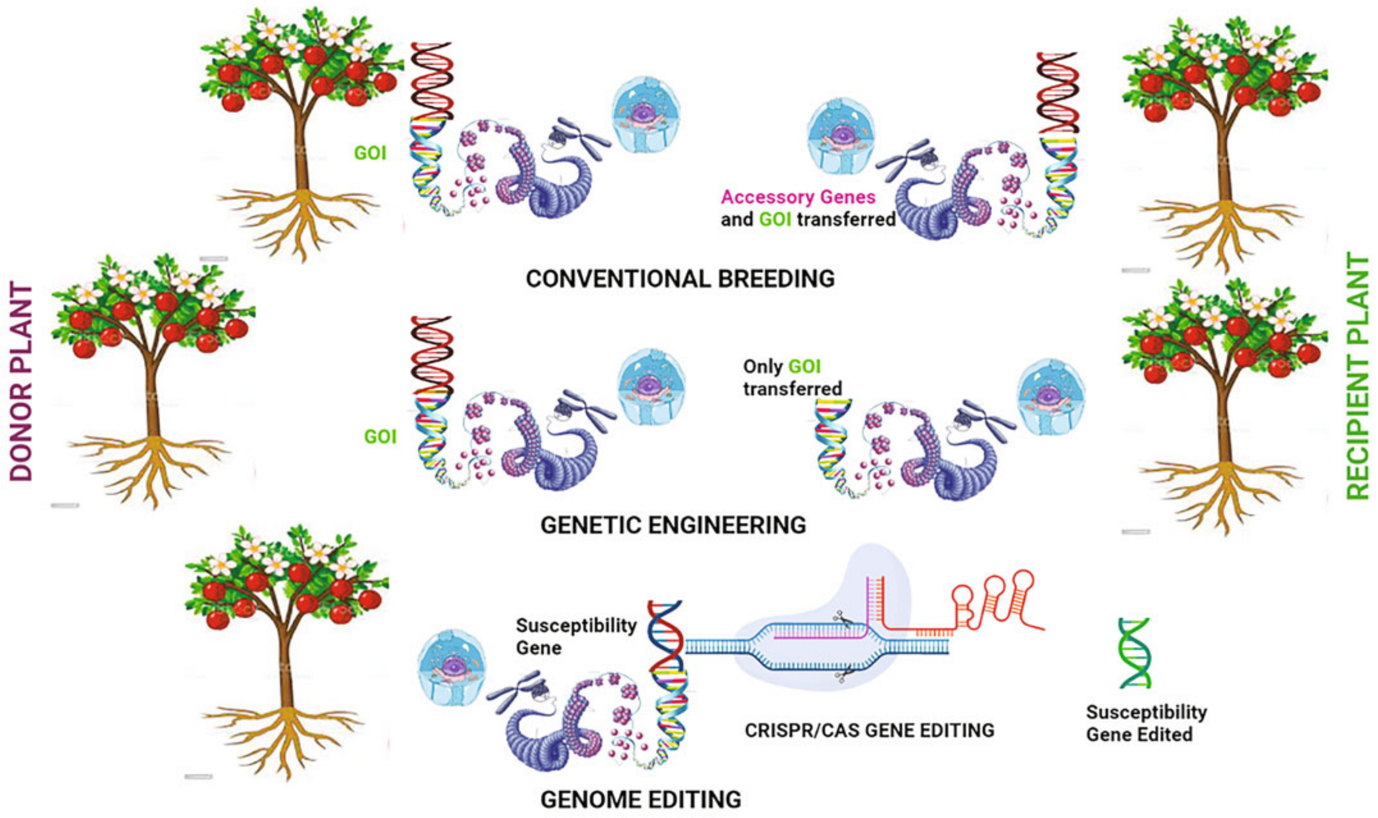

Fig. 13.1 Conventional breeding, genetic engineering, and genome editing

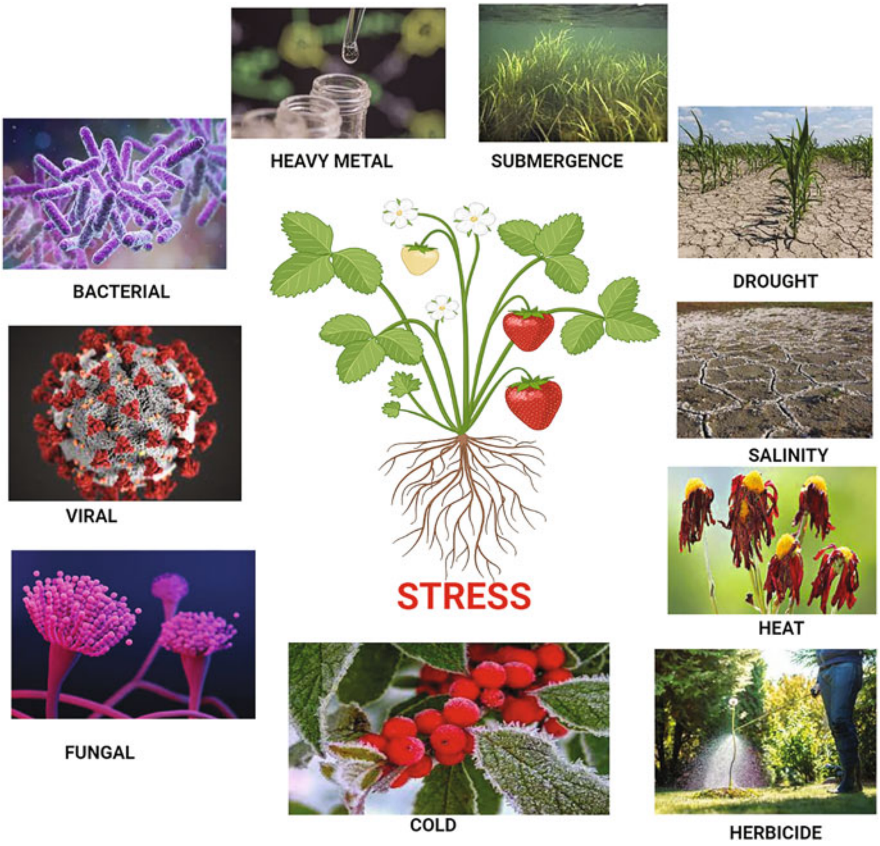

Fig. 13.2 Different stresses which affect plants

advances tolerance to abiotic stress in various species of grains, vegetable, fruits, and crop plants by interference of Se genes (Ahmad et al. 2021).

13.2.1 Drought Stress

Drought and salt tolerance (DST) gene was modified by CRISPR/Cas9, and 366 base pairs were removed from the coding sequence in indica mega rice cv. MTU1010. MTU1010 dst mutants generated showed resistance/tolerance to water deficiency, osmotic, and salt stresses because of decrease in density of stomata and expanded surface area of leaves for enhanced retention of water and light-use efficiency (Santosh Kumar et al. 2020). CRISPR/Cas-edited SAPK2 knockdown rice mutants were susceptible to water deficiency (Lou et al. 2017). CRISPR/Cas-modified drought-responsive OsDREB and salt-sensitive OsERF922 genes (Liu et al. 2012; Hoang et al. 2016). Editing of dehydration responsive element binding protein 2 (TaDREB2) and ethylene responsive factor 3 (TaERF3) enhanced tolerance to water deficiency in wheat (Kim et al. 2018). CRISPR/Cas editing system produced ARGOS8-v1 and ARGOS8-v2 lines with remarkable increase in yield of grains in ARGOS8 variants in *Zea mays* under drought and boosted tolerance to water deficiency (Shi et al. 2017). CRISPR/Cas9-edited transcription factor ZmWRKY40 conferred *Arabidopsis* tolerant to drought (Wang et al. 2018a). In tomato, CRISPR/Cas9-modified slnpr1 developed tolerance to drought conditions (Li et al. 2019a). CRISPR/Cas knockout lines in tomato produced by TEY (Thr-GluThy) motifs in mitogen-activated protein kinases 3 (SlMAPK3) imparted tolerance to water deficiency (Wang et al. 2017b). CRISPR/Cas9 mutation OST2 gene altered stomatal closing pattern and increased tolerance to water deficiency stress in Arabidopsis (Osakabe et al. 2016).

13.2.2 Heat Stress

Tomato has efficient transformation system for achieving improvements in fruit quality (Pan et al. 2016). The CRISPR/Cas-mediated modification of mitogen-activated protein kinase 3 (SlMAPK3) and sl Agamous-Like 6 (SlAGL6) enhances sensitivity in *Solanum lycopersicum* L. to high temperature stress and develops heat-tolerant tomato. The SlAGL6 gene editing resulted in generation of parthenocarpic fruits. ADP-ribosylation factor 4 (SlARF4) enhances salt sensitivity. CRISPR/Cas-modified plants can tolerate various stresses (Klap et al. 2017; Bouzroud et al. 2020). CRISPR-bzr1-mutant with reduced production of H_2O_2 and heat tolerance and BZR1-overexpressing lines of tomato with enhanced production of H_2O_2 and retrieval of thermo tolerance prove role of BZR1 in thermo tolerance through control of homologs of Feronia (Fer) (Yin et al. 2018). CRISPR-based heat-stress sensitive albino 1 (HSA1) deletion mutants show higher sensitivity to heat (Qiu et al. 2018). The CRISPR/Cas-based modification of thermosensitive genic male sterile 5 (TMS5) develops thermosensitive male sterile *Zea mays* (Li et al. 2017).

13.2.3 Salt Stress

In rice, OsBBS1 gene has a role towards salt sensitivity, and OsMIR528 gene acts as a positive regulator of salinity (Lan et al. 2019; Ganie et al. 2021; Sun et al. 2019). OsNAC041 and OsRR22 genes also enhance tolerance to salinity in *Oryza sativa* (Ganie et al. 2021; Lan et al. 2019; Sun et al. 2019). CRISPR/Cas-mediated knockdown of SnRK2, SAPK-1, and SAPK-2 genes confer resistance to salinity in *Oryza sativa* (Lou et al. 2017). SlMAP3 knockout in tomato decreases SlLOX, SlGST, and SlDREB expression and induces resistance to salinity (Wang et al. 2017b).

13.2.4 Cold Stress

In rice, B-amylase (BMY) genes control degradation of starch and maltose accumulation to provide protection against cold. MYB transcription factor (OsMYB30) and OsJAZ9 bind to promoter regions of B-amylase gene. CRISPR/Cas-edited OsMYB30 produced tolerance to cold in *Oryza sativa* lines (Zeng et al. 2020b). The CRISPR/Cas editing developed Ospin5b, gs3, and Osmyb30 mutants (Zeng et al. 2020b). Editing of annexin (OsANN3) conferred cold tolerance to japonica rice cultivar (Shen et al. 2017). The CRISPR/Cas9 editing of ABA activated protein kinase 2 (SAPK2) made rice resistant to cold stress (Lou et al. 2017). The CRISPR/Cas-generated cold-tolerant cbf1 mutants' accumulated higher amounts of indole acetic acid (IAA) and hydrogen peroxide in chilling sensitive plants of tomato (Li et al. 2018).

13.2.5 Herbicide Stress

Herbicide-tolerant crops are beneficial for contemporary agriculture. CRISPR/Cas9 and prime editing of acetolactate synthase 1 (ZmALS1) and acetolactate synthase 2 (ZmALS2) boosted tolerance to chlorsulfuron herbicide in *Zea mays* (Nuccio et al. 2021; Svitashev et al. 2015). Fusion of Cas9 and VirD2 enables cleavage of OsALS and synchronized HDR-mediated OsALS gene repair which lead to herbicide tolerance in *Oryza sativa* (Ali et al. 2020). In rice, acetolactate synthase (OsALS) precise nicking and HDR-mediated repair using prime editing has also improved tolerance to herbicides (Butt et al. 2020). CRISPR/Cas9 (nCas9)-generated mutations or nuclease-deficient Cas9 (dCas9) fused to Petromyzon marinus cytidine deaminase (PmCDA1) developed rice mutant lines showing herbicide tolerance (Shimatani et al. 2017). CRISPR-based editing of ALS1 improved herbicide tolerance in plants of *Brassica napus*, *Solanum lycopersicum*, and *Oryza sativa* (Wang et al. 2021). CRISPR/Cas alongside single-stranded oligonucleotides induces tolerance to herbicides in *Linum usitatissimum* (Sauer et al. 2016).

13.2.6 Water Submergence Stress

Natural floods also set limitations on yield of crops (Voesenek and Bailey-Serres 2015). Introgression of submergence 1 A-1 (SUB1A-1) in high-yielding rice varieties through marker-assisted breeding produced varieties tolerant to water submergence (Dar et al. 2018).

13.2.7 Heavy Metal Stress

Heavy metals (Khan et al. 2020) pose serious hazards to plants. Metals accumulate inside the grains and indirectly pose danger to human health. Plants can grow on contaminated soils by CRISPR/Cas-mediated prevention of transporter genes involved in heavy metal translocation. CRISPR/Cas mutation of natural resistance-associated macrophage protein 5 (OsNRAMP5) reduced Cd accumulation in *Oryza sativa* and enhanced its growth and development (Yang et al. 2019; Khan et al. 2020). CRISPR/Cas9 of Auxin-Regulated Gene Involved in Organ Size 8 (ARGOS8) enhanced drought resistance in *Zea mays* (Shi et al. 2017). Innumerable examples of CRISPR/Cas-based tolerance to plant abiotic stresses are précised in Table 13.1.

13.3 CRISPR/Cas-Mediated Genome Editing Technologies in Plants for Biotic Stress Resilience

Plant pathogens (bacterial, fungal, and viral) (Fig. 13.2) cause various plant diseases (Taylor et al. 2004) that decrease plant yield. Insect herbivores also decline yield by directly damaging and by acting as vectors of diseases. The changing climate undesirably affects plant tolerance to different biotic stresses. Direct crop loss (20–40%) is because of biotic stresses (Oerke 1994). CRISPR/Cas develops disease-resistant and insect-resistant plants (Ahmad et al. 2020). Crop protection can be achieved by introduction of dominant resistance (R) genes by breeding or transgenics. Susceptibility (Su) genes can be targeted by CRISPR/Cas in plant genomes. Su genes encode factors useful for infection to pathogens. Su gene knockout through targeted mutagenesis decrease susceptibility of disease (Ahmad et al. 2020). Su genes encoding sugar transporters can be targeted to enhance disease resistance. Bacterial blight is caused by *Xanthomonas oryzae* pv oryzae. During infection, transcription activator-like effectors (TALEs) escalate transcription of OsSWEET11, OsSWEET13, and OsSWEET14. CRISPR/Cas edits connection between TALEs and SWEET genes by mutation of TALE binding site in promoters of OsSWEET11, OsSWEET13, and OsSWEET14 (Zhou et al. 2015; Li et al. 2020a, b; Zeng et al. 2020a, b). Rice lines generated have broad-spectrum tolerance to bacterial blight (Oliva et al. 2019). Rice resistant to blast disease caused by *Magnaporthe oryzae* has been generated by knocking out ethylene-responsive factor (ERF) gene OsERF922 and Pi21 Su genes (Wang et al. 2016; Nawaz et al. 2020). Resistance to powdery mildew due to *Podosphaera xanthii* in Triticum was developed by target of Mildew Locus O (MLO) gene through CRISPR/Cas (Wang et al. 2014). Rice tungro disease is caused by rice tungro spherical virus, and resistance against this disease is governed by translation initiation factor 4 gamma gene (eIF4G). Rice tungro disease resistant lines were generated by CRISPR/Cas disruption eIF4G (Macovei et al. 2018). The resistant lines of Triticum to powdery mildew were produced by synchronized CRISPR/Cas editing of three mildew resistance locus (MLO) homologues (Wang et al. 2014; Zhang et al. 2017). Resistant plants had enhanced yield. Knockout of fungal pathogen genes of ALB1 (ALBINA 1), RSY1 USTA, and UvSLT2 of rice blast and false smut was generated through CRISPR/Cas (Foster et al. 2018; Liang et al. 2018). Cas variants of CRISPR/Cas systems can target viral genomes of both the DNA or RNA nature. The CRISPR/

Table 13.1 CRISPR/Cas-based designing of abiotic stress-resistant crop plants

Plant species	Targeted gene/s	Reference/s
Cold abiotic stress tolerance		
Oryza sativa	IFY1a, TIFY1b	Huang et al. (2017)
Oryza sativa	OsMYB30	Zeng et al. (2020a, b)
Oryza sativa	OsAnn3	Shen et al. (2017); Romero and Gatica-Arias (2019)
Drought abiotic stress tolerance		
Arabidopsis thaliana	AREB1 A.	Roca-Paixão et al. (2019)
Arabidopsis thaliana	Vacuolar H+pyrophosphatase (AVP1)	Park et al. (2017)
Arabidopsis thaliana	OST2	Osakabe et al. (2016)
Arabidopsis thaliana	miR169a	Zhao et al. (2016)
Brassica napus	BnaA6.RGA (DELLA Protein)	Wu et al. (2020a)
Cicer arietinum	Coumarate ligase (4CL) and Reveille 7 (RVE7)	Badhan et al. (2021)
Oryza sativa	OsDST	Santosh Kumar et al. (2020)
Oryza sativa	OsEBP89	Zhang et al. (2020b)
Oryza sativa	OsSAPK2	Lou et al. (2017)
Oryza sativa	OsSRL1, OsSRL2	Liao et al. (2019)
Oryza sativa	NAC14	Shim et al. (2018)
Oryza sativa	DERF1, MSH1, PMS3, MYB5, EPSPS, SPP	Zhang et al. (2014)
Oryza sativa	OsmiR535	Yue et al. (2020)
Solanum lycopersicum	NPR1	Wang et al. (2015); Li et al. (2019a)
Solanum lycopersicum	MAPK3	Wang et al. (2017b)
Triticum aestivum	DREB2, DREB3, ERF3	Kim et al. (2018)
Zea mays	ARGOS8	Shi et al. (2017)
Heat abiotic stress tolerance		
Solanum lycopersicum	SlMAPK3	Yu et al. (2019)
Solanum lycopersicum	SlAGL6	Klap et al. (2017)
Heavy metal stress tolerance		
Oryza sativa	OsNramp5	Tang et al. (2017)
Oryza sativa	OsLCT1, OsNramp5	Songmei et al. (2019)
Oryza sativa	OsNramp5	Yang et al. (2019)
Herbicide abiotic stress tolerance		
Brassica napus	BnALS1	Wu et al. (2020b)
Glycine max	ALS1	Li et al. (2015)
Linum usitatissimum	EPSPS	Sauer et al. (2016)

(continued)

Table 13.1 (continued)

Plant species	Targeted gene/s	Reference/s
Oryza sativa	OsALS	Zhang and Gao (2020)
Oryza sativa	OsALS (novel allele G628W)	Wang et al. (2020)
Oryza sativa	OsALS	Butt et al. (2020)
Oryza sativa	OsALS1	Kuang et al. (2020)
Oryza sativa	OsALS	Sun et al. (2016)
Solanum lycopersicum	SlALS	Danilo et al. (2019)
Solanum tuberosum	ALS1	Butler et al. (2016)
Triticum aestivum	EPSPS	Arndell et al. (2019)
Triticum aestivum	MS26, MS45	Svitashev et al. (2015)
Triticum aestivum	LIG1	Svitashev et al. (2015)
Zea mays	ZmALS1, ZmALS2	Jiang et al. (2020)
Zea mays	ZmALS1, ZmALS2	Li et al. (2020a, b)
Zea mays	ZmALS2	Svitashev et al. (2015)
Salinity abiotic stress tolerance		
Oryza sativa	GTγ-2	Liu et al. (2020)
Oryza sativa	PQT3	Alfatih et al. (2020)
Oryza sativa	BGE3	Yin et al. (2020)
Oryza sativa	SPL10	Lan et al. (2019)
Oryza sativa	DOF15	Qin et al. (2019)
Oryza sativa	OsRR22	Zhang et al. (2019)
Oryza sativa	OsDST	Santosh Kumar et al. (2020)
Oryza sativa	qSOR1	Kitomi et al. (2020)
Oryza sativa	OsSAPK2	Lou et al. (2017)
Oryza sativa	OsmiR535	Yue et al. (2020)
Oryza sativa	PIL14	Mo et al. (2020)
Solanum lycopersicum	SlARF4	Bouzroud et al. (2020)
Solanum lycopersicum	GGP1, WUS	Li et al. (2018)

Cas-based editing of replication of geminivirus resulted in tolerance against wheat dwarf virus in *Hordeum vulgare* (Kis et al. 2019). Multiplex genome editing enhanced resistance against *Sclerotinia sclerotiorum* by knockdown of BnWRKY11 (2 copies) and BnWRKY70 (4 copies) in *Brassica napus* (Sun et al. 2018). CRISPR/Cas9 altered promoter sequence of canker susceptibility gene CsLOB1 in citrus plant which leads to tolerance to canker (Jia et al. 2017; Peng et al. 2017). CRISPR/Cas9-edited SlMlo1 gene in transgene-free tomato known as Tomelo provides resistance to it from powdery mildew disease (Nekrasov et al. 2017). RNA targeting CRISPR/LshCas13a interferes with Turnip mosaic virus (TuMV) and mediates immunity against RNA-based viruses in plants (Aman et al. 2018a, b). Cas13d system target and interfere TMV, potato virus X (PVX), and TuMV (Mahas et al. 2019) (Table 13.2).

Table 13.2 CRISPR/Cas-based designing of biotic stress-resistant crop plants

Plant species	Biotic stress	Targeted gene/s	Reference/s
Bacterial biotic stress tolerance			
Citrus × paradisi	Citrus canker	CsLOB1	Jia et al. (2017)
Malus domestica	Fire blight disease (caused by Erwinia amylovora)	DIPM-1, DIPM-2, and DIPM-4	Malnoy et al. (2016)
Oryza sativa	Bacterial Leaf Blight	Xa13/host S gene	Li et al. (2020a, b)
Oryza sativa	Bacterial Leaf Blight	OsSWEET13/ host S gene	Zhou et al. (2015)
Oryza sativa	Bacterial Leaf Blight	OsSWEET14/ host S gene	Zeng et al. (2020a, b); Zafar et al. (2020b)
Solanum lycopersicum	Bacterial Speck	SIDMR6-1/host S gene	de Toledo Thomazella et al. (2016)
Solanum lycopersicum	Bacterial Speck	SlJAZ2/host S gene	Ortigosa et al. (2019)
Solanum lycopersicum	Bacterial Spot	SIDMR6-1/host S gene	Ortigosa et al. (2019)
Wanjincheng orange	Citrus canker	CsLOB1	Peng et al. (2017)
Fungal biotic stress tolerance			
Banana	Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (TR4)	RGA2, Ced9	Dale et al. (2017)
Oryza sativa	False Smut	USTA, UvSLT2/fungal gene	Liang et al. (2018)
Oryza sativa	Rice blast	OsERF922/host S gene	Wang et al. (2016)
Oryza sativa	Rice blast	OsPi21/host S gene	Nawaz et al. (2020); Li et al. (2019b, c)
Oryza sativa	Rice blast	OsALB1, OsRSY1/fungal gene	Foster et al. (2018)
Solanum lycopersicum	*Botrytis cinerea*	SlMYC2/host S gene	Shu et al. (2020)
Solanum lycopersicum	Phytophthora blight	SIDMR6-1/host S gene	Ortigosa et al. (2019)
Solanum lycopersicum	Powdery mildew	SlMlo1/host S gene	Nekrasov et al. (2017)
Solanum lycopersicum	Powdery mildew	PMR4/host S gene	Koseoglou (2017)
Triticum aestivum	Powdery mildew	TaMLO/host S gene	Wang et al. (2014)

(continued)

Table 13.2 (continued)

Plant species	Biotic stress	Targeted gene/s	Reference/s
Triticum aestivum	Powdery mildew	TaEDR1/host S gene	Zhang et al. (2017)
Vitis vinifera	Botrytis cinerea	VvWRKY52	Wang et al. (2017a)
Vitis vinifera	Powdery mildew	Mlo-7	Malnoy et al. (2016)
Viral biotic stress tolerance			
Arabidopsis thaliana	Beet severe curly top virus (BSCTV)	BSCTV genome	Ji et al. (2015)
Arabidopsis thaliana	Turnip mosaic virus	Virus RNA genome	Aman et al. (2018a, b)
Arabidopsis thaliana	Turnip mosaic virus	EIF4E	Pyott et al. (2016)
Banana	Endogenous banana streak virus	Virus sequences in plantain genome	Tripathi et al. (2019)
Banana	Clover yellow vein virus	Eif4e1	Bastet et al. (2019)
Cucumis sativus	RNA viral disease	elf4E/host S gene	Chandrasekaran et al. (2016)
Glycine max	Soybean mosaic virus	GmF3H1, GmF3H2, and GmFNSII-1	Zhang et al. (2020a)
Hordeum vulgare	DNA viral disease	MP, CP, Rep/Rep, IR/virus genome	Kis et al. (2019)
Manihot esculenta	African cassava mosaic virus	AC2 and AC3	Mehta et al. (2019)
Manihot esculenta	Cassava brown streak disease	nCBP-1, nCBP-2	Gomez et al. (2019)
Nicotiana benthamiana	Beet severe curly top virus (BSCTV)	BSCTV genome	Ji et al. (2015)
Nicotiana benthamiana	Tomato yellow leaf curl virus resistance	TYLCV genome	Ali et al. (2015)
Nicotiana benthamiana	Cotton leaf curl Multan virus	Rep and IR	Yin et al. (2019)
Nicotiana benthamiana	Chilli leaf curl virus	virus DNA Rep, IR, and Cp	Roy et al. (2019)
Oryza sativa	Rice Tungro disease	eIF4G	Macovei et al. (2018)
Solanum lycopersicum	PVX, TMV, TMV	DCL2/virus genome	Wang et al. (2018b, c)
Solanum lycopersicum	Tomato yellow leaf curl virus (TYLCV)	CP, Rep/virus genome	Tashkandi et al. (2018)
Solanum lycopersicum	Yellow leaf curl virus	IR and CP/virus genome	Faal et al. (2020)
Solanum lycopersicum	Pepper Mottle virus	eIF4E1/host S gene	Yoon et al. (2020)

13.4 Conclusion

CRISPR/Cas is a potential and desirable tool for introducing desired traits in economically important and commercial crop plants with greater efficiency and specificity and may provide food security in the challenging climate-changing scenarios. Several research studies discussed above show the success stories of CRISPR/Cas-mediated tolerance towards single and multiple abiotic and biotic stress factors. Thus, CRISPR genome editing tools can redesign the future of agriculture and provide a way forward to designing of climate-resilient smart crops.

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14 Decrypting Drought Stress Tolerance of Crop Plants via Photosynthesis and Antioxidative Defense Mechanisms

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Abstract

Drought is one of the hot topics needing urgent attention in the current era of climate change. Crop plants are frequently exposed to a range of abiotic and biotic stresses during the cropping season. Among the various abiotic stresses, drought stress is a key environmental constrain that has detrimental effect on plant growth and development. With the emergence of drought, plants give a plethora of responses for survival resulting in modifying a series of reactions for survival, resulting in changing physiological, molecular, and biochemical processes. Drought affects most physiological functions including reducing CO_2 assimilation rates and disrupting primary photosynthetic processes and pigments. It also accelerates reactive oxygen species production, which triggers the formation of an antioxidative mechanism. The purpose of this chapter is to provide insight on the effects of drought on plant growth and development, photosynthesis (decrease

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or increase photosynthesis directly influence the amount of assimilate production), antioxidative defense mechanism (enzymatic, nonenzymatic antioxidant, and other protective mechanisms), oxidative stress (elevated the formation of reactive oxygen species under drought stress), ROS as a signaling molecule, water relation of the plant (leaf water potential, water use efficiency), and the development of transgenic drought plants.

Keywords

Antioxidants · Biotic and abiotic stress · Photosynthetic rate · Water use efficiency · ROS · Osmolytes and drought

Abbreviations

ABA	abscisic acid
DNA	deoxyribonucleic acid
DREB	dehydration-responsive element binding proteins
ERF	ethylene-responsive element binding factor
EST	expressed sequence tags based markers
H_2O_2	hydrogen peroxide
HO	hydroxyl radical;
O_2	reactive singlet oxygen
O_2^-	superoxide
PETC	photosynthetic electron transport chain
PIP	protein intrinsic of plasma membrane
ROS	reactive oxygen species
TF	transcription factors
TPS	trehalose 6-phosphate synthase

Introduction

Drought is abiotic stress that significantly impacts plant growth and development, particularly at the seedling stage. Drought at the seedling stage has been linked to significant plant mortality events across the world (Carnicer et al. 2011; Martin-StPaul et al. 2017; Park Williams et al. 2012). Plants can only grow if they have the optimal combination of environmental variables, such as sunlight, temperature, air humidity, and water availability. The slight variations in these parameters could effect on growth and productivity.

Drought stress is a complicated abiotic stress factor that has a significant effect on plant metabolic activities, growth, and yield (Osakabe et al. 2014) and is implicated in crop yield reductions of up to 50% (Zlatev and Lidon 2012). Water stress is characterized by changes in water relations, physiological processes, modifications in cellular membranes and ultrastructure of cellular components, and membrane denaturation (Yordanov et al. 2003). Drought stress affects plants for two main reasons: (1) a lack of water availability to the roots and (2) excessive transpiration rates. The most common environments for these constrains are found in arid and semiarid regions (Rahdari and Hoseini 2012). Plants employ stress avoidance and stress tolerance mechanisms to cope with low water availability (Lawlor 2013).

Morphophysiological Responses of Plant Against Drought Stress

Drought is a major constraint in crop production and food security in modern agriculture worldwide. This stress forces the plant to modify its architecture (morphophysiological changes) and induces internal cellular mechanisms such as biochemical changes, which ultimately cause low productivity and limit plant growth and development. Drought stress restricts vegetative and reproductive plant growth due to loss of turgidity and thus causes adverse effects on yield. Moreover, the carboxylation efficiency, transpiration rate, water potential of leaves, stomatal conductance, and net photosynthetic rate also diminish significantly during water stress conditions (Fahad et al. 2017). The crop plant has acquired a wide range of modifications and adaptations such as chemical, molecular, and morphophysiological mechanisms to cope with the drought stress and other multiple abtiotic stresses. These adaptations enable the plant to survive under harsh environments and complex conditions of stress.

Generally, geneticists and breeders categorize drought tolerance mechanisms into four strategies: drought recovery, drought avoidance, drought tolerance, and drought escape. Drought tolerance and avoidance are the most effective mechanisms to cope with drought stress (Khan et al. 2018). Drought avoidance is important because it keeps physiological processes like plant root growth and stomatal regulation during short-term moderate water deficits. Many quantitative trait loci (QTLs) and genes for drought avoidance, escape, and tolerance-related phenotypes have been reported (Martignago et al. 2019). In contrast, severe dehydration may be endured by changing physiological processes such as osmotic modifications through osmoprotectants and osmolyte accumulation (Luo 2010). Drought escape refers to a plant's capability to modify its life cycle and growth period in order to avoid seasonal drought stress (Luo 2010; Bhatia et al. 2014). Drought recovery refers to a plant's capacity to restart growth performance after experiencing drought stress. Plants have developed various morphological and physiological approach for dealing with drought stress over time (Fig. 14.1).

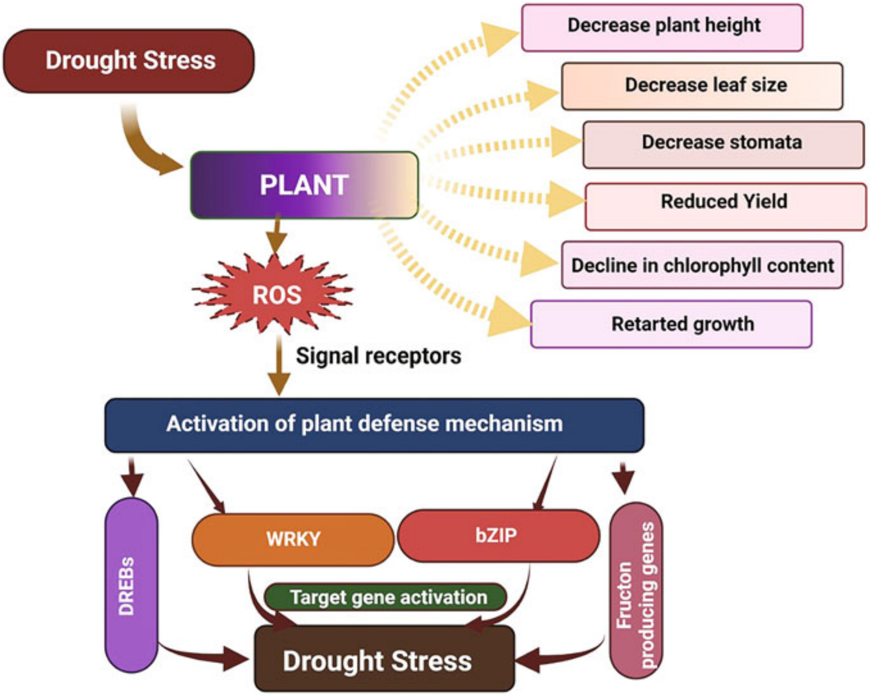

Fig. 14.1 Morphophysiological mechanisms to contend with the effects of the drought

Influence of Plant Growth and Water Relation in Response to Drought Stress

Drought is the most common stress that plants cope with; most of their yield is lost due to this stress (Ramegowda and Senthil-Kumar 2015). Drought stress substantially hindered growth parameters, which connected to a simultaneous drop in photosynthetic pigment concentrations at both mild and severe drought stress. Plants have less biomass and display less development due to the drought, which has also reduced photosynthetic efficiency, photosynthetic pigments, and the number of stomata in Tetraena Mandeville (Khan et al. 2019). Stomatal closure and reduction in the number of stomata cause a decrease in chlorophyll content, photosynthetic pigment, and stomatal opening, which also fetch change in the structure of chlorophyll (Jabeen and Ahmad 2017). Reduction in plant leaf size is observed as a result of drought stress which is directly proportional to yield losses (Khan et al. 2019). Drought stress also causes a reduction in leaf size which also causes a reduction in chlorophyll and hence causes a reduction in carbon assimilation (Bashir et al. 2020). Morphological parameters are also adversely affected by drought stress, including fresh and dry weights, root shoot length, root/shoot ratio and dry matter accumulation, leaf thickness, and leaf width and leaf area (Jabeen and Ahmad 2017). Abscisic acid, methyl jasmonate, and antioxidant system are generated to protect plants from various kinds of stresses, including drought stress (Saleem et al. 2020). Drought stress induces the production of ascorbic acid and GSH, which trigger plant stress tolerance by regulation of ROS homeostasis (Alharby and Fahad 2020). Osmoprotactants and ascorbate neutralize H_2O_2 and scavenge peroxides, and they are essential components of the glutathione cycle, particularly in redox reactions (Rana et al. 2020: Rehman et al. 2019). Drought stress also results in a decline in the uptake of essential nutrients such as phosphorous calcium and potassium.

Abiotic stress impairs antioxidant response induce overaccumulation of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in plants. The photosynthetic characteristics and biochemical indicators such as proline, soluble sugars, total soluble proteins, amino acids, and glycine betaine levels were drastically changed due to soil moisture deficit. The apoplastic pathway or the symplastic pathway is used to transport water in plant roots. The latter comprises the plasma membrane, where the transport is arbitrated by the expression of aquaporins (Wang et al. 2003). Plants go through a variety of modifications to prevent being harmed by water stress. Morphological changes include the growth of root hairs, the depth of roots, and the rolling of leaves (Taji et al. 2004). The net photosynthetic rate to transpiration rate ratio, or biomass production to transpiration rate ratio, is a common way to assess water usage efficiency (WUE). Drought stress is one of the most significant limitations of crop productivity, and stress tolerance can be improved by adjusting fundamental variables approximating osmotic pressure (Praba et al. 2009). One of the essential constituents of drought tolerance in agricultural plants is an osmotic adjustment, which includes the deposition of solutes. Due to osmotic adjustment, plants can retain a high relative water content (RWC) despite a low leaf water potential (Farooq et al. 2009a, b). Many crops have shown osmotic adjustment in their leaves, including *Triticum Aestivum* (Budak et al. 2013), *Zea mays* (Pei et al. 2010), *Sorghum bicolor* (Assefa et al. 2010), *Phaseolus vulgaris* (Guler et al. 2012), *Oryza sativa*, (Bunnag and Pongthai 2013), *Hordeum vulgare* (Witcombe et al. 2008), *Cicer arietinum* (Krouma 2010), *Pisum sativum* (Zlatev and Lidon 2012), *Helianthus* (Rauf and Ahmad Sadaqat 2008), and *Pennisetum glaucum* (Boyer et al. 2008).

Antioxidants such as catalase, peroxidase, and proline function as ROS scavengers help plants to cope with abiotic stresses by higher activity of these enzymes. Plants grown in well-watered conditions survived longer, although several researchers have found poor performance in reduced growth and yield under water stress conditions (Nezami et al. 2008). After nitrogen (N), phosphorus (P) is the second most important nutrient, and it is in short supply in Pakistani soils. Many field crops benefit from (P), which increases their development and output while boosting root growth in drought-stricken crops (Yaseen and Malhi 2009). The usage of phosphorus helps to mitigate adverse effects of drought stress and improve crop productivity (Singh et al. 2021).

Impact of Drought on Plant Photosynthesis and CO_2 Assimilation Rate

Water availability is critical for plant growth and development, and a decrease in water availability during critical growth stages of plants may impair plant growth and development. Another study found similar results when stomatal restriction was used under mild water stress and non-stomatal restriction was used under severe water stress circumstances (Misson et al. 2010). Low moisture conditions have a substantial effect on many metabolic activities, including carbon assimilation and biogeochemical processes. Water stress causes damage to the fundamental structure of macromolecules and food-producing machinery, limiting carbon fixation by photosynthesis (Golldack et al. 2011; Ali and Ashraf 2011).

Water deficit has a detrimental effect on the photosynthesis rate, water use efficiency, and transpiration rate of maize, brassica, and mung bean plants (Ahmed et al. 2002; Kauser et al. 2006; Ashraf et al. 2007). Carotenoids, on the other hand, are essential for water stress tolerance (Jaleel et al. 2009). A decrease in photosynthetic activity combined with increased absorption of light compromises the photosynthetic activities of plants. Therefore, this high light energy may result in an overaccumulation of reactive oxygen species (ROS) such as H_2O_2 and O_2•, thus impeding the synthesis of D1 (Murata and Takahashi 2008). This inhibition of D1 synthesis further reduces PETC activity, interfering with CO_2 fixation in many plants (Altaweel et al. 2007) (refer to Fig. 14.2).

Osmotic Adjustment

Water stress directly affects the internal mechanisms at the cellular level and modifies the turgidity and osmotic balance of the crop plants (Saud et al. 2017). The plant adapts to water stress conditions through osmotic adjustments to diminish the plant damage. In response to drought conditions, the plant defense mechanisms trigger the accumulation of osmoprotectants and osmolytes, which further regulate the water status and ROS homeostasis at the cellular level. Osmotic balance is negatively associated with drought stress (Ma et al. 2019).

Among osmoprotectants, organic substances such as sugar (e.g., trehalose, fructan, etc.), polyol, alkaloids, mannitol, sorbitol, betaines, polyamines, D-mannitol, ectoine, and proline are directly involved in osmotic adjustment in response to drought stress. Moreover, certain inorganic compounds and glycine also participate in this response (Ma et al. 2019; Mehrotra et al. 2014). These mentioned osmolytes assist the plant's internal cellular systems by protecting the plant's integral proteins. Moreover, membrane integration from the damage of higher concentrations of oxidative and inorganic ions relieves the plant from drought stress (Khan et al. 2019).

The contrasting role of osmoprotectants (like proline and glycine betaine) has been verified by exogenous application to improved drought stress tolerance (Caine et al. 2019). Furthermore, exogenous application of proline and GB has been

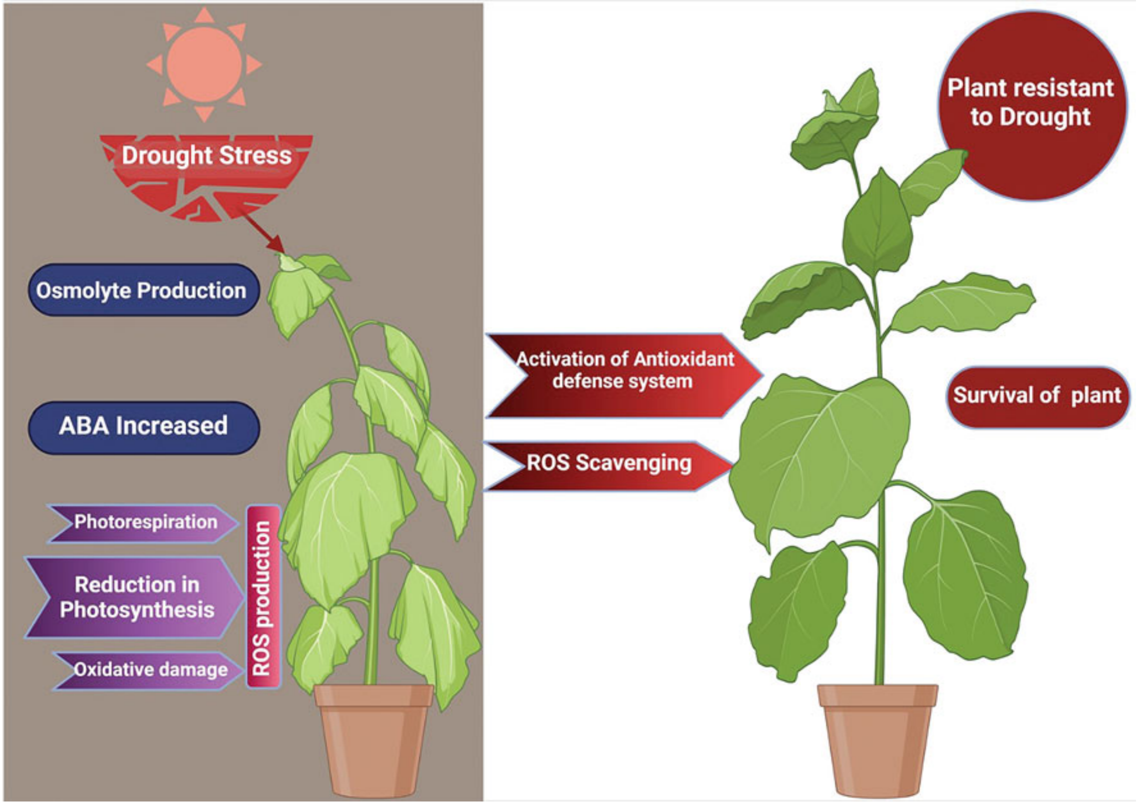

Fig. 14.2 The drought stress causes stomatal closure, which checks the CO_2 intake and reduces photosynthesis, affecting growth and yield. The antioxidant defense system maintains the plant's macromolecules by scavenging reactive oxygen species (ROS)

observed to upregulate the antioxidant-based plant defense mechanisms. These osmoprotectants also reduce the superoxide dismutase (SOD), glyoxalase II (Gly II), and glutathione S-transferase (GST) activities which elevate during the drought stress (Caine et al. 2019).

Various researchers have exploited the role of osmoprotectants and osmolytes in rice water stress tolerance to develop drought-tolerant genotypes (Li et al. 2018). The transgenic lines were more tolerant of drought. The OsERF71 is a localized nuclear protein that reduces water loss and regulates proline and ABA responses biosynthesis genes during drought stress in rice. Hence, it is evident that the transgenic approaches using such proteins can play a role in developing water stress-tolerant rice genotypes.

Role of Oxidative Stress in Plants

Oxidative stress is a secondary constraint that a plant faces as a result of basic stresses such as drought, salinity, extreme temperatures, results in overaccumulation of ROS in mitochondria, peroxisomes, and chloromycetin. ROS disrupts the plant's macromolecular processes, resulting in failure cellular functionalities (Demidchik 2015). Oxidative stress disrupts the equilibrium of components that decrease and oxidize (Potters and Horemans 2010). Cellular organelles are highly sensitive to overproduction of ROS by plants, which facilitate transmission of electron via peroxisomes, chloroplasts, and mitochondria in the photosynthesis (Triantaphylides and Havaux 2009; Gill and Tuteja 2010).

Abiotic stimuli induce oxidative stress, which affects photosynthesis by decreasing stomatal conductance and CO_2 absorption in plants, resulting in an increased rate of ROS production and accumulation in plants (Noctor et al. 2002). It has been observed that plants experiencing extreme moisture deficiency acquire a greater amount of H_2O_2 through photorespiration. By contrast, plants experiencing moderate moisture deficiency shut their stomata to minimize water loss through transpiration, reducing carbon dioxide input and delaying photosynthesis (Noctor et al. 2002; Nayyar and Gupta 2006). When ROS levels are low, they function as a signaling agent, activating a range of cellular processes, but when ROS levels are high, they cause damage to deoxyribonucleic acid (DNA), lipids, and proteins inside plant cellular organelles, resulting in reduced crop production (Sharma et al. 2012a, b).

As a significant stressor on plants, ozone promotes apoplast disintegration and stimulates O_2, H_2O_2, and HO (Fiscus et al. 2005). On the other hand, UV-B light causes oxidative stress by converting H_2O_2 to HO, which leads to a variety of plant metabolic disruptions and tissue damage. Ionic channels, mitogen-activated protein kinases, Ca2+-activated NADPH, transcription factors, calmodulin, and $Ca2^+$-dependent protein kinases are all triggered by overproduction of ROS. Furthermore, the antioxidant defense mechanism protects plant macromolecules by scavenging reactive oxygen species (ROS) (Demidchik 2015) (Fig. 14.2).

Effects of Antioxidants on Plants Under Drought Stress

Drought stress causes to increase the production of oxygen and nitrogen species in plants which initiates the antioxidant mechanisms by increasing the amount of antioxidant production. It is assumed that drought stimulates ascorbate-dependent antioxidant action of the plant compared to thiol-based redox switch and regulatory network (Laxa et al. 2019). Drought stress caused reduced turgor pressure and wilting by reducing water contents, stomatal closure, and reduced carbon fixation in plants. This depleted CO_2 causes ribulose bisphosphate to oxidize and produce internal H_2O_2 (Noctor et al. 2002). The unavailability of carbon dioxide, also known as an electron acceptor, leads to slow down the oxidation of nicotinamide adenosine dinucleotide phosphate in the Kelvin Benson physiological cycle of plants. Decreased NADP+ causes the increasing number of electrons leading to elevated ROS species (Tarafdar and Pula 2018). This type of stress-tolerant varieties produced more H_2O_2 to cope with the water stress via initiating the defense mechanism.

In contrast, reactive oxygen species are made in the apoplast and are closely linked to calcium signaling pathways. Some respiratory burst oxidase homolog (RBOH) proteins (calcium and phosphorylation-dependent proteins) are present on the plant plasma membrane, which is predominantly responsible for anionic peroxide and superoxide production in retort to drought and additional stresses (Evans et al. 2016: Sierla et al. 2016). The other signaling enzymes are also involved in phosphorylation, such as cell wall-associated kinases which participated in receiving the signal of change or decrease in turgor pressure of the plant. These kinases are directly linked to the release of the reactive oxygen species during the phosphorylation of RBOHS proteins (Sun et al. 2020). A particular class of plant proteins (SNF1-related kinases 2) is the primary regulators of plant stress and the slight change in osmotic pressure. MAPKKK stimulates the RAF-like proteins SNF1, which is involved in coping with the drought stress in the plant via a signaling mechanism (Soma et al. 2020).

The antioxidant system is necessarily stimulated by getting signals from drought stress in the form of kinases or other regulators. The plant has a defense system of antioxidants which keep regulated by reactive oxygen species. These antioxidants are small molecules, for instance, glutathione (GSH), tocopherol, ascorbate, and carotenoids. Enzymes are a minor proteomic nature having an antioxidant-based system including superoxide dismutase (SOD), catalase (CAT), thiol peroxidases, and glutathione peroxidases (Hussain et al. 2016). Drought stress caused the upregulation of all antioxidant enzymes. The upregulation of enzymes is depicted at post-transcriptional and transcriptional stages. This upregulation also stimulates the production of nonprotein enzymes during drought stress. Major antioxidant enzymes including CAT, APX, and GPX were produced during drought stress in plants. These enzymes play an important role in scavenging reactive oxygen species (Mittler and Zilinskas 1994). Among the most significant antioxidant compounds, ascorbate peroxidase is linked to the glutathione pathway and a major player to detoxify H_2O_2. The APX1 gene is responsible for the overexpression of ascorbate and seems to confer drought resistance to plants as reported in *Brassica juncea*

(Saxena et al. 2020). The detailed function of APX related to drought stress was reported in *Arabidopsis thaliana* (Kaur et al. 2021). Ascorbic acid and proline were the best antioxidants that help plants (*Echinochloa frumentacea*) survive under drought stress (Singh et al. 2021).

CAT is the iron-containing important antioxidant enzyme that stimulates the detoxification of H_2O_2 to water and oxygen. It plays a crucial role in drought stress at the post-transcriptional level (Luna et al. 2005); for instance, the CAT1 gene was observed in drought-grown wheat (Eftekhari et al. 2017). CAT1 expression is linked to MAPK cascade, an ABA (abscisic acid)-dependent stress-related hormone (Xing et al. 2008). Glutathione reductase, glutathione transferase, and CAT work together in drought stress to mitigate the effects of H_2O_2 (Lou et al. 2018). PRX are iron-containing enzymes called oxidases; these are produced under drought stress with co-release of stress-relating hormones like methyl jasmonate and abscisic acid, for instance, in tea plants (Li et al. 2020). SOD are metalloenzyme played an essential role in various abiotic stress including drought-type stress. SOD causes the dismutation of two molecules of negatively charged oxygen ions to produce water and neutral molecular oxygen. There are a lot of isoforms of SOD based on metal which is involved in forming complexes. These isoforms (Mn-SOD, Fe-SOD, Cu, Zn-SOD) are activated in stress conditions in different plants like *Triticum aestivum* (Khayatnezhad and Gholamin 2021), *Pisum sativum* (Mohammadi et al. 2020), *Zea Mays* (Moharramnejad et al. 2019), *Solanum lycopersicum* (Jangid and Dwivedi 2017), and cassava cultivars (Zhu et al. 2018).

Molecular Responses of Plant Against Drought Stress

As a major problem for crop productivity, drought causes a variety of stress-related reactions in plants at all levels, from cell to biochemical to physiological to molecular. Increased synthesis of a wide variety of reactive oxygen species (ROS) in various cellular compartments, such as mitochondria, peroxisomes, and chloroplasts, is induced by water stress (Kaur and Asthir 2017). Plants utilize their antioxidant defense systems as a defensive mechanism to regulate intracellular ROS homeostasis and maintaining cellular redox equilibrium. Overaccumulation of ROS is characterized as a signal to activate their defence system, with H_2O_2 acting as a secondary messenger. Similarly, $Ca2^+$ fluxes and abscisic acid (ABA) content regulate signaling against increased redox state (Kaur and Asthir 2017). Desiccation-related genes, dehydrins, transcription factors (TF), heat shock proteins, aquaporin, and late embryogenesis abundant proteins all play important roles in the acclimation process of plants when they are exposed to drought (Kaur and Asthir 2017). Drought-stressed plants receive and transmit signals that activate the defence system through osmotic adjustment and resynthesis of stress-responsive genes such as CBF and dehydration response element binding proteins (DREB), dehydrins, and NCED genes (Chen et al. 2016).

Transcriptome research showed that plants close their stomata to adapt in reduced moisture conditions by altering gene expression involved in glycan metabolism. The plant maintains its stress memory, and these genes respond to other stimuli, such as high temperature and intense light. Furthermore, analysis of 300 genes confirmed that all of these genes were involved/expressed under combined stress conditions (Anbazhagan et al. 2014). Numerous genes, such as the DRED transcription factors and the ERF/AP2 family, have been discovered to play a crucial role in plant growth and development and single and combined stressful environments.

Under moderate drought conditions, overexpression of DREB1A is reported in transgenic chickpea plants (Anbazhagan et al. 2014). The previous study has shown the effectiveness of rd29A: DREB1A on processes linked to transpiration efficiency, stomatal regulation gas exchange, root architecture, and water absorption under low moisture conditions, therefore indicating water stress resistance when compared to controls. Other drought stress studies revealed 106 EST-based markers, units markers, and SSR markers. These markers generate high-quality transcripts that may be used to identify various kinds of genes that improve drought resistance (Varshney and Dubey 2009). Correspondingly, a study of 147 stress-induced proteins and 205 protein spots in chickpea plants identified several additional drought-related proteins that are required for molecular chaperones, cell wall modification, nucleocytoplasmic transport, chromatin remodeling and gene transcription, the ROS pathway, and signal transduction under drought conditions. CaN-600 dehydrins were synthesized, which act as scavengers of reactive oxygen species and protect enzyme and cellular functions under stress environments (Liu et al. 2015).

The “dehydration-responsive element binding proteins” (DREB) family is a member of the “ethylene-responsive element binding factor” (ERF) family, and it was discovered that AREB and DREB regulate assimilate translocation in plants under drought stress (Agarwal et al. 2006). Under water stress conditions, plant molecular responses are primarily controlled by producing ABA-related element binding proteins and transcription factors which are associated with the “leucine zipper” gene subfamily (Delorge et al. 2014). Furthermore, certain enzymes, such as “trehalose 6-phosphate synthase” (TPS) and “protein intrinsic of plasma membrane” (PIP), play a role in reducing drought-induced cell death by controlling membrane pores and channels and regulating water and solute movements (Maurel et al. 2008).

Transgenic Approach to Cope with the Drought Stress in Plants

The plants alter the gene expression via upregulating or downregulating its gene expression to respond to various abiotic stress settings (Gao et al. 2018). This alteration in gene expression in retort to many stresses designates the drought tolerance (Khan et al. 2018). Thousands of genes are involved in coping with abiotic stresses, and several of them have a role in plant-drought resistance. However, the rice plant retorts to drought stress via downregulating more than 6000 genes and upregulating above 5000 genes (Todaka et al. 2015). Different metabolites are accumulated in the plant during drought stress in rice. The comparative studies showed that a higher expression level of genes encodes two compounds, that is,

Table 14.1 Genes involved in conferring drought resistance in different crops

Abbreviations of genes and transcription factors	Full name	Characters offered to plant for drought tolerance	Crop to which providing tolerance	Reference
DREB	Drought response element binding	Reduction of membrane damage and enhanced osmotic pressure, increased yield	Rice, *Arabidopsis* Moso Bamboo Wheat	Kudo et al. (2017); Zhou et al. (2020)
WRKY	Tryptophan-arginine-lysine-tyrosine (WRKY) transcription factors	Lower membrane damage greater profiles of antioxidant enzymes and proline following ABA pathway Accumulate osmolytes soluble sugar, root growth	Chrysanthemum, glycine max, alfalfa, and *Arabidopsis*, cotton	Martignago et al. (2020); Sharma et al. (2012a, b)
SST, *Ta6-SFT*, and *Ta1-FFT*	Fructan-producing genes	Increase fructan content as osmoregulators to induce drought tolerance	Tobacco	Pilon-Smits et al. (1995)
bZIP or SlbZIP	Basic leucine zipper transcription factor SlbZIP	Enhanced ABA content, CAT enzyme, and MDA content Increased cholorophyl content Following ABA pathway	Tomato *Arabidopsis*	Zhu et al. (2018); Zhong et al. (2015)
(AtTGA4)	(TGACG motif-binding factor 4)	Drought tolerance was aided by increased nitrogen transport and absorption activities in the ABA pathway	*Arabidopsis* Tomato	Wang et al. (2016); Zhong et al. (2015)

isocitrate lyase and malate synthase, under drought stress in the glycolate cycle with glucose accumulation in rice but not observed in *Arabidopsis*. In contrast, the downregulation of genes reduces cytochrome P450 735A which is directly associated with the cytokinin levels in rice but not *Arabidopsis* (Todaka et al. 2015) (Table 14.1).

Although certain transgenic plant types are drought resistant, many plants suffer a reduction in height and crop production due to drought stress (Kudo et al. 2017). Multiple genes also provide resistance to drought stress. For instance, drought-response element binding (DREB)-like transcription factors can substantially improve plant water stress tolerance. DRAB1A gene is inserted to make the plant variety drought resistant, as in rice (Kudo et al. 2017). DREB also conferred drought resistance in transgenic wheat plants has shown reduced membrane damage, enhanced osmotic pressure, and enhanced production as equated to non-transgenic plants (Zhou et al. 2020). GsWRKY20 genes offer resistance to drought in glycine max, alfalfa and *Arabidopsis* under cauliflower mosaic virus p32S promoter control. Transgenic plants have shown lower membrane damage and greater profiles of antioxidant enzymes, and proline were screened in transgenic plants as compared to non-transgenic (Martignago et al. 2020) (Table 14.1).

Fructan-producing genes are inserted in tobacco for resistance against drought stress conditions (Pilon-Smits et al. 1995). Primary leucine zipper transcription factor SlbZIP confers drought tolerance in tomatoes by enhancing plant stress-related physiological states by elevated ABA content, CAT enzyme, and MDA content (Zhu et al. 2018). Wheat was transformed with TaWRKY2 and exhibited high proline, soluble sugar, and chlorophyll profiles and conferred drought resistance (Table 14.1).

Conclusions

Drought stress is one of the problematic environmental situations that limit the yield of agricultural plants worldwide. The primary issue is improving plant productivity as water scarcity decreases, which can be consummate. Understanding how variations in osmotic potentials activate various metabolic and molecular responses involved in plant defense under diverse environmental circumstances is one way of solving the problem.

Drought stress tolerance in agricultural plants is further closely linked to the plants' ability to adjust to the changes of the environment and the litheness of their cellular metabolism under stress conditions. ROS regulates many cellular functions associated with environmental changes. Initially, ROS were thought to be harmful by-products of aerobic metabolism that were removed by two types (enzymatic and nonenzymatic antioxidants). With the exception of the fact that they are toxic, ROS are important signaling molecules in many processes, including growth, development, adaptations to abiotic and biotic stress stimuli, and programed cell death. Like calcium signaling, which is organized by storage and release, ROS signaling is measured by the balance of their synthesis and scavenging. A few lineages are still unknown, such as the routes that maintain an optimal level and the role of reactive oxygen species (ROS) in arbitrating plant defense systems.

Furthermore, the function of ROS in cell-to-cell contacts, network assimilation linked to the abiotic stress retort, and potential interactions among ROS and reactive nitrogen species remain unsolved. Focusing on these features will provide complete knowledge on drought tolerance mechanisms, and applying these parameters to crop plants worldwide will result in the creation of drought-tolerant agricultural plants. ROS regulates a variety of cellular activities that are involved in responding to environmental changes.

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15

Role of Brassinosteroids (BRs) in Modulating Antioxidative Defense Mechanism in Plants Growing Under Abiotic and Biotic Stress Conditions

Nandni Sharma, Sandeep Kour, Deepak Kumar, Ravinderjit Kaur, Anjali Khajuria, and Puja Ohri

Abstract

Brassinosteroids (BRs) are a group of steroidal phytohormone that plays an essential role in regulating various chemical and physiological processes involved in plant growth and development. Furthermore, physiological and molecular studies of BRs have revealed their potential of enhancing yield and productivity of crops by regulating variety of genes. But as per current scenario, variety of abiotic and biotic environmental stresses acts as major constraints in yield and productivity of crops. Furthermore, BRs act as nontoxic, environmentally safe steroidal compounds that has the potential in modulating plant responses against abiotic and biotic stresses. When applied exogenously at specific dose and at particular developmental stage of plant, they are known to enhance both quality and quantity of the crop plants. Moreover, BRs are also known to have antifungal, antiviral, and anti-ecdysteroidal properties, which make them potential alternate of chemical fungicide, pesticide, and herbicides. Therefore, keeping in view all these properties of BRs, the current book chapter focuses on the role of BRs in modulating enzymatic and nonenzymatic antioxidant defense mechanism of plants under abiotic and biotic stress conditions.

Keywords

Brassinosteroids · Antioxidants · Defense system · Abiotic stress · Biotic stress

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15.1 Introduction

Various abiotic and biotic stresses elicit threatening impact on productivity and yield of crops. Various abiotic stresses include thermal (high and low temperature), water (drought and flooding), heavy metal toxicity, salinity, and UV radiation stresses, and the biotic stress includes pathogenic stress. All these stresses have contributed in converting arable lands to unproductive barren lands, thus ultimately resulting in economic loss in agricultural field (Ahanger et al. 2018). All these stresses affect germination, growth, and various physiological processes in plants, and moreover, it has been predicted that if the similar situation continues, there will be scarcity of staple crops for human population (which is growing at an alarming rate) by 2050 (Ahanger et al. 2014). Keeping in mind, all these changes, biologists are trying to meet all these challenges by using various environmentally stable strategies like the use of biological components, drainage, water management, etc. One such strategy is the application of phytohormones, since various phytohormones play an essential role in regulating the normal and developmental processes and are also involved in combatting the effects caused due to various biotic and abiotic stresses by modulating several signaling pathways to evoke plants responses.

Among all plant hormones, brassinosteroids (BRs), a group of steroidal hormones found in lower as well as higher plants, are involved in regulating various mechanisms involved in growth and developmental processes in plants (Liu et al. 2017). BRs act as an essential regulator involved in photosynthesis, antioxidant defense system, and plant-water relation under normal as well as stress conditions, thus ultimately regulating the growth and developmental processes under normal as well as stress conditions. Furthermore, in the case of plants having mutations in BR biosynthesis, abnormal developmental phenotypes are generated, thus confirming the potencies of BRs (Sahni et al. 2016). Moreover, BR-induced ameliorating stress responses have been reported in various plants that were exposed to thermal, water, heavy metal, and pathogen stress (Hayat et al. 2010; Singh et al. 2012; Talaat et al. 2015; Zhao et al. 2016; Jasrotia and Ohri 2017a). BRs are applied exogenously to plants and are reported to have ability of mitigating different stresses in concentration-dependent manner and also on the developmental stage of plants as well as on the treated plant organs (Bao et al. 2004). So, in the current book chapter, efforts have been made to examine the potential of BRs in modulating antioxidant defense in plants growing under stress conditions. Moreover, cross talks of BRs with other phytohormones have also been summarized here.

15.2 Plant Responses to Environmental Stresses

Plants respond to various environmental stresses which include abiotic factors like heavy metals, drought, wounding, salinity, changes in temperature and light, pesticides, and nutrient stress and biotic factors such as pest and pathogen attack (Gull et al. 2019). Abiotic and biotic stresses induce morphological, biochemical, molecular, and physiological changes in plants. Extreme temperature, salinity

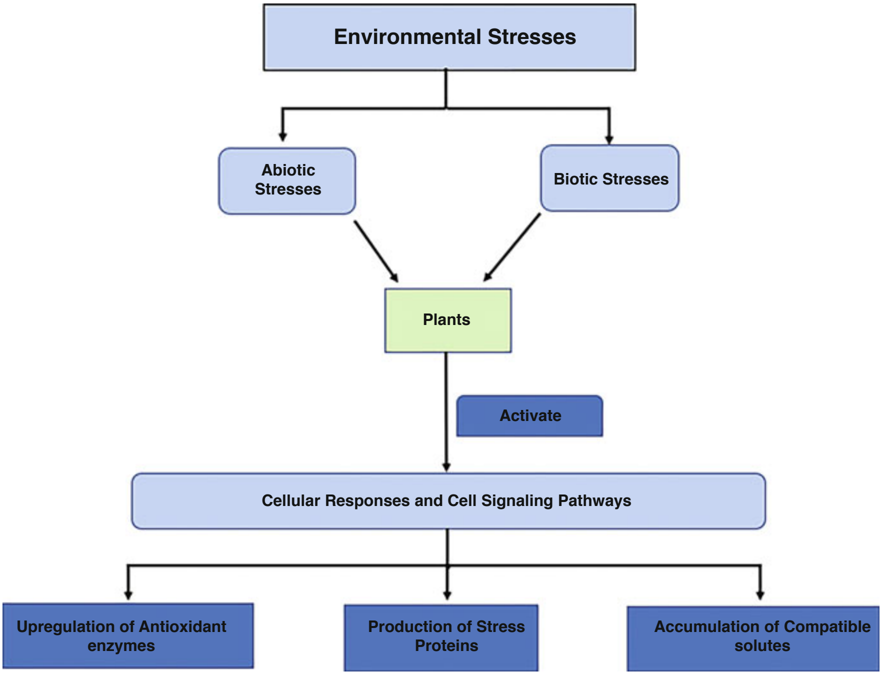

Fig. 15.1 Response of plants to different environmental stresses

drought, and oxidative stress are often interconnected and may lead to similar cellular damage. For example, salinity and drought condition are primarily evidenced as osmotic stress, leading to disruption of ion homeostasis in the cells (Samynathan et al. 2021). Denaturation of structural and functional proteins is caused by oxidative stress, which often accompanies high temperature, drought, or salinity stress (Chaki et al. 2020). As a result, various environmental stresses often activate similar cellular responses and cell signaling pathways (Sewelam et al. 2016), such as accumulation of compatible solutes, upregulation of antioxidants, and production of stress proteins (Kosová et al. 2018; Dumont and Rivoal 2019; Hasanuzzaman et al. 2020). Plants also undergo certain biochemical adaptations which involve various changes in cell biochemistry. These changes include detoxification mechanism, synthesis of special proteins, evolution of new metabolic pathways, accumulation of the metabolites, and changes in phytohormone level (Fujita et al. 2006) (Fig. 15.1).

15.3 Biosynthesis of BRs

The pathway leading to the biosynthesis of BRs and different genes involved in BR biosynthesis has been identified in *Arabidopsis* as well as in rice and tomato (Divi and Krishna 2009). Initially, BR-biosynthetic pathway was established by feeding

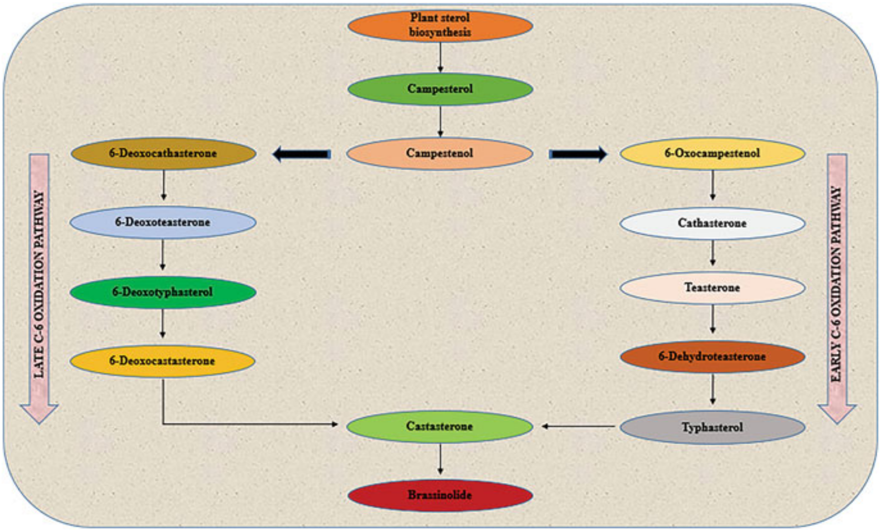

Fig. 15.2 Biosynthesis of brassinosteroids

cultured cells of *Catharanthus roseus* and *Arabidopsis* seedlings with deuterium-labeled substrates followed by identification of various metabolites and reaction sequences involved in biosynthetic pathway by utilizing gas chromatography-mass spectrometry (GC-MS) (Choi et al. 1996; Fujioka et al. 2000; Noguchi et al. 2000). The precursor for the biosynthesis of brassinolide (BL), the most active BR, is campesterol (CR). Earlier, BRs were thought to be biosynthesized from two parallel pathways, namely, early C-6 and late C-6 oxidation pathways (Fujioka et al. 1998). According to these pathways, CR is first converted to campesterol (CN), then to castasterone (CS), and finally to BL (Fig. 15.2). In early C-6 oxidation pathway, CN is first converted to 6-oxocampestanol, then to cathasterone, teasterone, 3-dehydroteasterone, typhasterol, and then CS. In late C-6 oxidation pathway, CN is first hydroxylated at C-22 to form 6-deoxocathasterone and is then converted to corresponding intermediates as in early C-6 oxidation pathway but in C-6 deoxy forms. These two pathways ultimately converge at CS, which is eventually converted to BL (Zhao and Li 2012). Another branching pathway termed as CN-independent pathway, which is an early C-22 oxidation branch, has been reported (Fujioka et al. 2002). Recently, a shortcut route involving C-23 hydroxylation leading to the conversion of CR to 6-deoxytyphasterol has been described (Ohnishi et al. 2006). Experimental data on different plant species have revealed that the CN-independent and late C-6 oxidation pathways are the predominant BR-biosynthetic pathways (Zhao and Li 2012). Different genes involved in BR-biosynthesis are *constitutive photomorphogenesis and dwarfism (CPD), de-etiolated-2 (DET2)*, and *DWARF4 (DWF4)* (Bartwal and Arora 2020). Constitutive expression of these genes can be modulated to regulate the endogenous levels of BR in plants (Fig. 15.2).

15.4 Role of BRs in Plant Growth and Development

BRs are steroidal phytohormones that are analogous to animal steroidal hormones. Mass spectrophotometric analysis such as UHPLC-ESI-MS/MS determined a total of around 22 natural BRs in a minute sample of plant tissue which exhibited a highly significant growth-promoting influence in plants (Tarkowska et al. 2016). They play imperative roles in divergent aspects of plant biology ranging from elongation and division of cell, root growth, photomorphogenesis, stomatal and vascular differentiation, seed germination, plant immunity, and its reproduction (Gudesblat and Russinova 2011; Vardhini and Anjum 2015; Wei and Li 2016) (Table 15.1). Besides, BRs regulate the production and oxidation of radicals and root gravitropic response and mediate plant responses to environmental cues (Krishna 2003; Bajguz and Hayat 2009; Vardhini 2019). Table 15.1 describes the physiological role of BRs in growth and development of different plant species.

15.5 Cross Talk of BRs with Other Plant Hormones

Several stress-responsive phytohormones act as a molecular regulatory element that assist sessile plants to maintain their growth plasticity and provide ability to adapt in tough environmental conditions. A cascade of interactions (occur mainly through phosphorylation/a common second messenger) that helps in regulating signaling network and persists among varied plant hormones which alter cellular dynamics is known as cross talk. This cross talk between phytohormones helps in revealing and targeting host resistance mechanisms under stress (Kohli et al. 2013; Wani et al. 2016; El-Esawi 2017; Li et al. 2021). On the basis of their action, phytohormones are grouped into two main categories: First group includes auxins (AUX), gibberellins (GA), brassinosteroids (BRs), strigolactones (SL), and cytokinins (CK) that coordinate during plant growth and development, while others play a vital role under environmental cues, namely, abscisic acid (ABA), brassinosteroids, ethylene (ET), jasmonic acid (JA), and salicylic acid (SA) (Pieterse et al. 2009; Santner et al. 2009; Denance et al. 2013; Fahad et al. 2015). Thus, BRs play a dual role in plants both under stress as well as during normal growth.

15.5.1 Interplay Between Brassinosteroids and Auxins

BRs and auxins are master hormones with coordinated effects on innumerable phases of plant growth and developmental pathways including the biosynthesis of BRs mediated by auxins (Yoshimitsu et al. 2011; Hao et al. 2013; Chaiwanon and Wang 2015). Physiological and genetic assays demonstrate opposite role of BR and AUX for controlling root growth by directing the expression of DWF4 (DWARF4) and BZR1 (BRASSINAZOLE-RESISTANT 1). On one hand, in roots where auxins enhance DWF4 expression, BRs suppress it through feedback mechanism (Yoshimitsu et al. 2011; Chaiwanon and Wang 2015). Also, during lateral root

Table 15.1 Effect of BRs on the various physiological aspects of plant growth and development in different plant species

Physiological parameters	Plant species	BR analogues/inhibitors used	Reference
Seed germination/ seedling growth	*Brassica juncea L.*	24-Epibrassinolide (EBL); 28-Homobrassinolide (HBL)	Sirhindi et al. (2009, 2011), Kumar et al. (2012), Soares et al. (2020)
	Raphanus sativus L.	EBL; HBL	Mahesh et al. (2013)
	Leymus chinensis	EBL	Guo et al. (2014)
	Acer pseudoplatanus L.; *Fraxinus excelsior* L.	EBL	Procházka et al. (2015)
	Tobacco	EBL	Bukhari et al. (2016)
	Picea abies, *Pinus sylvestris*, *Pseudotsuga menziesii*, and *Quercus robur*	2α,3α,17β-trihydroxy-5α-androstan-6-one (a Synthetic BR)	Kuneš et al. (2016)
	Solanum lycopersicum L.	EBL	Ahammed et al. (2012a), Shu et al. (2016)
	Solanum melongena	EBL	Xue-Xia et al. (2011), Wu et al. (2015), He et al. (2016), Wu et al. (2016)
	Cucumis sativus	EBL	Yuan et al. (2012a)
	Arabidopsis thaliana	Brassinazole	Yamagami et al. (2017)
	Vigna radiata L.	BL	Lalotra et al. (2017)
	Oryza sativa L.	7,8-Dihydro-8α-20-hydroxyecdysone (αDHECD; a BR mimic)	Sonjaroon et al. (2018)
	Pisum sativum	BL; EBL	Jiroutová et al. (2019)
	Triticum aestivum L.	BL	Toman et al. (2019)
	Gossypium hirsutum	EBL	Chakma et al. (2021)
Shoot and root growth	*Arachis hypogaea* L.	BR	Verma et al. (2012)
	Capsicum annuum L.	EBL	Abbas et al. (2013)
	Phaseolus vulgaris	EBL	Cheng et al. (2014)
	Oryza sativa	BR	Fahad et al. (2016)

(continued)

Table 15.1 (continued)

Physiological parameters	Plant species	BR analogues/inhibitors used	Reference
	Potatoes	BL	Hu et al. (2016)
	Arabidopsis thaliana	BL; Brassinazole	Vragovic et al. (2015), Yamagami et al. (2017)
	Vigna radiata L.	BL	Lalotra et al. (2017)
	Carica papaya L.	BR	de Assis-Gomes et al. (2018)
	Gossypium hirsutum	EBL	Chakma et al. (2021)
	Zea mays	EBL	Trevisan et al. (2020)
	Chenopodium quinoa Willd	BR	Sadak et al. (2020)
	Solidago canadensis	BR	El-Sayed et al. (2020)
	Pyrus ussuriensis	BL	Zheng et al. (2020)
	Solanum lycopersicum L.	EBL	Shu et al. (2016); Nazir et al. (2021)
	Berberis thunbergii L.	BL; EBL	Pacholczak et al. (2021)
Photomorphogenesis	*Arabidopsis thaliana*	BL	Kim et al. (2012); Zhiponova et al. (2013); Youn et al. (2016)
	Solanum melongena	EBL	Xue-Xia et al. (2011)
	Capsicum annuum L.	EBL	Abbas et al. (2013)
	Camellia oleifera	BL	Zhou et al. (2013)
	Solanum lycopersicum L.	EBL	Xia et al. (2014), Li et al. (2015), Nazir et al. (2021)
	Dwarf pear	BL	Chen et al. (2014)
	Leymus chinensis (Trin.) Tzvel.	BL	Niu et al. (2016); Wang et al. (2016)
	Oryza sativa	EBL; BR; 7,8-Dihydro-8α-20-hydroxyecdysone (αDHECD; a BR mimic)	Sun et al. (2015), Tong and Chu (2016), Fahad et al. (2016), Sonjaroon et al. (2018)
	Brachypodium distachyon L.	24-Epicastasterone	Xu et al. (2015)
	Chenopodium quinoa Willd	BRs	Sadak et al. (2020)
	Tobacco	EBL	Zhang et al. (2021)

(continued)

Table 15.1 (continued)

Physiological parameters	Plant species	BR analogues/inhibitors used	Reference
Photoperiodism and flower development	*Arabidopsis thaliana*	BR	Domagalska et al. (2010)
	Cucurbita pepo	Brassinozole	Manzano et al. (2011)
	Brassica napus	BL	Belmonte et al. (2011)
	Arachis hypogaea L.	BR	Verma et al. (2012)
	Solidago canadensis	BR	El-Sayed et al. (2020)
Photosynthesis	*Carica papaya* L.	BR	de Assis-Gomes et al. (2013)
	Secale cereale L.	EBL	Pociecha et al. (2016)
	Pisum sativum	EBL	Dobrikova et al. (2013)
	Helianthus annuus L.	EBL	Filova et al. (2013), Kaplan-Dalyan and Sağlam-Çağ (2013)
	Vigna radiata	HBL	Yusuf et al. (2014), Alyemeni and Al-Quwaiz (2016)
	Oryza sativa L.	BL; BR; 7,8-Dihydro-8α-20-hydroxyecdysone (αDHECD; a BR mimic)	Cao and Zhao (2008); Fahad et al. (2016); Sonjaroon et al. (2018)
	Glycine max L.	EBL	Bariş and Sağlam-Çağ (2016)
	Triticum aestivum L.	EBL; BL	Sağlam-Çağ (2007); Toman et al. (2019)
	Capsicum annuum L.	EBL	Yang et al. (2019)
	Solidago canadensis	BR	El-Sayed et al. (2020)
	Chenopodium quinoa Willd	BRs	Sadak et al. (2020)
	Solanum lycopersicum L.	EBL	Carvalho et al. (2013), Shu et al. (2016), Nazir et al. (2021)
Nitrogen metabolism	*Cajanus cajan* (L.) Millsp.	EBL	Dalio et al. (2013)
	Vigna radiata L.	HBL	Yusuf et al. (2014)
	Solanum lycopersicum L.	EBL	Shu et al. (2016)
	Arabidopsis thaliana	BL	Zhao et al. (2016)

(continued)

Table 15.1 (continued)

Physiological parameters	Plant species	BR analogues/inhibitors used	Reference
	Capsicum annuum L.	EBL	Yang et al. (2019)
	Vigna unguiculata L.	EBL	Cardoso et al. (2019)
Senescence	*Triticum aestivum* L.	EBL	Sağlam-Çağ (2007)
	Helianthus annuus L.	EBL	Kaplan-Dalyan and Sağlam-Çağ (2013)
	Citrus unshiu	EBL	Zhu et al. (2015)
	Solanum lycopersicum L.	EBL	Carvalho et al. (2013); Nazir et al. (2021)
	Glycine max L.	EBL	Bariş and Sağlam-Çağ (2016)
	Pisum sativum L.	EBL	Fedina et al. (2017)
	Carica papaya L.	BR	de Assis-Gomes et al. (2018)
	Capsicum annuum L.	EBL	Yang et al. (2019)
	Lilium orientalis	BR	Nergi and Ali (2020)

formation, BIN2 (BRASSINOSTEROID-INSENSITIVE2; a key component that mediate BR and auxin signaling during root development) plays an important role in auxin signaling, but BR signaling retards BIN2 activity which in turn couldn't activate transcription factors BES1 (BRI1-EMS-SUPPRESSOR 1) and BZR1 leading to downstream control of plant growth and development. But BIN2 causes the phosphorylation of ARF7 and ARF19 (AUXIN RESPONSE FACTOR), thereby enhancing their DNA-binding capacity at lower levels of BR (He et al. 2002; Yin et al. 2002; Cho et al. 2014). In shoot elongation, brassinosteroid treatment downregulates transcription factor ARF genes ARF4 and ARF8 in *Arabidopsis* wild-type (WT) seedlings in contrast to increased level of expression in BR-deficient mutants (Jung et al. 2010); however, the overexpression of ARF8 could suppress growth of hypocotyl resulting into a weaker apical dominance (Tian et al. 2004; Peres et al. 2019) (Fig. 15.3). These outcomes clearly indicated an elaborated and a dynamic interaction of ARFs through BZR1 and BIN2 both transcriptionally and post-transcriptionally to regulate plant growth and its development via BR-auxin cross talk (Peres et al. 2019).

15.5.2 Interplay Between Brassinosteroids and Gibberellins

A cooperative and an interdependent relationship exists between BRs and GAs, with multiple layers that interact in a species, tissue, and in a dose-dependent manner. The

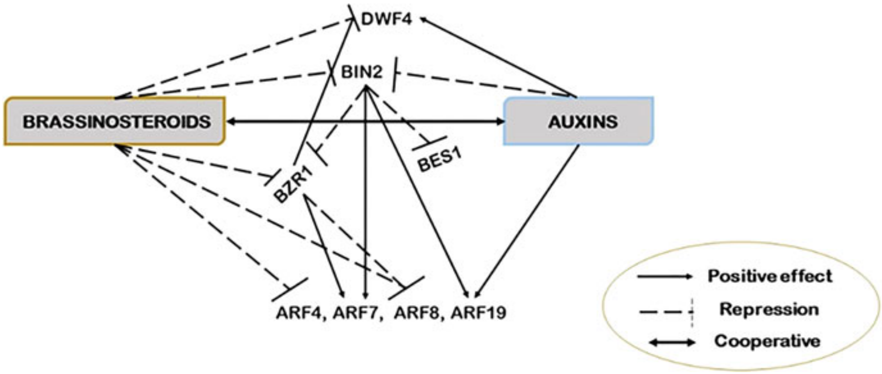

Fig. 15.3 A schematic model showing different signaling and biosynthesis genes during brassinosteroid-auxin interaction

studies revealed that the DELLA proteins (a key negative regulator of gibberellin signaling) have a suppressing effect on BZR1 transcriptional activity while interacting with BZR1/BES1 (Bai et al. 2012; Gallego-Bartolome et al. 2012; Li et al. 2012a; Peres et al. 2019). In *Arabidopsis* seedlings with lower levels of BR biosynthesis, hypocotyl elongation was promoted by GA or brassinazole treatment revealing the cooperative role of both hormones though it depends on the stage of growth, physiological conditions, and on the branched BR-regulated GA pathway (Gallego-Bartolome et al. 2012; Stewart Lilley et al. 2013; Unterholzner et al. 2015). BR influences GA biosynthesis not only in dicots but also in monocot plants as evident through the bioinformatics, chromatin immunoprecipitation (ChIP), and in vitro DNA binding studies, suggesting a direct binding of BZR1/BES1 to the target expression levels of two genes GA20ox, GA3ox, and GA2ox (encode enzymes in the rate-limiting step of GA production) from *Arabidopsis* and rice plants, respectively (Tong et al. 2014; Unterholzner et al. 2015; Peres et al. 2019). Thus, as per the postulates of the proposed model of BR-GA signaling involving the interaction between BZR1/BES1 and DELLA, BZR1/BES1 are activated post-translationally by BR to promote GA biosynthesis; further the escalated GA enhances DELLA degradation and releases the BZR1/BES1 activity. The stability of this proposed interaction is dependent upon the phosphorylation state of BZR1/BES1 proteins and the cellular localization of these processes (Ross and Quittenden 2016; Tong and Chu 2016; Unterholzner et al. 2016; Allen and Ptashnyk 2017) (Fig. 15.4). Additionally, the homeostasis between BR-GA is also affected during biotic stress where the biotic agents cause an interruption in the interplay between the hormones by producing hormonal mimicking signals for their own survival thereby disarming the immunity of their host (De Vleesschauwer et al. 2012; Peres et al. 2019).

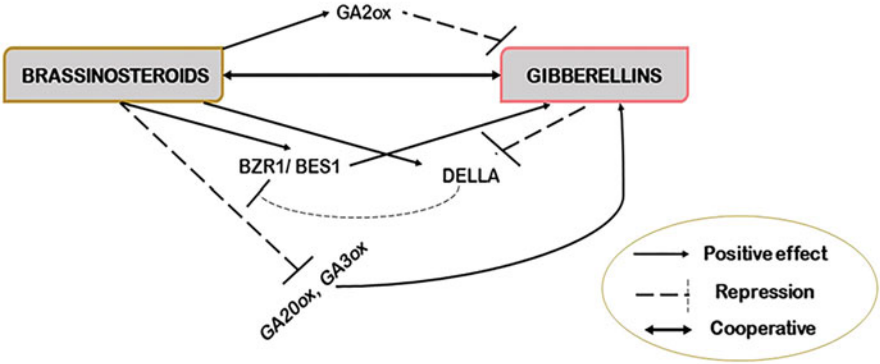

Fig. 15.4 A schematic diagram showing interaction between different signaling and biosynthesis genes during brassinosteroid-gibberellin cross talk

15.5.3 Interplay Between Brassinosteroids and Cytokinins

An indirect cross talk exists between brassinosteroids and cytokinins to regulate growth and development of plants. During lateral root formation, auxin transport is employed, and at molecular level, BR induces the expression of PIN genes (auxin efflux carriers) required for the development of root primordium, while CK suppresses its establishment by downregulating the expression of PIN genes, thus disturbing the auxin accumulation (Bao et al. 2004; Benjamins and Scheres 2008; Vercruyssen et al. 2011). Enzymatic targets of BR-mediated responses such as isopentenyl transferases (IPTs) and CKXs (CK oxidases/dehydrogenases) are responsible for the biosynthesis of bioactive cytokinins as well as its inactivation respectively. For example, in *Arabidopsis*, CKX3 gene directs the breakdown of CKs, and its overexpression under PYK10 (a root-specific promoter) reduces the levels of CKs in roots, causing minimal leaf and root growth. Reversibly, ectopic expression of CKX3 and BRI1 showed synergistic elevation in the leaf and root growth of plants (Werner and Schmülling 2009; Werner et al. 2010; Vercruyssen et al. 2011). In the regulation of several stress responses, negative role of CKs has been observed stating the gain and loss of function of CKX and IPT. On one hand, the overexpression of CKX implicated a deficiency of CK along with an elevated tolerance for drought and salinity; the suppression of IPT resulted in reduced levels of bioactive CK with enhanced stress tolerance. This negative relation may further be attributed to much repression of CK signaling pathway and inducing ABA signaling marker genes (such as AIL1, COR47, RAB18, RD29B, and SAG29) during cross talk between ABA and CK where ABA demonstrated similar results of increased stress tolerance via exogenous application (Nishiyama et al. 2011, 2012; Peres et al. 2019). Alternately, the role of BR can also be seen in drought stress such as in transgenic rice where it depends upon the physiological state of plant. The transgenic lines with IPT driven by PSARK (a stress- and maturation-induced promoter) showed increased CK levels before the start of senescence and BR

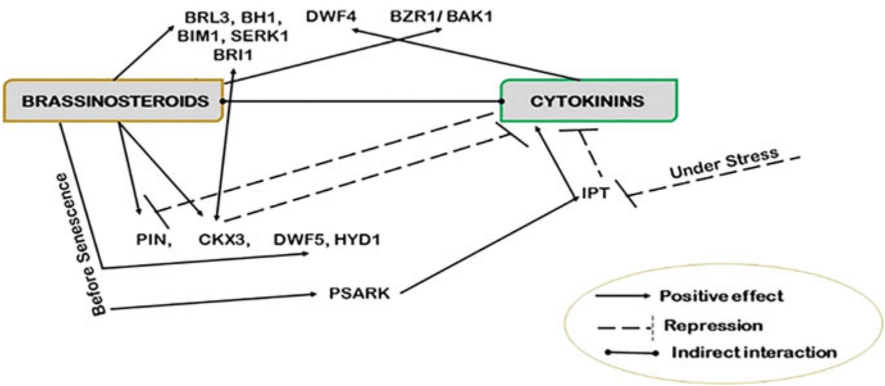

Fig. 15.5 Diagrammatic representation showing different signaling and biosynthesis genes during brassinosteroid-cytokinin interplay

signaling genes (BRL3, BRI1, BH1, BIM1, and SERK1) and its biosynthesis genes [DWF5 and HYD1 (HYDRA1)] were also upregulated under/no stress (Peleg et al. 2011) (Fig. 15.5).

15.5.4 Interplay Between Brassinosteroids and Ethylene

Cross talk between brassinosteroid and ethylene suggested indirect controls of different facets of plant growth and development. On one side, BR negatively regulates shoot gravitropism, and ethylene promotes shoot gravitropic reorientation through the involvement of auxin signaling genes (Guo et al. 2008; Vandenbussche et al. 2013). This is mainly achieved by activating and inhibiting negative and positive auxin signaling genes such as AUX/IAA and ARF7 and AR F19, respectively. However, ethylene works antagonistically by enhancing ARF7 and ARF19 and suppresses AUX/IAA to control shoot gravitropic responses (Vandenbussche et al. 2013). In case of root gravitropic responses also, the two hormones interact in opposite ways where BR increases root gravitropism while ET retards it by revamping auxin transport in the BR and ET mutants (Buer et al. 2006; Kim et al. 2007; Vandenbussche et al. 2013). Besides it, exogenously applied BR increases ET production in *Arabidopsis* seedlings by upregulating the expression of its key gene ACS (1-aminocyclopropane-1-carboxylate synthase) and stability of its proteins (mainly ACS5, ACS6, and ASC9) during external and internal stimuli thereby adjusting the ethylene synthesis in the plant tissues (Hansen et al. 2009; Muday et al. 2012). During root cell elongation, BRs and ET interaction has been observed in the root hair as well as the non-hair cells. In the case of root hair cells, the targeted expression of BRI1 activates the cell elongation in all tissues; however, it is retarded in non-hair cells due to elevation in the expression of two ACS genes: ACS5 and ACS9. Consequently, ACS genes catalyze the rate-limiting step of ET synthesis by forming ACC (1-aminocyclopropane-1-carboxylate) that accumulates and enhances

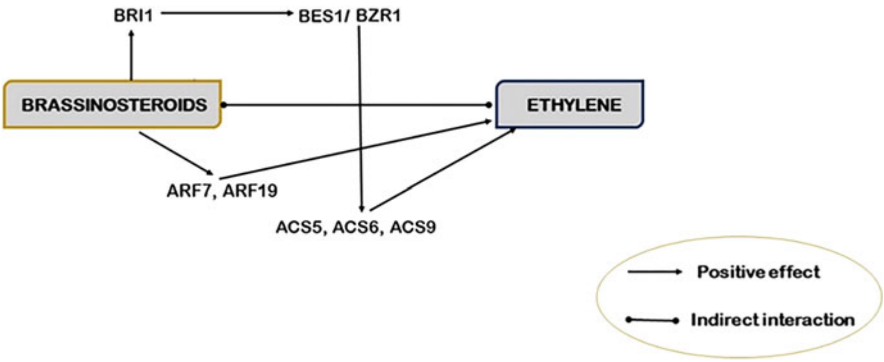

Fig. 15.6 A schematic representation of different signaling and biosynthesis genes in brassinosteroid-ethylene relationship

ethylene signaling, thereby inhibiting unidirectional cell expansion (Fridman et al. 2014; Zhu et al. 2016). On the contrary, synergistic relationship also exists between BR and ET in controlling hyponastic growth being employed by plants to cope the environmental strains. Here, ET is the main regulator and in turn is regulated by BR. C-23 hydroxylation of BR synthesis is mediated by ROT3 (ROTUNDIFOLIA3/CYP90C1), and any change in it impairs local cell expansion and inhibits BR synthesis which further lowers ethylene-induced upward leaf movement (Polko et al. 2013) (Fig. 15.6).

15.5.5 Interplay Between Brassinosteroids and Abscisic Acid

In plants, seed germination, root elongation, and even during stomatal closing, plants with defective BR signaling show enhanced sensitivity for ABA, thus showing antagonistic relationship between BRs and ABA (Steber and McCourt 2001; Zhang et al. 2009; Li et al. 2012b; Wang et al. 2020). During signaling process also, BR signaling opposes the ABA biosynthesis. This is evident through the removal of BSK5 (a positive regulator of BR signaling) which causes the induction of ABA3 and NCED3 (ABA biosynthesis-related genes) (Ha et al. 2016; Ha et al. 2018). Additionally, during BR-ABA cross talk, upstream of BIN2 kinase causes the downstream of BR receptor complex. In this, two negatively regulating ABA genes, ABI1 and ABI2, interact as well as dephosphorylate BIN2 (a negative regulator of BR signaling) to further regulate the phosphorylation of BES1. However, an in vitro ABA signal transduction mimicking showed that ABA through its receptors inhibit ABI2 which further promotes BIN2 phosphorylation (Zhang et al. 2009; Wang et al. 2018; Bulgakov and Avramenko 2020). Even under drought stress, BES1 impedes ABA induction of a drought-related transcription factor RD26 (RESPONSIVE TO DESICCATION 26) and it reciprocatively exhibits antagonism by modulating BES1-regulated transcription which hinders brassinosteroid-regulated growth (Chung et al. 2014; Ye et al. 2017). Moreover, early signaling of ABA is modified

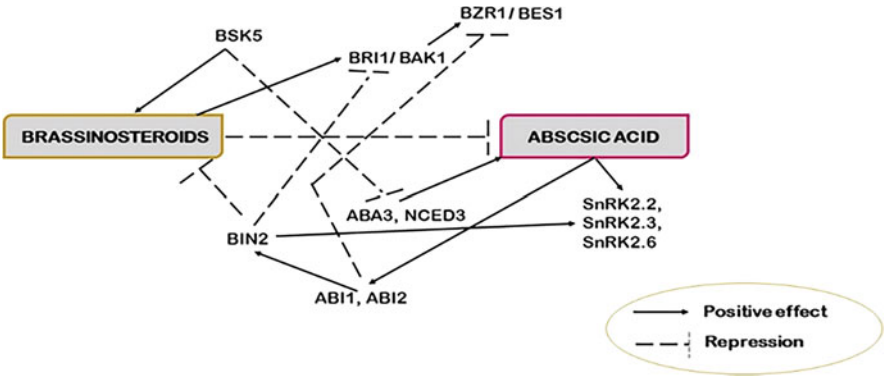

Fig. 15.7 Diagrammatic model showing interaction of signaling and biosynthesis genes during brassinosteroids-abscisic acid interlinkage

by BR as in the case of *Arabidopsis*, by directly affecting phosphorylation of the active ABA signaling participants such as SnRK2.2, SnRK2.3, and SnRK2.6. Here too, BIN2 kinase signaling acts as an interacting protein of SnRK2.2 causing its phosphorylation along with SnRK2.3 (Belin et al. 2006; Yoshida et al. 2010; Fujita et al. 2013; Cai et al. 2014) (Fig. 15.7).

15.5.6 Interplay Between Brassinosteroids and Salicylic Acid

The existence of the cross talk between BR and SA plays a key role in plants under a variety of environmental constraints. During biotic stress, though BR acts as an enhancer in vast range of disease resistance, this BR-mediated boosted resistance does not depend upon SA. However, the joint effect of BR and SAR (systemic acquired resistance) provides an additive protection against pathogens (Nakashita et al. 2003; Saini et al. 2015). Under biotic stress, APETALA2/ETHYLENE-RESPONSIVE FACTOR gene GhTINY2 is strongly enhanced. Its overexpression boost the plant's tolerance, and its underexpression makes the plant susceptible to infection. This is mainly because of more SA accumulation and its signal transduction through WRKY51 (WRKY transcription factor 51). However, the overexpression of GhTINY2 retards growth, knockdown of genes induced by BRs, and upregulation of BR-repressed genes. This occurs because of its interaction with BZR1 along with restraining of the transcriptional activation of IAA19 (INDOLE-3-ACETIC ACID INDUCIBLE 19) (Xiao et al. 2021). NPR1 (NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES1) regulate BR signaling genes; BIN2 and BZRI induces stress tolerance in plants, and the interplay between BR and SA may be due to the NPR1 gene which stimulates expression of the SA-related genes involved in plant defense (Divi et al. 2010; Ohri et al. 2015). Additionally, negative cross talk also exists between SA and BR signaling pathways that resulted in the immune-suppressive effect of BR. Moreover, the external

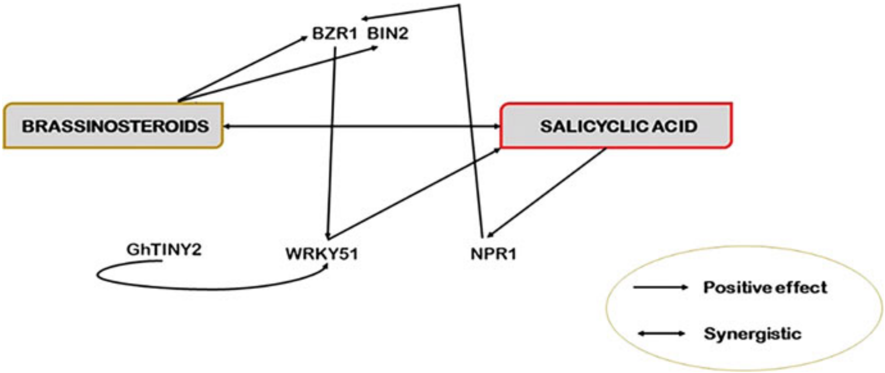

Fig. 15.8 A simplified model showing different signaling and biosynthesis genes during brassinosteroids-salicylic acid interaction

application of BRs activated the master defense regulators of SA pathway such as NPR1 and OsWRKY45 (WRKY transcription factor) (De Vleesschauwer et al. 2012) (Fig. 15.8).

15.5.7 Interplay Between Brassinosteroids and Jasmonic Acid

BR modulates JA signaling and inhibits JA-dependent growth and plays a vital role in both abiotic/biotic stresses in plants. Induced *OPR3* (encodes 12-oxophytodienoic acid reductase) jointly by BRs and JA signifies a potential integration node between BR action and JA synthesis (Zhang et al. 2009; Saini et al. 2015). In *Arabidopsis*, restoration of the sensitivity and hypersensitive reaction of JA was seen during a leaky mutation of DWF4 in coi1 mutant and a hypersensitive reaction in the wild type toward JA. But when BRs were applied exogenously, it mitigated root growth inhibition of JA because of downregulation of DWF4 in a COI1-dependent (CORONATINE INSENSITIVE1) manner by jasmonate (Ren et al. 2009; Jang et al. 2020). Jasmonate-induced anthocyanin accumulation is a hallmark of jasmonic acid-induced responses which reduces in BR-biosynthetic mutants, but the BR application helps plants accumulate anthocyanin. This occurs because of the minimal expression of MYB (transcription factor) genes PAP1 and PAP2 (PRODUCTION OF ANTHOCYANIN PIGMENT1) (Peng et al. 2011; Song et al. 2011; Wasternack and Hause 2013). In rice, thionin genes known for encoding antimicrobial peptides were greatly induced by JA but were enhanced by BR during stress (Kitanaga et al. 2006). Against insect herbivory also, BR-JA cross talk showed the defensive role of JA for anti-herbivory, while BR impedes it (Campos et al. 2009). In *Oryza sativa*, exogenous application of JA decreased expression of BR signaling gene, OSBRI1 and BR biosynthesis gene, and OsDWF4, during nematode infection revealing antagonistic interplay between JA and BR (Nahar et al. 2013) (Fig. 15.9).

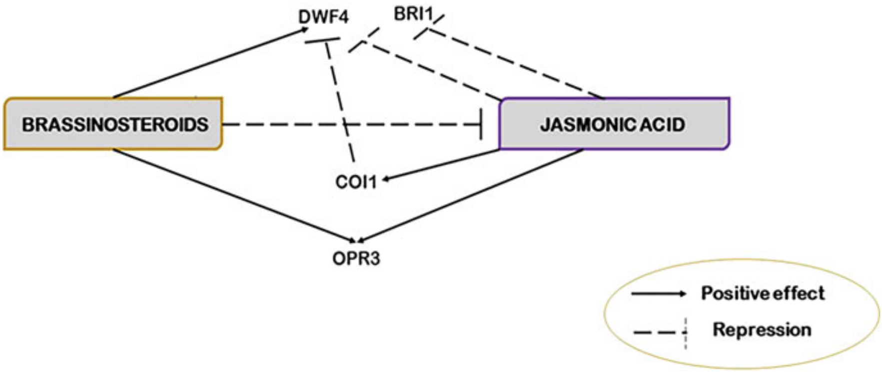

Fig. 15.9 A schematic representation showing different signaling and biosynthesis genes of brassinosteroids-jasmonic acid cross talk

15.5.8 Interplay Between Brassinosteroids and Strigolactones

Cross talk between BR and SL is still in its budding stage, and the inter-hormonal interaction pathways have been demonstrated recently (Faizan et al. 2020). Both BRs and SL help in regulating shoot branching in plants. This is achieved through the SL's key signaling component E3 ubiquitin ligase MAX2 (a shoot branching inhibitor) that continuously interacts with BR's transcription factors BZR1 and BRI1 EMS SUPPRESOR1 (BES1) by binding directly with them and causing their degradation. However, MAX2-mediated degradation of BR transcription factors is promoted by SL signaling resulting in suppressed shoot branching (Yin et al. 2002; He et al. 2005; Kim and Wang 2010; Wang et al. 2013). However, this has been contradicted in *bes1-D* mutant, where the role of BES1 was re-examined. The chosen phenotype with enhanced shoot branching does not show any association with the characteristic *bes1-D* leaf phenotype, thereby advocating that the branching defect described earlier might be wrongly referred to as a mutation in BES1 only (Bennett et al. 2016). Further, it is expected that advances in the studies of this new class of phytohormone will help in explaining the key underline players of the hormonal cross talk between BRs and SLs (Fig. 15.10).

15.6 BR-Mediated Modulation of Plant Antioxidant Defense System Under Abiotic Stress

Reactive oxygen species (ROS) are key regulatory and signaling molecules that play important role in plant growth and development. ROS, such as superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) production, elevates when plant is exposed to certain stressed conditions. Brassinosteroids improve the scavenging mechanism of these ROS by modulating the antioxidative as well as

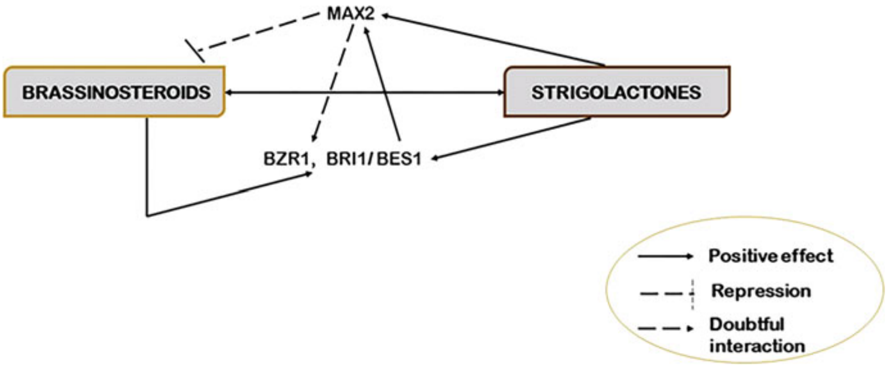

Fig. 15.10 Diagrammatic representation showing signaling and biosynthesis genes during brassinosteroid-strigolactone interaction

non-antioxidative system, thus playing an essential role in plant stress tolerance (Table 15.2).

15.6.1 Thermal Stress

In present scenario, with the increase in global greenhouse effect, changes in climatic conditions lead to rise in temperature, which has become one of the major detrimental stresses amid of constantly fluctuating environmental factors (Luo and Lau 2019; Karwa et al. 2020). Transcriptomic studies reveal that thermal/heat stress causes downregulation of critical gene(s) involved in the synthesis of cell wall, carbon assimilation, transport and accumulation of starch, and many metabolic pathways (Kothari and Lachowiec 2021). Plants in the environment are inevitable to such conditions but undergo some series of mechanisms to cope up with increased temperature, namely, osmoprotectants, ion transporters, antioxidant system, late embryogenesis abundant (LEA) proteins, heat shock proteins, signaling messenger, and factors of transcriptional machinery (Rodríguez et al. 2005). Various reports are available which depict BR-induced heat tolerance in plants. Though the underlined mechanism activated by BR for providing thermal stress tolerance is still not so clear, but different studies suggest that a signaling cascade is initiated by BR application which activates and brings together the small polypeptides and proteins, such as heat shock proteins (HSPs) or stress-induced proteins, to alleviate stress conditions (Bhandari and Nailwal 2020). BRs are believable to act as immunomodulators, protecting plants from injuries of HT stress. Several reports showed that BRs increase the production of heat shock proteins (HSPs) under thermal stress, thereby protecting proteins against irreversible heat-induced damage by preventing denaturation and facilitating the refolding of damaged proteins (Chauhan et al. 2011). BRs elevate the activities of various enzymes involved in the ascorbate–glutathione (AsA-GSH) cycle and maintain the homeostatic redox

Table 15.2 Role of BRs in modulating antioxidative defense system of plants under abiotic stress conditions

Type of abiotic stress	BR source	Mode of treatment	Plant species	Effect	References
Drought stress	BR	Seedling treatment	*Zea mays*	Enhanced water stress tolerance by increasing ABA biosynthesis	Zhang et al. (2011)
	BL	Soaking of roots	*Xanthoceras sorbifolia*	Increased leaf water content, relative water content, soluble sugar and protein content, SOD, POD, CAT, APOX activities	Li and Feng (2011)
	EBL	Foliar spray	*Capsicum annuum*	Enhanced antioxidant activity and prevented drought-induced inhibition photoinhibition	Hu et al. (2013)
	EBL HBL	Seed priming	*Raphanus sativus*	Increased levels of nucleic acids and soluble proteins, decreased activities of RNase and lowered lipid peroxidation and MDA content	Mahesh et al. (2013)
	BR	Seed priming	*Arachis hypogaea*	Improved oxidative enzyme levels and enhanced polyphenol and relative water content	Savaliya et al. (2013)
	HBL	Foliar spray	*Helianthus annuus*	Increased protein synthesis and acid inverters activity	Filova (2014)
	EBL	Foliar spray	*Capsicum annuum*	Elevation of stress-related transcription factors and antioxidant enzymes (*cAPX* and *MDAR*)	Hu et al. (2015)
	EBL	Foliar spray	*Lycopersicum esculentum*	Decreased ion leakage, lipoxygenase activity and ethylene production and increased SOD activity in leaves	Behnamnia (2015)
	EBL HBL	Seed priming	*Cajanus cajan*	Increased ABA, glycine betaine, proline accumulation, and decreased H_2O_2 and MDA content	Shahana et al. (2015)
	EBL + spermine	Foliar spray	*Zea mays*	Enhanced activities of dehydroascorbate reductase and monodehydroascorbate reductase	Talaat et al. (2015)
	EBL	Foliar spray	*Vigna unguiculata*	Improved photosystem II efficiency, gas exchange, and increased SOD, CAT, APX, and POX activities	Lima and Lobato 2017
	EBL	Seedling treatment	*Vitis vinifera*	Reduced the H_2O_2 content and superoxide radical (O_2^{*-} $^{-}$) production rate and enhanced content and activities of antioxidants	Wang et al. (2019)

Salinity stress	EBL + putrescine	Foliar spray	*Cucumis sativus*	Improved growth, chlorophyll content, and photosynthetic efficiency and promoted the activities of CAT, SOD, and POD	Fariduddin et al. (2013)
	EBL + putrescine	Foliar spray	*Lycopersicon esculentum*	Decreased MDA content and lipid peroxidation	Slathia et al. (2013)
	EBL + SA (salicylic acid)	Seed priming	*Phaseolus vulgaris*	Enhanced concentration of osmoprotectants and improved leaf water status and integrity of plasma membrane	Semida and Rady (2014)
	EBL + methyl jasmonate	Foliar spray	*Vitis vinifera*	Improved transpiration rate, pigments, photosynthesis, and proline content	Seif et al. (2014)
	EBL	Foliar spray	*Pisum sativum*	Improved osmolytes concentration and plant biomass.	Shahid et al. (2015)
	EBL + spermidine	Foliar spray	*Vigna radiata*	Increased activities of POX, SOD, and CAT and enhanced proline content, glycine betaine content, and gas exchange characteristics	Mir et al. (2015)
	EBL + ABA	Foliar spray	*Solanum tuberosum*	Enhanced various antioxidative defense enzyme activities	Upadhyaya et al. (2015)
	EBL	Seed priming	*Gossypium hirsutum*	Enhanced activities of GPX and SOD and improved proline content and pigments	Surgun et al. (2015)
Heat stress	EBL	Foliar spray	*Zea mays*	Alleviated protein degradation and enhanced cell membrane stability due to increased CAT, SOD, and POX activities	Yadav et al. (2016)
	BL	Foliar spray	*Leymus chinensis*	Enhanced biosynthesis of proline, sugars, and protein content and activation of antioxidant defense machinery	Niu et al. (2016)
	EBR	Spraying	*Solanum melongena* L.	Enhanced photosynthesis, seed germination, yield, and quality of crop	Wu et al. (2014)
	BRs	Seed priming	*Pinus sylvestris* L.	Enhanced seed germination	Cukor et al. (2018)
Zinc stress	EBL	Dipping	*Solanum nigrum*	Redox homeostasis	Sousa et al. (2020)
Cadmium stress	EBR	Spraying	*Vigna unguiculata* L.	Enhancement in leaf, root, and total dry matter. Elevated chlorophyll and gas exchange parameters	Santos et al. (2018)

(continued)

Table 15.2 (continued)

Type of abiotic stress	BR source	Mode of treatment	Plant species	Effect	References
Iron stress	EBR	Dipping	*Oryza sativa* L.	Enhanced electron transportation, photosynthetic activity	Tadaiesky et al. (2021)
Chromium stress	EBL	Seed priming	*Oryza sativa* L.	Improved ROS scavenging and maintain homeostasis	Basit et al. (2021)

potential during thermal stress in plants. BRs enhanced the expression of various genes encoding these enzymes (Zhang et al. 2014; Yadava et al. 2016; Kaur et al. 2018; Li et al. 2018; Surgun-Acar and Zemheri-Navruz 2019). Brassinosteroids alleviate the heat stress by regulating the glyoxylate and antioxidative system in the case of *Ficus* seedlings (Jin et al. 2015; Anwar et al. 2018). A study conducted by Sonjaron et al. (2018) revealed that 7,8-dihydro-8a-20-hydroxyecdysone (aDHECD), a mimic of brassinosteroid, improve the photosynthetic activity and carbohydrate content in rice seedlings under high temperature conditions. The mechanism of BR that contributes to plant heat stress (HS) tolerance is mediated by various essential complicated processes, namely, enhancing photosynthetic efficiency by maximizing the rate of carboxylation by Rubisco and improving the efficiency of PSII photochemistry; elevating photosynthetic pigments, stomatal conductivity, and membrane stability; activating antioxidant mechanisms; and maintaining redox homeostasis. Contrary to this, reduction in lipid peroxidation and production of ROS is observed (Hayat et al. 2010; Kaur et al. 2018; Kaya et al. 2019). Although a large number of studies demonstrated the heat stress-protective role of BR using exogenous applications, only a small number of studies are focused on molecular mechanism involved in heat stress tolerance (Ahammed et al. 2014; Zhou et al. 2014). It has been reported that a transient H_2O_2 production in the apoplast functions as a critical signal to mediate BR-induced heat stress tolerance in tomato (Zhou et al. 2014).

15.6.2 Heavy Metal Stress

Presently, heavy metals (HM) are regarded as major pollutants in the environment due to their toxic effect at very low concentration. “HM” is collective term, which applies to the group of metals and metalloids with greater atomic density than 4 g/cm^3, or five times, greater than water (Hawkes 1997; Gjorgieva Ackova, 2018). A number of HMs include cobalt (Co), nickel (Ni), lead (Pb), silver (Ag), iron (Fe), cadmium (Cd), chromium (Cr), zinc (Zn), arsenic (As), and the platinum group elements that are present in the environment affecting all its living components (Nagajyoti et al., 2010). Plants being sessile are exposed to various stress conditions in the environment, and HM constitutes one of the major obstacles in growth and development of plants. Anthropogenic activities and improper use of fertilizers and pesticides, urbanization, industrialization, and fossil fuel combustion have led to tremendous increase in concentrations of various hazardous chemicals in agricultural soils (Chen et al. 2015; Zhao et al. 2018). Toxic effect of HMs varies according to plant species, their concentrations, and chemical nature. HM reduces the rate of photosynthesis and the required precursors for the process. Also, there is significant reduction in quality and quantity of yield cultivated in polluted area with high risk of chemical consumption by human population (Wu et al. 2017; Hasan et al. 2019).

Recently, BRs, as an alternate eco-friendly tool for improving heavy metal (HM) stress tolerance in plants, have gained momentum (Bücker-Neto et al. 2017). BRs are reported for assimilation and metabolizing capacity for these chemicals (Santos et al. 2018). Plant exposed to HMs showed varied responses at the morphological, cellular, and molecular levels, and to study the complex mechanism underlining the improved tolerance by application of BRs**,** various studies have been conducted by worldwide scientists showing ameliorating effect of BRs during HM stress (Nawaz et al. 2017; Santos et al. 2018; Sharma et al. 2018; Wu et al. 2019; Ahammed et al. 2020; Soares et al. 2020; Betti et al. 2021). Study conducted by Jakubowska and Janicka (2017) decipher the potent role of BRs to provide stress tolerance during cadmium stress in cucumber plants. It has been observed that the BR stress tolerance is induced by the induction of plasma membrane NADPH oxidase and H^+ ATPase pump enzyme in cucumber during Cd stress. Similarly, BRs improve the tolerance against Cd in cowpea plants (Santos et al. 2018).

Exogenous application of EBL enhances the lead tolerance in both seeds and seedlings of *Brassica juncea* L. EBL alters the antioxidative enzyme activity and enhances the rate of ROS scavenging by their increased activity (Soares et al. 2020). Similar results have also been obtained by Wu and his coworkers (2019) in which EBL showed ameliorative effect against metalloid stress. Exogenously applied EBL (foliar spray) altered various enzymes and decreased the ROS level in wild *Arabidopsis thaliana* seedlings which improves stress tolerance against antimony. Similarly, EBL plays an important role in providing strength to rice plants against iron stress. Exogenous application of EBL can alter the light-capturing capacity and stomata conductance, increase thickness of epidermis of leaves, and maintain membrane integrity of leaves under Fe toxicity (Tadaiesky et al. 2021). Similarly, application of 28-Homobrassinolide improves the stress tolerance against Pb, Cd, and Zn and attenuates their toxic effect on growth and development of seedlings (Xu et al. 2019). Tolerance to high level of Zn has been reported in *Solanum nigrum* L by the foliar application of EBL, contributing in better physiological status and redox homeostasis in Zn-stressed seedlings (Sousa et al. 2020). BRs in combination with calcium play important role in amelioration of aluminum stress in plants (Ashraf et al. 2019). Improved root/shoot length and enhanced carotenoid, glutathione, ascorbic acid, and tocopherol content were reported with elevation in the expression catalase, peroxidase, glutathione reductase, and glutathione-S-transferase genes by EBL application during lead stress (Kohli et al. 2018). In conclusion, exogenous application of BR induce enhancement of tolerance to heavy metals is their involvement in substantial improvement in carbon metabolism, photosynthetic pigment content, antioxidative defense system, ROS scavenging capacity, glutathione content etc. (Choudhary et al. 2012; Rajewska et al. 2016). Though there are many reports available for stress tolerance properties of steroidal hormone (brassinosteroids), but there is still uncertainty about endogenous BR levels being modulated by exogenous BR under heavy metal stress.

15.6.3 Drought Stress

Water scarcity, which is one of the most deleterious of all environmental stresses, checks the growth of many crop varieties and declines the quality and quantity of crop production (Todorova et al. 2016). Severe drought stress conditions lead osmotic stress due to overproduction of reactive oxygen species (ROS) thereby reducing photosynthetic rate; revamping nitrogen and antioxidant metabolism, secondary metabolite accumulation, and mineral nutrition; and ultimately causing growth reduction in plants (Jatav et al. 2014; Ahanger et al. 2015; Ahanger et al. 2018). Various studies have reported that the exogenous application of plant growth regulators like BRs can mitigate the drought-induced adverse effects on the growth and metabolism of plants (Behnamnia 2015; Nawaz et al. 2015; Talaat et al. 2015; Ahanger et al. 2018). They enhance the antioxidative defense of plants to combat water deficiency. Exogenous application of BRs to plants suffering from drought stress causes reduction in H_2O_2 (hydrogen peroxide) and MDA (malondialdehyde) contents as a result of scavenging activities of antioxidative enzymes such as peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) (Li and Feng 2011; Vayner et al. 2014; Nawaz et al. 2017). Foliar application of EBL (0.01 mg/L) can also improve the antioxidant activity and drought-induced inhibition of photosynthetic functioning in *Capsicum annuum* (Hu et al. 2013).

It has been observed that exogenously applied BRs increase the concentration of abscisic acid and negate the toxic effects of water stress on plants (Wang et al. 2019). Supplementation of BRs (24-EBL and 28-HBL) alleviates the toxic effects of polyethylene glycol-6000 (PEG)-induced drought stress by enhancing seed germination, seedling length, and biomass (fresh and dry weight) in *Cajanus cajan* by increasing abscisic acid, glycine betaine, and proline accumulation (Shahana et al. 2015). It has been observed that the application of EBL and HBL to drought-stressed pigeon pea significantly decreases H_2O_2 and MDA accumulation by increasing antioxidative activities of SOD, CAT, POD, APX, and GR (glutathione reductase) (Shahana et al. 2015). Earlier, both EBL and HBL treatments have been found to reverse the inhibitory effects of PEG-6000-induced water stress on radish seedlings by increasing seed germination and seedling growth associated with enhanced levels of nucleic acids and soluble proteins and decreased activities of RNase. They also maintained the membrane integrity by lowering lipid peroxidation and MDA content (Mahesh et al. 2013). Recently, Tanveer et al. (2019) discussed the potential role of EBL in improving drought stress tolerance in plants. EBL ameliorates the negative effects of water stress by increasing carbon assimilation rate, perpetuating balance between ROS and antioxidants, and accumulating solutes especially proline (Tanveer et al. 2019). It enhances photosynthesis and other leaf gas exchange traits by protecting the ultrastructure of photosynthetic pigment apparatus from degradation (Tanveer et al. 2019).

BRs mediated plant defense mechanism against oxidative stress by maintaining the expression of genes involved in encoding xyloglucan endotransglucosylase/hydrolases (XTHs) or by escalating the activity of H^+-ATPase, sucrose synthase, and cellulose synthase (Clouse 2011; Nawaz et al. 2017). Application of BRs transmutes the expression of genes responsible for encoding both structural and regulatory proteins (Ahammed et al. 2020). It was studied that the overexpression of *Arabidopsis* BR biosynthetic gene *DWF4* in *Brassica napus* increased seed yield, root biomass, and length and enhanced stress tolerance caused by dehydration (Sahni et al. 2016). Duan et al. (2017) cloned a BR biosynthetic gene, *SoCYP85A1*, from *Spinacia oleracea* and studied its effect on abiotic stress tolerance in tobacco. They found that overexpression of the cloned gene improved drought tolerance and resulted in longer primary root and more lateral roots in transgenic tobacco as compared to wild types by eliminating ROS and MDA accumulation and improving proline content along with antioxidant enzyme activities (Duan et al. 2017).

15.6.4 Salinity Stress

Salinity is a major abiotic stress that renders most of the agricultural lands as barren wastelands resulting in food scarcity. It affects the water absorption capacity of plants that leads to overall decrease in plant growth (Bartwal and Arora 2020). Salinity stress, often termed as physiological drought, negatively affects plant development and productivity by inducing osmotic and ionic imbalances (Ahanger and Agarwal 2017; Kaur et al. 2018; Ahammed et al. 2020). Salt stress-induced toxic effects on plants include osmotic stress, ionic toxicity, truncated nitrogen metabolism, increased production of ROS that leads to oxidative damage, retarded photosynthetic functioning, and hindrance in uptake and translocation of mineral nutrients (Ahmad et al. 2010; Iqbal et al. 2015; Ahanger and Agarwal 2017). It is well established that exogenous application of BRs can reverse the negative effects induced by saline conditions on growth and development of plants. Time and again various studies have reported the role of BRs in mitigating the toxic effects of salt stress in wide range of plants including *A. thaliana,* rapeseed (*Brassica juncea*), mustard (*B. napus*), eggplant (*Solanum melongena*), pepper (*Capsicum annuum*), cucumber (*Cucumis sativus*), maize (*Zea mays*), and common bean (*Phaseolus vulgaris*) (Yuan et al. 2012a; Yue et al. 2018; Ahammed et al. 2020).

BRs help plants to cope up with salt stress by regulating antioxidative defense system. They activate the antioxidative defense machinery by influencing or controlling the transcription and/or translation of specific genes to improve the oxidative stress tolerance potential of plants (Cheng et al. 2015; Fariduddin et al. 2014). In a study, it was found that 24-EBL application enhanced growth, soluble proteins, and antioxidant activities of *Solanum tuberosum* suffering from salinity stress (Khalid and Aftab 2016). In another study, it was observed that exogenous application of BR conferred tolerance and mitigated the negative effects of NaCl-induced salt stress in cucumber by improving growth, chlorophyll content, and photosynthetic efficiency; promoting the activities of antioxidative enzymes, namely, CAT, SOD, POD; and increasing proline content (Fariduddin et al. 2013). In the same plant NaCl-induced production of ROS like O_2^- and H_2O_2 was mitigated by the enhancement of antioxidative defense system by EBL application (Lu and Yang 2013). Supplementation of watermelon with 24-EBL increased its salt tolerance by enhancing CO_2 accumulation and water use efficiency; BRs were suggested to promote photosynthesis through activation of photosynthetic enzymes like Rubisco under salt stress conditions (Cheng et al. 2015).

It is well documented that salinity stress reduces the percentage of seed germination and overall biomass of crops, but after treating stressed plants with brassinolide, an increase in germination rate as well as root and shoot length was seen, and nitrate reductase activity was also increased as compared to stressed plants (Lalotra et al. 2017). Apart from exogenous application of BRs, harmful effects of high salt content have been ameliorated, and stress tolerance has been improved by employing seed priming techniques (Ahanger et al. 2018). In an experiment Zhang and his co-workers pre-treated the seeds of *Medicago sativa* with EBL and then exposed them to saline conditions. They noticed that under the influence of EBL, seed germination increased and oxidative damage reduced by enhanced activities of antioxidant enzymes, namely, SOD, POD, and CAT (Zhang et al. 2007). It has been seen that BRs regulate DNA methylation that plays a pivotal role in salinity tolerance. In a study, cytosine DNA methylation was found to be decreased in *Linum usitatissimum* (flax) upon NaCl (150 mM) exposure; however, seed priming with 24-EBL induced total methylation and enhanced salt tolerance, suggesting its role in epigenetic modification under salinity stress (Amraee et al. 2019).

15.6.5 Other Major Abiotic Stress

Aside from the above discussed major abiotic stressors, BRs and related compounds can also play notable roles in plants to cope up with other abiotic stressors like pesticides, photoinhibition/light stress, nutrient stress, and water-logging/water-flooding stress (Ahammed et al. 2012b; Sharma et al. 2013, 2017; Xia et al. 2006; Ogweno et al. 2010; Ahanger et al. 2018; Janeczko et al. 2010; Kang et al. 2006, 2009; Liang and Liang 2009; Lu et al. 2006; Lu and Guo 2013). 24-Epibrassinolide can enhance the tolerance of *Oryza sativa* and *Brassica juncea* to stress generated by pesticide imidacloprid (IMI) by decreasing lipid peroxidation via enhanced activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APOX), guaiacol peroxidase (GPOX), glutathione reductase (GR), and monodehydroascorbate reductase (MDHAR), upregulating the expression of some genes like Fe-SOD, Mn-SOD, Cu/Zn-SOD, CAT, APOX, and GR (Sharma et al. 2013; Sharma et al. 2017). In Ca $(NO_3)_2$-exposed *Cucumis sativus,* EBL upregulated the ROS-scavenging metabolism of antioxidant enzymes and protected the photosynthetic membrane system (Yuan et al. 2012b). Mitigation of impacts caused by pyrene and phenanthrene toxicity in tomato has been observed as result of EBL-mediated decreased content of MDA and increased activity of CAT, APOX, GPOX, and GR (Ahammed et al. 2012a). Application of EBL decreased the lipid peroxidation and increased H_2O_2 metabolism via enhanced activity of GST and GSH content which were asserted to help *Solanum tuberosum* to counteract phenanthrene-accrued consequences (Ahammed et al. 2012b, c). In tomato exposed to phenanthrene and Cd co-contamination, decrease in lipid peroxidation and enhanced antioxidant defense system by EBL were reported by Ahammed et al. (2013a). Recently, Ahammed et al. (2013b) evidenced that EBL benefited the tomato to maintain photochemical quenching coefficient (Pq), quantum efficiency of PSII phytochemistry {(PS II)}, and photochemical efficiency of PSII (Fv/Fm) under polychlorinated biphenyls induced oxidative stress by enhancing the activities of antioxidant enzymes. Phytotoxicities of nine pesticides (abamectin, chlorpyrifos, Cuproxat, cyazofamid, Fluazifop-p-butyl, flusilazole, haloxyfop, imidacloprid, and paraquat) had been alleviated by pre-treating *Cucumis sativus* with EBL. EBL increased the CO_2 assimilation capacity and antioxidant enzyme activity in *C. sativus* (Xia et al. 2006). Application of EBL under light stress in tomato (*Lycopersicon esculentum*) enhanced the activity SOD, CAT, APOX, and GPOX enzymes and benefited the plants to maintain photochemical quenching (qP), quantum efficiency of PSII (PS II), and net photosynthetic rate (Pn) by decreasing lipid peroxidation (Ogweno et al. 2010). Significant role of EBL in enhancing the activity of antioxidant enzymes was evidenced in plants exposed to chlorpyrifos (Xia et al. 2009). Similarly, application of 24-epiBL to *Arachis hypogea* mitigated the oxidative stress induced by Fe-deficiency and reduced ROS production by enhancing activity of nitrate reductase, antioxidant system, and osmolyte accumulation (Song et al. 2016). Exogenous application of EBL (soaking and foliar) reduced uptake of iron and sodium and increased uptake of magnesium, calcium, and potassium in wheat (Janeczko et al. 2010). Foliar spray of EBL in *C. sativus* mitigated toxic effects of excess calcium on the uptake of necessary mineral elements such as potassium, magnesium, phosphorus, and manganese (Yuan et al. 2015). BRs and related compound were evidenced to furnish tolerance to water-logging/water-flooding stress to different crops such as oilseed rape (Liang and Liang 2009), soybean (Lu et al. 2006), and cucumber (Kang et al. 2006, 2009; Lu and Guo 2013) mainly by decreasing oxidative damage via increased activities of SOD and POD (peroxidase).

15.7 BR-Mediated Modulation of Plant Antioxidant Defense System Under Biotic Stress

Under natural conditions, plants are exposed to both abiotic stress (discussed above) and biotic stress (viruses, bacteria, fungi, insects, nematodes, parasites, and weeds). Plants use inducible defense mechanism to effectively tolerate different types of stress. Induced defense mechanism of plants against biotic stress is similar to defense induced against abiotic stress (Anwar et al. 2018). BRs and related compound not only help the plants to cope up with abiotic stresses but also play an important role to enhance the tolerance against biotic stresses (Krishna 2003; Ali et al. 2007; Jager et al. 2008; Bajguz and Hayat 2009; Nawaz et al. 2017) (Table 15.3). They involve complex signaling cascade to positively regulate antioxidant defense metabolism (Belkhadir et al. 2012) and induce innate immune response to protect the cells from different biotic stresses (Wang et al. 2012). Application of brassinolide (BL) in *A. thaliana* infected with cucumber mosaic virus (CMV) increased the activity of antioxidant enzymes like CAT, SOD, POD, and APOX; decreased photosystem damage; and modulated expression of genes related to defense (Zhang et al. 2015). Growth and activity of antioxidant enzymes in tomato also enhanced by

Table 15.3 Role of BRs in modulation of plant antioxidant defense system under various biotic stress

Type of biotic stress	BR source	Mode of BR treatment	Plant species	Effects	References
Bacteria	EBL	Injected in leaves	*Arabidopsis thaliana*	Inhibition of FLS2-mediated immune signaling	Albrecht et al. (2012)
Fusarium culmorum	EBL	Soil and foliar application	*Hordeum vulgare*	Inhibition of *Fusarium* head blight	Ali et al. (2013)
Oidium sp. and *Pseudomonas syringae and* TMV	EBL	Hydroponic system	*Hordeum vulgare*	Increased resistance against powdery mildew fungus *Oidium* sp. bacterium *Pseudomonas syringae* and TMV	Ali et al. (2014)
TMV	BL	Leaf treatment	*Nicotiana benthamiana*	Increased systemic TMV resistance	Deng et al. (2016)
Fusarium	EBL	Root and foliar application	*Cucumber sativus*	Reduced infection of *Fusarium* wilt	Ding et al. (2009)
Verticillium dahlia	BL	Soil application	*Gossypium barbadense* and *Gossypium hirsutum*	Enhanced resistance against wilt causing fungus	Gao et al. (2013)
Meloidogyne incognita	EBL	Seed dipping	*Solanum lycopersicum*	Increased tolerance against *M. incognita* by enhancing activity of antioxidant enzymes	Jasrotia and Ohri (2014, 2017a, b)
Meloidogyne graminicola	BL	Foliar application	*Oryza sativa*	Enhanced innate immunity against *M. graminicola*	Nahar et al. (2013)
Meloidogyne incognita	HBL EBL	Seed treatment	*Brassica juncea*	Increased tolerance against *M. incognita*	Ohri and Kaur (2011); Ohri et al. (2011)

(continued)

Table 15.3 (continued)

Type of biotic stress	BR source	Mode of BR treatment	Plant species	Effects	References
Pythium graminicola	BL	Media augmentation	*Oryza sativa*	Inhibited infection caused by root oomycete *Pythium graminicola*	De Vleesschauwer et al. (2012)
Cucumber mosaic virus (CMV)	BL	Foliar spray	*Arabidopsis thaliana*	Enhanced tolerance against CMV	Zhang et al. (2015)
Citrus disease	EBL	Fruit dipping	*Citrus unshiu*	Improved disease resistance and postharvest quality	Zhu et al. (2015)

28-homobrassinolide (HBL) application and resulted in reduced oxidative stress caused by nematodes (Kaur et al. 2013, 2014). In rice, induced systemic defense against nematode stress was stimulated by exogenous application of epibrassinolide (Nahar et al. 2013). At low temperature, management of *Botrytis cinerea* in postharvest grapes was reported by Liu et al. (2016) when EBL was applied exogenously. Similarly, Zhu et al. (2010) reported that exogenously applied brassinosteroid solution enhanced the activity of antioxidant enzymes like CAT, SOD, and POX and inhibited the development of blue mold decay caused by *Penicillium expansum* in harvested jujube fruit. Additionally, improved fruit quality and delayed senescence due to reduced ethylene production were observed suggesting that BR-mediated detain in fruit decay might be related with induction of disease resistance. *Citrus unshiu* fruit dipped in EBL manifested enhanced resistance against citrus disease due to increased activity of stress-related metabolites (Zhu et al. 2015). Reduced susceptibility against leaf blight and rice blast diseases in barley seedlings grown under hydroponic system containing EBL was also observed (Ali et al. 2014). Moreover, resistance in uzu barley lines against powdery mildew fungus *Oidium* sp., bacterium *Pseudomonas syringae* pv. Tabaci and tobacco mosaic virus (TMV) was also induced by EBL. Application of BL in tobacco and rice showed similar effects (Nakashita et al. 2003). Application of BR-containing extract of *Lychnis viscaria* seeds caused an enhanced resistance of tomato, cucumber, and tobacco to fungal and viral pathogens (*Botrytis, Sphaerotheca fuliginea,* and TMV, respectively) (Roth et al. 2000).

15.8 Conclusion

It is a well-established fact that different environmental stressors (biotic and abiotic) are responsible for negatively affecting the agricultural economy by directly reducing the productivity of different crop plants. Moreover, this decline in productivity is becoming more severe with passing times. Since, these stressors generate ROS in affected plants, this further leads to destructive effects on physiological and metabolic processes of plants. In order to overcome this ROS generation, various strategies have been developed, and one such strategy is the application of plant growth regulators, which can act as a feasible environmentally safe alternative. In this continuation phytohormones like BRs and its associated components have been reported to induce antioxidant defense system of plants under different stressful conditions. Furthermore, BRs have also the efficiency of interacting with other phytohormones under normal and stressed conditions thus making them more potent for the resistance in plants against different environmental stresses.

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Selenium-Mediated Regulation of Antioxidant Defense System and Improved Heavy Metals Tolerance in Plants

16

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Abstract

Being stalkless organism, agricultural crops are constantly confronted by environmental stresses such as heavy metals (HMs) which severely affect the growth, productivity and thus yield losses. To minimize the HMs-induced phytotoxic effects and improve the HM tolerance by plants, the utilization of beneficial elements such as selenium (Se) as antioxidant defense agent can be effective solutions. At low concentrations, Se compounds (selenite or selenate) can promote plant growth and stress tolerance. The key mechanism is that lower Se doses can activate the antioxidant defense capacity (by stimulating the activities of

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enzymatic or nonenzymatic antioxidants); reduce the extra accumulation of reactive oxygen species, malondialdehyde (stress marker); and scavenge associated damages due to oxidative stress as well as membrane lipid peroxidation, ultimately enhancing the plant tolerance against HMs stress. Here, we have overviewed the available literature concerning the physiological roles of Se species via the involvement of enzymatic or nonenzymatic antioxidant systems in the alleviation of HMs-induced toxic effects in plants. The aim of the present chapter is to apprise our understandings regarding the beneficial and protective roles of inorganic Se forms (selenite and selenate) on the mitigation of HM stress (via involving antioxidant defense systems) in different plants. In this way, the exploitation of Se as antioxidative agent in HMs-susceptible crops can be ideal strategy to ameliorate HMs stress and Se-exposed plants can display improved growth under environmental stress conditions. Hence, Se-supplementation should be focused to develop stress-tolerant genotypes.

Keywords

Selenium · Heavy metals · Plants · Antioxidants · Oxidative stress

16.1 Introduction

Edaphic selenium (Se) exists in different states such as nano-selenium (Se°), elemental Se [selenide (Se^{-2})], inorganic Se [selenite (Se^{+4}) and selenate (Se^{+6})], and organic Se [selenomethionine (SeMet), selenocysteine (SeCys), methylselenol, and Se-methylselenocysteine (MetSeCys)] in plants (Wu et al. 2016). The distribution and bioaccumulation of above-reported Se forms in plant tissues are decided by specific plant (Yin et al. 2019). Soil pH, organic matter, redox capacity, and clay levels governed the Se bioavailability (Dhillon and Dhillon 2003). Lower adsorption capacity of Se^{+6} mediates higher solubility in water than Se^{+4} (Mayland et al. 1991). This indicated that Se^{+4} is dominant species in acidic soils or reducing conditions and alkaline soils or oxidizing conditions favor Se^{+6}. Therefore, a higher bioavailability/mobility was noticed in oxidizing conditions and thus lower mobility in reducing conditions.

Mostly plants take up Se in the form of inorganic Se such as Se^{+4} and Se^{+6}, and then convert them into organic Se forms such as SeMet and SeCys (Gupta and Gupta 2016). Se speciation may differ in absorption capacity from soils and transport pathways. Plants are unable to take up elemental Se forms due to their insolubilities in water systems, while organic forms have relatively higher solubilities and thus can easily be assimilated by plants (Natasha et al. 2018). Se^{+4} competes for phosphorus ions for their uptake by plant roots, thus transporting via phosphate transporters and utilizing passive transport for absorption (Li et al. 2008; Wan et al. 2016; Ulhassan et al. 2019b). Se^{+6} (via active transport) competes with sulfate (S) for its uptake by plants which suggested the involvement of sulfate transporters in its transport mechanism (Gupta and Gupta 2016). Largely, Se has been regarded as essential element for plants having higher Se accumulation capacity and easily assimilates due to chemical similarity with sulfur (Terry et al. 2000). Numerous indications suggested that Se is required for Se accumulators which are prevalent in Se-rich soils (Terry et al. 2000; Gupta and Gupta 2016). Se essentiality in higher plants is still controversial and debatable.

In response to adverse environmental conditions such as heavy metals stress, plants boost their endogenous defense system (Hassan et al. 2017a, b; Ali et al. 2018a, b; Mwamba et al. 2020; Hussain et al. 2021a, b; Sheteiwy et al. 2021; Yang et al. 2021; Zhang et al. 2021). Usually, hyperaccumulators had potential to stimulate the antioxidant defense systems to cope with the extra accumulation of reactive oxygen species (ROS) caused by excessive Se levels in cellular organs. The excessive accumulation of Se in plant tissues can desynchronize the cellular redox status by overproducing the ROS that impairs the protein structures, lipid peroxidation, and oxidative injuries (Gupta and Gupta 2016; Mostofa et al. 2017, 2020; Ulhassan et al. 2018, 2019a, b). In contrary, low Se levels can enhance the plant defense system by minimizing the abiotic stress-induced oxidative stress (Hasanuzzaman et al. 2012; Malik et al. 2012; Feng et al. 2013a; Ulhassan et al. 2019c). Previous studies documented that Se is involved in antioxidant defense metabolism in hyperaccumulators (Freeman et al. 2010; Hasanuzzaman et al. 2012; Malik et al. 2012; Ulhassan et al. 2019a, c). It was noticed that hyperaccumulator plants such as *Spondias pinnata* had 1.5-fold higher antioxidant potential than non-hyperaccumulators such as *Stanleya albescens* (Freeman et al. 2010). Being a micronutrient, Se exerts beneficial effects at low concentrations (Ulhassan et al. 2019a, c) and can promote plant growth and development against harsh environmental conditions (Feng et al. 2013a, 2021; Handa et al. 2019; Ulhassan et al. 2019c). In this chapter, we have discussed the growth-enhancing roles of Se and its involvement in improving the heavy metals tolerance in plants by reducing the oxidative stress and modulating the antioxidant defense system (mainly). The outcomes of this chapter can be helpful to boost the remediation efficacy of heavy metals-contaminated soils and thus improves the cultivation of plants in these contaminated areas.

16.2 Physiological Roles of Selenium in Plants and Involvement of Antioxidant Defense Machinery

The extra production of ROS indices such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet-}$), hydroxyl free radical (OH^-), singlet oxygen, and lipid peroxidation free radicals is the ultimate outcome of heavy metals stress (Ahmad et al. 2017; ul Hassan et al. 2017a, b; Ali et al. 2018c; Aslam et al. 2021; Salam et al. 2021). The overgeneration of ROS caused membrane lipid peroxidation as MDA which is a marker of lipid damages. To fight against oxidative stress in plant metabolism, plants stimulate their intrinsic antioxidant enzyme activities such as superoxide dismutase, catalase, glutathione reductase, and ascorbate peroxidase (Silva et al. 2018; Nazir et al. 2020). Low Se levels improve the plant defense mechanisms by the detoxification of oxidative free radicals and increase the enzymatic and nonenzymatic antioxidant enzymes which help in the reduction of ROS and induced oxidative stress (Schiavon and Pilon-Smits 2017; Ulhassan et al. 2019c). The key involved mechanisms are the regulation of enzymatic and non-enzymatic antioxidant defense systems, direct scavenging of ROS, improving the photosynthetic apparatus and mitigation of ROS via the dismutation of $O_2^{\bullet -}$ into H_2O_2 (Silva et al. 2020).

Nonenzymatic antioxidants such as glutathione peroxidase (GSH), ascorbate (AsA), phytochelatins (PCs), carotenoids, flavonoids, proline, and alkaloids also minimized the extra production of ROS (Foyer and Noctor 2012; Nazir et al. 2020). The activation of antioxidant and H_2O_2 reduction efficacy of Se are mediated by GSH via using AsA. A key enzyme, GSH-Px, is involved in the activation of plant defense systems and sequestration of H_2O_2, $O_2^{\bullet -}$ and MDA (Hartikainen et al. 2000). The forms of AsA displayed multifunctional role in plants such as induction of secondary metabolites and osmolyte accumulation (Foyer and Noctor 2012). The lower levels of Se can increase the AsA level that results in the reduction of H_2O_2 via maintaining enhanced activity of antioxidant enzymes and reduction in the biosynthesis of zeaxanthin and α-tocopherols (Hasanuzzaman et al. 2019). Vitamin E, α-tocopherol, can minimize the accumulation of $O_2^{\bullet -}$ and lipid peroxidation free radicals (Hartikainen et al. 2000). In this way, α-tocopherol prevented the peroxidation of lipid membranes.

16.3 Selenium Mediated Alleviation of Heavy Metals Toxicity in Plants

Sessile plants are encountered by various forms of environmental stress factors including heavy metals which are phytotoxic especially at excessive concentrations. Plants utilize different strategies to mitigate their inhibitory effects and maintain cellular homeostasis (Xie et al. 2019). In the following section, the potential beneficial roles of Se in alleviating the heavy metals-induced phytotoxic effects have been documented. For this purpose, the involvement of antioxidant defense systems will be mainly targeted.

16.3.1 Cadmium

Cadmium (Cd) is the third most toxic element and carcinogenic for human health (Clemens et al. 2013). Numerous studies confirmed that Cd causes severe damages to morphological, physio-biochemical, and cellular attributes (Rizwan et al. 2019; Mwamba et al. 2020; Zwolak 2020; Hamid et al. 2021; Riaz et al. 2021). In response to Cd-facilitated extra production of ROS, plants activate their antioxidative defense system in the form of antioxidant enzyme activities (Riaz et al. 2021). It has been documented that Se can mitigate the oxidative damages induced by Cd (Wan et al. 2019; Zwolak 2020; Riaz et al. 2021). Recent investigation suggested that exogenous supply of Se stimulated the activities of superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), and ascorbate peroxidase (APX) enzymes and minimized the over accumulation of ROS in the tissues of rice plants (Qingqing et al. 2019; Wan et al. 2019).

In *Brassica campestris*, the exogenous applications of Se increase the activities of catalase (CAT), key antioxidant enzyme, by scavenging the excessive generation of ROS within cells (Ding et al. 2017). In rice plants, the supplementation of Se enhanced the Cd tolerance by increasing the levels of glutathione and phytochelatins and reducing the ROS and MDA contents in both shoots and roots (Huang et al. 2021). Tang et al. (2020) determined that foliar spray of Se as sodium selenite can reduce the ROS and MDA contents by activating the SOD, POD, and CAT activities in spinach. In the shoots and roots of wheat plants, the foliar applications of Se as sodium selenate upregulated the SOD, POD, CAT, and APX activities and reduced the MDA contents under Cd exposed soil conditions (Wu et al. 2020). In Cd-treated mustard families including *Brassica napus* and *Brassica juncea*, the supplementation of Se IV or Se VI notably enhanced plant tolerance. Interestingly, Cd alone applications enhanced the SOD and POD activities but declined the CAT activity, which was reversed by Se treatments in the leaves and roots of both *Brassica* species. Their findings suggested that plants (mediated by Se) greatly activated their defense system to handle oxidative or membrane damages (induced by ROS and MDA) and maintain redox balance against Cd stress (Zhang et al. 2020). These above-reported observations suggested that both inorganic Se forms can be effective in the detoxification and remediation of Cd toxicity and contamination, respectively.

16.3.2 Chromium

Chromium (Cr) exists in trivalent and hexavalent ionic forms, and its excessive amounts in soil environment caused severe toxic effects on plant growth and development. The key mechanism of Cr phytotoxicity is the overproduction of ROS and lipid peroxidation as MDA, thus causing oxidative damages and injuries to cellular membranes (Ahmad et al. 2017; Handa et al. 2019; Singh and Prasad 2019; Ulhassan et al. 2019c). To cope with these Cr-triggered ROS or MDA-governed cellular or membrane damages, various approaches in the form of antioxidants such as biomolecules, osmoprotectants, and enzymatic or nonenzymatic activities have been well documented. Here, we will discuss the potentials of beneficial elements such as Se to mitigate the Cr toxic effects in different plant species. In *Brassica napus*, the supplementation of Se as sodium selenite mitigated the Cr toxicity by activating the enzymes of AsA-GSH cycle (SOD, CAT, APX, GR, DHAR, and MDHAR), glyoxalase defense system (Gly I and Gly II), and GSH and AsA contents and reducing the accumulation of ROS (H_2O_2 and $O_2^{\bullet-}$), MDA, and methylglyoxal contents in both leaves and roots. In addition, Se (IV) improved the other defense systems such as carotenoids and stimulated the thiol biosynthesis such as essential amino acids in order to enhance the Cr tolerance (Ulhassan et al. 2019c). Handa et al. (2019) investigated that exogenous supply of Se as sodium selenate alleviated the Cr toxicity in the tissues of *Brassica juncea* by upregulating the gene expressions of *SOD*, *POD*, *CAT*, *GR*, and *GST* enzymes and reducing the oxidative damages by minimizing the generation of ROS (H_2O_2 and $O_2^{\bullet-}$) and lipid peroxidation as MDA. Moreover, Se (VI) applications elevated the levels of secondary metabolites such as carotenoids, total phenols, flavonoids, and anthocyanins as well as transcript levels of phenylalanine and chalcone synthase and thus help to improve the Cr tolerance.

16.3.3 Lead

Lead (Pb) is classified as harmful contaminant for soil, plant, and human health (Aslam et al. 2021). Balakhnina and Nadezhkina (2017) explained that alone Pb treatments intensified the rates of peroxidation. As compared to non-stress conditions, Se supply reduced the oxidative stress or degradation and enhanced the activities of APX, GR, and GPX enzymes in the leaves and roots of *Triticum aestivum* under Pb stress. Their findings confirmed the Se-mediated adaptive responses against Pb stress and indicated that soil Pb levels decide the plant adaptive potential. Under alone Pb-treated *Brassica napus* seedlings, SOD and GPX activities were depressed in leaves and roots. The addition of Pb-containing nutrient solution displayed positive effects on these activities (Wu et al. 2016). Mroczek-Zdyrska and Wójcik (2012) explained that alone applications of Pb decreased the guaiacol peroxidase (GPOX) and increased the glutathione peroxidase (GSH-Px), and no difference in the activity of CAT enzyme was noticed in the tissues of *Vicia faba*. The exogenous supplementation of Se in Pb-contaminated Hoagland solution reversed the trends of these enzyme activities, increased the synthesis of thiol compounds, and reduced the extra accumulation of lipid peroxidation and $O_2^{\bullet-}$. Their outcomes suggested that low concentrations of Se act as antioxidant enzyme against Pb-triggered peroxidation and oxidative stress. In *Zingiber officinale* plants, alone treatments of Pb in soil conditions drastically overproduced the oxidative stress and membrane damages by inducing the production of ROS and MDA along with drastic changes in the activities of antioxidant enzymes. Their findings confirmed that Se reduces the accumulation of ROS (H_2O_2 and $O_2^{\bullet-}$) and MDA while maintaining the ROS balance or antioxidant defense system by improving the activities of SOD, POD, and CAT enzymes and thus reduced the Pb-induced toxic effects in *Zingiber officinale* (Chen et al. 2019).

16.3.4 Mercury

Mercury (Hg) has great bioaccumulation for living organisms and persistently reside in environment mainly from anthropogenic activities (Chang et al. 2009). Overall, a few studies have been reported concerning the potential roles of inorganic Se forms in alleviating the Hg-induced toxic effects (via involvement of antioxidant enzymes) in different plant species. Li et al. (2018) investigated the molecular elucidation of antagonistic effects between Se and Hg in the tissues of 2-week-old rice seedlings. Their outcomes revealed that the addition of Se modulated the proteins interlinked with Hg stress responses, DNA replication, cell cycle, energy, and carbohydrate metabolisms, demonstrating that these proteins facilitated the antagonistic effects of Se in response to inorganic Hg toxicity. In the tissues of Hg-exposed rice seedlings, the applications of Se increased the activities of SOD, POD, CAT, APX, glyoxalase I, and glyoxalase II and levels of proline and soluble sugar and decreased MDA and $O_2^{\bullet-}$ contents. The key mechanism is that Se effectively improved the antioxidant capacity and osmotic adjustments by decreasing the oxidative stress, membrane damages, and stimulation in the activities of protective enzymes, thus minimizing the Hg-mediated toxic effects (Zhou et al. 2017). Besides the involvement of antioxidant enzyme activities, Tran et al. (2018) reported that Se IV and Se VI effectively inhibited the Hg toxicity by reducing the bioaccumulation and bioavailability or absorption of total Hg levels in the shoots and roots of pak choi (*Brassica chinensis*). In this way, both inorganic Se forms can be utilized as beneficial Se fertilizer to mitigate the toxic effects of Hg in *Brassica chinensis* or possibly other plants in dry lands.

16.3.5 Antimony

Antimony (Sb) is pervasive toxic metal ion. The cultivation of crops, especially in mining areas, contains excessive amounts of Sb in their edible parts, thus causing human health problems (Feng et al. 2013a, b). It has been suggested that Se alleviates Sb-mediated toxic effects in rice seedlings by reducing the extra accumulation of ROS (H_2O_2 and $O_2^{\bullet-}$) and lipid peroxidation as MDA in plant tissues. Relatively higher activities of CAT and APX enzymes suggested their involvement in Sb tolerance mechanisms. Overall, SOD and POD activities displayed decreasing trends in values, indicating their nonavailability in Sb resilience in rice plants (Feng et al. 2016). In addition to few studies about the participation of antioxidant enzyme activities, various other mechanisms have been reported in order to detoxify Sb toxicity by inorganic Se forms. Moreover, Se mediated increase in the synthesis of pectin, lignin, and hemicellulose and formation of iron plaque in roots (Liu et al. 2019) and decrease the subcellular distribution of Sb in cell wall and cytosol (Ding et al. 2015) to regulate or mitigate Sb toxic effects in rice seedlings. These observations suggested that certain concentrations of inorganic Se can effectively alleviate the Sb-induced phytotoxic effects via diverse mechanisms. The recent reports on the potential roles of selenium in enhancing the heavy metals tolerance via the involvement of antioxidant enzyme activities are documented (Table 16.1).

Table 16.1 The potential roles of selenium in improving the heavy metals tolerance by the involvement of antioxidant enzyme activities

Applied dose of Se species	Plant species	Heavy metals stress	Modulation in antioxidant enzyme activities	References
0,10, 20, and 40 mg/L Se (VI)	*Triticum aestivum*	Cd-contaminated soils	↑ SOD, POD, CAT, and APX ↓ MDA levels	Wu et al. (2020)
0.02 (w/v) Se (IV)	*Spinacia oleracea*	Cd-contaminated soils	↑ SOD, POD, and CAT ↓ MDA	Tang et al. (2020)
Soil (0.5–1 mg/kg), foliar (0.125–0.250 mM) Se (IV)	*Triticum aestivum*	Cd-contaminated soils	↑ SOD and POD ↓ MDA	Zhou et al. (2021)
0, 1, 5, and 10 uM Se (IV)	*Solanum lycopersicum*	0.50 mM Cd	↑ SOD, APX, CAT and GR ↓ H_2O_2, MDA	Alves et al. (2020)
2.5 μM Se (IV)	*Pfaffia glomerata*	50 μM Cd	↑ SOD, guaiacol peroxidase (GPOX) ↓lipid peroxidation	Pereira et al. (2018)
0, 5 and 10 μM Se (IV)	*Brassica napus*	100 μM Cr	↑ Enzymes of AsA-GSH cycle, carotenoids, proline, GSH and AsA ↓ ROS (H_2O_2 and $O_2^{\bullet -}$) and MDA	Ulhassan et al. (2019c)
0, 2, 4, and 6 μM Se (VI)	*Brassica juncea*	300 μM Cr	↑ lipid-water antioxidants, carotenoids, anthocyanins, free proline, glycine betaine, trehalose, non-protein thiols ↓ROS (H_2O_2 and $O_2^{\bullet -}$) and MDA	Handa et al. (2018)
0, 2, 4, and 6 μM kg^{-1} Se (VI)	*Brassica juncea*	300 μM kg^{-1} Cr	↑ SOD, POD, CAT, GR, GST, total phenols, flavonoids and anthocyanins ↓ROS (H_2O_2 and $O_2^{\bullet -}$) and MDA	Handa et al. (2019)
0.4 and 0.8 mg Se (VI)	*Triticum aestivum*	50 and 100 mg kg^{-1} Pb	↑ APX, GR and GPX ↓ Peroxidation rate as thiobarbituric acid reactive substances (TBARs)	Balakhnina and Nadezhkina (2017)
5, 10 and 15 mg kg^{-1} Se (IV)	*Brassica napus*	300 and 500 mg kg^{-1} Pb	↑ SOD and GPx ↓ ROS (H_2O_2 and $O_2^{\bullet -}$) and lipid peroxidation	Wu et al. (2016)

(continued)

Table 16.1 (continued)

Applied dose of Se species	Plant species	Heavy metals stress	Modulation in antioxidant enzyme activities	References
1.5 and 6 μM Se (IV)	*Vicia faba*	50 μM Pb	↑ Total sulfhydryl group, guaiacol peroxidase (GPOX) ↓ CAT activity, lipid peroxidation as MDA, $O_2^{\bullet-}$	Mroczek-Zdyrska and Wójcik (2012)
1 ml kg^{-1} Se (VI)	*Zingiber officinale*	500 mg kg^{-1} Pb	↑ SOD, POD and CAT activity ↓ ROS (H_2O_2, OH^- and $O_2^{\bullet-}$) and lipid peroxidation	Chen et al. (2019)
0, 1, 2, 3, and 4 μM/L	*Oryza sativa*	0.5 mg/L Hg	↑ SOD, POD, CAT, APX, Gly I and Gly II activity ↓ ROS (H_2O_2 and $O_2^{\bullet-}$) and lipid peroxidation	Zhou et al. (2017)
0.1, 1, and 5 mg/L Se (IV)	*Oryza sativa*	5 mg/L Sb	↑ Biomass accumulation, Sb-detoxification ↓ membrane lipid peroxidation as MDA	Feng et al. (2011)
0.1, 0.305, 0.8, and 1.295 mg/L Se (IV)	*Oryza sativa*	1, 2.171, 5, 7.829, and 9 mg/L Sb	↑ CAT and APX activity ↓ ROS (H_2O_2 and $O_2^{\bullet-}$) and cellular membrane lipids	Feng et al. (2016)

16.4 Conclusions and Future Prospects

A number of investigations have targeted the physiological importance of Se and its governed protective roles against abiotic stress mainly HMs. Inorganic Se forms (selenite and selenate) are considered essential micronutrients and beneficial elements mainly at low concentrations with optimal Se doses varied with target plant species. Recently, the roles of Se as antioxidant agent have gained worldwide interest. Se confers beneficial effects on plants under HMs stress and activates antioxidant defense machinery to restore the plants against oxidative stress or induced cellular damages. Still the antioxidant role of Se is unpredictable and furtive. Possibly, Se shuttles metabolic equivalents during HMs stress and triggers antioxidant defense machinery which helps to reduce the oxidative damages. A thorough research elucidating the molecular mechanisms of antioxidative defense system of both inorganic Se forms is perquisite, which can broaden our knowledge concerning the management of oxidative stress. The essentiality of Se as micronutrient is still controversial as it can be phytotoxic at particular higher doses. Therefore, selection of particular Se level is crucial, and risk-free supply of Se species should be targeted in future research. More rigorous research related to the participation and interaction of Se with plant metabolism can open new avenues regarding human nutritional status or quality.

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Antioxidant Defense System in Plants Against Biotic Stress

17

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Abstract

Globally, biotic stress has led to massive damage to the agricultural products and increases the risk of hunger in many regions. To counteract biotic stress, plants have evolved a defense mechanism via various pathways, which mainly includes pathogen-associated molecular patterns (PAMPs) and plant resistance proteins. The defense machinery in plants is influenced by both abiotic and biotic factors and draws impact on crop yield and quality. Like abiotic stress, biotic factors of plant stress impart a crucial role in enhancing the generation of ROS in plant system. ROS as a consequence of stress metabolism causes damage because of oxidative stress. However, the significance of ROS as signaling molecule in controlling various biological activities associated with growth and development and other pathways that have a role in adaptation to various biotic stresses has also been studied. To limit invasion of pathogens, a balance of ROS-scavenging and ROS-producing pathways is necessary for proper defense mechanism. Additionally, plants have evolved nonenzymatic and enzymatic components for survival against stress. Both these antioxidant systems work in coordination, and the proteins generated are overexpressed during attack by pathogens to ensure defense response in tolerant plant species.

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Antioxidants · Biotic stress · Reactive oxygen species · Defense · Pathogen

17.1 Introduction

Plants show certain physiological and molecular responses to overcome the stress conditions, under biotic stress such as attack by phytophagous insects or pathogen infections (virus, fungi, and bacteria) that could have damaging effect on agriculture as it restricts the growth of plants and reduces the yield. Therefore, it is important to understand the impact of biotic stress in plants and defense strategies that plants utilize to counter attack these unsuitable conditions. The plants recognize pathogens by pattern recognition receptor present on plant cells that detects pathogen-associated molecular patterns (PAMPs) associated with pathogens. This type of immunity is termed as PAMP-triggered immunity (PTI) (Monaghan and Zipfel 2012). The second type of immunity is termed as effector-triggered immunity (ETI), where plant resistance proteins detect pathogen-specific effectors (Avr proteins) of pests or pathogens. ETI also activates hypersensitive response (HR) which causes apoptosis of cells at infection site and cells of its surrounding areas. Both ETI and PTI induce release of phytohormones like salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). SA hormone activates systemic acquired resistance (SAR), while JA and ET activate induced systemic response (ISR) (Grant and Lamb 2006; Spoel and Dong 2012; De Vleesschauwer et al. 2014).

Cells maintain redox homeostasis for normal functioning, as exaggerated reduction and oxidation are detrimental for cellular components (Foyer and Shigeoka 2011). Plants have evolved antioxidant defense system inside them as an adaptation to counteract generation of reactive species. Protection from reactive species varies in plants from species to species (Díaz-Vivancos et al. 2010). Antioxidants are those molecules that have an ability of quenching or inhibiting reactive radical species and therefore preventing the damage to cellular components (Dumont and Rivoal 2019). The prominent antioxidants are glutathione (GSH), tocotrienols, tocopherols, carotenoids, ascorbate (Asc), and phenols and are among the low molecular mass-based antioxidants. These molecules self-react with free radicals and aid in the removal of reactive species, but those reactions catalyzed by enzymes like superoxide dismutase (SOD), ascorbate oxidase, ascorbate peroxidase [APX (EC 1.11.1.11)], catalase (CAT), and glutathione peroxidase (GPX) are more efficiently removed than those without enzyme involvement (Noctor et al. 2018). Antioxidants having low molecular mass remove the reactive species via direct and indirect mechanisms. Indirect method involves transition metal chelation, which inhibits the involvement within Fenton or Haber-Weiss reaction (Oztetik 2011), while direct method involves receiving and donating of electrons, free radical scavenging, and thus preventing their interaction with biomolecules.

ROS are by-products of metabolic pathways like photorespiration, photosynthesis, and respiration. Reduction of molecular oxygen (O_2) to water (H_2O) promotes the production of ROS. The ROS that induces oxidative damage includes $^1O_{2,}$ O_2^{-}, $H_2O_{2,}$ $\dot{O}H$, $RO^{\cdot}$, $ROO^{\cdot}$, and ROOH (Bhattacharjee 2005). ROS production in response to elicitors and pathogens has their role in various defensive processes which include lignifications, formation of papillae, and cross-linking of hydroxyproline glycoproteins (HPGP) (Olson and Varner 1993). Following generation of ROS in plants, various metabolites and enzymes are released to quench ROS to protect the cell from damage. In this chapter, we have highlighted the role of antioxidants and ROS in the defense against biotic stress as well as interplay between ROS-generating and ROS-scavenging pathways.

17.2 Plant Defense System Against Biotic Stress

Biotic stress is a term that describes the damage that is caused by live organisms such as bacteria, viruses, fungi, insects, nematodes, and weeds. The severity of the stress depends on environmental conditions, casual organisms, and the level of crops (Walters 2010). To cope up with the biotic stress, plants have physical barriers in the form of waxes, trichomes, and cuticles to stop pathogenic invasion. Besides that, plants produce chemicals to shield themselves against pathogens and herbivorous. Furthermore, plants recognize pathogens through two pathways that stimulate immune responses. The first is receptor recognition of pathogens known as pathogen-associated molecular patterns (PAMPs), which includes lipopolysaccharides (LPS), flagellin, peptidoglycan, and fungal chitin. This type of defense system is called as PAMP-triggered immunity (PTI) (Monaghan and Zipfel 2012). The second mechanism is effective triggered immunity (ETI), where there occur plant resistance proteins that identify pathogen-specific effectors known as Avr proteins. This pathway stimulates hypersensitive response (HR) which causes apoptosis of localized and its surrounding cells (Spoel and Dong 2012).

Signal transduction, whether ETI or PTI induced, activates many phytohormones which include salicylic acid (SA), ethylene (ET), and jasmonic acid (JA). Salicylic acid pathway stimulates the defense against biotrophic and hemi-biotrophic pathogens, while jasmonic acid and ethylene exhibit defense against necrotrophic pathogens and also against chewing insects in *Arabidopsis* (De Vleesschauwer et al. 2014).

For plant pathogens, there are two effective defense mechanisms: One is systemic acquired resistance (SAR), and the other is induced systemic resistance (ISR). In SAR, there occur increased levels of salicylic acid, when a necrotizing pathogen infects plants (Grant and Lamb 2006). In ISR, activation of ET and JA signaling takes place, due to specific strains of root-colonizing bacteria (nonpathogenic) (Van Loon et al. 1998). Additionally, plants recognize herbivore-associated elicitors (HAE) released by phytophagous insects that cause them to exhale certain volatiles that tend to attract their enemies and also warn the neighboring plants to the approaching threats (Santamaria et al. 2013).

17.3 ROS Signal Transduction Under Biotic Stress

ROS are by-products of metabolic pathways like photorespiration, photosynthesis, and respiration. Stepwise reduction of oxygen molecule (O_2) because of leakage of electrons from mitochondria, chloroplast, and plasma membrane electron transport results in the production of toxic ROS (Alscher et al. 1997; Bhattacharjee 2005) in non-stressed cells of plants, which gets intensified under abiotic and biotic stimuli. The ROS that induces oxidative damage includes $^1O_2$, O_2^-, H_2O_2, $O\dot{H}$, $RO^\cdot$, $ROO^\cdot$, and ROOH. Reduction of molecular oxygen (O_2) to water (H_2O) promotes the production of ROS (Bhattacharjee 2005). Consequent to ROS production, plants utilize several metabolites and enzymes to quench ROS to protect the cellular compartments of cell. The plants have evolved multiple defense response pathways against biotic stress by expressing diverse types of genes, which are modulated by surrounding microclimate, humidity, and temperature. Among various types of pathways that are linked to the regeneration of ROS, NADPH oxidase is regarded as a vital enzyme in controlling the pathway. NADPH oxidase is stimulated by Ca^{2+} and provokes synthesis of O_2^- and is transformed into H_2O_2 in cell membrane by abiotic factors (Fraire-Velázquez et al. 2011). Studies revealed that the concentration of Ca^{2+} ion and ROS component within the cell is seen to be higher and is known to cause activation of signaling pathways under biotic and abiotic stimuli especially by phytophagous animals (biotic). Thus the influx of Ca^{2+} ion is raised high to a level where it is involved in signaling through other parallel molecular events by associating with Ca^{2+} sensor proteins especially calcineurin B-like proteins (CBLs) and calcium-dependent protein kinases (Kim et al. 2009).

Attack of pathogens on plants activates the first level of defense response, that is, hypersensitive response (HR). In this response system, H^+ and Ca^{2+} influx is equilibrated at higher rate with the efflux of Cl^- and K^+ ion due to which reactive nitrogen species (RNS) and reactive oxygen species (ROS) are generated. The ROS so generated is involved in diverse functions as a part of HR such as microcidal activities and cell wall lignification process. NO and H_2O_2 produced by RNS and ROS, respectively, activate the gene expression of some viral defense responsive genes such as glutathione S transferase (GST), phenylalanine lyase (PAL), and other pathogenesis related genes (PR genes), when both these species are available simultaneously in proper balance (Desikan et al. 1998; Delledonne et al. 1998; Hancock et al. 2002).

During oxidative stress, ROS consumes electrons from target molecules which cause delirious impact on macromolecules in cells. ROS has negative impact on proteins, unsaturated lipids, and DNA and also modulates their structure and function. This negative impact leads to dysfunction of cell and then death (Taylor et al. 2002). Besides that, ROS also acts as a secondary messenger in signaling pathways. This depends on the interaction with cell sensors that controls various biological processes like transcription and translation. This sensor system affects not only growth and development but also apoptotic signals in response to abiotic and biotic stimuli (Kovtun et al. 2000).

17.4 Role of ROS in Plant Pathogenesis

The consumption of O_2 to generate ROS is known as oxidative burst, which is known to be the primary cellular response during recognition of pathogens and activation of defense machinery of plant. Avirulent pathogens cause induction of biphasic ROS production with short transient first phase succeeded by continuous phase of higher magnitude that is correlated with resistance to disease. In some fungal studies tri-phasic ROS production has also been observed. These variations can be associated with the complicated development of pathogens and effect of host genotype. Pathogens that are virulent. Virulent pathogens suppress or avoid host recognition, activates only first phase of ROS production. Elicitors of defense responses that are termed as PAMPs also cause oxidative burst (Shetty et al. 2003; Hückelhoven and Kogel 2003; Chisholm et al. 2006). Production of ROS by peroxidases or NADPH oxidases after pathogen infection is a well-recognized process. NADPH oxidase, a plasma membrane-bound enzyme, causes generation of superoxide anions by electron transfer process from NADPH present in the cytoplasm to the O_2 available in apoplastic site which then dismutate to O_2^- and H_2O_2 (Bolwell et al. 2002). Peroxidases that are present in cell wall have ROS-scavenging activity; in addition to that, they act as NADPH oxidases and cause formation of H_2O_2 and O_2^- (Baker and Orlandi 1995). Production of ROS in response to elicitors and pathogens has been implicated in many defensive processes related to interaction of pathogen to their host plant. ROS has been demonstrated to have antimicrobial effects during defense responses, important being H_2O_2 (Walters 2003; Custers et al. 2004). However in plant-pathogen interaction, ROS toxicity depends on pathogen sensitivity to the concentration of ROS present (Levine et al. 1994). The levels of H_2O_2 generated rely on many factors which include age, type of plant species, and nature of elicitor (Legendre et al. 1993; Nürnberger et al. 1994; Shetty et al. 2007).

ROS generation during initial phases of oxidative burst is involved in the strengthening of cell wall. Process of cell wall strengthening is described by papilla formation, lignifications, and cross-linking of (hydroxyproline-rich glycoprotein HPRG) monomers (Olson and Varner 1993). Papilla serves as a barrier to penetration of pathogens. Studies indicated that protein cross-linking in cell wall is induced by elevated production of ROS during pathogen attack (Kumudini and ShettyA 2002). Cross-linking network of HRGP monomers provides anchorage for lignifications that might block formation of haustoria and lead to nutrient shortage which may be disadvantageous for biotrophic pathogens (Shailasree et al. 2004). Besides that, cell wall strengthening checks the movement of pathogen toxin inside the cells of plants and blocks the availability of nutrients and also limits the spread of infection.

Hypersensitive response (HR) is characterized by sudden cell death of the host cell at the site of infection (Lam et al. 2001). HR is achieved by activation of resistance genes, observed in plant species against microbial pathogenic attack (van't Slot and Knogge 2002; Hammond-Kosack and Parker 2003). HR has proven to be productive against biotrophic pathogens, and because of nutrient shortage, death of host cell occurs. It is reported that HR is not effective against fungal necrotrophic pathogens as the dead cell facilitates pathogenesis that results in increased colonization of necrotrophs (Greenberg and Yao 2004). HR is characterized by oxidative burst followed by cellular lesions. The mechanism of HR involves various steps including condensation of chromatin, membrane blebbing, DNA cleavage, disruption of cell membrane, and finally release of cellular contents. Various studies have proposed the correlation between HR and ROS, but the exact mechanism is unclear. It is postulated that there occurs a cooperation between ROS and phytohormones such as ET, JA, SA, and ABA which influences the induction of HR (Shetty et al. 2003).

ROS particularly H_2O_2 in coordination with salicylic acid was reported to enable the initiation of SAR (Durrant and Dong 2004). The SA and SAR are linked with the expression of defense-related genes which include PR proteins. It is believed that ROS mediates SAR by associating with non-expressor of PR-1(NPR1) and controls the systemic responses by regulating NPR1 redox state. SAR furnishes persistent, broad spectrum resistance caused by broad range of elicitors or pathogens (Ryals et al. 1996; Mou et al. 2003; Kanzaki et al. 2003). Activation of signaling occurs at the primary site of infection, which then translocate to other uninfected areas. The involvement of SA in generation of H_2O_2 has been established by various studies showing the negative impact of SA on catalase (CAT) activity (Chen et al. 1993). In SAR, the activity of SA is mediated likely by heightened levels of H_2O_2. On the other side, activation of SA synthesis by H_2O_2 was also demonstrated by various studies, indicating that H_2O_2 may act upstream of SA (Leon et al. 1995).

17.5 Interplay Between ROS-Producing and ROS-Scavenging Pathways

Rapid ROS production has an important part in disease resistance responses. Excess ROS can lead to cellular injury or death. To mitigate the harmful effect of this "double-edged sword," nature has maintained their homeostasis by an equal scavenging process known as antioxidant defense system which basically is enzyme mediated consisting of SOD, CAT, APX, and GPX and is needed to maintain steady-state concentration of ROS (Apel and Hirt 2004). APX-1 enzyme in *Arabidopsis* mutant was confirmed to be an important element of ROS-scavenging gene products leading to the raised levels of H_2O_2, oxidation of proteins, and stunted growth of plant (Caverzan et al. 2016). This antioxygen defense machinery comprises of two branches, enzymatic antioxidants and nonenzymatic antioxidants (Das and Roychoudhury 2014) (Fig. 17.1).

17.5.1 Different Antioxidants Produced in Plants

The enzymatic defense systems include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase

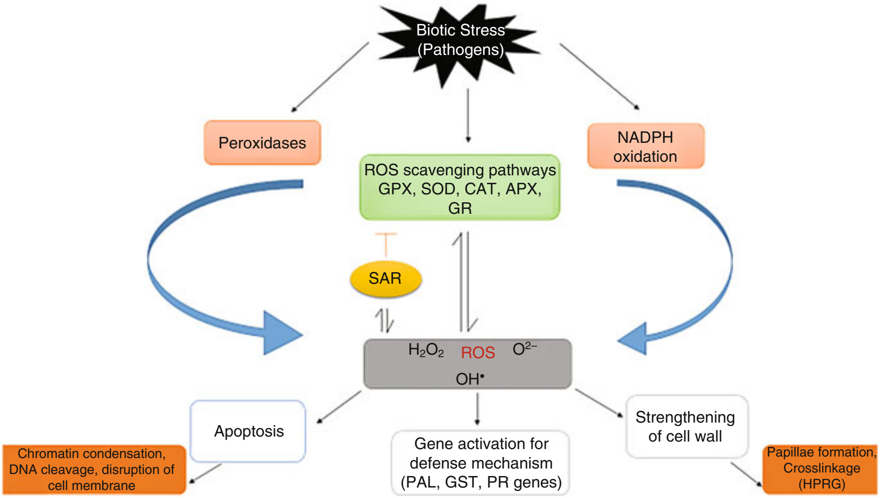

Fig. 17.1 Interplay between ROS-producing and ROS-scavenging pathways [Abbreviations *SAR* systemic acquired resistance, *GPX* glutathione peroxidase, *SOD* superoxide dismutase, *CAT* catalase, *APX* ascorbate peroxidase, *GR* glutathione reductase, *ROS* reactive oxygen species, *PAL* phenylalanine lyase, *GST* glutathione S transferase, *HPRG* hydroxyproline-rich glycoprotein]

(GR), glutathione peroxidase (GPX), superoxide, and nonenzymatic metabolites, namely, ascorbate (ASC), coordination in giving protection against the toxic effect of ROS (Tarafdar et al. 2018; Mithöfer et al. 2004; Navrot et al. 2006). The role of these enzymatic and nonenzymatic defense machinery is to convert these free radicals into reduced forms like water (H_2O) which no longer have a potential to cause cellular damage (Hasanuzzaman et al. 2020). This operating machinery are basically the subprocesses of some cellular pathways like Asada–Halli well cycle or AsA-GSH cycle, peroxidase cycle or water-water cycle, photorespiration, and the processes involving β-oxidation of fatty acids (Biswas et al. 2020). The information about them is briefed as under:

Enzymatic Antioxidant Machinery

1. **Superoxide dismutase (SOD):** It is the first line of defense operating during ROS damage and functions in dismutation of O_2^- to H_2O_2 through cyclic oxidation and reduction of metal ions such as zinc, copper, and manganese (McCord and Fridovich 1969). This enzyme is found in all aerobic cells and usually exists in three metallic forms:
    - Cu/ZnSOD: found in stroma, chloroplast, cytosol, peroxisomes, and apoplast
    - Mn-SOD: found in peroxisomes, mitochondria, and also in apoplast and cell wall
    - Fe- SOD: found to some extent in chloroplast and stroma

These SODs convert superoxide radical O_2- into H_2O_2 which is further converted into H_2O via CAT, APX, and GPX in AsA–GSH cycle (Biczak et al. 2016; Luis et al. 2018).

$$O_2^{\bullet -} \underset{\rightarrow}{\mathrm{SOD}} H_2O_2$$

$$H_2O_2 \underset{\rightarrow}{\mathrm{CAT, APX, GPX}} H_2O$$

2. **Catalases (CATs):** It is generally found in peroxisomes and mitochondria. It was considered that this enzyme is not found in chloroplast, but recent studies have also found this enzyme to be present in chloroplast though in minute quantities. CAT also eliminates H_2O_2, by its conversion into H_2O and $O_{2.}$ (Frugoli et al. 1996). The cellular pathways in which ROS scavenging is predominant are photorespiration and β-oxidation of fatty acids. They contain four heme subunits Fe^{2+}reducing H_2O_2 into H_2O and O_2. These are the only antioxidant enzymes which do not have any reducing equivalent in ROS-scavenging process making them unique in this activity (Arora et al. 2002). It has been seen that CATs have low affinity toward organic peroxides compared to enzyme peroxidase, which can remove H_2O_2 even if present in minute concentrations. The processes again involve the coordination of nonenzymatic defense machinery like GSH and Asc which act as electron donors removing one H_2O_2 molecule by two molecules of GSH (Kohen and Nyska 2002).
3. **Ascorbate peroxidase (APX):** Its role can be summarized in AsA–GSH cycle in which APX acts as a key enzyme in reducing H_2O_2 to water (Asada 1992). What catalase does in peroxisomes APX do it in chloroplast due to their lower ability in the later. Thus, it is the only enzyme responsible for scavenging H_2O_2 into H_2O in chloroplast using ascorbic acid as the reducing agent a nonenzymatic antioxidant. Their importance within the plants can be seen by the presence of their family consisting of five classes of these peroxidases. These isoforms are distributed throughout the plant organs in different compartments, namely: stroma (sAPX) and thylakoids (tAPX) in chloroplasts, microbodies (including glyoxysomes and peroxisomes; mAPX), cytosol (cAPX), and mitochondria (mitAPX, as a membrane-bound form, which respond in different manner to different physiological and environmental stresses (Gangwar et al. 2014).

Nonenzymatic Defense Machinery

1. **Ascorbic acid:** Among nonenzymatic defense system, it plays the predominant role than others due to its diverse electron donating capacity (Barnes et al. 2002). The AA in plants is present in mitochondria, cytosol, and apoplast, but they are not present in vacuoles. It is usually synthesized via basic pathway called Smirnoff-Wheeler pathway catalyzed by L-galactano-γ-lactone dehydrogenase and D-galacturonic acid (Das et al. 2014). It can donate two electrons: One leads

to the formation of ascorbate, and other leads to the formation of DHA. AA has the affinity for H_2O_2, OH•, $O^{-}{}_{2}O\bullet_{-\ 2}$ there by reacting with these free radicals it can generate α-tocopherol eventually protecting the membranes from oxidative damage (Shao et al. 2008).

2. **Reduced glutathione (GSH):** It is almost found in all cellular compartments like cytosol, mitochondria, ER, vacuoles, chloroplasts, peroxisomes, and in the apoplast. The reducing power of GSH is attributed to its central cysteine residue. The GSH prevents the cell in oxidative damage by two ways, through metal ion chelation which is another source of ROS in plants and by acting as an excellent scavenger of the free radicals like $H_2O_{2,}$OH•, and $O\bullet^{-}$ (Zlobin et al. 2017; Krasnovsky Jr 1998). In order to prevent the plant cell due to harmful effects of ROS, the balance between GSH and GSSG (glutathione disulfide) is an utmost precondition. This is done by regenerating AA (ascorbic acid) and yielding GSSG which is further converted into GSH by GR (glutathione reductase). Thus, it has been seen that GSH not only acts as a good free radical scavenger but also helps in the generation of AA another key player of nonenzymatic defense machinery. Furthermore, it is one of the major antioxidant molecules in antioxidant defense system through AsA–GSH cycle. Apart from preventing cell in oxidative damage, it also helps in general growth and development of plant cells (Mullineaux and Rausch 2005).
3. **Phenolic compounds:** Phenols are diverse chemical compounds, which have versatile affinity free radicals thanks to their unstable benzene rings with the power of donating an electron. This group consists of secondary metabolites like esters, lignin, tannins, and flavonoids. They can form complexes with metal ions restricting the formation of ROS and thus hampering Haber-Weiss reaction (Fenton 1894). Phenolic compounds have been seen to hinder the processes like lipid peroxidation, H_2O_2 capture cascade and Asc, phenol synergestic generation pathways involved in free radical capture thus preventing the cell from oxidative damage (Takahama and Oniki 1992; Sakihama et al. 2000; Sharma et al. 2012).

17.6 Conclusion

This chapter gives a description related to how plant's defense machinery responses to biotic stress. A number of experimental evidences revealed that a diverse variety of enzymes (regulatory and antioxidants) and functional proteins works in a coordinated manner to respond to biotic stress via ROS and other signaling pathways of hormone involvement. The combined effect of antioxidants and other metabolites of non-antioxidant nature leads to diminished cellular damages caused by ROS molecules generated because of pathogenic attack as well as by other biotic stimuli. Additionally, damaging impact of ROS triggers hypersensitive response (HR) as first level of defense response. ROS also acts as a second messenger in the establishment of communication between pathogen avirulent genes (avr) and disease resistance (R) genes enhancing disease response. Notwithstanding the intercommunication between ROS-scavenging and ROS-producing network systems, the interconnection of ROS with other regulatory network is also well elucidated and needs to be discovered more to understand the key secrets of their equilibrium at molecular level.

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Revisiting the Crucial Role of Reactive Oxygen Species and Antioxidant Defense in Plant Under Abiotic Stress

18

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Abstract

Global climate change and abiotic stresses, like waterlogging, salinity, heavy metals, high temperature, etc., greatly affect plant growth, development, and ultimately crop yield. Oxygen radicals and their derivatives produced by plant cells, known as ROS, result in abiotic stress. Plants contain complicated antioxidative defense mechanism, consisting of nonenzymatic and enzymatic components, which check ROS accumulation and induce plant defense. This chapter focuses on deleterious effects of ROS and antioxidant defense mechanism under various abiotic stresses responsible for ROS detoxification and transcription factors associated with ROS and micro-RNA production under abiotic stress. In addition, it also focuses on crop engineering for abiotic stress resistance in relation to antioxidant machinery and reactive species.

Keywords

ROS · Antioxidant systems · Cross tolerance · Stress signaling · Abiotic stress

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18.1 Introduction

Abiotic stresses like drought, salinity, metals toxicity, waterlogging, high temperature, etc. are worldwide problems which arise due to harsh climatic conditions (Raza et al. 2019). The sustainable crop production may have serious threats due to environmental stresses. Excessive ROS accumulation, including free radicals (superoxide anion, O_2, H_2O_2, hydroperoxyl radicals), adversely affects the plants and leads to oxidative stress like OH, alkoxy radical, and non-radical molecules (Hasanuzzaman et al. 2019). ROS production occurs mainly in chloroplasts, peroxisomes, apoplast, mitochondria, and plasma membranes (Singh et al. 2019). Though ROS are produced inside plants as part of normal cellular metabolism, its reactive nature and excessive accumulation lead to cellular component breakdown like carbohydrates, DNA, proteins, and lipids (Raja et al. 2017). It triggers nitrogen-activated protein kinase pathway and transmits signals through redox reactions in nucleus to develop various abiotic stress resistance, and ROS help in plants' acclimation process under environmental stimuli (Singh et al. 2019).

Both antioxidant methods (nonenzymatic and enzymatic) work in elimination or detoxification of the excess ROS. Antioxidant enzymes are catalase (CAT), superoxide dismutase (SOD), enzymes of AsA-GSH (ascorbate-glutathione), guaiacol peroxidase (GPX), tocopherols, glutathione (GSH), ascorbate peroxidase (APX), and ascorbate (AsA), while carotenoids and phenolic compounds are powerful cellular nonenzymatic antioxidants. High antioxidant capacity maintenance eliminates lethal ROS that is related to plant resistance against environmental stresses (Jaspers and Kangasjärvi 2010a, b). The various levels of ROS cause oxidative stress, which destroys lipids, proteins, and DNA. Conversely, it has been observed in recent two decades that physiological and biological processes are also regulated by ROS signaling molecules. It has been found that, in early stages of evolution, ROS act as a signaling mechanism to adapt under changed environmental conditions. Prokaryotes possess a well-developed mechanism through which ROS directly activate transcription factors to adapt under stress condition (Liu et al. 2007; Jaspers and Kangasjärvi 2010a, b).

Transcription factors (TFs) regulate stress-induced gene expression involved in domestication and stress resistance through interchange of gene cis-elements. Microarray studies revealed that TFs are induced by 32 different kinds of oxidative stress treatments that were fivefold upregulated. From these, seven belongs to NAM/NAC, six to WRKY family, and eight to AP2/ERF (Gadjev et al. 2006; Liu et al. 2007). In addition, some TFs are found to modulate abiotic stress response (Jaspers and Kangasjärvi 2010a, b). This chapter summarizes harmful effects of ROS and focuses on molecular methods that enhance ROS-mediated antioxidant defense and survival mechanism against various environmental stress.

18.2 Reactive Oxygen Species (ROS) Signature Molecules for Plant Acclimation to Stress

ROS is also directly involved in regulating the redox state of the cell by modifying its proteins and transcription and translation resulting in the instigation of an acclimation response that would alleviate the effects of stress on metabolism and reduce the level of metabolically produced ROS (Choudhury et al. 2017). The two main cradles of ROS during stress, metabolic and signaling ROS, are shown to interact and form a ROS signature that controls plant acclimation to stress through redox reactions that regulate transcription and translation of stress acclimation proteins and enzymes (Fig. 18.1). Some ROS are produced as a result of destructions in the metabolic activity (metabolic ROS) and consequent signaling as an integral process to respond against abiotic stress (signaling ROS). Metabolic ROS could unswervingly modify the redox active status of some rate-limiting enzymes and control metabolic fluxes in the cell (flux control), thus altering various metabolic reactions in order to hostage the effect(s) of stress. Moreover, it also activates transcription and/or translation process by transforming the function of key regulatory proteins (Foyer and Noctor 2016). On the other hand, signaling ROS is accumulated as a reaction to stress perception by stress sensor molecules (e.g., cyclic nucleotide-gated channels activated by heat stress (Mittler et al. 2012) and is mediated by calcium- and/or phosphorylation-derived activation of NADPH oxidases (RBOH) at the plasma membrane (PM) (Gilroy et al. 2014).

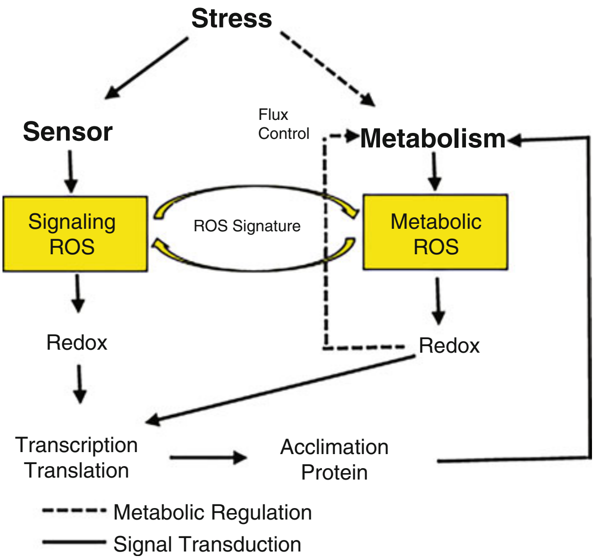

Fig. 18.1 The mechanism of reactive oxygen species (ROS) in acclimation against abiotic stress

18.3 Subcellular Localization of ROS

Metabolic and signaling ROS could be generated in many subcellular compartments (e.g., metabolic ROS mainly in the chloroplast and signaling ROS at the apoplastic region). Nonetheless, they can have an impact on each other and even move between compartments (e.g., H_2O_2 that can move across membranes in a regulated process via aquaporins (Tian et al. 2016). ROS are produced enzymatically and nonenzymatically in plants. Plasma membrane is equipped with essential players like NADPH oxidases (NOXs), also recognized as respiratory burst oxidase homologues (RBOHs), against ROS. RBOHs quench electron from cytoplasmic NADPH and transfer to O_2 to generate anionic form of oxygen ($O_2^{\bullet -}$), which upon activation of superoxide dismutase (SOD) in the apoplastic region rapidly converts to H_2O_2 (Suzuki et al. 2011; Chen and Yang 2020). Recent discoveries reveal that under stress, chloroplast, peroxisomes, and mitochondria can outspread membrane structures that will commence the nuclear envelope and could directly modify the ROS status of the nuclei (Foyer and Noctor 2016). However, in the presence of labile cytosolic Fe^{2+}, ROS (H_2O_2) can interact with it to convert into highly toxic hydroxyl radical, resulting in oxidative damage at cellular level. Despite the fact that vacuole occupies a comparatively large volume of the plant cell and could have a significant buffering capacity of ROS, the role of the vacuole in ROS signaling and metabolism is currently unknown (Fig. 18.2).

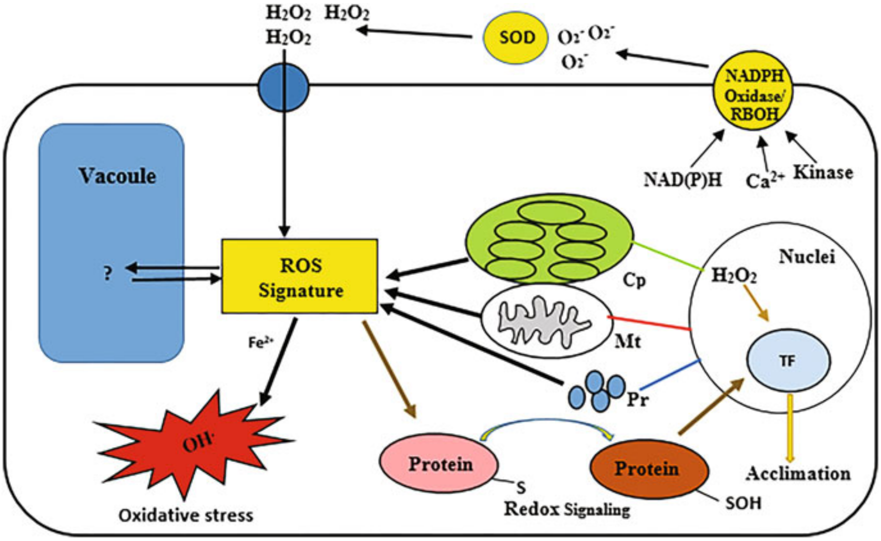

Fig. 18.2 The interaction of ROS generated in different compartments during abiotic stress. Abbreviations: *Cp* chloroplast, *Mt* mitochondria, *Pr* peroxisome, *RBOH* respiratory burst oxidase homolog, *TF* transcription factor, *SOD* superoxide dismutase

18.4 Oxidative Stress Under Abiotic Stress

A number of abiotic stresses like heat stress induce oxidative stress through excessive ROS production. Chloroplasts, peroxisomes, mitochondria, plasma membranes, and apoplast are cellular sites for ROS formation, but main production sites of ROS are chloroplasts (Singh et al. 2019). Mostly abiotic stresses lower CO_2 availability, restrain carbon fixation, and resulted in molecular oxygen reduction. This ultimately leads to ROS synthesis, which affects chloroplast performance and disturbs photosynthetic processes (Gill and Tuteja 2010). Furthermore, ROS production varies within genotypes, plant species, stress duration, and stress tolerance level.

18.4.1 Salinity Induced Oxidative Stress

Salinization influences plant growth by disturbing ion toxicity, nutrient deficiency, osmotic stress, and genetic toxicity, which lead to excessive ROS production and cause stress. For example, Rehman et al. (2019) observed an increase in H_2O_2 production by 2.5 and 3 times at concentrations of thiobarbituric acid reagents (TBARS) below 100 mM and two to three times at a sodium chloride concentration of 200 mM. However, under salt stress, oxidative stress varies within plant tissues, for example, root tissue is more affected by salt oxidative stress. Cheng et al. (2020) reported that under salt stress in rice root tissues, lipid peroxidation, total ROS, and electrolyte leakage (EL) increased two times as compared to control condition. In another study, Ahanger et al. (2020) reported the accumulation of $O_2^{\bullet -}$ and H_2O_2 in the different parts of the plants which caused to increase the contents of malondialdehyde and EL. This confirmed that salinity (100 mM NaCl) causes oxidative stress in tomatoes. Likewise, in sweet pepper, under salt stress (0.4%), MDA and EL increased by two times (Abdelaal et al. 2020), while MDA, EL, and H_2O_2 increased by twofold and in mung bean $O_2^{\bullet -}$ contents which were found under salinity stress (Ahmad et al. 2019). Moreover, in maize, MDA increased by 25% and H_2O_2 by 50% contents under 120 mM NaCl salt stress as compared to control condition. Though oxidative stress varies by genotypes within a species, Lalarukh and Shahbaz (2020) found that under 120 mM NaCl salt stress, H_2O_2 concentration increases (by 78%) in genotype FH-572 and decreased in genotype in FH-621 (by 20%). Tariq and Shahbaz (2020) analyzed two *Sesamum indicum* genotypes (TS-5 and TH-6) resistant to salinity with 70 mM NaCl and observed that the “TS-5” genotype was salt tolerant as compared to control (TS-6). This clearly revealed that different plant species respond differently under salt-induced oxidative stress conditions.

18.4.2 Chilling Induced Oxidative Stress

In plants, overproduction of ROS caused by chilling/low temperature (LT) leads to EL and lipid peroxidation and resulted in retardation of photosynthetic apparatus activity, degradation of membrane fluidity, and imbalanced ROS scavenging (Repetto et al. 2012). Han et al. (2017) showed an increase in the content of EL (by 49%) and MDA (by 180%) in 14-day-old rice plantlets exposed to cold stress (12 °C, 6 days). Similarly, significantly high concentrations of MDA and H_2O_2 were found in the cold-sensitive genotype *Solanum lycopersicum* (Jinpeng No. 1) under LT stress (15 °C/8 °C day/night; 24 and 48 h), as controls (Liu et al. 2020). Wild type (WT) of *Ammopiptanthus mongolicus* when exposed under the influence of LT stress in a controlled system showed H_2O_2 accumulation compared to with transgenic plants (G-1 and G-2) (Xue et al. 2019).

18.4.3 Heat Induced Oxidative Stress

High temperature (HT) decreases cellular metabolism, inhibits oxygen-evolving complex (OEC), damages cellular membranes through rapid lipid peroxidation and DNA damage, and ultimately causes the death of the cell (Hasanuzzaman et al. 2013). Moreover, it retarded PSII and electron flux, decreased quantum efficiency (Fv/Fm), and reduced PSII photochemistry in plants under high temperature fractions (35 °C/32 °C day/night) (Fahad et al. 2016). Overproduction of $O_2^{\bullet -}$ radical by 79.9% and 50% drives to heat-induced oxidative damages in tobacco and cucumber seedling under heat temperature (i.e., 35 °C and50°C), respectively (Ding et al. 2016). Similarly, in sorghum, heat stress also enhanced O_2 level by 2.3- and 3.5-fold in pistils and pollen (Djanaguiraman et al. 2018). In contrast, rice grain did not exhibit any remarkable changes in O_2 content and MDA content under high temperature stress, but H_2O_2 content increased under HT stress as compared to control (Rasheed et al. 2018).

18.4.4 Waterlogging/Waterflooding Induced Oxidative Stress

Flooding or waterlogging (WL) induces anoxic or hypoxic conditions which are consequences of the accumulation of toxic compounds in the different parts of the plant which impose the influence on plant metabolism and overproduction of ROS, thus resulting in oxidative stress (Loreti et al. 2016). In sorghum bicolor WL-sensitive genotypes (JN31), MDA content accumulation increased compared with WL-tolerant genotypes (JN01) under various conditions of waterlogging at intervals of 6, 9, and 12 days (Zhang et al. 2019). Likewise, Anee et al. (2019) also observed remarkable increase of MDA and H_2O_2 contents in *Sesamum indicum* L. cv. BARI Til-4 (WL-sensitive) under WL stress of different durations (i.e., 2, 4, 6, and 8 days). However, waterlogging was also associated with higher production oxidative stress, as reported in *lycopersicum* and an Antarctic plant called *Deschampsia Antarctica* (Park and Lee 2019).

18.5 Antioxidant Pool in Plants

A complex defense antioxidant system is observed in plants that contain enzymatic and nonenzymatic constituents that help to eliminate ROS. ROS production occurs in various plant cell organelles, like peroxisomes, chloroplasts, and mitochondria, and various cellular compartments (Pang and Wang 2008). The synthesis of various toxic oxygen metabolites at low levels and ROS production and quenching balance exist under normal conditions. This production of ROS and quenching balance get disturbed by number of environmental factors that increase intracellular ROS levels and resulted in oxidative destruction to various biochemical molecules (Sharma et al. 2010).

The ascorbate (AsA), cellular redox buffer glutathione (GSH) and carotenoids, tocopherol, and phenolic compounds are the various components of nonenzymic antioxidative defense system. These components are associated with various cellular components and directly associated with plant development and growth by regulating processes of elongation, mitosis, cell death, and aging (De Pinto and De Gara 2004). Mutations with reduced content of nonenzymatic antioxidant revealed by stress hypersensitivity (Semchuk et al. 2009).

18.5.1 Ascorbate and Glutathione Cycle

The antioxidant ascorbate (AsA) performs a very important role to cope the oxidative stress caused by elevated level of ROS and protects macromolecules from oxidative damage. It is a dominant antioxidant and directly related to boost up antioxidant reactions (enzymatic and nonenzymatic) by donating electrons. AsA also influences the physiological processes of plant such as in metabolism, growth, and development. The Smirnoff-Wheeler pathway introduced AsA in plants which flows through GDP-L-galactose, L-galactose, GDP-D-mannose, L-galactono-1, and 4-lactone (Wheeler et al. 1998). This pathway also involved to convert D-the galacturonic acid to L-galactonic acid with the help of galacturonic acid reductase and finally L-galactone-1,4-lactone, L-galactono-1, 4-lactone oxidized to AsA by the enzyme L-galactono-1, 4-lactone dehydrogenase (GALDH). In mitochondria, the transportation of L-galactono-γ-lactone dehydrogenase by electroporation facilitated diffusion of protons to other components of the cell. These enzymes are also present in various parts of plants including organelles, apoplast, cell types, and abundantly in photosynthetic tissues (Shao et al. 2008). Apoplastic ASA involved to defend plants against a variety of external deleterious oxidants (Barnes et al. 2002). Under normal condition, AsA are present in low level in chloroplast and effect as cofactor (violaxanthin de-epoxidase) which directly influence the indulgence of extra excitation energy (Smirnoff 2000). Protects membranes by reacting with $O_2^{\bullet-}$, H_2O_2 and regenerates α-tocopherol of the toco-pheroxyl radical protect the function of prosthetic transition metal ions containing enzyme. AsA plays a key role in the removal of H_2O_2 through the AsA GSH cycle (Pinto et al. 2003). MDHA is a short-lived radical which degrades into DHA and AsA or be reduced to AsA by

NADP (H)-dependent enzyme MDHAR spontaneously. Above the pH 6.0 DHA is very unstable and degrades into oxalate and tartrate (Miyake and Asada 1994). To keep away from this, DHA is rapidly reduced to AsA by the catalyst (DHAR utilizing lessening reciprocals) from GSH (Asada 1996). AsA level changes in response to various stresses (Srivastava and Dubey 2011). Under stress condition, AsA level depends on balance of performance, and rate of AsA biosynthesis and turnover depend on the need for antioxidants (Chaves et al. 2002). The excess AsA biosynthesis is related with tolerance to abiotic stresses in plants, ranging from GDP-mannose to GDP-L-galactose, a significant stage in the pathway of AsA biosynthesis according to Smirnoff-Wheeler in higher plants. Ascorbate accumulation increased as result of overexpression of GME family, which was found in tomato plants to improve resistance against abiotic stresses (Zhang et al. 2011). In Arabidopsis, AsA accumulation increases under oxidative stress and leads to tolerance. The vtc-1 mutant lacks the activity of GDP-mannose pyro-phosphorylase, an enzyme found in the ascorbate biosynthetic pathway, because it involved in ascorbate synthesis (Wang et al. 2010).

18.5.2 Phenolic Compound Activation

Phenolic compounds are the secondary metabolites found in the plants and involved in various antioxidant activities. The tannins, hydroxycinnamate esters, lignin, and flavonoids are some phenolic compounds which have antioxidant properties. Polyphenols are composed of aromatic rings with substituents -OH or -OCH_3 that increase biological activity, including antioxidant activity. Polyphenols inhibit lipid peroxidation by chelating transition metal ions to scavenge free radicals and trapping lipid alkoxyl radicals. They decrease membrane fluidity and modify lipid packing order (Arora et al. 2000). These modification and changes limit the peroxidation reaction by checking the diffusion off free radicals. In plants multiple stresses resulted in phenolic metabolism induction as a response. Janas et al. (2009) also perceived that the activity of ROS as a signal for adaptation to abiotic stress especially Cu^{2+} stress leads to the accretion of total phenolic compounds in the lentil roots which grown in the dark. Defects of one gene in the Arabidopsis L. mutants showed significantly improved sensitivity to UV-B radiation as compared to control. High flavonoid concentrations in transgenic potato plants showed further developed antioxidant capacity (Lukaszewicz et al. 2004).

18.6 Enzymatic Components

Enzymatic components of antioxidant defense system include catalase (CAT), monodehydroascorbic acid reductase (MDHAR), superoxide dismutase (SOD), ascorbic acid glutathione (AsAGSH), and cycle ascorbic acid peroxidase (APX). It is composed of guaiacol peroxidase (GPX), glutathione reductase (GR), and dehydroascorbic acid reductase (DHAR) and various antioxidant enzymes (Noctor and Foyer 1998). With the emergence of oxidative stress, the plant defense mechanism activates the gene expression and synthesis enzymes in various subcellular compartments to cope the stress. Different plant-based antioxidant enzymes perform vital roles in limited stress-induced ROS.

18.6.1 Superoxide Dismutase

Superoxide dismutase (SOD) is directly involved to cope the oxidative stress of aerobic organisms. (Scandalios 1993). The SOD enzyme is the member of metalloenzyme group and involved in various catalytic activities such as the breakdown of $O_2^{\bullet -}$ to O_2 and H_2O_2. It is located in subcellular compartments of the plant and produces activated oxygen. SOD is targeted to each intracellular compartment by a nuclear-encrypted amino-terminal targeting sequence. Mn-SOD was found to be present in mitochondria and chloroplast of Fe-SOD. Three isoforms of Cu/Zn-SOD are present in various parts of plants including chloroplast, cytosol, peroxisome, and mitochondria (Bowler et al. 1992). Eukaryotic Cu/Zn-SOD is present in dimer form in plants and sensitive to cyanide, while Mn-SOD and Fe-SOD are found in dimer or tetramers and not sensitive to cyanide (Scandalios 1993). The activity of SOD exceeded in plants under different environmental stresses like metal toxins and drought (Mishra et al. 2011). The plant shows the tolerance against stress when SOD activities elevated. Oxidative stress enhanced with SOD concentration in plants; therefore it cannot be used as a direct selection criterion for drought tolerance plant material (Zaefyzadeh et al. 2009).

18.6.2 Guaiacol Peroxidase

Guaiacol peroxidase (GPX) is a protein-containing heme which involved to oxidize aromatic electron donors (guaiacol and pyrogallol) at the expense of hydrogen peroxide. It is mainly present in microorganisms, animals, and plants. These enzymes contain two structural Ca^{2+} ions and four conserved disulfide bonds (Schuller et al. 1996). In plant tissues a number of GPX isoenzymes are present and localized in cell wall, cytosol, and vacuoles. Various biosynthetic processes are associated with GPX, like cell wall lignification, wound healing, and biosynthesis of ethylene which involved to protect plants during abiotic and biotic stresses and degradation of IAA (Kobayashi et al. 1996). GPX plays a role in effectively removing reactive forms of O_2 and radical peroxides under stress conditions. Under stress conditions, it induces GPX activity (Han et al. 2009). Radotic et al. (2000) found that the elevated activity of GPX is associated with metal toxicity and suggest its potential as a biomarker of metal toxicity to plants. Moreover, Tayefi-Nasrabadi et al. (2011) observed that under salinity condition, salt-resistant safflower plants perform best and least damaged due to salt-induced oxidative damage results, through increasing the activity of GPX, catalytic efficiency, and specific isoenzyme induction compared to salt-sensitive plants.

18.7 Antioxidant-Based Abiotic Stress Tolerance

Plants usually generate antioxidants (enzymatic/nonenzymatic) to trigger oxidants produced during unfavorable condition (Farhat et al. 2021). Plants like *Phaseolus vulgaris* (Zlatev et al. 2006), *Oryza sativa* (Sharma and Dubey 2005), and *Trifolium repens* L. (Chang-Quan and Rui-Chang 2008) regulate their antioxidant (SOD) response to attain survival under drought stress. Similar trend was observed in chickpea (Kukreja et al. 2005) and tomato Gapiñska et al. 2008), under saline stress. UV-B irradiation enhanced SOD activity in Arabidopsis, pea, rice, and wheat, but has no or little effect on cereals and soya bean. In a field experiment, UV-B-induced stress substantially enhanced SOD activity in wheat and chickpeas and soybean varieties (Agrawal et al. 2009). Catalase (CAT) activity showed similar response under drought and salt stress in wheat (Simova-Stoilova et al. 2010) and chickpea (Kukreja et al. 2005; Eyidogan and Öz 2007), respectively. Oxidative damage in *Nicotiana tabacum* is effectively overcome by higher accumulation of *chl*APX under both drought and saline stress (Badawi et al. 2004). Roychoudhury et al. (2012) reported the salt-sensitive and salt-tolerant rice cultivars against Cd stress and also observed the antioxidative enzyme activities; however, salt-tolerant cultivars showed more accumulation of antioxidants. Contrary to other antioxidants, CAT activity showed marked reduction under Cd-induced stress in salt-sensitive rice cultivars. In another study, Roychoudhury and Ghosh (2013) suggested a gradual enhancement of the catalase and peroxidase action with the swelling level of $CdCl_2$ in *Vigna radiate*. *Vaccinium myrtillus* L. considered as tolerant plant by colonizing successfully heavy metal-contaminated soil. A careful examination of plants grown in contaminated soil, a high pool GSH contents, and non-protein thiol, proline, and GPX activities were observed. GPX activity seems to be mutual, complex, and closely related to heavy metal stress response (Kandziora-Ciupa et al. 2013). MDHAR overexpression in tobacco and of DHAR in Arabidopsis correlated to enhanced salt tolerance (Eltayeb et al. 2007). Moreover, salt-tolerant plant species show more accumulation of GR and APX than salt-sensitive varieties (Aghaei et al. 2009). The exogenous application of AsA enhanced photosynthetic metabolism of wheat under limited supply of water (Malik and Ashraf 2012). Similarly, the foliar treatment of AsA on tomato leaves mitigated the adverse effects of salt and ensured the long-term survival (Shalata and Neumann 2001). Remarkable reduction in GSH/GSS ratio revealed that susceptible varieties (IR-29 and Pusa Basmati) are more sensitive to moisture stress than the tolerant variety of Pokkali (Basu et al. 2010). Increased values of AA and GSH in salt-tolerant cultivar Pokkali were observed than those of sensitive cultivar Pusa Basmati (Vaidyanathan et al. 2003). Arsenic (III) is mitigated by phytochelatins. It remarkably reduces the GSH contents in rice roots. Due to anti-oxidative property, GSH addition suppresses MDA contents and ultimately resulted in protection and restoration of growth against arsenic stress (Roychoudhury and Basu 2012). Wheat plants exposed to heat stress caused rapid accumulation of reduced form of glutathione (GSH) by upregulating GSH gene expression (Hasanuzzaman et al. 2013). Transgenic tobacco plants having tocopherol gene (VTE1) reduced impact of lipid peroxidation by maintaining the membrane integrity and H_2O_2 balance and enhancing photosynthetic activity compared to parents and wild relatives (Liu et al. 2008). In Arabidopsis, it has been proved that vte1 and vte4 mutants were susceptible to salt stress by showing poor growth due to oxidative stress. α-Tocopherol regulates intracellular Na+/K+ translocation and hormone balance (Ellouzi et al. 2013). Lack of α-tocopherol in plants caused more production of polyunsaturated fatty acids in response to UV-B radiations. Carotenoid molecules per chlorophyll positively correlates with drought-mediated resistance in plants (Jain et al. 2003).

Two isolines of soybean with medium to low flavonoid contents irradiated with natural UV-B levels exhibited an increase of both enzymatic and nonenzymatic antioxidant (APX, CAT, GR) activities but reduced its SOD and ascorbate activity (Xu et al. 2008). Proline performs vital role in osmoprotection and absorb energy to regulate redox potentials under different moisture regimes. Drought-resistant chickpea cultivars respond to different moisture conditions with rich pool of proline than sensitive cultivars (Mafakheri et al. 2010). Among rice seedlings exposed to high salt (200 mM NaCl) stress, the salt-sensitive cultivar showed tolerance by accumulating proline and anthocyanin compared to the salt-tolerant cultivars (Roychoudhury et al. 2008). It was suggested that the flavonoids and proline contents have fundamental significance in regulating antioxidant response to tolerant and sensitive rice cultivars against salt, which can be attributed to the reduction of excessive membrane permeability (Chutipaijit et al. 2009) (Table 18.1).

18.8 Transcription Factors for Regulating Oxidative Stress

Transcription factors (TF) manipulate the expression pattern of stress-related genes against ROS. Expression patterns showed that multiple genes are associated to cope the abiotic stress tolerance process in plants. The microarray analysis revealed 32 transcription factors (TFs), common to many abiotic stresses. Among 32 transcription factors, six belongs to WRKY, seven to NAM/NAC, and eight to AP2/ERF family (Gadjev et al. 2006). In addition, many endoplasmic reticulum-associated TFs have been discovered which are associated to cope the environmental stress in plants (Jaspers and Kangasjärvi 2010a, b). AtbZIP17 and AtbZIP28 genes upregulated during salinity and heat stress, respectively (Liu et al. 2007). In addition, redox transcription factors 1 (RRTF1) and AtERF6 are also involved in activating immune system of plants by activating reactive oxygen species, bind to the promoter region (GCC), which leads to changes in gene expression (Wang et al. 2013; Matsuo et al. 2015). An AP2/ERF TF, a cytokinin reaction factor 6, suppresses the cytokinin-associated genes which are responsible to cause oxidative stress (Zwack et al. 2016). Some more transcription factors such as zinc finger TFs, WRKY family NAM or NAC (no apical meristem), RAV, Zat, Myb, and GRAS families mediated ROS response (Chen et al. 2010). Although Zat10 is a gene repressor to suppress the activity of ROS (Mittler 2006), Zat12 and Zat6 positively influenced in the expression of Apx1 (Shi et al. 2014). LSD1 and LOL1 paralogs of zinc finger limit the accumulation of O2 and SOD (Epple et al. 2003). In addition, the transcription

Table 18.1 Antioxidant response of different plant species under abiotic stress with and without elicitors

Plant species	Stress	Oxidative stress	Elicitor used	Antioxidant response	Reference
Triticum aestivum L.	NaCl	Increase MDA, H_2O_2 and O_2^-/NaCl	Nitrogen (N) supplementation	SOD, CAT, APX, GR, MDHAR, DHAR, and the synthesis of AsA and GSH compared to stressed plants without N	Ahanger et al. (2019)
Nicotiana benthamiana		Enhanced MDA/150 mM NaCl	Acetylcholine (ACH)	Superoxide dismutase and peroxidase increased compared to stressed plants without ACH	Qin et al. (2020)
Solanum lycopersicum		H_2O_2, TBARS/50 μM NaCl	Vanillic acid (Vin)	AsA/DHA, GSH/GSSG, MDHAR, GR, GST, SOD, and CAT increased compared to stressed plants without Vin	Parvin et al. (2020)
Medicago sativa		MDA and H_2O_2	Melatonin	Increased the activities of CAT, POX, and Cu/Zn-SOD compared to stressed plants without melatonin	Cen et al. (2020)
Cucumis sativus		MDA and H_2O_2	Melatonin	Increased the activities of CAT, POX, and Cu/Zn-SOD compared to stressed plants without melatonin	Zhang et al. (2020)
Brassica juncea		MDA and H_2O_2	Nitric oxide (NO)	Increased SOD, CAT, APX, and GR activity compared to stressed plants without NO	Jahan et al. (2020)
Zea mays	Drought	MDA, O_2^-, H_2O_2TBARS	No elicitor	Increased activities of APX, MDHAR, and DHAR, SOD, AsA and DHA contents	Anjum et al. (2017)
Oryza sativa var. japonica cv. Nipponbare		$O_2^{\bullet-}$ and H_2O_2 MDA	No elicitor	Decreased SOD, APX, and CAT activities, but POD activity increased by 59% compared to control	Liu et al. (2019)
Glycine max *G. tomentella*		MDA	No elicitor	A substantially increased SOD, GR	Hamim et al. (2017)
Brassica napus cv. Binasarisha-3		MDA, H_2O_2	NO pretreatment (500 μM sodium nitroprusside)	Increased MDHAR, DHAR, GR, GST compared to stressed plants without NO	Hasanuzzaman et al. (2017b)

Vigna radiata cv. BARI Mung-2		H_2O_2, $O_2^{\bullet -}$, LOX	Spermine (Spm, 0.2 mM)	Decreased AsA/DHA ratio, CAT, MDHAR but increased APX, GR compared to stressed plants without Spm	Nahar et al. (2017a)
Solanum lycopersicum cv. Login 935		$O_2^{\bullet -}$, H_2O_2, MDA	No elicitor	Enhanced SOD, CAT, APX, AsA, GSH, α tocopherol contents	Rady et al. (2020)
Brassica napus Cv. BINA Sharisha-3	Cd	$O_2^{\bullet -}$, H_2O_2, MDA	No elicitor	Reduction of AsA, CAT, MDHAR, DHAR Increased GSSG, GST, APX, GR activity	Hasanuzzaman et al. (2017a)
Vigna radiata cv. BARI mung-2	Al	H_2O_2, $O_2^{\bullet -}$, LOX	Spd (0.3 mM spermidine)	Reduction of AsA, MDHAR, DHAR, and CAT but increased GSH, GSSG, SOD, GST, GPX, APX, GR activity compared to stressed plants without Spd	Nahar et al. (2017b)
Triticum aestivum cv. Pradip	Pb	$O_2^{\bullet -}$, H_2O_2, MDA	Glutathione (GSH, 1.0 mM)	APX activity increased, but MDHAR, DHAR, CAT, GPX decreased; GR increased initially and then declined compared to stressed plants without glutathione treatment	Hasanuzzaman et al. (2018)

Abbreviations: *Al* aluminum, *APX* ascorbate peroxidase, *Cd* cadmium, *GPX* guaiacol peroxidase, *GR* glutathione reductase, H_2O_2 hydrogen peroxide, *MDA* malondialdehyde, *MDHAR* monodehydroascorbate reductase, $O_2^{\bullet -}$ superoxide radicle, *GST* glutathione S transferase, *Pb* lead, *TBARS* thiobarbituric acid reactive substances factors (NAC) produce resistance to environmental stress by the ABA signaling pathways in Arabidopsis (Puranik et al. 2012; Vermeirssen et al. 2014). Gene expression patterns vary in response to deviations in redox properties which are attained through the TF's activation and suppression with stress-sensitive/stress-tolerant cis-acting factors. To activate transcriptional factors, sense kinases are contained in the autophosphorylating plasma membrane in response to an external stimulus, after which they are supplied with a phosphoryl group, which leads to conformational changes in TFs, allowing them to bind to promoter of the cis-element and gene expression. Since various types of reactive oxygen species respond to various cysteinyl residues, the same transcriptional factor can influence the gene regulation of various genes under stress. Tyrosine phosphatase (PTP1) is regulated by the concentration of H_2O_2 in Arabidopsis via upregulation of MPK6 (Gupta and Luan 2003).

18.9 Impact of Micro-RNA to Cope Abiotic Stress

MicroRNAs are involved in the plant's development and adaptation to different stresses by regulating interactions between plants and environmental components (Ferdous et al. 2015). The molecular mechanism of miRNA action on abiotic stress is a powerful tool for plant breeding, especially in changing climatic conditions (Shriram et al. 2016).

18.10 Impact of Transgenic Approaches to Explore Reactive Species and Antioxidant Machinery Abiotic Stress in Plants

The plant faced various stresses especially abiotic stress which leads to the modification in stress-sensitive metabolites, genes, and proteins that regulate the growth of plant and cope the harmful effects of stress. Therefore, understanding molecular or biochemical mechanisms under stressful environmental conditions may be the key to creating stress-resilient plants. Traditional molecular breading strategy involves identifying QTLs related to stress resistance. However, traditional disadvantages of molecular breading include agronomic transmission of unwanted traits. Transgenic approaches also have negative impact on the plant and influence the gene expression antioxidant enzymes in plants and also limit the activity osmoprotectants. To explore this activity, it needs to observe the single gene expression for detoxification enzymes, osmolyte biosynthesis pathway enzymes, aquaporins or osmolyte biosynthesis pathway enzymes, or transfer of several genes that activate TF to regulate abiotic stress expression (Wani et al. 2016). It is important to use them as effective targets for engineering crops against abiotic stress. APX, CAT, and SOD are important antioxidant stress-responsive genes (MDAR, POX, GR, GPX, and DHAR.) in crops. In transgenic peas Mn-SOD overexpression resulted in drought tolerant (Wang et al. 2005). The overexpression of APX and Cu-Zn-SOD gene fescue plants resulted in ROS removal and increase resistance against various stresses (Lee et al. 2007). The simultaneous overexpression of NDPK2, APX, and Cu-Zn-SOD enabled the plant to survive under harsh and stressful environment (Kim et al. 2010). The overexpression of various enzymes including aldehyde reductases (ALR), aldose reductase (ADR), aldo–keto reductases (AKRs), and glyoxalases detoxifies RCS. PsAKR1, MG, and OsALR1 MDA overexpression detoxifies under saline condition in rice and tobacco (Nisarga et al. 2017). By detoxifying reactive aldehyde, the overexpression of AKR4C8 and AKR4C9 in Arabidopsis increases oxidative stress resistance (Simpson et al. 2009)]. The overexpression of ALDH3H1, ALDH7B4, and ALDH3I1 decline the lipid peroxidation rate and enhanced the resistance against abiotic stress in Arabidopsis (Kotchoni et al. 2006). The UV-B irradiation and drought methyl viologen (MV) increase with the overexpression of AKR and MsALR in the cytotoxic RCC. Glyoxalase pathway improves resistance to abiotic stress by the reduction of oxidative damage through rapid MG formation through overexpression of (Gly I, II, and III) genes. Similarly, the overexpression of ZmLEA3 enhances the tolerance against osmatic and oxidative stress in tobacco plants (Liu et al. 2016). In Arabidopsis overexpression of AtRZFP showed higher superoxide dismutase and peroxidase activity, reduced ROS accumulation, and increased the contents of proline and soluble sugars because AtRZFP associated directly with osmotic stress and salinity (Zang et al. 2016).

Likewise, mi-RNAs play an important role in regulating gene expression that is involved in osmotic regulations and stress responses in recent years, and miRNAs have been used to developing abiotic stress-resistant crops (Shriram et al. 2016). The H_2S downregulates the transcription levels of miR393a and miR393b, while NaHS treatment showed different miRNA expression related to drought stress of miRNA398, miRNA167, and miRNA396 at seedling stage in Arabidopsis. In maize the overexpression of miRNA and ZmmiR156 induced the tolerance against oxidative stress in tobacco plants under drought and salinity stress. The high concentration of SOD, CAT, and APX and the lower levels of MDA were observed in miRNA and ZmmiR156 transgenic maize plant (Kang et al. 2020). The overexpression of free radical in transgenic plant scavengers showed better stress tolerance, and some contradictory results have been obtained, maybe due to reduction of radical scavenging machinery. McKersie et al. (1996) describe that overexpression of Mn-SOD in alfalfa increased dis-mutation of superoxide radicals and improves plant survival at low temperature but disease resistance decreased. However, Creissen et al. (1999) studied that transgenic tobacco plant showed more capacity of glutathione biosynthetic in chloroplasts which lead to oxidative stress. Light et al. (2005) describe that a genetically modified cotton line (*Gossypium hirsutum* L.) indicating tobacco GST, that results in salinity, cold and herbicides resistance.

18.11 Conclusion

Abiotic stresses are main limiting factor affecting plant growth and development all over the world. To decipher biochemical, physiological, molecular, cellular mechanisms of abiotic stress reaction and tolerance development is need of the time to develop sustainable agricultural production. Abiotic stress leads to ROS gathering, which resulted in plant oxidative damage. Primarily, ROS were considered lethal molecules, and aerobic metabolites are present in various subcellular compartments. ROS metabolism is essential under stress condition, for plant adaptation, survival, growth, and development. Under stress conditions, antioxidant systems of enzymatic and nonenzymatic sustain and balance among detoxification and ROS production. In contradiction, ROS are observed as double function in plant biology as result of molecular cross-talk with other signaling molecules, that is, RSS and RNA. They perform signaling functions at low concentrations, but high oxidative stress may result in cell death, and as result of signaling pathway activation, ROS toxicity obviously kills cells. In addition, modern genome editing tools such as CRISPR/Cas are helping tools for mutant plant development for one or more genes (ROS detoxification regulators) to improve antioxidant defense system activity and increase development and growth of plants. Recently, rapid propagation is powerful tool for plant development and growth acceleration under required conditions. Therefore, genome editing and rapid plant breeding techniques can be used to develop genetically modified plants with stress-induced antioxidant.

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Plant Life Under Changing Environment: An Exertion of Environmental Factors in Oxidative Stress Modulation

19

Sabreena and Shahnawaz Hassan

Abstract

The environment entails set of relationships between biotic and abiotic modules that are impeccably well adjusted by various natural processes. Being static, land floras are wide open to an array of harsh environmental settings particularly abiotic stresses (salinity, drought, temperature fluctuations, heavy metal, ozone, ultraviolet radiation, as well as nutrient deficits). Oxidative stress promulgated by the environmental stressors has been considered as a serious constraint that impacts the development, growth, and reproduction in plants. Due to upsurged and alarming setup of global warning, the frequency, duration, and intensity of hostile environmental conditions are projected to be enhanced that will ultimately have a deleterious bearing on yield and world food production. Plants subjected to contrary environmental factors promote oxidative stress through reactive oxygen species (ROS) generation that activates signaling pathways impacting physiological, biochemical, and molecular processes. In order to maintain a proper physiological level in plants, ROS is crucial which in turn heavily relies on enzymatic and nonenzymatic antioxidant operations. So, reinforcing the understanding and comprehension of oxidative stress and antioxidant system in plants can aid in improving its tolerance toward punitive environment. In this book chapter, contemporary outcomes on the metabolism of ROS and anti-oxidative defense of plants will be elucidated in addition to antioxidant regulation under adverse environmental factors.

Keywords

Combined abiotic stress · Salinity · Drought · Nutrient deficiencies · UV radiation · Heat stress · Cold stress · Antioxidative modulation

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19.1 Introduction

Natural processes flawlessly balance the environment, which is made up of a system of relationships between living and nonliving entities. Each species has an impact on its surroundings and is influenced by it. Salinity, drought, harsh temperatures, metal toxicity, air pollution, UV light (Choudhury et al. 2013), excessive pesticide levels, and pathogen infection are all examples of environmental variables that can cause oxidative stress (OS) (Fig. 19.1) in plants (Al-Gubory and Laher 2018; Foley et al. 2016). OS is triggered by either direct or indirect environmental stresses, such as the production and buildup of reactive oxygen species (ROS), which harm a cell prior to its removal. To avoid stimuli, animals have the capacity to migrate and flee. As sessile animals, plants, on the other hand, have evolved sophisticated stress-relieving methods. The plant cells exhibit the state of "oxidative stress" if the amount of ROS generated surpasses the internal defense systems.

Superoxide radical (O_2), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and others are examples of reactive oxygen species (ROS) (Khan and Khan 2017). They are made in cellular parts such as chloroplasts, peroxisomes, mitochondria, and plasma membrane and are considered natural byproducts of the aerobic pathway (Apel and Hirt 2004). The fact is that the rise in ROS levels impacts cellular, physiological, and biochemical processes (S. Li et al. 2018; Martínez et al. 2017; Van Ruyskensvelde et al. 2018). This has a substantial effect on crop productivity and value (Singh et al. 2017). Taking an example such as more expression of AtCYP21-4, a protein associated with oxidative stress tolerance, leads to heftier tubers in potatoes (*Solanum tuberosum* L.) (Park et al. 2017). Furthermore, overexpression of CitERF13 in the citrus fruit crust of sweet oranges

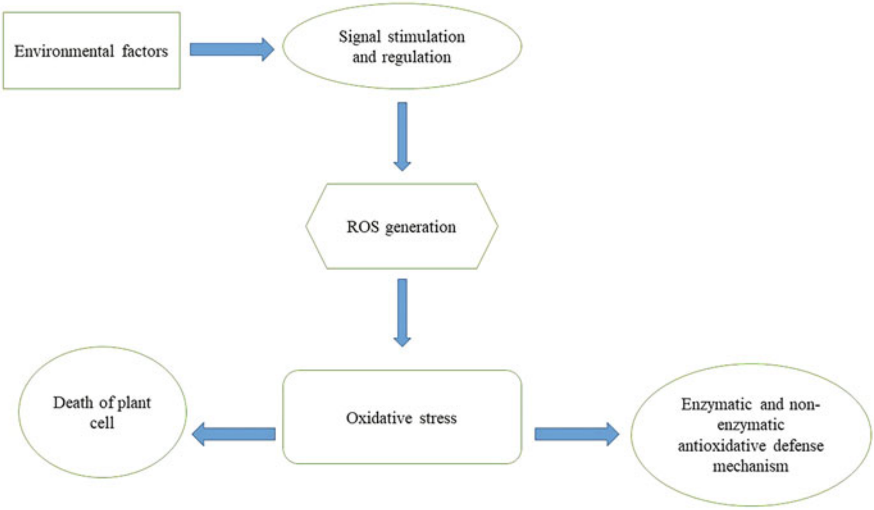

Fig. 19.1 Environmental factors and oxidative stress in plants

(*Citrus sinensis* L. Osbeck) stemmed in fast chlorophyll decay besides materialization of ROS (Xie et al. 2017). Similarly, mutations of the singlet oxygen ($^1O_2$) overgenerating flu and chlorina1 (ch1) in Arabidopsis (*Arabidopsis thaliana*) have revealed that $^1O_2$ caused alterations in gene expression (Shumbe et al. 2016). To finish, we can state that ROS has a major effect on crop productivity as well as crop quality.

Over time, oxidative stress exploration has primarily concentrated on *Escherichia coli*. However, in the last 10 years, it has shifted from mammals (such as humans) to plants, predominantly model crops (e.g., *Arabidopsis thaliana*, rice). This led to a noteworthy improvement in our knowledge about the functioning and working of oxidative stress toward developmental, defensive, and environmental factors (G. Guan and Lan 2018). Over the counteraction of ROS results in the shortfall of an essential intracellular signaling molecule, henceforth plants developed an antioxidant defense system to retain a dynamic balance of ROS.

This chapter focuses on the ROS metabolism in plants besides providing a quick overview of ROS types, production sites, and induced oxidative stress. Then, we'll go through the antioxidative defense machinery's role in preventing overproduction of ROS in adverse conditions, as well as a review of recent studies on several environmental factors that affect oxidative stress regulation.

19.2 The Metabolism of ROS in Plants

19.2.1 Production of ROS

Out of ROS types, H_2O_2 has garnered a lot of research attention as it is has been found extremely important for regulations of processes such as senescence (Jajic et al. 2015), functioning of stomata (Rodrigues et al. 2017), cell wall cross-linking (J. Li et al. 2017a), controlling of cell cycle (Pokora et al. 2017), photosynthesis (Exposito-Rodriguez et al. 2017), stress tolerance (Lv et al. 2018), and antioxidative defense (Liu et al. 2016). H_2O_2 has also been shown to interact with other signal molecules critical for plant development and senescence, for instance, abscisic acid, auxin, brassinosteroid (BR), and ethylene (Alqurashi et al. 2017). Response meticulousness allows diverse signaling transduction routes to work in response to perceived environmental cues, besides changes in H_2O_2 generation and accumulation are thought to affect the physiological status of the plants. H_2O_2 can trigger several acclamatory reactions with respect to stress signal which strengthen endurance to diverse stressors, according to several recent studies.

Both stressed and unstressed plant cells generate reactive oxygen species (ROS). The generation of highly reactive ROS is caused by the gradual depletion of oxygen caused by high-energy contact or electron transferal processes. The triggering of ROS in plants is reliant on energy and requires a continuous outflow of electrons from electron transport actions. These electron transport activities take place in numerous metabolic conduits positioned in special cellular sections (Das and Roychoudhury 2014; Gupta et al. 2015). In the presence of light, chloroplasts and peroxisomes are the primary ROS generators, whereas mitochondria are the primary ROS producers in the absence of light (Choudhury et al. 2013). The effective light-capturing photosynthetic machinery is housed in the chloroplast, which is made up of an extremely systematized structure of thylakoids. PS I and PSII are the principal foundations of ROS making in thylakoids and comprise the basis of their light-harvesting systems (Khan and Khan 2017). When chlorophyll is overexcited under stress conditions, O_2 may generate $^1O_2$ adjacent the response spots of PSII. Many additional cellular sites also play a role in the production of reactive oxygen species. Localized NADPH-dependent oxidase transports electrons from NADPH on the cytoplasmic side to O_2, resulting in O_2 at the plasma membrane, which is critical for environmental sensing (P. Sharma et al. 2012). ER is also involved in Cyt P450's generation of O_2. During unfavorable climatic conditions, stress signals combined with ABA render the apoplast for H_2O_2 production (Hu et al. 2006).

19.3 Oxidative Damage

Reduced ROS levels mediate events like stomatal closure, programmed cell death (PCD) (Petrov et al. 2015), gravitropism (Wassim et al. 2013), and stress tolerance (Nath et al. 2017) in plant cells. Plants produce excess ROS as a result of constant environmental stressors, which will not be entirely disposed of by the active oxygen scavenging mechanism. As a result, important physiological actions such as lipid peroxidation, nucleic acid oxidation, protein denaturation, enzyme inhibition, as well as PCD pathway activation should be performed (Das and Roychoudhury 2014; Nath et al. 2017).

Lipids and proteins in plant cells are primary recipients of oxidative harm due to ROS. Lipid peroxidation, or the oxidative degradation of polyunsaturated lipids in the plasma membrane, takes place in each organism and is often used as a marker to measure levels of lipid injury during severe settings (Gaschler and Stockwell 2017). It has recently been established that lipid peroxidation initiates a cascade of reactions that can produce other reactive chemicals, for example, ketones, aldehydes, and hydroxyl acids, as well as change proteins through the oxidation of certain amino acid residues (Reginato et al. 2015). Modifications like glutathionylation, carbonylation, nitrosylation, and disulfide link development affect the protein's activity (Grimm et al. 2012).

19.4 Antioxidative Defense System in Plants

Increased ROS production in plant cells is caused by salinity, drought, cold, metal toxicity, air pollution, UV-B radiation, and excessive pesticide concentrations as well as pathogen presence (Molassiotis et al. 2016). Numerous studies (Cavallini et al. 2016; Delaunay-Moisan and Appenzeller-Herzog 2015) have established the importance of intracellular antioxidant defense mechanisms in the face of various stresses. This antioxidant defense mechanism, which works in diverse subcellular sections to scavenge ROS, is made up of enzymatic and nonenzymatic components (Sharma et al. 2012).

Due to the limitations of the experimental settings, the majority of research on the antioxidative defense system originally focused on enzymatic features. SOD, APX, CAT, and other antioxidative defense enzymes have all been studied extensively to get a better knowledge of how the body responds to oxidative stress caused by a range of environmental conditions. Many transgenic plants have been created in recent years with altered antioxidant enzyme expression, leading in improved tolerance to salt, high temperatures, and drought stress, including Arabidopsis, tomato, rice, tobacco, and maize (Khan and Khan 2017). Plants may enhance their resistance to a range of stressors by overexpressing a single gene, and transgenics that overexpress SOD to improve stress tolerance have been investigated (Cavallini et al. 2016).

Many plant species have had their genes producing antioxidative defense enzymes investigated extensively. The majority of research on oxidative stress-related transcription factors including AP2/ERF, NAC, MYB, and the bHLH family (Marinho et al. 2014) has concentrated on antioxidant enzyme transcriptional regulation. Overexpression of the buckwheat (*Fagopyrum tataricum*) FtbHLH3 gene increased drought tolerance in Arabidopsis, which was ascribed to reduced H_2O_2 levels, greater SOD and CAT activities, and improved photosynthetic efficiency in transgenic lines compared to WT (Yao et al. 2017). In response to high levels of H_2O_2, rice miR529a overexpression resulted in enhanced seed germination, increased SOD and POD activity, and decreased leaf rolling rate and chlorophyll content (Yue et al. 2017). However, the fundamental regulation systems for antioxidant enzymes are unknown and must be investigated.

19.5 Exertion of Environmental Elements in Oxidative Stress Modulation

19.5.1 Salinity

In several dry and semidry areas globally, soil salinity remains a chief concern which inhibits the yield and superiority of cultivated harvests. Hypersaline environments have a variety of effects on stressed crops, including oxidative stress, genotoxicity, ionic unevenness plus toxicity, nutrient shortage, and osmotic stress, all of which contribute to the plants' poor health (Shah et al. 2017). Plant cells reduce photosynthetic electron transport thereby producing disproportionate ROS (Fig. 19.2). Plants establish multiple means to avert the negative impacts listed above, containing salt compartmentalization and elimination (W.-H. Wang et al. 2016).

In plants, each enzymatic scavenger works together to combat salt stress and promote better growth and development. Following NaCl treatment, the activities of CAT and DHAR rose in all structures of salt-treated maize sprouts, including roots and developed and underdeveloped leaves, while SOD, APX, GST, and GR increased mainly in the roots (AbdElgawad et al. 2016). Two native wheat

Fig. 19.2 Exertion of environmental factors in oxidative stress modulation

salt-tolerant cultivars, BARI Gom 27 and 28, exhibited lower H2O2 accretions and higher CAT, peroxidase, and APX activities than salt-sensitive cultivars due to decreased oxidative damage (Siddiqui et al. 2017). The increased expression of enzymatic antioxidants caused by salt therapy in the studies above implies that salt treatment is an effective strategy to reduce saline toxicity. However, several investigations have found that the expression levels of these enzyme genes vary depending on the salt level, exposure time, and plant developmental stage (Cunha et al. 2016).

Because antioxidant enzymes are so important, genetic engineering may be utilized to increase salt tolerance in a variety of plants by overexpressing antioxidant enzyme pathway genes (Cunha et al. 2016). For example, STRK1 activates Tyr-210, a critical phosphorylation site in CatC. (receptor-like cytoplasmic kinase). Furthermore, STRK1 overexpression phosphorylated and activated CatC, enhancing salt and oxidative tolerance while also regulating H_2O_2 homeostasis. Rice seedling growth was boosted by overexpression of STRK1, while rice seedling development was hampered by deletion of STRK1 (Cunha et al. 2016). In *Puccinellia tenuiflora,* long-term exposure to NaHCO3, NaCl, H_2O_2, and PEG6000 increased PutAPX expression. Transgenic Arabidopsis plants overexpressing PutAPX showed improved salt toxicity tolerance and lower levels of lipid peroxidation when cultivated with 150 or 175 mM NaCl (Q. Guan et al. 2015).

19.5.2 Drought

Drought is a significant environmental stress for plant development in a constantly warming world and leads to a decline in crop output, particularly for saleable crops such as rice, wheat, and maize (Hossain et al. 2016). Plants, on the other hand, have evolved a variety of ways to mitigate the effects of drought (Shah et al. 2017). The generation of reactive oxygen species (ROS) is shown to be a fundamental mechanism in plants' physiological response to drought, causing increasing oxidative damage, restricted development, and final cell death as soon as ROS levels approach a particular onset (Molassiotis et al. 2016).

ROS scavenging related genes/enzymes with increased expression has been shown in many studies to assist in the development of long-term drought tolerance (W.-B. Wang et al. 2009). Activating 10 ROS scavenging related genes in the OE lines, and downregulating their expression in the RNAi lines, for example, improved rice drought tolerance via changing ROS homeostasis (Xiong et al. 2018). Drought treatment reduced leaf water loss, depleted H_2O_2 concentration, increased leaf water matter, and enhanced POD and CAT activities in Arabidopsis ZAT18 (a C2H2 zinc finger protein) OE plants (Yin et al. 2017).

Oxidant enzymes have a major role in ROS scavenging, according to studies using transgenic plants. In comparison to wild-type (WT) leaf slices, overexpression of pea (*Pisum sativum*) MnSOD in rice decreased electrolyte outflow following polyethylene glycol 6000 usage, which can cause drought stress (Wang et al. 2005). Overexpression of APX and Cu/ZnSOD in sweet potato chloroplasts enhanced drought resistance and retrieval, according to another research. When compared to WT, it also exhibited enhanced photosynthetic activity when exposed to drought stress (Lu et al. 2010).

19.5.3 Chilling

Crop development, output, and distribution are all hampered by chilling stress. Crop chilling tolerance must therefore be improved in order to increase crop yields. Chilling tolerance is generally linked to antioxidant enzyme activity augmentation plus equivalent H_2O_2 accretion drop since chilling promotes oxidative stress resulting in lipid peroxidation, chlorophyll deterioration, and so on.

As typical oxidoreductases, glutaredoxins (GRXs) primarily use glutathione's reducing capacity to break disulfide bonds in substrate proteins as well as uphold cellular redox evenness. The expression of AtGRXS17 in tomato has been shown to confer transgenic tomato chilling stress resistance lacking several developmental abnormalities. When exposed to cold, tomato plants expressing AtGRXS17 have reduced ion outflow and higher utmost photochemical efficacy than wild-type plants (Hu et al. 2006). In such transgenic tomato plants, soluble sugar accumulates at a faster rate as well. To improve resistance to oxidative stress initiated via chilling stressors, it has been reported that co-expression of MeCu/ZnSOD and MeAPX2 in cassava (*Manihot esculenta* Crantz). increased concentrations of antioxidative enzyme activity in addition to reduced quantities of chlorophyll deficit, lipid peroxidation, and H_2O_2 buildup (Xu et al. 2014). Similarly, in *Brassica rapa*, co-expression of BrMDHAR and BrDHAR increased plant resilience to freezing via hybridization (Shin et al. 2013).

19.5.4 Metal Toxicity

Heavy metal contamination has become so significant since the industrial revolution that a growing number of scientists are doing important scientific research. Heavy metals' harmful effects on plants and the environment are usually determined by their concentrations (Stankovic et al. 2014). At normal concentrations of heavy metals, plants demonstrate their ability to prevent negative consequences (Juknys et al. 2012). Excessive levels of heavy metals have previously been shown to disrupt homeostasis and enhance ROS generation in plant cells (Shahid et al. 2014).

Heavy metals ingested by plants are engaged in numerous pathways which generate free radicals due to their redox activity. Iron (Fe), copper (Cu), chromium (Valko et al. 2005), and other redox-active elements can take part in a redox-cycling reaction, causing creation of deadly hydroxyl radicals that cause substantial impairment to existing cells. When wheat (*Triticum aestivum* L.) is stressed by Cr, mannitol has the capability to stimulate an antioxidant enzyme that may aid to alleviate pathological symptoms (Adrees et al. 2015). Other metals with no redox capability, for example, lead, cadmium, mercury, zinc, and nickel, depress the antioxidative system by exhausting glutathione and binding sulfhydryl clusters of antioxidative enzymes such as reductases, superoxide dismutases, and catalases (Fryzova et al. 2017). They similarly tamper by means of photosynthetic processes (Sharma and Dietz 2009). Several writers have pointed out that the severity of oxidative stress caused by heavy metals differs by species plus among genotypes, tissues, and/or developing phases. Metal prone vegetation shows severe signs when exposed to oxidative strain, whereas metal-resilient vegetation shows relatively minor to no oxidative damage (Juknys et al. 2012).

A number of compounds have been found that may minimize heavy metal uptake and alleviate oxidative stress in plants, in addition to antioxidant responses in plants. *Abelmoschus esculentus* was suppressed by biochar made from *Citrus epicarp* (L.). Copper toxicity in rice seedlings was reduced by reducing Cu absorption and oxidative harm using exogenous SNP (sodium nitroprusside) and GSH (glutathione)

(Mostofa et al. 2014). Furthermore, some fungi can protect plants through mycorrhization (Schutzendubel and Polle 2002).

19.5.5 UV-B Radiations

UV-B radiation acts as a preordained abiotic component for photosynthetic plants on exposure to sunshine. On exposure to excessive quantities of UV-B radiation, many cellular constituents, especially macromolecules (DNA and protein), are disrupted, resulting in the formation of oxygen radicals in plants. Crop plants subjected to physical radiations, such as ionizing and nonionizing UV-B, have been known for many years to create disproportionate free radicals, which induce chromosomal changes in the plants (Agrawal et al. 2009). Almost all UV-B irradiation dosages interfered with meiotic-pollen mother cells and pollen grains in *Vicia faba* L. (Abdel Haliem et al. 2013), resulting in a genotoxic effect. Because it acts in the UV-B-sensing conduit in the roots, the RUS1/RUS2 (Root UV-B Responsive) complex in Arabidopsis is engaged in seedling morphogenesis and development during the early stages of development. Seedling development is hampered in the absence of the RUS1/RUS2 complex because photoreceptors produce a considerably enhanced signal after UV-B sensing (Leasure et al. 2009).

19.5.6 Pathogens

Plant diseases and epidemics caused by pathogen infection have posed a threat to plant development, agricultural productivity, and food security around the world. Pathogens are one of the most dangerous hazards to plants because of their diverse and constantly developing characteristics. Sessile plants, unlike vertebrates, evolved a conserved, unique, and complex immune system to battle invading diseases. The majority of microorganisms are dealt with by physical and chemical barriers, while the rest are dealt with by particular resistance responses known as host resistance. Plants respond to PAMPs (pathogen-associated molecular patterns) by triggering a variety of immunological responses in the cells (Luo et al. 2017).

The role of RBOH in stress feedback has received a lot of attention. ACD11, for example, controls the ROS-related defense response with Arabidopsis BPA1 and its homologs as binding partners. Those binding allies are capable of being guided by RxLR207, a *Phytophthora capsici* effector, resulting in ROS-mediated cell death, which is essential for *P. capsici* pathogenicity (Li et al. 2019).

One more strategy to improve plant disease tolerance is by stopping peroxidase-catalyzed H_2O_2 breakdown and enhancement of ROS spurt to exterminate attacking pathogens. A spontaneous alteration of the transcription element which lowers peroxidase production besides imparts broad-spectrum blast resistance in rice has been discovered recently (Li et al. 2017b).

Even though ROS bursts plus buildup injure plant cells, ROS production is necessary for plant immunity. Interim oxidative stress is used by the plant immune system to defend against pathogens because of the signaling and bactericidal activities of ROS. Plants can detect pathogen invasion thanks to ROS' dual involvement in signal transduction. However, in order to govern ROS function, its production must be strictly regulated.

Swift systemic signaling is critical for plant tolerance to abiotic stressors, which is aided by ROS and Ca^{2+} waves (Gilroy et al. 2014). Abiotic stress factors can also reduce or increase plant tolerance to infections (Bai et al. 2018). After immunogenic treatment, ROS production spikes in a matter of minutes; consequently, this biological course may be monitored to determine the role of plant constituents in the spurt of beforehand immunological feedbacks (Zipfel 2014).

19.6 Conclusions

During their growth, plants are exposed to a range of environmental stresses which help them to attune to those settings by making molecular, biochemical, and physiological changes, particularly through antioxidant systems. Due to exceedingly responsive characteristic and quick half-life of ROS, it is yet unknown in what way plants perceive pressures then plan for approaching threats. Genetically engineered plants with overexpressed functional genes have showed promise in mitigating oxidative stress in recent research. Furthermore, attempts need to increase in order to develop transgenic florae that co-express numerous beneficial genes in order to attain high tolerance against various adverse situations.

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Beneficial Role of Phytochemicals in Oxidative Stress Mitigation in Plants

20

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Abstract

Both biotic and abiotic stresses cause a great harm to plants by hampering growth and reducing yield. Various unfavorable conditions such as drought and salinization lead to an increased production of free radicals ($^{\bullet}OH$, $O_2^{\bullet-}$, $^{\bullet}NO$) and imbalances in cellular redox homeostasis. This imbalance results in oxidative stress and subsequent stress responses in the plant. Oxidative stress can be the cause of oxidative damage to the biomolecules like proteins, lipids, and deoxyribonucleic acid (DNA); photosynthetic systems; and cell death. The stress responses include diverse signaling pathways leading to various molecular, physiological, biochemical, and morphological adaptations that help the plants to withstand the stress. In addition to primary metabolites, plants produce various secondary metabolites that aid in plant survival during stress. Secondary metabolites such as polyphenols, flavonoids, carotenoids, phenolic acids, terpenoids, and alkaloids enhance the plant survival by acting as antioxidants, direct free radical scavenging, indirect ROS signaling, UV absorbing, and improving structural and functional stabilization and anti-proliferative and defense against bacteria, fungi, and viruses. Terpenes directly detoxify ROS, cause membrane stabilization, and lead to stress-induced senescence. Alkaloids have an antioxidant potential of scavenging free radicals and inhibit H_2O_2-induced oxidation. Phenolic compounds efficiently scavenge ROS and act as potential nonenzymatic antioxidants. In addition to this, these improve plant metabolism, growth and development, seed germination, and biomass

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accumulation. Increase in secondary metabolite levels in response to various stresses has been reported in various plants, for example, *Catharanthus roseus*, *Hypericum perforatum*, *Artemisia annua*, *Rauvolfia tetraphylla*, *Solanum nigrum*, and *Achillea fragrantissima.*

Keywords

Phytochemicals · Secondary metabolites · Reactive oxygen species · Oxidative stress · Mitigation

20.1 Introduction

The recent times have aimed at production of more food and prevention of crop losses in order to meet the demands of ever-increasing human populations. Abiotic stresses (salinity, drought, heat, cold, UV radiation, heavy metal toxicity) affect a large portion of arable land, and these are expected to increase due to the impact of climate change and anthropogenic activities. Abiotic stresses affect plant growth and yield. These result in imbalances of cellular redox homeostasis with excessive reactive oxygen species (ROS) production. The balance between ROS generation and scavenge is disturbed in addition to an accelerated ROS propagation. This leads to the damage of vital macromolecules (nucleic acids, carbohydrates, proteins, and lipids) and eventually cell death. Protein damage by ROS is caused by amino acid oxidation resulting in disulfide bond formation (cysteine), irreversible carbonylation in side chains (arginine, lysine, and threonine), and methionine sulfoxide formation (methionine). ROS production hampers the CO_2 fixation in the chloroplasts and also reacts with chlorophyll resulting in the formation of chlorophyll triplet state which can rapidly give rise to singlet oxygen ($^1O_2$), thereby damaging the photosynthetic complexes (Hasanuzzaman et al. 2020; Sharma et al. 2019b). Phytochemicals are the biologically active components of plants that are derived from different parts of the plant such as leaves, barks, seed, seed coat, flowers, roots, and pulps and are used for various medical purposes (Ingle et al. 2017). These naturally occurring chemical compounds provide the plants with protection from various diseases and damage. Besides contributing to the plant's color, flavor, and aroma, these also protect the plants from environmental hazards such as various stresses including drought, salinity, UV exposure, pathogen attack, and pollution (Koche et al. 2016). Among the tens of thousands of phytochemicals, a small number have been identified and isolated. These phytochemicals include primary metabolites (amino acids, sugars, proteins, purines, and pyrimidines) and secondary metabolites such as polyphenols, flavonoids, lignans, plant steroids, carotenoids, phenolic acids, terpenoids, isoflavones, phenylpropanoids, and so on (Xiao and Bai 2019).

Secondary metabolites are powerful hydrogen-donating antioxidants that scavenge ROS and are accumulated in plants in response to various stresses such as ultraviolet B, salt, drought, and cold (Kusvuran 2021). Secondary metabolites play a photo protective role due to the presence of conjugative double bonds (Edreva et al.

2008). Secondary metabolite synthesis and concentration are affected by plant physiology, stage of development, genotype, and environmental factors. Secondary metabolite biosynthesis is accelerated during various potential stresses. Reportedly more than 100,000 secondary metabolites are synthesized in plants under various unfavorable conditions (Khare et al. 2020). Major secondary metabolites such as alkaloids, phenolics, terpenoids, and tannins are potential antimicrobial, antioxidant, anti-inflammatory, and anti-cancerous agents (Koche et al. 2016). Secondary metabolites help the plants in surviving various abiotic stresses such as drought and salinity. Salinization is one of the most destructive abiotic stresses which induces various morphological, physiological, and biochemical processes in a plant. Salinization results in high production of ROS leading to oxidative stress. In order to scavenge ROS, plants produce a large number of secondary metabolites (Yang et al. 2018). The activation of downstream pathways by drought stress is mainly the result of phytohormone synthetic networks that initiate the secondary metabolite biosynthesis. The drought stress is sensed by roots, and the signal is transmitted to the aboveground tissues that induce efficient phytohormone signaling. In addition to the scavenging of ROS, drought-induced secondary metabolites also trigger various mitigation functions in plant tissues. Rise in secondary metabolite levels in response to drought stress has been reported in various medicinal plants, for example, *Catharanthus roseus*, *Hypericum perforatum*, and *Artemisia annua* (Jogawat et al. 2021).

Water scarcity led to an increase in the quantity of secondary metabolites artemisinin in *Artemisia* and betulinic acid, quercetin, and rutin in *Hypericum brasiliense*. Water deficiency decreased the total flavonoid content in *Glechoma longituba*. Salinity causes both ionic and osmotic stress in plants and leads to an increase or decrease of secondary metabolites. Salt stress increased the reserpine and vincristine (alkaloids) concentration in *Rauvolfia tetraphylla* and *C. roseus*, respectively. An increase in alkaloid content in *Solanum nigrum*, *C. roseus*, and *Achillea fragrantissima* due to salt stress has been reported. Increased salinity resulted in an increase in phenolic acid in *A. fragrantissima* (Verma and Shukla 2015). A significant contribution of various flavonoids (catechin, naringenin, ruitin, quercetin) in the upregulation of enzyme activity of Halliwell-Asada pathway specially glutathione reductase (GR) and dehydroascorbate reductase (DHAR) has been reported in salt-tolerant rice cultivar. This resulted in better ROS scavenging and mitigation of oxidative stress (Banik and Bhattacharjee 2020). *Casuarina glauca* can thrive under extreme environments like high salinity. It was seen that in addition to an effective antioxidant machinery, a flavonoid-based secondary antioxidant system was activated by *C. glauca* plants in order to cope with the oxidative stress thereby enhancing the plant tolerance (Jorge et al. 2019).

20.2 Phytochemicals

On the basis of biological requirements, phytochemicals are broadly classified as primary metabolites and secondary metabolites. The primary metabolism in plants leads to the production of primary metabolites like fats, carbohydrates, amino acids, and nucleic acids. Primary metabolites are required for important biological functions such as growth, development, and reproduction of plant cell. Secondary metabolites are produced via secondary metabolism processes, and they in return provide the plant with protection against pathogens, herbivores, and a number of abiotic stresses (Velu et al. 2018). Secondary metabolites are low molecular weight compounds that are abundant in the plant kingdom. Secondary metabolites are very specific and are found in large numbers among the various groups of plants. These are synthesized from primary metabolites in different chemical configurations by regulating the primary pathway metabolism. The three major classes of secondary metabolites present in edible plants are terpenoids, phenolic compounds, and nitrogen- and sulfur-containing compounds like alkaloids and glucosinolates. Phytochemicals like anthocyanins give color, and volatile phytochemicals like monoterpenes, sesquiterpenes, and some phenylpropanoids are responsible for taste and aroma (Holopainen et al. 2018).

Terpenes also known as terpenoids are the largest class of secondary metabolites that are generally insoluble in water. The basic structural element of terpene is known as an isoprene unit, and all the terpenes are derived from the union of a five-carbon atom having a branched carbon skeleton. Phenolic compounds contain a phenol group-hydroxyl group attached to a carbon atom in a benzene ring. The various derivatives of phenolic compounds are simple phenylpropanoid, benzoic acid derivatives, anthocyanin, isoflavones, tannins, lignin, and flavonoid compounds. Secondary metabolites having nitrogen in their structure include alkaloids, cyanogenic glucoside, and glucosinolate. In alkaloids the nitrogen atom is usually a part of the heterocyclic ring containing both nitrogen and carbon atoms (Anulika et al. 2016). Secondary metabolites do not play any role in the primary metabolic requirements of the plant, but they play an important role in increasing the plant's ability to withstand the environmental challenges. These play protective roles such as antioxidative, free radical scavenging, UV absorbing, and antiproliferative and defense against bacteria, fungi, and viruses (Kennedy and Wightman 2011). Plant's interaction with their living environment takes places via secondary metabolites. For example, polyphenols help in nutrient mobilization and also in root to shoot signal transduction. Phenolic compounds in the root exudates alter the physiochemical properties of the rhizosphere. Phenolics help in humus formation, nitrogen fixation, and nutrient uptake via the chelation of metallic ions; enhanced active absorption sites; and accelerated mobilization of elements calcium (Ca), magnesium (Mg), potassium (K), zinc (Zn), iron (Fe), and manganese (Mn). Flavonoids play an important role in the maturation of the pollen (Sharma et al. 2019a). In the plant, secondary metabolites can be present in the root, stem, leaves, or the bark, depending on the type of secondary metabolite. Secondary metabolites have a restricted distribution in the plant kingdom, that is, a particular secondary metabolite can be one in a single or related group of species (Anulika et al. 2016). Secondary metabolites are translocated from one cell to another via xylem or phloem. These are accumulated in vacuoles, idioblasts, laticifers, trichomes, and ducts (Hadacek 2002). In plants the biosynthesis and accumulation of secondary metabolites are influenced by various genetic, ontogenic, morphogenetic, and environmental factors (light, temperature, salinity, soil water, soil fertility) which imply toward their role in the adaptation and survival of the plant during various environmental stresses (Yang et al. 2018). Secondary metabolite biosynthesis is organ, cell, or development dependent, and the regulation of genes involved in the biosynthesis is linked to various environmental, seasonal, and external factors. Alkaloids, quinolizidines, caffeine, and some terpenes are reportedly synthesized in the chloroplast, while the biosynthesis of lipophilic compounds is linked to endoplasmic reticulum. After the synthesis in a single organ such as roots, roots, or leaves, secondary metabolites are transported within the plant via xylem and are stored according to their polarity. Alkaloids, glycosylates, and tannins being hydrophilic compounds are stored in vacuoles or idioblasts, while terpene-based lipophilic compounds are stored in trichomes, glandular hairs, resin ducts, thylakoid membranes, or on the cuticle. Some defense-related compounds (alkaloids, flavonoids, cyanogenic glycosides, coumarins) may be stored in the epidermis itself. The storage can be tissue or cell specific. Reportedly in annual plants secondary metabolites are mostly stored in flowers, fruits, and seeds. On the other hand, bulbs, roots, rhizomes, and barks in perennial species contain high levels of secondary metabolites (Acamovic and Brooker 2005). Secondary metabolites provide the plants with adaptive capacity by the production of complex chemical types and improving structural and functional stabilization. Plant bioactive compounds are high in nutritional value and are used as flavoring agents and pharmaceuticals having industrial value. Recent studies have suggested the antioxidant and anti-radical role of plant secondary metabolites in oxidative stress defense response. This involves the production of lipoic and ascorbic acid, flavonoids having o-dihydroxy group (carotenoids, arylamines, quercetin), and various aliphatic and unsaturated fatty acids. The defense response system in the family Solanaceae leads to the production of sesquiterpenes; Brassicaceae members produce glucosinolate-myrosinase; Fabaceae produce isoflavones; Rutaceae and Meliaceae produce limonoids. Flavonoids, phenolics, and polyphenolics play a vital role in plant antioxidant responses, signaling, pigment, and lignin biosynthesis (Isah 2019). Various elicitors or signal molecules and abiotic stresses induce the secondary metabolite production. Elicitors are biotic or abiotic factors that induce physiological and morphological responses in the plants and accumulation of secondary metabolites such as phytoalexin. The abiotic elicitors comprise of various chemicals, metal ions, and inorganic compounds, while the fungi, bacteria, viruses, and herbivores are biotic elicitors. Elicitors can act as avirulent determinants and are recognized by plant receptors (R-proteins) that are localized on the plasma membrane or cytoplasm. Elicitors cause changes in receptor conformation leading to the activation of their corresponding effectors (ion channels, G-proteins, lipases, and kinases). These initiate signaling pathways and various defense reactions such as synthesis of defense secondary metabolites (Zhao et al. 2005). More than 100,000 secondary metabolites are produced by the limited taxonomic groups of the plant kingdom. On the basis of photosynthetic pathway, these are grouped into terpenes (or isoprenoids), phenolic compounds (phenylpropanoids and flavonoids), and nitrogen-containing compounds (alkaloids, glucosinolates, and cyanogenic glycosides). The factors influencing the concentration of secondary metabolites are broadly divided into four groups: (1) genetic, (2) ontogenic, (3) morphogenetic, and (4) environmental. The environmental factors include several abiotic factors like temperature, drought, salinity, seasonality, circadian rhythm, altitude, light, UV radiation, metal ions, wounding, and nutrient deficiencies and biotic factors (pathogen attack) (Verma and Shukla 2015).

20.2.1 Terpenoids

Terpenes or terpenoids are the largest group of secondary metabolites, comprising more than 25,000 substances. These are generally insoluble in water and are synthesized from primary metabolites by two pathways, that is, mevalonate pathway (joining three molecules of acetyl-CoA) and methylerythritol 4-phosphate (MEP) pathway. Terpenoids are polymeric isoprene derivatives that are synthesized from acetyl coenzyme-A (acetyl Co-A). The number of isoprene units (C5 units) that are linked in head to tail fashion is the basis for their classification. Monoterpenes (ten carbons or two C5 units), sesquiterpenes (three C5 units), diterpenes (four C5 units), triterpenes (six C5 units), tetraterpenes (eight C5 units), and polyterpenoids ([C5]n). Terpenes include menthol (monoterpenes); gibberellins (diterpenes); sterol, digitoxin, digoxin, gitoxin, and brassinosteroids (triterpenes); carotene (tetraterpenoids); and rubber (terpene polymers) (Bhatla 2018). Essential oils are chiefly composed of monoterpenes. These are the major components of Angelica species (73%), hyssop (70%), rose (54%), and mint (45%). In essential oils monoterpene hydrocarbons (α- and β-pinene, limonene, Δ3-carene, and myrcene) are present as complex mixtures. Like monoterpenes, sesquiterpenes are also essential oil components and are predominantly found in cedarwood (98%), vetiver (97%), sandalwood (90%), patchouli (71%), myrrh (62%), and ginger (59%). These are most prevalent in the family Asteraceae. Comparatively diterpenoids have a limited distribution. Gibberellic acid and phytol are the two universally distributed diterpenes. A relatively smaller group of terpenes are the sesterterpenoids which have both biomedical and synthetic applications. Triterpenes are of widespread distribution and include α- and β-amyrin and the derived acids, ursolic, and oleanolic acids. These are present in the waxy coatings of leaves and also on fruits. Tetraterpenoids and carotenoids function as accessory pigments in photosynthesis and also give color to fruits (mostly orange or red) and flowers (mostly yellow). Natural rubber is composed of polyterpenoids and is present as latex for example, rubber tree (*Hevea brasiliensis*, Euphorbiaceae) (Ludwiczuk et al. 2017). In plants volatile terpenes help in the mitigation of oxidative stress by modulating the oxidative status of plants. High reactivity of some monoterpenes and sesquiterpenes with ozone suggested the role of volatile organic compounds (VOC) in alleviation of oxidative stresses (Holopainen and Gershenzon 2010). One of the most prominent responses of stressed plants is the volatile emission profile. The reaction of terpenes with other oxidants mediates the abiotic stress protection in plants and is also involved in indirect ROS signaling. In response to photoinhibition, antioxidants such as tocopherol and carotenoids (zeaxanthin, neoxanthin, and lutein) can directly detoxify ROS. In stress conditions volatile terpenes in association with phytohormones mediate a process called premature or stress-induced senescence which may lead to senescence, apoptosis, and abscission in plants in addition to the economization of available resources. Due to their amphipathic nature, isoprene causes membrane stabilization by transiently inserting in the membrane thereby enhancing the hydrophobic interactions of large protein complexes between themselves or with membrane lipids (Boncan et al. 2020). Solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) analysis of volatile terpenes produced by rice seedlings subjected to oxidative stress by various abiotic factors revealed a mixture of monoterpenes (limonene, sabinene, myrcene, α-terpinene, β-ocimene, ɣ-terpinene, and α-terpinolene) emitted by rice seedlings in a time-dependent manner indicating toward the antioxidative roles of terpenes (Lee et al. 2015). A higher accumulation of oleuropein (terpene family secondary metabolite) in leaves and roots of salt-stressed olive tree suggests its role in the mitigation of oxidative stress. Diterpene, and carnosic acid in association with α-tocopherol and ascorbate (low molecular weight antioxidant molecules) protect the Labiatae species from oxidative damage induced by water stress (Akhi et al. 2021).

In plants, terpenes are involved in the adaptation to various biotic and abiotic stresses. Being volatile, these help the plants in communicating with neighboring plants, pollinators with the help of airborne infochemicals (Kabera et al. 2014). Aromatic monoterpenes are widely present in Asteraceae, Apiaceae, Burseraceae, Dipterocarpaceae, Lamiaceae, Myricaceae, Myristicaceae, Poaceae, Rutaceae, Verbenaceae, and resin of conifers. In flowering plants these are used to attract the pollinating arthropods. These are extracted from plants as essential oils and are used in perfumes and phytomedicine for treating rheumatism, infections (bacterial, fungal), cold, and intestinal spasms. Carotenoids are the most important tetraterpenes which are used as accessory pigments for photosynthesis, provide the plants with protection against UV light, and are powerful antioxidants (Wink 2015). Isoprene and monoterpene substantial emissions protect various vascular and nonvascular plants from thermal stress. These volatile compounds are present in an intercalation with the photosynthetic membranes and hence enhance the membrane functionality. Volatile monoterpenoids are known to protect photosynthetic tissues of *Chrysanthemum morifolium* via their herbivore deterrent effects. Volatile terpene mixtures can also act as interspecific, intraspecific, and intraplant signals in order to induce defense responses in tissues of the same plant or neighboring plants (Tholl 2015).

20.2.2 Alkaloids

The most active secondary metabolites include the alkaloids which are widely distributed in the plant kingdom especially in the angiosperms. These are composed of one or numerous nitrogen atoms. When the alkaloids are derived from amino acids and the nitrogen atoms are arranged in a ring structure, these are known as true alkaloids (e.g., nicotine, atropine), while when present in a side chain, without an amino acid origin, these are known as pseudoalkaloids (caffeine, theobromine, etc.). True alkaloids are further divided into several subtypes on the basis of the ring structure (Wink 2015). Contrary to other secondary metabolites, alkaloids have independent biosynthetic pathways and may originate from different amino acids. Alkaloids play a defensive role in plants as they are generally toxic and inhibit herbivory. Some important alkaloids like morphine, berberine, vinblastine, and scopolamine are known to have important medicinal properties, while others like cocaine, caffeine, and nicotine are used as stimulants or sedatives (Olivoto et al. 2017). Approximately 5500 alkaloids are known by far, and these are included in the most efficient, diverse, and therapeutically important plant substances. Flowering plants, that is, angiosperms, are the major sources of alkaloids, and as low as 20% plant species contain alkaloids. These are extremely toxic and bitter but have a remarkable therapeutic effect when used in small quantities. They protect the plants against various microorganisms (bacteria and fungi), provide feeding deterrence against various insects and herbivores, and act as allelochemicals against other plants. Biosynthetic precursor and heterocyclic ring system classify alkaloids into various categories such as indole, piperidine, tropane, purine, pyrrolizidine, imidazole, quinolizidine, isoquinoline, and pyrrolidine alkaloids (Roy 2017). Alkaloids have an antioxidant potential of scavenging free radicals and inhibit hydrogen peroxide (H_2O_2)-induced oxidation (Hasanuzzaman et al. 2020). Root alkaloids in *Catharanthus roseus* (L.) (rosea variety) increased significantly under drought stress (Jaleel et al. 2008a). *C. roseus* is a source of about 130 terpenoid indole alkaloids including vincristine and vinblastine, which are used as anticancer drugs. Chromium toxicity in the plant generated oxidative stress and resulted in antioxidant responses. This included an increase in concentration of vincristine and vinblastine alkaloids, which shows their good antioxidant potential (Rai et al. 2014). Drought conditions led to oxidative stress in *Catharanthus roseus* (L.) and subsequent antioxidant responses. The alkaloid ajmalicine significantly increased in drought-stressed *C. roseus* plants (Jaleel et al. 2008b). Drought stress in *Senecio jacobaea* and *Senecio aquaticus* led to the accumulation of pyrrolizidine alkaloids in the plants (Akhi et al. 2021).

20.2.3 Phenolics

A variety of secondary metabolites produced by the plants containing a hydroxyl functional group into an aromatic ring are known as phenolic compounds. This large group of compounds can be divided into five subgroups: coumarins, lignins, flavonoids, phenolic acids, and tannins. Among these lignins and flavonoids are the most widespread in plants. Phenols are synthesized by two basic metabolic pathways: (1) shikimic acid/phenylpropanoid pathway and (2) malonic acid pathway. Majority of the phenolic compounds are synthesized from phenylalanine which is a product of the shikimic acid pathway. As a result, these are also known as phenylpropanoids. Phenylalanine is formed by the combination of erythrose 4-phosphate with phosphoenolpyruvate (PEP). It is then converted into *trans*-cinnamic acid by phenylalanine ammonia-lyase (PAL). Further this pathway also leads to the formation of various other phenolic compounds such as flavonoids, coumarins, lignans, hydrolysable tannins, monolignols, and lignins. Flavonoids are potential antioxidants and are further classified into anthocyanins, flavones, flavonols, and isoflavones. Under optimal and suboptimal conditions, phenolics produced by the plants play important roles in plant developmental processes such as cell division, photosynthetic activity, hormonal regulation, reproduction, and nutrient mineralization. In addition to this plant phenolics impact various physiological processes that enhance tolerance and adaptability of plants under stressed conditions (Olivoto et al. 2017; Sharma et al. 2019a; Cheynier et al. 2013). Abiotic stresses affect plant growth and result in reduced crop production and crop yield by 50% and 70%, respectively. Secondary metabolism stimulation in plants improves crop performance, and plants accumulate various polyphenols in order to sustain various unfavorable environments. Phenolic concentration in plant tissue can be used as a good indicator to determine the abiotic stress tolerance in plants. Phenols improve plant metabolism, growth and development, seed germination, and biomass accumulation. Accumulation of phenolic compounds is a consistent feature of stress conditions which improves tolerance and adaptability (Sharma et al. 2019a).

Phenolic compounds efficiently scavenge ROS and act as potential nonenzymatic antioxidants. Owing to their ideal chemical structure, polyphenols are more efficient antioxidants in vitro than tocopherols and ascorbate. The antioxidative properties of polyphenols are attributed to their high reactivity as hydrogen and electron donors, chain breaking function (stabilization and delocalization of the unpaired electrons by the polyphenol derived radicals), and chelation of the transition metals (Fenton reaction). The oxidation of lipids and other molecules is prevented by phenolic antioxidants (PhOH) by rapid donation of hydrogen atoms to radicals, that is, $ROO\bullet + PhOH \rightarrow ROOH + PhO\bullet$. The phenoxy radical thus formed is relatively stable and does not further initiate radical reactions. Flavonoids on the other hand are important phenolic compounds that have the ability to prevent peroxidation by modifying the lipid packing order and decrease the fluidity of the membranes. This causes a delay in the diffusion of free radicals, thereby restricting peroxidative reactions. The functionality of flavonoids depends on the number and arrangement of their hydroxyl groups attached to ring structures (Tiwari and Sarangi 2015). Study on *Moringa oleifera* provided a reaffirmation on the involvement of phenolic compounds as the first line of defense against oxidative stress (Ramabulana et al. 2016). Arsenic (As) phytotoxicity in *Oryza sativa* L. was ameliorated by selenium by the modulation of phenolic compounds and enhanced uptake of nutrient elements. The increased phenolic compounds include gallic acid, protocatechuic acid, ferulic acid, rutin, and thiol metabolism-related enzymes (Chauhan et al. 2017). The toxicity of free radicals is significantly reduced by phenolic compounds. Nitric oxide applied to salt-stressed broccoli plants enhanced the total phenolics, chlorophyll a, glycine betaine, and activities of antioxidant enzymes. This resulted in lowering of H_2O_2 and malondialdehyde (MDA) levels (Akram et al. 2020). The use of phenolic acids (gallic acid, ferulic acid, p-coumaric acid, caffeic acid, and salicylic acid) as priming agents had stress alleviating effects on water-stressed wheat seedlings. These lead to an upregulation of antioxidant enzyme activity with an increase in total phenolic content resulting in a decrease in H_2O_2 and MDA levels and alleviating oxidative damage induced by drought stress (Bhardwaj et al. 2017).

20.3 Oxidative Stress in Plants

Plants are sessile and are exposed to a number of environmental stresses throughout their life span. These environmental constrains include pathogen attack, temperature fluctuations, water scarcity, salinization, and high light intensity. When these factors exceed a certain limit, plants undergo stress. This stress leads to reduced plant growth and lower productivity. Environmental stresses are responsible for crop losses worth billions of dollars. Environmental stresses can either be biotic or abiotic. Biotic stress includes pathogenic microorganisms, predators, fungi, etc. Abiotic stress is caused by air pollutants, drought, salinity, high light intensity, and extreme temperatures. Abiotic stress causes various physiological and molecular changes in the cells including reduced photosynthetic activity, excessive ROS production, turgor loss, changes in membrane fluidity, composition, concentration of solutes, accumulation of organic solutes, and specific osmolytes. ROS detoxification and cell homeostasis are maintained by accumulation of osmoprotectants (amino acids, polyols, quaternary ammonium, and tertiary sulfonium compounds) (Bartwal et al. 2013). Biotic and abiotic signal perception by plant cells leads to the activation of signaling pathways resulting in phosphorylation cascades, accumulation of jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), ethylene, nitric oxide (NO), and ROS generation. Phytohormone ABA plays important role in abiotic signaling, while JA, SA, NO, and ethylene are involved in biotic signaling (Fujita et al. 2006). Ca^{2+} and ROS are the secondary messengers that play important roles in stress signaling and secondary metabolite production (Qiao et al. 2021). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) include radical species such as hydroxyl radical ($^\bullet OH$), superoxide radical anion ($O_2^{\bullet -}$), and nitric oxide radical ($^\bullet NO$), having unpaired electrons and exhibiting short biological half-lives as well as non-radical species like singlet oxygen ($^1O_2$), peroxynitrite ($ONOO^-$), and hydrogen peroxide (H_2O_2), which can also be transformed into some of the mentioned radical species. When the production of ROS and RNS exceeds the antioxidant capacity of cells, it leads to oxidative stress. Oxidative stress can be the cause of oxidative damage to the biomolecules like proteins, lipids, and deoxyribonucleic acid (DNA) (Tavares and Seca 2019). When the excessive production of reactive species exceeds antioxidant defenses, the redox balance is disturbed, and this gives rise to oxidative stress. Ion imbalance due to environmental challenges leads to high energy state electrons transferred to molecular oxygen giving rise to reactive forms such as singlet oxygen, hydroxyl radicals, superoxide ions, hydroxyl radicals, and peroxides (H_2O_2). Antioxidant defenses enzymatic and nonenzymatic components. Ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, and glutathione reductase (GR) form the enzymatic component. Nonenzymatic component comprises of vitamin E, glutathione, ascorbic acid, and a number of secondary metabolites that help in the mitigation of oxidative stress by scavenging excessive ROS (Matsuura et al. 2014). It has been reported that abiotic stresses cause more than 50% loss of average yields in major cereal crops, with 21% reduction reported in maize and 40% in wheat. Drought tolerance involves better osmoprotection and antioxidant capacity. Secondary metabolites and osmoprotectants reduce the detrimental effects of stress-induced ROS. During drought stress signaling networks in plants initiate secondary metabolite biosynthesis that scavenge the ROS and prevent lipid peroxidation. The drought-induced secondary metabolites also alert other plant tissues in order to perform stress mitigating functions. Secondary metabolites significantly enhance the plant growth and survival chances during stress conditions. In stressed environmental conditions such as drought, pyruvate and other intermediates of glycolysis pathway are diverted for SM synthesis via shikimic acid, melonic acid, and methylerythritol phosphate pathway (Jogawat et al. 2021). Highly active cell organelles like chloroplasts, mitochondria, and peroxisomes produce 1–2% of ROS out of total consumed $O_{2.}$ Chloroplasts are the main sites for ROS generation were increased ROS limits CO_2 fixation. ROS reaction with chlorophyll results in the generation of ($^1O_2$), which in turn damages photosynthetic systems. One to five percent of consumed O_2 in mitochondria leads to H_2O_2 formation which leads to the detrimental •OH production. Peroxisomes have a 2- to 50-fold higher concentration of H_2O_2 as compared to chloroplasts and mitochondria. Increased ROS production overwhelming the defense system results in oxidative stress, cell damage, and cell death (Das and Roychoudhury 2014).

20.4 Role of Phytochemicals in Oxidative Stress Mitigation

Secondary metabolites reportedly play an important role in plant tolerance and adaptation to various environmental stresses. Environmental factors such as altitude, salinity, high/low temperature, drought, and light conditions significantly influence the quality and quantity of secondary metabolites in plants. Combination of various environmental stresses disrupts cell homeostasis leading to an increase of reactive oxygen species (ROS) and ultimately oxidative stress. Oxidative stress causes oxidative damage to the plant, and many plant enzymes such as ascorbate peroxidase, catalase, superoxide dismutase, and glutathione reductase with antioxidative activity scavenge different ROS (superoxide, hydroxyl radicals, and singlet oxygen). Qualitative and quantitative increase in secondary metabolites (flavonoids, phenolic compounds, alkaloids, carotenoids, steroids, tannins, and terpenoids) was observed in endemic species grown at higher altitudes, where these withstand various abiotic stresses. Phenolic antioxidants trapped free radicals inhibiting lipid peroxidation, prevented the free radical diffusion, and restricted the peroxidative reactions. Tannins minimized the oxidative damage by scavenging the free radicals and thus increasing the adaptation to the oxidative stress (Hashim et al. 2020). The antioxidant and antiradical functions of secondary metabolites are attributed to the available –OH, –NH2, and –SH groups; aromatic nuclei; and unsaturated aliphatic chains. Flavonoids such as quercetin chelate the transition metals (Fe) and thus interfere with the production of ROS via Fenton reaction. Tobacco and bean when subjected water stress and heat shock showed accumulation of polyamines and phenyl amide formation with ROS scavenging ability. Similarly, anthocyanin accumulation in cotton leaves suffering Na/K imbalance results in higher ROS scavenging (Edreva et al. 2008). Tryptophan and terpene condensation by strictosidine synthase leads to the production of monoterpene indole alkaloids (MIA) which are important bioactive compounds. These include ajmalicine, camptothecin, vincristine, and vinblastine. Reportedly oxidative stress is directly liked to MIA biosynthesis. Osmotic stress in *C. roseus* cells leads to higher ajmalicine content, which implies that oxidative stress is significantly correlated with alkaloid production. Hence, MIA can play antioxidant responses in stressed plants (Matsuura et al. 2014). Increasing salinity led to a significant increase of phenolic compounds and flavonoids in black mustard. There was an increase of about 159% and 103% phenols and flavonoids, respectively, as compared to the non-saline condition (Ghassemi-Golezani et al. 2020). *Brassica juncea* under oxidative stress due to copper (Cu) toxicity showed a significant increase in the ascorbate and flavonoid levels. This indicates that these antioxidants act as internal chelators and stabilize toxic metal ions (Sharma et al. 2019c). Drought stress in safflower resulted in increased PAL activity which is a key enzyme for phenolics biosynthesis such as flavonoids and anthocyanins. The chemical nature of phenolic compounds protects the cells against oxidative stress by metal chelation and free radical binding. Flavonoids inhibit lipid peroxidation and increase membrane fluidity and stability hence preventing the ROS release (Chavoushi et al. 2020). The increased phenolic and flavonoid contents in two pepper genotypes (BIB-6 and BIB-8) can be linked to their higher salt tolerance (Kusvuran et al. 2021).

Reportedly isoprene biosynthesis in plants is activated by high temperature, solar radiance, and water scarcity. Isoprene is capable of quenching various ROS and NOS species and enhancing the thylakoid membrane stability. O_3 stressed *Salvia officinalis* plants showed enhanced biosynthesis of phenylpropanoids especially rosmarinic acid which serves as an effective antioxidant because of its two catechol groups in the benzene rings. It is concluded that amplified biosynthesis and accumulation of phenolic compounds may serve as secondary antioxidant defense system and compliment the primary antioxidants in order to protect the plants against the oxidative injury. Superior heat tolerance in transgenic sweet potato plants was attributed to higher carotenoid content especially β-carotene and cryptoxanthin. β-carotene is suitable for singlet oxygen scavenging, while cryptoxanthin maintains thylakoid membrane stability (Guidi and Tattini 2021). Drought and heat stress affect plant growth and productivity, and this combination of stress is one of the most recurring stresses in the natural environment. Two citrus species *Carrizo citrange* and *Cleopatra mandarin* withstanding heat and drought conditions showed the accumulation of various metabolites. In response to stress, *Cleopatra* showed increased accumulation of phenylpropanoids, flavonols, and glycosylated and polymethoxylated flavones (tangeritin). Accumulation of sinapic acid and sinapoyl aldehyde, which are the precursors of lignin, was seen in stress-tolerant *Carrizo*. The observed metabolite changes suggested the accumulation of antioxidative and photoprotective secondary metabolites in order to mitigate the detrimental effects of stress (Zandalinas et al. 2017). Barium toxicity leads to oxidative stress in plants. Barium stress in *Brassica juncea* and *Cakile maritima* leads to the activation of antioxidant defense system including the antioxidant enzymes and secondary metabolites. The metal chelating and ROS scavenging properties of phenols enable them to slow down the free radical formation. A significant increase in total phenols and flavonoids was seen especially in the aerial parts of the plants in order to overcome barium toxicity (Bouslimi et al. 2021). Melatonin application to drought-stressed Moldavian balm plant mitigated the oxidative stress directly and indirectly by increased concentration of nonenzymatic antioxidants (flavonoids, polyphenol compounds, anthocyanin) and enhancement of enzyme activity of phenylalanine ammonia-lyase and polyphenol oxidase (Naghizadeh et al. 2019). Stressful environments reportedly witnessed an upregulation of genes of key phenolic biosynthetic enzymes such as *PAL*, *C4H* (cinnamate 4-hydroxylase), *4CL* (4-coumarate: CoA ligase), *CHS*, *CHI* (chalcone isomerase), *F3H* (flavanone3-hydroxylase), *F3′H* (flavonoid3′ hydroxylase), *F3′5′H* (flavonoid 3′5′-hydroxylase), *DFR* (dihydroflavonol 4-reductase), *FLS* (flavonol synthase), *IFS* (isoflavone synthase), *IFR* (isoflavone reductase), and *UFGT* (UDP flavonoid glycosyltransferase) (Sharma et al. 2019a). Triterpenes, ursolic acid (UA), oleanolic acid (OA), phenolic acid, and rosmarinic acid (RA) increased in *Prunella vulgaris* L. species under drought stress which indicates toward and important antioxidant response toward drought stress (Chen et al. 2011). Increased phytoene synthase and β-lycopene cyclase expression led to an increased accumulation of carotenoids in *Daucus Carota* and *Bixa Orellana* in response to salt stress. Salinity stress in cotton showed an increase in tannic acid, flavonoids, and gossypol. *Rauvolfia tetraphylla* and *Catharanthus roseus* showed an accumulation of alkaloids reserpine and vincristine under salt stress. Cu toxicity in *Panax ginseng Meyer* and *Withania somnifera* L. led to an increase in phenolic and lignin components. Drought-caused oxidative stress in willow leaves led to an increase in flavonoids and phenolic acids (Khare et al. 2020). Foliar spray of ascorbic acid on water stressed common bean (*Phaseolus vulgaris* L.) helped in the mitigation of adverse drought effects by enhancing photosynthetic pigments (carotenoids and chlorophyll) and secondary metabolites content (flavonoids, phenolics and tannins) (Gaafar et al. 2020). Monoterpene indole alkaloid, brachycerine, is an antioxidant which is induced by osmotic stress, wounding, metal stress, and abscisic acid. Heat stress in *Psychotria brachyceras* led to a 4.5-fold increase in brachycerine concentration which mitigated oxidative stress by reducing lipid peroxidation and hydrogen peroxide concentration (da Silva Magedans et al. 2017). The salt tolerance mechanism in safflower (*Carthamus tinctorius* L.) is attributed to increased antioxidant enzyme activities, osmolytes, and accumulation of secondary metabolites (flavonoids) (Gengmao et al. 2015). Volatile isoprenoids provide the plants with protection against the oxidative stress caused by high light, temperature, drought, and oxidizing conditions of the atmosphere. These help the plants in withstanding the stress by (1) stabilization of membrane, (2) direct scavenging of oxidizing species by isoprenoids, and (3) indirect role in ROS signaling (Vickers et al. 2009).

20.5 Conclusion

Various biotic (herbivores, pathogens) and abiotic factors such as drought, salinity, and extreme temperatures are potential factors that affect the pathways of several plant secondary metabolites. In response to stress, plants synthesize various secondary metabolites that aid in plant adaptation. This can be attributed to their structural diversity and function. Recent literature reports suggest that plant secondary metabolites play a crucial role in stress responses and act as potential antioxidants that help in coping with oxidative stress. Secondary metabolites play an active role in the inhibition of photooxidation of chlorophyll, detoxification of free radicals and hence provide protection against lipid peroxidation. Manipulation of production and action of secondary metabolites by the improved use of biotechnology can help in enhancing the tolerance level and adaptiveness of sensitive plants under stress.

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