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Reactive Oxygen Species: Generation, Damage, and Quenching in Plants During Stress

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Introduction

Oxygen in the Earth's atmosphere is vital for the existence of most life forms, although in its ground state O_2 is relatively unreactive. During normal metabolic activities it is changed into reactive oxygen species (ROS). ROS are generated by normal cell metabolism, but production is accelerated under stress conditions and therefore equilibrium between ROS generation and removal may be disturbed (Apel and Hirt, 2004). Numerous defense strategies are employed by organisms to cope with the various stresses to which they are subject, and plants have evolved a range of physiological and biochemical adaptations to enable them to endure those stresses (Hirayama and Shinozaki, 2010). ROS are utilized for various cellular metabolic activities such as photosynthesis and respiration, in chloroplasts, peroxisomes, and mitochondria. Stress may arise from natural as well as anthropogenic activities, and a common feature of different stresses is increased ROS production. In plants, besides normal cellular metabolism, large quantities of ROS are generated as a result of numerous biotic and abiotic stress factors such as ozone pollution, ultraviolet-B radiation, drought, extreme temperatures, salinity, heavy metals, and pathogens (Arora *et al.,* 2002; V.P. Singh *et al*., 2011; Tripathi *et al.,* 2012a,b, 2016a,b, 2017a–c; S. Singh *et al.,* 2015, 2017; R. Singh *et al.,* 2016). ROS damage plant function by causing lipid peroxidation, protein oxidation, and damage to nucleic acids, enzyme suppression, and stimulation of programmed cell death. Under normal conditions, ROS generation in cell compartments remains low. However, during stress ROS generation can exceed a certain level, thereby disrupting cellular homeostasis and enhancing ROS production (Sharma *et al.,* 2012).

Plants are naturally equipped with antioxidative defense systems consisting of an array of enzymatic and non-enzymatic antioxidants capable of dissipating ROS. For the maintenance of plant vigor, equilibrium between ROS production and their detoxification is required. Imbalance between ROS production and detoxification results in oxidative damage particularly to DNA, proteins, and lipids (Tripathy and Oelmüller, 2012). However, ROS are not simply a harmful product generated during stress and makes the system imperfect

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rather acts an important role in the signaling (Reczek and Chandel, 2015). ROS function as important signaling molecules in regulating various processes such as growth, development, reaction to biotic and abiotic stresses, and programmed cell death (Ahmad *et al.,* 2008). Sensing of ROS occurs in various cellular compartments, and the interaction of ROS with their target molecules stimulates the expression of genes involved in the signal pathways (Laloi *et al.,* 2004).

Abiotic stress involves environmental stress factors such as ozone pollution and increased ultraviolet-B radiation. Other stress factors like heavy metals, salinity, extreme temperature, drought, and pathogens are also potential generators of ROS. The chief sites of ROS production in plants are mitochondria, chloroplasts, and peroxisomes. Apart from reporting the destructive roles of ROS, this chapter elaborates the quenching and combating of ROS-induced oxidative stress. We provide a comprehensive overview of the plant's antioxidative defense system, involving antioxidants (enzymatic and non-enzymatic) capable of detoxifying ROS by acting as an integrative network, using a series of redox reactions. Enzymatic antioxidants comprise of catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GP), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). Non-enzymatic antioxidants include ascorbate (AsA), glutathione (GSH), carotenoids, tocopherols, and phenolics. Normal plant functioning is achieved by maintaining the equilibrium between production of ROS and the plant's antioxidative capabilities. The versatile character of ROS is also overviewed to depict their role as ubiquitous "signaling molecules." The signaling functions of ROS and ROS-induced redox reactions are also described along with the gene expression. ROS signaling is assimilated with many signaling networks in plants, such as calcium signaling, protein kinase networks, cellular metabolic networks, and redox responses (Mittler *et al.,* 2011).

Types of ROS

ROS are derivatives of O_2 and are mainly produced in mitochondria, peroxisomes, and chloroplasts of plant cells. ROS play both harmful and beneficial roles, depending upon their formation and accumulation rates in plant cells. Increased ROS generation is liable to cause damage to plant cells; however, low concentrations lead to the initiation of numerous beneficial reactions in plants. In plants, out of the total O_2 consumed, about 1% is destined for production of the various ROS (Asada and Takahashi, 1987). When O_2 absorbs adequate energy, its unpaired electron gets activated and starts reverse spin leading to formation of singlet oxygen, $^1O_2$, while monovalent reduction leads to formation of the superoxide radical, $^{\cdot}O_2^-$, hydrogen peroxide, H_2O_2, and the hydroxyl radical, $^{\cdot}OH$ (Apel and Hirt, 2004). Here, we provide details of the various types of ROS formed inside plant cells.

Superoxide Radical ($O_2^{\cdot-}$)

Superoxide radical is mainly produced in the thylakoid membrane of chloroplasts by photosystem system I (PSI). Reaction of O_2 with cytochrome *c* oxidase and the alternative oxidase leads to release of H_2O and transfer of four electrons. However, when O_2 reacts with other components of the electron transport chain (ETC) only one electron is transferred, leading to $O_2^{\cdot-}$ formation (Puntarulo *et al.,* 1988). This $^{\cdot}O_2^-$ is further responsible for $^{\cdot}OH$ and $^1O_2$ formation (Elstner, 1987; Halliwell, 2006). After protonation this $O_2^{\cdot-}$ forms HO_2^-, which

attacks polyunsaturated fatty acids (PUFA) causing lipid peroxidation (Bielski *et al.,* 1983). $O_2^{\cdot-}$ is also transformed into $^{\cdot}OH$ via the Haber–Weiss and Fenton reactions.

Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide in plants is generated mainly during photosynthesis and photorespiration. H_2O_2 is relatively stable and plays very important roles inside plant cells. Slesak *et al.* (2007) speculated on the involvement of H_2O_2 in the evolution of photosystem II (PSII) on Earth. At low concentrations, H_2O_2 triggers tolerance mechanisms in plants against various stress factors, whereas programmed cell death (PCD) is the result of higher H_2O_2 accumulation (Quan *et al.,* 2008).

Hydroxyl Radical ($^{\cdot}OH$)

Hydroxyl radicals are highly reactive ROS and can easily react with lipids, proteins, and DNA. ·OH can also be produced by the transformation $O_2^{\cdot-}$ and H_2O_2 in the manifestation of some transition metals like Fe, through Fenton's reaction (Gill and Tuteja, 2010). As plants lacks the enzymatic mechanisms for quenching $^{\cdot}OH$, it can ultimately lead to cell death (Gill and Tuteja, 2010).

Singlet Oxygen ($^1O_2$)

This is an excited state of oxygen not related to electron transport to O_2. The chlorophyll triplet state due to uneven energy dissipation during photosynthesis is liable to cause $^1O_2$ formation (Gill and Tuteja, 2010). Also low concentration of intercellular CO_2 inside chloroplast could also leads to $^1O_2$ formation (Gill & Tuteja, 2010). The entire photosynthetic machinery, along with PSI and PSII, experiences severely damaging effects due to $^1O_2$. It has the capacity to react with different biomolecules such as proteins, lipids, nucleic acids, pigments, and so forth, and can alter PSII activity, finally causing cell death (Wagner *et al.,* 2004; Krieger-Liszkay *et al.,* 2008). Tocopherol, plastoquinone, and β-carotenes are well recognized quenchers of $^1O_2$. When this ROS is not quenched inside plant cells, it upregulates various defense genes against photo-oxidative damage (Krieger-Liszkay *et al.,* 2008).

ROS Production in Plants

Photosynthesis in sunlight is the main source of energy for plants. Oxidative strain is a usual phenomenon for all living beings. Plants are at risk of oxidative damage mainly due to the presence of photoreceptors, oxygenic environments, and PUFA in the chloroplasts (Gill and Tuteja, 2010). During light conditions chloroplasts and peroxisomes are key sites of ROS production (Foyer and Noctor, 2003), while mitochondria are chief sources during darkness (Møller, 2001). In this section, we discuss the various sites of ROS production.

Chloroplasts

The chloroplast is well organized and equipped with thylakoid membranes and light-harvesting complexes (Pfannschmidt, 2003). ROS ($O_2^{\cdot-}$, $^1O_2$, and H_2O_2) are generated in the chloroplast at various sites, but PSI and PSII related to ETCs are target sites. Various stress conditions lead to CO_2 limiting conditions, mainly liable for ROS generation. During normal conditions, electrons flow from excited photosystems to $NADP^+$, reducing it to NADPH, which

subsequently reduces the ultimate electron acceptor, CO_2, in the Calvin–Benson cycle. During stressed conditions electron transport flows through ferredoxin to O_2, and this diverted flow of electrons leads to $O_2^{\cdot-}$ formation via the Mehler reaction (Wise and Naylor, 1987; Elstner, 1991). Further PSII studies have confirmed the participation of quinone A (QA) and quinone B (QB) in $O_2^{\cdot-}$ production (Takahashi *et al.,* 1988). Under low light conditions $^1O_2$ is the regular by-product at PSII (Buchert and Forreiter, 2010). H_2O_2 formation at the stromal membrane is the result of $O_2^{\cdot-}$ dismutation by Cu/Zn-SOD (Takahashi *et al.,* 1988). Further, H_2O_2 may be transformed to $\cdot OH$ depending upon the availability of Fe^{2+} at Fe-S centers with the help of the Fenton reaction.

Peroxisomes

Peroxisomes are small, spherical organelles bounded by a single lipid bilayer. They are concerned with the oxidation of long-chain fatty acids, and are major sites for ROS generation in plants. There are two main sites of $O_2^{\cdot-}$ production in the peroxisome, within the organelle's matrix and on the peroxisome membrane (Del Río *et al.,* 2002). In the matrix $O_2^{\cdot-}$ is produced due to the action of xanthine oxidase (XOD) during the tranformation of xanthine as well as hypoxanthine to uric acid (Corpas *et al.,* 2001). Generation of $O_2^{\cdot-}$ on the peroxisome membrane involves cytochrome *b* and NADH (with flavoprotein) along with the participation of MDHAR (Del Río *et al.,* 2002). H_2O_2 generation in peroxisomes is mainly dependent on the glycolate oxidase response, the β-oxidation of fatty acids, and the reactions of flavin oxidases and imbalance of $O_2^{\cdot-}$ (Huang *et al.,* 1983; Del Rio *et al.,* 2002, 2006).

Mitochondria

Mitochondria are the cell's "powerhouses" and are also major sites of ROS production, mainly at various sites in the ETC. ROS generation inside mitochondria is a general phenomenon, but various stresses causes modification of electron carriers in the ETC leading to enhancement of ROS formation (Noctor *et al.,* 2007; Blokhina and Fagerstedt, 2010). Complex I and III are identified as a major site for $O_2^{\cdot-}$ production in mitochondrial ETC, and this $O_2^{\cdot-}$ is further reduced to H_2O_2 by SOD activity (Raha and Robinson, 2000; Sweetlove and Foyer, 2004; Quan *et al.,* 2008). According to Møller (2001) about 1–5% of O_2 consumption by mitochondria is related with H_2O_2 generation, which further generates highly toxic $^{\cdot}OH$ by reaction with Fe^{2+} and Cu^+; these $^{\cdot}OH$ radicals are capable of migration from mitochondria through membrane penetration (Sweetlove and Foyer, 2004; Rhoads *et al.,* 2006). Any stress can change the physiology of plants, and mitochondria can play a principal role in controling ROS generation by energy dissipating systems (Gill and Tuteja, 2010).

Other Sources

There are also various other known sites of ROS formation in plants, like endoplasmic reticulum, plasma membrane, cell wall, and apoplast. In endoplasmic reticulum, cyt P450 with flavoproteins leads to ROS formation (Dybing *et al.,* 1976). Oxidoreductases, which take part in electron transport, are commonly present on plasma membranes and are mainly responsible for ROS production. NADPH oxidase has a major role in ROS metabolism during stress conditions in plants (Kwak *et al.,* 2003; Apel and Hirt, 2004). Cell walls are also a potential site, where NADH and peroxidases lead to ROS formation (Gross, 1977). Apoplastic ROS generation depends on enzymes of the cell wall (Apel and Hirt, 2004; Heyno *et al.,* 2011), for instance, oxalate oxidase leads to H_2O_2 generation from oxalic acid in the apoplastic region (Wojtaszek, 1997). Further studies have confirmed that generation of $\cdot OH$ in the apoplast is mainly dependent on cell wall peroxidases (Heyno *et al.,* 2011).

Various Stresses Generate ROS in Plants

ROS formation is a normal phenomenon for all living beings throughout their life cycle. Plants subject to stress conditions undergo increased ROS formation (Sharma *et al.,* 2010). Numerous stresses causes oxidative stress in plants and consequent stimulation of the antioxidative defense system (enzymatic and non-enzymatic) to provide protection against ROS (Figure 5.1). ROS production under several stress factors is discussed in this section.

UV-B Stress

UV-B radiation coming from the Sun to the Earth's surface is detrimental to plant growth, physiology, and yield (Agrawal and Rathore, 2007; Choudhary and Agrawal, 2015). Decreases in leaf size, chlorophyll content, photosystem II activities, stomatal conductance, and chlorophyll fluorescence have been observed (Choudhary and Agrawal, 2014a; Kakani *et al.,* 2003).

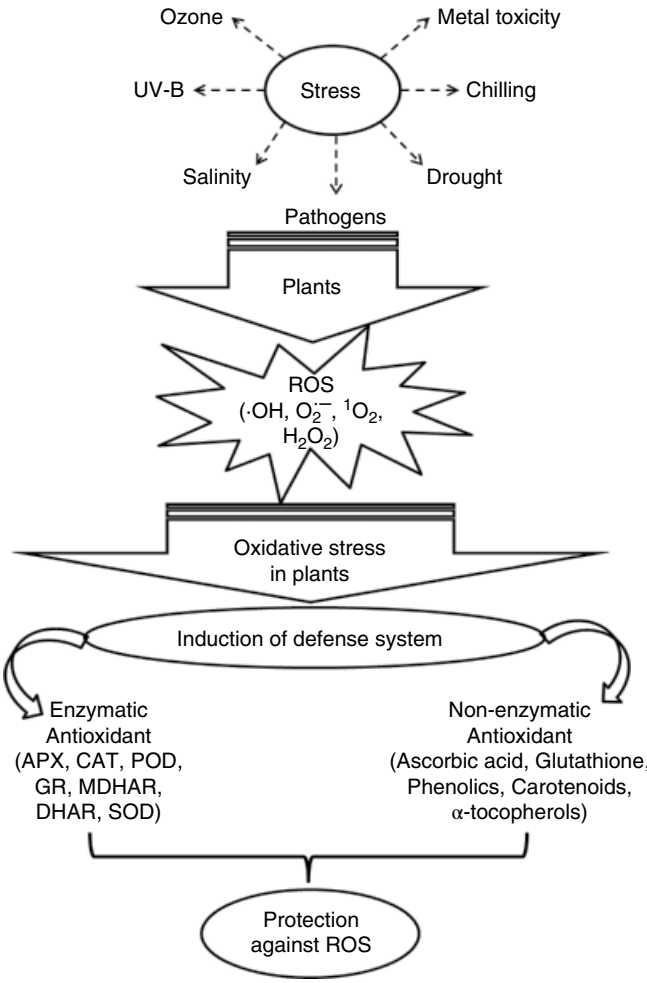

Figure 5.1 An overview of reactive oxygen species (ROS) production and its quenching in plants. APX, ascorbate peroxide; CAT, catalase; DHAR, dehydroascorbate reductase; GR, glutathione reductase; MDHAR, monodehydroascorbate reductase; POD, peroxidase; SOD, superoxide dismutase.

Elevated UV-B is known to cause increases in lipid peroxidation, solute leakage, and formation of ROS (e.g., $O_2^{\cdot-}$, $^1O_2$, H_2O_2, and $^{\cdot}OH$). Reduction in photosynthetic pigments and protein content, and increase in phenolics and non-photosynthetic pigments are well known UV-B effects on plants. Plants exposed to UV-B radiation often display low photosynthetic rates due to impaired functions of photosystems, especially PSII reactions in thylakoid membranes, a decrease in rubisco activity and the enzymatic processes of the Calvin–Benson cycle, ATPase activity, and stomatal limitations to CO_2 diffusion (Surabhi *et al.,* 2009). PSII is a complex of pigments and proteins; the core is made up of D1 and D2 proteins (Barber *et al.,* 1997). Degradation of D1 and D2 is driven even at UV-B fluence rates as low as 1 $\mu mol\, m^{-2}\, s^{-1}$ (Jansen *et al.,* 1998; Bouchard *et al.,* 2006). A detailed analysis of different PSII properties revealed that donor and acceptor sites of the PSII reaction center were affected by UV-B, with the primary target for PSII damage by UV-B at acceptor sites (QA, QB, and plastoquinone pool) (Apostolova *et al.,* 2014). Photo-oxidative stress under UV-B could potentially disturb CO_2 fixation and reduce the content and activity of $NADP^+$ and ribulose bisphosphate carboxylase/oxygenase (rubisco). Consequently, the photosynthetic ETC is over-reduced, forming superoxide radical and singlet oxygen in the chloroplasts. To overcome these negative effects, plants induce antioxidative defense mechanisms (Agrawal *et al.,* 2009). Increased activities of different antioxidative enzymes (SOD, POX, APX, CAT, GR, etc.) enable ROS scavenging (Choudhary and Agrawal, 2014b; Takshak and Agrawal, 2014).

Ozone Stress

In the troposphere, O_3 (ozone) is an important pollutant and reported to be harmful to plants. Ozone penetrates leaves through their open stomata and follows the same path as carbon dioxide. Ozone is a powerful oxidizing agent able to react with molecules on cell walls and activate the production of the ROS, which causes numerous destructive effects inside cells, affecting lipids, proteins, and nucleic acids (Iriti and Faoro, 2008; Chaudhary and Agrawal, 2013; Singh *et al.,* 2014; Rai *et al.*, 2015). In a cell O_3 itself infrequently gets far-off and being a strong oxidant, can interact with components of the apoplast to produce ROS such as H_2O_2, $O_2^{\cdot-}$, $^{\cdot}OH$, and $HOO^{\cdot}$ radicals (Heath, 2008). Production of these ROS induced by exposure to O_3 seems to an example of an "oxidative burst" in the O_3 affected cells; therefore the appearance of symptoms and ROS accumulation at the injured sites in plants are detected along with localized cell death (Pasqualini *et al.,* 2003). Accumulation of H_2O_2 has been confirmed in tobacco leaves (Schraudner *et al.,* 1998), while in *Arabidopsis* the accumulation of both $O_2^{\cdot-}$ and H_2O_2 has been observed by Rao *et al.* (2000). ROS accumulation at definite sites and the visible lesions that develop suggested that ROS act as the key regulators of cell death (Overmyer *et al.,* 2000). Chronic dosage of O_3 is also capable of generating lesions in leaves, as observed by H_2O_2 localization in mung bean cultivars, although this is suppressed by a foliar spray of 10 M ascorbic acid (Chaudhary and Agrawal, 2014a,b). Maize cultivars exposed to ambient + 30 ppb of O_3 showed the localization of both H_2O_2 and $O_2^{\cdot-}$ (Singh *et al.,* 2014). Besides the localization of ROS, increments in the content of ROS (H_2O_2 and $O_2^{\cdot-}$) were observed in crop plants exposed to the elevated dose of O_3, such as mung bean, clover, and linseed under field conditions (Chaudhary and Agrawal, 2013; Tripathi *et al.,* 2011; Mishra and Agrawal, 2015).

Chilling Stress

Under low-temperature conditions, plants undergo various modifications such as the accumulation of soluble sugars, RNA chaperones, dehydrins, and ROS, and undergo numerous physiological disturbances. They may go on to develop chilling symptoms leading to the plant's death. Greater ROS production under chilling conditions is mainly related to the imbalance of absorption and use of light in the Calvin–Benson cycle (Logan *et al.,* 2006). Significant

reductions in rubisco content and its activity have been observed in cucumber plants under chilling conditions (Zhou *et al.,* 2006). Lipid peroxidation along with oxidation of proteins by H_2O_2 and $O_2^{\cdot-}$ are common phenomena under chilling stress (Prasad, 1997; Zhang *et al.,* 2008a). To combat oxidative stress under chilling conditions, plants show enhanced activities of antioxidant enzymes (APX, GR, SOD, DHAR, MDHAR, etc.) (Fryer *et al.,* 1998; Zhang *et al.,* 2008b). Non-enzymatic antioxidants (GSH, AsA, carotenoids, and α-tocopherol) also have significant roles in overcoming chilling stress (Radyuk *et al.,* 2009). Under severe and long-duration cold stress plants are unable to scavenge ROS efficiently, which may lead to death of the plants (Zhang *et al.,* 2008a).

Drought Stress

Drought is also an important environmental constraint that increases oxidative load on plant species in many ways. During drought, CO_2 inhibition leads to disturbances in activities of the photosystems and ETC inside the chloroplast, resulting in increased ROS generation (Asada, 1999). Also, excess light energy dissipation toward PSII and antenna pigment leads to greater ROS formation (Foyer and Harbinson, 1994). Production of $^{\cdot}OH$ through H_2O_2 reduction with the help of Fe, by SOD and AsA in chloroplast, is also a potential threat under drought. Increased ROS under drought led to increased activities of SOD, APX, POD, DHAR, GR, AsA, and GSH (Wang *et al.,* 2012).

Salinity Stress

Salinity stress also leads to more ROS like $O_2^{\cdot-}$, $^1O_2$, H_2O_2, and $^{\cdot}OH$ by the impairment of ETCs in chloroplasts and mitochondria. Inhibition of carbon fixation due to reduced availability of CO_2 after stomatal closure is a common phenomenon under salinity stress. A low CO_2 to O_2 ratio in chloroplast leads to increased production of H_2O_2 (Hernandez *et al.,* 2000). However, under high CO_2 conditions low ROS generation resulted due to higher rates of assimilation and lowered photorespiration (Perez-Lopez *et al.,* 2009). Lipid peroxidation and oxidation of proteins and nucleic acids are common damaging effects of ROS under salinity stress (Hernandez *et al.,* 2000; Tanou *et al.,* 2009; Karray-Bouraoui *et al.,* 2011). SOD, CAT, GR, APX, and GP are common antioxidant enzymes that combat the negative effects of high salt conditions (Mishra *et al.,* 2013). Upregulation of many genes at proteomic and genomic levels has been observed under salinity stress (Wang *et al.,* 2008).

Heavy Metal Stress

Agricultural soils, on a global scale, are slightly to moderately polluted by heavy metals like Cd, Cu, Zn, Ni, Co, Cr, Pb, As, and so forth. The formation of ROS, like $O_2^{\cdot-}$, H_2O_2, and $^{\cdot}OH$, is the primary response of plants against heavy metal stress. Several metals take part in ROS generation through the Haber–Weiss reaction (Mithofer *et al.,* 2004), by the disruption of ETC (Qadir *et al.,* 2004), and CO_2 fixation (Moustakas *et al.,* 1994). Oxidative injury to nucleic acids, lipids, and proteins is a common phenomenon under metal stress. Higher activities of GP, SOD, GR, APX, DHAR, MDHAR, and non-enzymatic antioxidants have been observed in different plant species under metal stress (Gallego *et al.,* 1996; Yamamoto *et al.,* 1997; Sharma and Agrawal, 2005; Maheshwari and Dubey, 2009).

Pathogens

Pathogens also affect plant metabolism and productivity by the excess production of ROS. Plasma membrane-associated NADPH oxidase leads to an oxidative burst during incompatible plant–pathogen communication (Bhattacharjee, 2012). During pathogen attack, $O_2^{\cdot-}$ and

H_2O_2 generation in the apoplastic region have been observed in potato (Doke, 1983; Grant *et al.,* 2000). Although the primary ROS production site is the apoplast, ROS may be produced in chloroplasts and mitochondria after pathogen attack (Abdollahi and Ghahremani, 2011). ROS generation during pathogen interaction causes necrosis; also, SIPK/Ntf4/WIPK activation leads to ROS formation in chloroplast, which later participates in cell death signaling in plants (Liu *et al.,* 2007). Salicylic acid (SA) also plays a significant role in ROS accumulation by regulating CAT and APX activities during pathogen infection (Mittler *et al.,* 1999; Klessig *et al.,* 2000). Enhanced SOD, POD, CAT, and APX activities have been observed in pathogen infections in plants (Radwan *et al.,* 2010; Ashry and Mohamed, 2012).

Quenching of ROS in Plants

Plants exposed to different stress conditions like high or low temperature, heavy metals, drought, air pollutants, pathogens, salinity, and so forth generate increased concentrations of ROS such as $O_2^{\cdot-}$, $^1O_2$, H_2O_2, and $^{\cdot}OH$ (see Figure 5.1). As a protection mechanism against these toxic radicals, plants possess antioxidant defense systems, which comprise of enzymatic and non-enzymatic antioxidants. Enzymatic defense includes CAT, APX, SOD, GR, DHAR, and MDHAR, whereas non-enzymatic antioxidants are carotenoids, GSH, AsA, and tocopherols (Mittler *et al.,* 2004).

Enzymatic Defense System

Catalase (CAT; 1.11.1.6)

Catalase is universal enzyme present in all living organisms that utilize oxygen, whether animals, plants, or bacteria. An important enzyme, it plays a vital role in protecting plant cells against oxidative damage caused by ROS. Moreover, it was the first enzyme to be discovered and characterized (Sharma *et al.,* 2012). Its main role is in the decomposition of H_2O_2 to H_2O and O_2 with the help of its heme cofactor. One molecule of catalase has the capacity to convert about six million H_2O_2 molecules to H_2O and O_2 per minute. Three classes of catalase have been described to date. Class I is mainly present inside photosynthetic tissues and its regulation is light dependent. Class II is found in the vascular tissues, whereas class III occurs in young seedlings (Willekens *et al.,* 1995). Also, three catalase isoforms have been located in maize plants, namely CAT1 and CAT2 in peroxisomes and cytosol, and CAT3 in mitochondria (Scandalios, 1990). Scavenging of peroxisomal H_2O_2 is mainly done during photorespiratory oxidation, fatty acid β-oxidation, and purine catabolism. Increased H_2O_2 concentrations in stressful conditions is a common phenomenon in plants. To combat these increments of H_2O_2, alteration in catalase activity has been described under UV-B (Choudhary and Agrawal, 2014b; Takshak and Agrawal, 2014) and Cd (Agrawal and Mishra, 2007) stress.

Ascorbate Peroxidase (APX; 1.1.11.1)

Ascorbate peroxidase mainly detoxifies H_2O_2 in plant cells by utilizing ascorbate as a substrate and forming DHA and H_2O as products. APX is the central component of the ascorbic acid-glutathione (AsA-GSH) cycle, having an important role in controlling ROS production. Five different isoforms of APX are known in plants, located in cytosol, peroxisomes, mitochondria, thylakoid, and stroma (Gill and Tuteja, 2010). APX is known to have much better affinity to H_2O_2 as compared to CAT and POD, which makes it an efficient H_2O_2 scavenger (Wang *et al.,* 1999). Under pathogen attack, it has been shown that higher APX activity leads to increased

POD activity, which boosts tolerance to oxidative damage and pathogens (Sarowar *et al.,* 2005). Higher APX activities have been reported under various stress factors, including UV-B (Choudhary and Agrawal, 2016), ozone (Chaudhary and Agrawal, 2013), and metal toxicity (Agrawal and Mishra, 2007).

Superoxide Dismutase (SOD; 1.15.1.1)

SOD is the most important metalloenzyme present in all aerobic organisms, which provides defense against $O_2^{\cdot-}$ under various stresses (Scandalios, 1993). Cu/Zn-SOD, Mn-SOD, and Fe-SOD are the three known isoforms in plants, all leading to the dismutation of $O_2^{\cdot-}$ to O_2 and H_2O_2 (Fridovich, 1989). Fe-SOD and Mn-SOD are present in chloroplasts and mitochondria, respectively however, Cu/Zn-SOD is located in chloroplasts, mitochondria, peroxisomes, and cytosol (Sharma *et al.,* 2012). Various reports have suggested that Cu/Zn-SOD is present in dimer forms only and is cyanide sensitive, whereas Fe-SOD and Mn-SOD can be in dimer or tetramer form and are cyanide insensitive (Scandalios, 1993; Del Rio *et al.,* 1998). Increased SOD activity is a common defense strategy adopted by plants against different biotic and abiotic stresses. Increased SOD production by plants was observed under UV-B (Takshak and Agrawal, 2014; Choudhary and Agrawal, 2016) and ozone (Chaudhary and Agrawal, 2013).

Glutathione Reductase (GR; 1.6.4.2)

GR is the flavoprotein oxidoreductase, generally present in all eukaryotes and prokaryotes. It plays a vital role in ROS defense as a central component of the ascorbate-glutathione (AsA-GSH) cycle. In plant cells it is found chiefly in chloroplasts, but also occurs in mitochondria and cytosol in small amounts (Gill and Tuteja, 2010). GR catalyzes the formation of glutathione (GSH) through NADPH-dependent reduction of oxidized glutathione (GSSG) (Reddy and Raghavendra, 2006). GSSG consists of two GSH molecules joined together with disulfide bond, which can be transformed to GSH by GR activity. The balance between GR and GSH is an important factor in determining the tolerance of plants against any environmental stress (Chalapathi Rao and Reddy, 2008). The transformation of GSSG to GSH with GR involves two reactions (Ghisla and Massey, 1989). First, the flavin part of GSSG is reduced by NADPH, and after the flavin is oxidized the disulfide bridge gets reduced to produce thiolate and cysteine anion. The second step leads to GSSG reduction through interchange reactions of thiol disulfide. Induction of GR activity under environmental stress has been explained in various studies and well correlated with the defense mechanism of plants (Chaudhary and Agrawal, 2013; Choudhary and Agrawal, 2014a; Takshak and Agrawal, 2014)

Dehydroascorbate Reductase (DHAR; 1.8.5.1)

Dehydroascorbate reductase is a monomeric thiol found mainly in roots and green shoots as well as in dry seeds. DHAR is of utmost importance to maintain reduced AsA inside plants cells. It catalyzes the transformation of dehydroascorbate (DHA) to ascorbic acid (AsA) through utilization of GSH as substrate (Ushimaru *et al.,* 1997). DHAR overexpression leads to plants' tolerance against various stresses. During AsA regeneration from monodehydroascorbate (MDHA) via enzymatic or non-enzymatic pathways, there is always the possibility of DHA formation. This DHA has a very short life inside plant cells, and is rapidly transformed to AsA by the action of DHAR, or to 2,3-diketogulonic acid via irreversible hydrolysis (Sharma *et al.,* 2012). Also, DHAR overexpression is related to enhancement and maintenance of the AsA pool size (Chen *et al.,* 2003; Qin *et al.,* 2011). Plants with DHAR overexpression exhibited tolerance against metal toxicity (Yin *et al.,* 2010), ozone stress (Chen and Gallie, 2005), and salinity (Ushimaru *et al.,* 2006).

Monodehydroascorbate Reductase (MDHAR; 1.6.5.4)

This enzyme belongs to the oxidoreductase family, and is mainly present in chloroplasts, mitochondria, peroxisomes, and cytosol of plant cells. MDHA produced after the APX reaction has a very short lifetime; if not reduced rapidly, it will be transformed to AsA and DHA (Ushimaru *et al.,* 1997). MDHAR shows high affinity toward MDHA as electron acceptor; NADH is its first choice as electron donor, compared to NADPH. It is the only known enzyme that utilizes MDA (organic radical) as substrate and also reduces phenoxyl radicals produced after H_2O_2 and horseradish peroxidase reaction (Sakihama *et al.,* 2000). MDHAR in chloroplasts has two major roles; the first is AsA generation from MDHA, and the second is reduction of O_2 to $O_2^{\cdot-}$ in the absence of MDHA (Miyake *et al.,* 1998). Overexpression of MDHAR provides tolerance against chilling stress in tomato (Stevens *et al.,* 2008) and salinity stress in potato (Eltayeb *et al.,* 2007).

Guaiacol Peroxidase (GP; 1.11.1.7)

This enzyme contains heme protein and is present in plants, vertebrates, and fungi. It contains a prosthetic group (ferriprotoporphyrin IX) and oxidizes numerous aromatic substrates like guaiacol and pyragallol by utilizing H_2O_2 (Vianello *et al.,* 1997). Isozymes of GP are present mainly in vacuoles, cytosol, and cell wall (Asada, 1992). GP plays diverse roles inside plant cells, including cell wall lignification, ethylene biosynthesis, indole acetic acid degradation, wound healing, and tolerance against various stress factors (Kobayashi *et al.,* 1996). GP is well recognized for effective scavenging of reactive forms of oxygen and peroxy radicals under different stresses (Vangronsveld and Clijsters, 1994). Induced activity of GP is well correlated with its defensive role against ROS, under metal (Radotic *et al.,* 2000) and salinity (Tayefi-Nasrabadi *et al.,* 2011) stress.

Non-enzymatic Defense System

Ascorbic Acid (AsA)

Ascorbic acid, also called vitamin C, is an organic compound having antioxidant properties. Highest concentrations are found in mature leaves with completely developed chloroplasts (Smirnoff *et al.,* 2004). AsA is a low molecular weight and very efficient ROS quencher, because of its tendency to donate electrons easily in enzymatic and non-enzymatic reactions (Sharma *et al.,* 2012). AsA plays a vital role in removal of H_2O_2 through the AsA-GSH cycle, where AsA is transformed into MDHA and then to DHA (Pinto *et al.,* 2003). MDHA and DHA are highly unstable compounds; MDHA can be reduced to AsA with the help of MDHAR (Miyake and Asada, 1994) while DHA can be transformed to tartarate and oxalate at pH greater than 6.0 (Noctor and Foyer, 1998) or it can be transformed to AsA by DHAR (Asada, 1996). Most of the AsA is located in the cytoplasm, but some is present in the apoplast where it acts as first line of defense against external ROS (Barnes *et al.,* 2002). Membrane protection is also provided by AsA, as it reacts directly with H_2O_2 and $O_2^{\cdot-}$ to generate α-tocopherol (Noctor and Foyer, 1998). Modifications in the level of AsA in the face of various stresses have been described in several studies (Chaudhary and Agrawal, 2013; Maheshwari and Dubey, 2009).

Glutathione (GSH)

Glutathione, an important metabolite present in plants, acts as a strong antioxidant. GSH is capable of avoiding damage to cellular components due to $^1O_2$, H_2O_2 and $^{\cdot}OH$. It is a tripeptide with gamma peptide linkages (γGlu-Cys-Gly). Studies have demonstrated its presence in various cellular organelles and compartments, including chloroplasts, peroxisomes, mitochondria, cytosol, apoplast, vacuoles, and endoplasmic reticulum (Mittler and Zilinskas 1992; Jimenez

et al., 1998). Besides signal transduction, it also participates in gene expression under stress and sulfate transport (Mullineaux and Rausch, 2005; Rausch and Wachter, 2005); it also plays a vital role in cell differentiation, resistance against pathogens, cell death, senescence, and enzymatic regulation (Xiang *et al.,* 2001). GSH acts as substrate for various cellular reactions that produce the oxidized form of glutathione (GSSG). The balance between the utilization and production of GSH and GSSG is vital for the proper functioning of plant cells (Foyer and Noctor, 2005). Role of GSH in counteracting ROS production is reflected in several studies examining several stress factors (Xiang *et al.,* 2001; Agrawal, 2007).

Carotenoids

Carotenoids are the pigments mainly present in chloroplasts and chromoplasts of plants, and also in some photosynthetic microorganisms. Carotenoids have a photoprotective role through the dissipation of extra energy as heat, by scavenging ROS and reducing lipid peroxidation. Carotenoids also participate in light harvesting by absorbing light of 400 and 550 nm and transferring this energy to chlorophyll (Sieferman-Harms, 1987). They scavenge triplet sensitizer (3Chl*; an excited triplet configuration of chlorophyll), $^1O_2$, and other free radicals as antioxidants to prevent damage to the photosynthetic apparatus (Collins, 2001). Carotenoids also act as signaling molecules for the stability of PSI, light-harvesting complex, and thylakoid membrane stabilization (Niyogi *et al.,* 2001). The prevention of triplet chlorophyll formation is based on the chemical properties of carotenoids; however, the presence of an isoprene chain permits the easy uptake of energy and its dissipation as heat from the excited molecules (Mittler, 2002). An increased carotenoid content has been shown to be beneficial under different environmental stresses (Gomathi and Rakkiyapan, 2011; Choudhary and Agrawal, 2016).

Tocopherols

These are also major potential ROS scavengers in the plant cell, being mainly involved in the quenching of ROS such as $^1O_2$ and peroxy lipid radicals (Diplock *et al.,* 1989). Four isomers of tocopherol (α, β, γ, and δ) have been described in plants, of which α-tocopherol posseses maximum antioxidant capacity owing to the three methyl groups in its structure (Fukuzawa *et al.,* 1982). Only photosynthetic organisms can make tocopherols, which occur mainly in green parts of the plant. Tocopherols are very useful for the protection of PSII, as they interact chemically and physically with ROS to prevent lipid membranes (Ivanov and Khorobrykh, 2003). A single molecule of tocopherol has the capacity to quench about 220 molecules of $^1O_2$ (Munné-Bosch, 2005). The benzoquinone ring and phytyl chain of tocopherol play a significant role in the quenching of $^1O_2$ (Fryer, 1992). Studies have suggested that tocopherols accumulate under different stresses like chilling, drought, and salinity (Yamaguchi-Shinozaki and Shinozaki, 1994; Munné-Bosch *et al.,* 1999; Bafeel and Ibrahim, 2008).

Phenolics

Phenolics are widely dispersed and the most abundant secondary metabolites in the plant kingdom. Flavonoids, phenolic acids, and tannins are common phenolics present in plants. Plant phenolics are mainly derivatives of cinnamic acid and benzoic acid. The antioxidant properties of phenolics have two aspects, scavenging and suppressing ROS formation (Grace and Logan, 2000). These compounds are free radical acceptors and inhibit oxidation of lipids and other molecules by donation of hydrogen molecules. Under adverse environmental conditions, flavonoids are involved in neutralization of ROS before they prove harmful to plant cells (Løvdal *et al.,* 2010). Polyphenols can also decrease membrane fluidity by modifying the lipid structure (Arora *et al.,* 2000). Induction of phenolics under different stresses has been observed in several studies (Michalak, 2006; Choudhary *et al.,* 2013; Choudhary & Agrawal, 2014a).

ROS Induce Signaling in Plants

The production of ROS in plants is stimulated by various environmental stresses, which disturb the balance between production of ROS and their detoxification by enzymatic and non-enzymatic antioxidants that diminish the oxidative stress (Apel and Hirt, 2004). High levels of ROS formation lead to photo-oxidative damage to proteins, DNA, and lipids and eventually cell death occurs. But apart from these damaging consequences of ROS, they also act as signaling molecules required for normal cellular function, including developmental processes, the hypersensitive reaction, systemic acquired resistance against pathogens, stress hormone production, acclimation, and PCD (Bhattacharjee, 2005). ROS are a central component of the stress response, and the amounts of ROS decide their function – high concentrations lead to cell death whereas low concentrations initiate expression of the defense genes (Das and Roychoudhury, 2014) (Figure 5.2). ROS influence various processes in cells, and at different developmental stages of plants. The ROS-induced biological reactions depend on the ROS dose, intensity of the signal, production site, and the plant's development stage (Zaninotto *et al.,* 2006). In the process of signal transduction ROS play a major role and alter cellular components as they are extremely reactive molecules able to oxidize cellular components and cause damage. To combat any stress numerous enzymes required in the scavenging of ROS are influenced, either downregulated or overexpressed (Ahmad *et al.,* 2008).

When plants encounter different stress factors, cells generate the capacity to rapidly produce and scavenge diverse kinds of ROS in a synchronized way, facilitating fast and vigorous fluctuations in the amount of ROS (Mittler *et al.,* 2011).

Additional advantageous functions of ROS are to disseminate the signal automatically for long distances throughout the plant (Mittler *et al.,* 2011). The different molecular properties of ROS determine their mobility within cells. The superoxide radical is not able to cross membranes, but can change into H_2O_2, which transfers through membranes and acts as a

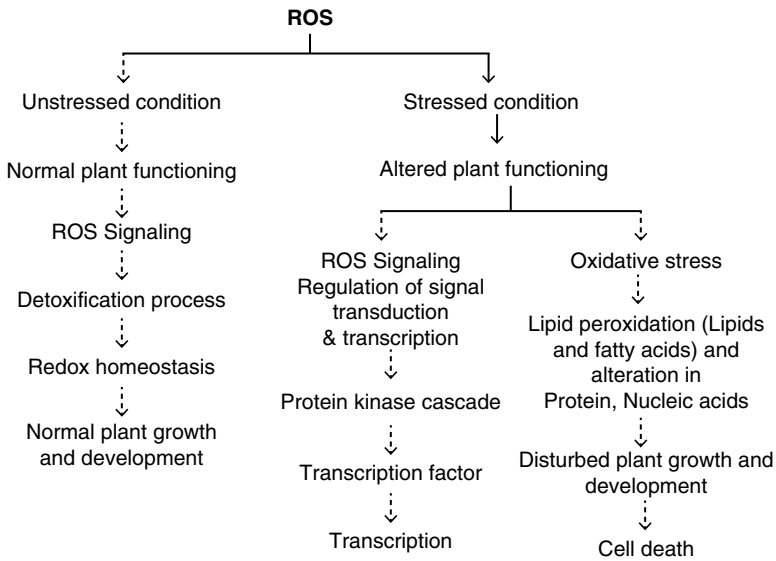

Figure 5.2 Role of reactive oxygen species (ROS) and the biological consequences with and without stress.

stable intermediate of ROS metabolism; it performs as a second messenger, since it can diffuse from the site of production (Varnova *et al.,* 2002). Furthermore, cells have evolved numerous mechanisms for prompt and controllable ROS production and exclusion (Grether-Beck *et al.,* 2000).

Signaling Functions of ROS

Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is uncharged and relatively stable compared to other ROS. It is capable of traveling long distances and penetrating membranes, so can act as an efficient messenger within or between cells (Mullineaux and Lawson, 2009). In plant responses to stress, H_2O_2 activates genes encoding many antioxidant and signaling proteins leading to stimulation of APX, GR, CAT, mitogen-activated protein kinase (MAPK), and phosphatases. H_2O_2 production initiates the signalling from chloroplasts to nucleus through activation of MAP kinase cascade (Pfannschmidt *et al.,* 2009).

Singlet Oxygen

Singlet oxygen possesses a short lifespan, therefore its action requires the participation of other signaling components such as the proteins Executer 1 and 2. Singlet oxygen induces lipid peroxides, and their derivatives function as signal molecules releasing from chloroplasts (Kreslavski *et al.,* 2012). It also exits from chloroplasts into cytosol and even reaches the nucleus to stimulate expression of the nuclear *GPXH* gene encoding glutathione peroxidase. Therefore, singlet oxygen ($^1O_2$) not only causes oxidation of PUFA but also lipid oxidation of thylakoid membrane (Valdivieso and Mullineaux, 2010).

Superoxide Anion

Superoxide anion also has a short life and shows similar signaling characteristics as H_2O_2. Signal transduction by superoxide anion ($O_2^{\cdot-}$) has been shown when plants are deficient in Cu/Zn-SOD (Rizhsky *et al.,* 2003). Superoxide anion reacts rapidly with NO, producing peroxynitrite in chloroplasts, also fulfilling signaling functions (Kreslavski *et al.,* 2012).

Redox Responsive Proteins

Plants are able to sense, transduce, and translate the signals induced by ROS into suitable cellular reactions. This process involves redox-sensitive proteins (RSPs) that can undergo reversible oxidation-reduction depending on the redox state of the cell (Foyer and Noctor, 2003). Glutathione or thioredoxins are abundant redox-sensitive molecules, able to control the redox state in the cells of higher plants (Foyer and Noctor, 2005).

Plants naturally encounter various stresses and can deploy a repertoire of protective responses involving various cellular functions stimulated by ROS signaling. Plants commonly experience excess photochemical energy, therefore chloroplasts stimulate RSPs in the photosynthetic electron transport chain of cytochrome *bf* complex. Free radicals generated during light harvesting leads to oxidative stress, if not balanced through utilization and dissipation might leads to cell death. However, ROS can show an affirmative function in the response to excess photochemical energy through photoinhibition and by introducing proliferation in the degradation rate of D1 protein at photosystem II (PSII) reaction center (Karuse, 1994). Phosphorylation is another method for controlling the dissipation of surplus light energy from PSII. RSP initiates the signals in the cytochrome bf complex which stimulates kinase activation and activation of

phosphorylation proceeded by the plastoquinone pool (Vener *et al.,* 1998). Therefore, ROS are significant contributors to cellular redox status, which participates in processes of controlling damage by acting as an "alarm" that initiates defense responses.

Depending on the degree of oxidative stress, linked signal transduction pathways are stimulated that can lead either to stress acclimation or to cell death (Shao *et al.,* 2008). Plant vigor may be affected by numerous biotic and abiotic stresses that precede signal transduction in plants. Initially, signaling comprises ion fluxes through the plasma membrane, increasing Ca^{2+} levels in the cytosol and activating MAPKs. The plasma membrane permits inward calcium flux as Ca^{2+} channels are transiently open. This Ca^{2+} signal might be related to regulatory mechanisms of ROS-producing enzymes (Mittler *et al.,* 2012).

ROS and Redox Signaling

Numerous signaling actions in cells are based on redox reactions, and ROS are directly associated with the cellular redox metabolism. ROS cause oxidation of lipids, DNA, and proteins besides other components, hence the need for redox homeostasis to be maintained by the cell's antioxidants and antioxidative enzymes (Shao *et al.,* 2008). Increasing ROS concentration disturbs the redox equilibrium in the cell by conversion of antioxidants into their oxidized state, which are normally found in the reduced state and utilized for signaling resulted into the unbalanced redox metabolism of cell (Tripathy and Oelmüller, 2012). The mechanism is unclear, but an example in plastids shows the influence of ROS on the redox situation. Increased light intensity induces production of ROS and decline in plastoquinone pool, leading to stimulation of protein kinase and photoacclimation process along with activation of various antioxidative genes (Apel and Hert, 2004; Mittler *et al.,* 2004).

Mechanism of ROS Signaling

In the presence of stress, plants have developed an integral mechanism for ROS sensing, transduction, and translation of signals into suitable cellular responses. This requires the presence of redox-sensitive proteins for oxidation and reduction reactions (Shao *et al.,* 2006). ROS facilitate the molecular mechanisms of redox-sensitive regulation of proteins mediated by signals comprising MAP kinase regulated protein phosphorylation, heterotrimeric G-proteins, and protein Tyr phosphatases (Foyer and Noctor, 2005).

ROS Generation and Signaling Sites

ROS generation and scavenging in plants under stress (abiotic/biotic) depends on the nature of ROS signaling, which takes place inside cells between different organelles, and between cells over long distances. Plants have the capability to scavenge ROS for long-distance signaling, and this is possible only when uninterrupted ROS generation takes place. ROS are mainly used as a general signal to activate the cellular signaling networks, while other signals function together with ROS to convey specific signals to combat the stress (Mittler *et al.,* 2011).

ROS generated in chloroplasts and mitochondria induce alterations in the nuclear transcriptome, influencing gene expression by modifying transcription factors (Choudhury *et al.,* 2013). ROS induce expression of numerous genes, signifying that ROS act as biological signals to regulate stress (Laloi *et al.,* 2004). When plants encounter stress (abiotic/biotic) the ROS concentration is increased, which alters gene expression. Changes at the gene level occurs via oxidation of components stimulating the signaling pathways resulting in the activation of

transcription factors or possibly those transcription factors that are redox sensitive (Choudhury *et al.,* 2013). Signaling components in the chloroplast are plastoquinone (PQ), ascorbate, glutathione, and ROS along with ferredoxin or thioredoxin (Pfannschmidt *et al.,* 1999).

In peroxisomes high amounts of H_2O_2 are generated naturally when plants encounter high light energy or any other stress, capable of stimulating the antioxidative enzymes such as catalase and ascorbate peroxidase. Photorespiration contributes to the scavenging causing a decline in catalase activity and leading to the accumulation of oxidized glutathione. Therefore the catalase deficiency is balanced by ascorbate and glutathione involving the signal transduction from chloroplast to peroxisomes (Foyer and Noctor, 2003).

ROS production in chloroplasts and peroxisomes is relatively high compared to mitochondria, although the quantity of oxidized protein is high in mitochondria due to its susceptibility to ROS (Møller & Sweetlove, 2010). Increased production of ROS is responsible for more oxidized proteins in mitochondrial electron transport complex which leads to programmed cell death (Tiwari *et al.,* 2002).

Gene Expression

In plants, stress generates ROS and appropriate amounts of ROS benefit plants in their responses to the stresses. Hydrogen peroxide is specifically involved in plant defense, stimulating both gene expression and via the activities of proteins such as MAP kinase, regulating transcription (Desikan *et al.,* 2000) (Figure 5.3). Gene expression in plant cells affected by ROS-stimulated signal transduction and altered redox state involves transcription factors, as promoter elements and DNA-binding factors acting as redox response elements to control the plants' gene expression.

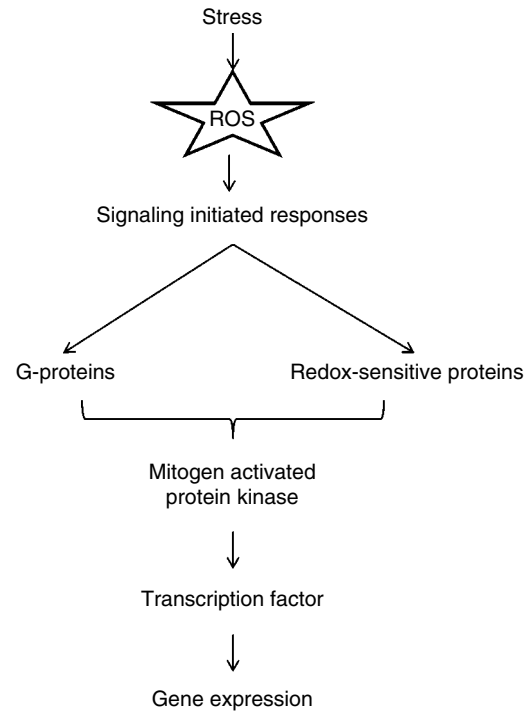

Figure 5.3 Reactive oxygen species (ROS) influence gene expression.

For example, glutathione *S*-transferase (GST) catalyzes the conjugation of GSH and stimulates the cellular detoxification pathway. GST gene expression is regulated by the pro-oxidant state of the cell, resulting in reduced GSH content (Daniel, 1993). Similarly, the oxidation of thiol group proteins and oxidation of Fe-S clusters are also integral to the function of redox-sensitive proteins. Another example of gene expression is observed during the photo-oxidative damage caused by the high light energy able to alter utilization, dissipation, and generation of the toxic radicals. This mechanism induces the signal transduction which leads to reduction in the plastoquinone pool along with kinase activation that changes the Fe-S protein linked with cytochrome *cf* complex (Vener *et al.,* 1998; Pfannschmidt *et al.,*1999).

Role of ROS Signaling

Signaling facilitated by ROS in plants induced by stress (biotic/abiotic) involves heterotrimeric G-proteins and protein phosphorylation controlled by definite MAP kinases and protein Tyr phosphatases (Foyer and Noctor, 2005; Pfannschmidt *et al.,* 2009). ROS, particularly H_2O_2, activates signaling protein kinases, especially MAPK; a protein kinase can phosphorylate a transcription factor and further regulate gene expression (Foyer and Noctor, 2003; Apel and Hirt, 2004).

ROS Induce Activation of MAPK Signaling Pathways

Mitogen-activated protein kinases are a class of plant serine/threonine protein kinases that perform an essential role in the transduction of numerous stress signals. They typically function as a cascade where MAPK is phosphorylated and activated by MAPK kinase (MAPKK), which is activated by MAPKK kinase (MAPKKK). These three kinases are interlinked and known as extracellular receptor kinases (Ahmad *et al.,* 2008) (Figure 5.4). Therefore, the signal transduction unit is conceived to stimulate active MAP kinase kinase kinase (MAPKKK), MAP kinase kinase (MAPKK), and MAP kinase (MAPK). A sequential phosphorylation-activation process initiates and transmits the signal from the MAPKKK to the target, in the form of a transcription factor (TF) whose activity and localization are influenced by phosphorylation. The proportions of phosphorylation activation and transmission designate that MAPKKKs can be stimulated by particular stimuli and the signaling pathways may assemble at the MAPKK level of the cascade. A single MAPKK could then phosphorylate several MAPKs. Signaling through MAPKKKs and MAPKKs might continue through other mechanisms as well besides phosphorylation of their direct downstream targets. MAPKs are controlled by protein phosphatases and able to target modification by ROS induced changes in protein phosphatase activity through MAPK signaling (Brosché *et al.,* 2010).

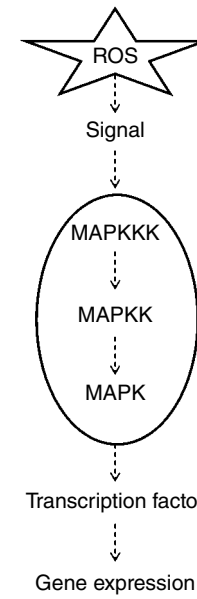

Figure 5.4 Signaling mechanisms through the mitogen-activated protein kinase (MAPK) pathway.

Therefore, MAPK signaling components are involved in eliciting responses to several signals including oxidative stresses. Activation of MAPK by phosphorylation normally leads to nuclear localization (Ahlfors *et al.,* 2004). Abiotic stress leads to the expression of genes affected by these kinase classes and stimulates the antioxidative defenses (Samuel *et al.*, 2000).

ROS Signaling Perspectives

Normally ROS are generated and induce metabolic activities by signaling the activation of plant metabolic pathways. Under stress condition ROS

generation is accelerated in different cell organelles, such as chloroplasts, peroxisomes, and mitochondria. Oxidative stress is generated due to the accumulation of the ROS, with consequent impairment of cell membranes, nucleic acids, proteins, and lipids. Normally, plants are endowed with an array of enzymatic and non-enzymatic antioxidants such as CAT, SOD, MDHAR, DHAR, GR, glutathione peroxidase, α-tocopherols, ascorbic acid, glutathione, carotenoids, and flavonoids to manage stress. ROS perform a significant role in signaling between various metabolic pathways; however, their dual role, determined by their concentration in the cell, means they can have a good or bad effect on the plant. The consequent signaling affects several downstream processes, leading to the induction of stress-responsive genes. The role of ROS as second messengers has raised considerable interest, and understanding the integration of ROS signaling is a powerful tool for influencing plant growth and development in the presence of stress.

ROS Induce Programmed Cell Death (PCD) in Plants

PCD is a vital process for a plant's normal growth and function, for example, in trichome formation, floral organ abscission, embryo formation, leaf development, and so forth. The proportion of PCD and proliferation of cells influences the growth and development of the plant (Gadjev *et al.,* 2008). Programmed cell death in plants is significantly caused by high concentrations of ROS, while lower doses induce signals that mediate lowering of oxidative stress (Miller *et al.,* 2010).

Excess production of ROS alters the functioning of mitochondria and chloroplasts, which play a significant role in PCD during photosynthesis; these organelles transmit signals to the nucleus, which regulates expression of various genes according to the redox situation and activates PCD. Endoplasmic reticulum also takes part in PCD, mostly by provoking the mobilization of Ca^{2+} from ER to mitochondria (Suzuki *et al.,* 2012). Therefore, PCD is an active process controlled genetically, and its execution takes place in a well-organized, stepwise manner intended to destroy an individual cell without affecting other cells (Petrov *et al.,* 2015).

Conclusions

ROS production and elimination are common processes in plants, whether in normal or stressed situations. However, several environmental stresses (such as drought, chilling, UV-B, ozone, salinity, metal toxicity, and drought) lead to increased generation of ROS, and are generally responsible for the oxidative stress leading to cell death. Widespread overproduction of ROS is the result of disturbed metabolism in mitochondria, chloroplasts, plasma membrane, and peroxisomes, or various altered metabolic activities in different components of the plant cell. Besides ROS causing damage under abiotic/biotic stress conditions, they also function as signaling components to encourage prevention of damage. The role played by ROS depends upon the extent of production; at low concentrations they play the role of signaling molecules that induce various beneficial responses in plants. However, at higher concentrations ROS are contributors to oxidative damage to DNA, proteins, and lipids, finally leading to cell death.

In order to protect themselves, plants are equipped with enzymatic (e.g., SOD, GR, CAT, APX, GP, DHAR, and MDHAR) and non-enzymatic (e.g., ascorbic acid, carotenoids, glutathione, tocopherols, and phenolics) defense systems. Numerous studies on ROS production and scavenging show that overexpression of antioxidant enzymes leads to enhanced scavenging of ROS in plants against various stresses (biotic and abiotic). Detailed studies are required

to elucidate the particular mechanisms of ROS production and scavenging, and the characterization of genes involved in cellular signaling, to develop plants with better tolerance to harsh environmental conditions.

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