ABSTRACT

Reactive oxygen species (ROS) are a by-product of normal cell metabolism in plants; however, the balance between production and elimination is disturbed under stress conditions. Several reactive oxygen species are continuously produced in plants as secondary products of aerobic metabolism. Depending on the source of the ROS species, some of them are highly toxic. Plants cellularly use various enzymatic and nonenzymatic mechanisms for detoxify ROS species. Enhanced level of ROS and absence of detoxify systems, can cause damage to biomolecules such as lipids, proteins and DNA and eventually cause to cell death. Despite their destructive activity, they are second messengers in a variety of cellular processes, including conferment of tolerance to various environmental stresses. This review paper describes the Variety of Reactive oxygen species, sources and roles of ROS in plants.

INTRODUCTION

Environmental stresses such as extremes of temperature, salinity, drought, heavy metals, herbicides and pathogens, greatly affect plant metabolism and productivity (Mittler and Blumwald, 2010).

The accumulation of molecular oxygen (O\(_2\)) in Earth’s atmosphere allows aerobic organisms to use O\(_2\) as the terminal electron acceptor during cellular respiration, which provides a higher yield of energy than fermentation (Bolwell and Woftastek, 1997) Ground state O\(_2\) is relatively unreactive. However, during normal metabolic activity such as respiration and photosynthesis unavoidably led to the production of reactive oxygen species (ROS) in some cellular organelles such as mitochondria, chloroplasts, and peroxisomes. In other hand Reactive oxygen species (ROS) are a by-product of normal cell metabolism in plants. The production of ROS is an unavoidable consequence of aerobic respiration (Puntarulo and Boveris, 1988) however, under stress conditions, the balance between production and elimination is disturbed. (Karuppanapandian and et al., 2011, Vellosillo and et al, 2010).

Reactive oxygen species (sometimes also referred to as AOS, active oxygen species, or ROI, reactive oxygen intermediates) is the term used to describe forms of oxygen that are energetically more reactive than molecular oxygen. Typically ROS are molecular species that have undergone electron addition(s) and are thus reduced forms of oxygen. When the terminal oxidases—cytochrome c oxidase and the alternative oxidase—react with oxygen, four electrons are transferred and water is the product. However, occasionally oxygen can react with other electron transport components. Here only one electron is transferred, and the result is the superoxide anion, O\(_2^-\). In plant tissues it has been estimated that 1–2 percent of oxygen consumption leads to superoxide formation (Puntarulo and Boveris, 1988, Chen and Dickman, 2005).

The major cause of loss of crop productivity worldwide is due to the Accumulation of ROS as a result of various special environmental condition (Mittler, 2002, Gill and et al, 2011). Several reactive oxygen species are continuously
produced in plants. Depending on the nature of the ROS species, some are highly toxic. Whereas a common feature among the different ROS types is their capacity to cause rapidly inactivate enzymes, damage vital cellular organelles in plants, and destroy membranes by inducing the degradation of pigments, proteins, lipids and nucleic acids which ultimately results in cell death (Foyer and Noctor, 2005). To survive plant have developed a complex signaling network involving different endogenous growth regulators that sense and protect them from environmental stresses. (Bhattacharjee, 2010, Miller and et al, 2009). In recent years, it has become apparent that in other circumstances plants purposefully generate ROS as signaling molecules to control various processes including: growth, development, pathogen defense, programmed cell death, stomatal behavior and response to other biotic and abiotic environmental condition. It is important to note that whether ROS will act as damaging, protective or signaling factors depends on the delicate equilibrium between ROS production and scavenging at the proper site and time (Gratao and et al, 2005). The cell response is strongly dependent on several factors.

ROS diffuses only a very short distance before reacting with a cellular molecule, so the subcellular location for formation of an ROS may be especially important for a highly reactive ROS (Gill and et al, 2011, Mittler and et al, 2004).

This review paper describes the Variety of Reactive oxygen species, sites of production in plant cells and their reactivity in various cellular components

1. Variety of Reactive oxygen species
Although, atmospheric oxygen is relatively non-reactive, it can give rise to ROS (Scandalios, 2005). The O₂ molecule is a free radical, as it has two unpaired electrons that have the same spin quantum number. This spin restriction makes O₂ prefer to accept its electrons one at a time, leading to the generation of the ROS (Navrot and et al, 2007, Del Rio and et al, 2006) (Fig. 1).

Oxygen, therefore, got converted to ROS by univalent reduction (transfer of electron) or by energy transfer. The common ROS produced in plant include superoxide (O₂⁻⁻). Through a variety of reactions, superoxide leads to the formation of perhydroxyl radical (HO₂⁻⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (OH), alkoxy radical (RO), peroxy radical (ROO), singlet oxygen (¹O₂), organic hydroperoxide (ROOH), and so forth (Miller and et al, 2009).

1.1. Superoxide
The single electron reduction of O₂ results in the generation of the O₂⁻⁻. Superoxide radical is generated in plant cell at the onset of oxidative burst of cell (Bhattacharjee, 2010, Elstner, 1991). However, occasionally O₂ can react with other ETC components. Here, only one electron is transferred, and the result is the O₂⁻⁻ a moderately reactive ROS with a half-life of approximately 1 μs (Jabs, 1999, Halliwell and Gutteridge, 2000). Therefore, it cannot cross biomembranes and is easily dismutated to H₂O₂. O₂⁻⁻ can also react with another very influential signaling free radical species, NO⁻ to give rise to peroxynitrite (ONOO⁻). HO₂⁻ is formed from O₂⁻⁻ by protonation in aqueous solutions. HO₂⁻ can cross biomembranes and subtract hydrogen atoms from PUFAs and lipid hydroperoxides, thus initiating lipid auto-oxidation (Gutteridge, 2000). It has been well established that ROS appear continuously during photosynthesis in the chloroplasts by partial reduction of O₂ molecules or energy transfer to them. The major site of O₂⁻⁻ production is the thylakoid membrane-bound primary electron acceptor of PSI (Puntarulo and Boheris, 1988).

When the terminal oxidases-cytochrome c oxidase and the alternative oxidase-react with O₂, four electrons are transferred and H₂O is released. It has been noted that O₂⁻⁻ is usually the first ROS to be generated (Puntarulo and Boheris, 1988). Recently, in an interesting work C3 and C4 photosynthesis under salinity was studied and it was found that Amaranth plants, unlike wheat, were able to
detoxify the $O_2^-$ by SOD and low-molecular-weight antioxidant aamarathine and reduced the intensity of LPO. A compensatory relation between SOD activity and amaranthine content in amaranth leaves under salt stress has also been noted (Gao and Zhang, 2008). Protonated form of $O_2^-$, $H_2O_2$ is more reactive than superoxide itself, but in plant cells at physiological pH, a very small proportion of $O_2^-$ would be in this form (Eltsner, 1987). However, superoxide can dismutate to form $H_2O_2$. Much more reactive OH can be formed from $O_2^-$ and $H_2O_2$ through Fe catalyzed Haber-Weiss reaction (Bhattacharjee, 2010, Eltsner, 1987). $O_2^-$ cannot pass through biological membranes as it is readily dismutated to $H_2O_2$ (Halliwell and Gutteridge, 2000).

1.2. Hydrogen peroxide

At low pH, dismutation of $O_2^-$ is unavoidable, with one $O_2^-$ giving up its added electron to another $O_2^-$ and then with protonation resulting in the generation of $H_2O_2$.

Hydrogen peroxide can react with other molecules in sites different from those where it has been produced for its capability to cross biomembranes, probably through aquaporins of cellular membranes. $H_2O_2$ may inactivate enzymes by oxidizing their thiol groups. Hydroxy radicals with half life of 1 μs is the most harmful ROS, because its strong instability leads to combine rapidly with whatsoever cellular component present in vicinity (Del Rio and et.al, 1992). The most cellular compartments have the potential to become the source of ROS. The peroxysomal and chloroplastic $H_2O_2$ may be 30 to 100 times faster than the formation of $H_2O_2$ in mitochondria as evident from whole leaf point of view (Bhattacharjee, 2010, Del Rio and et.al, 1992). Photosynthesizing plant cells show particularly high rates of $H_2O_2$ production. The rates of $H_2O_2$ production were estimated to account for $80-160 \text{ M}_2\text{s}_{-1}$ in chloroplasts (Asada, 1999) or for up to $10 \text{ M}_2\text{s}_{-1}$ in photosynthesing leaves (Foyer and Noctor, 2003). Photosynthesis is rapidly inactivated by low concentrations of $H_2O_2$ because $CO_2$ fixation is inhibited (Asada, 1999). A concentration of $10 \text{ M} H_2O_2$ caused a 50% inhibition of $CO_2$ assimilation in isolated chloroplasts (Kaiser, 1976). $H_2O_2$ is a less reactive oxidant when compared to other ROS. Nevertheless, $H_2O_2$ may be particularly harmful, because it is relatively stable and may therefore spread within or among cells by diffusion. $H_2O_2$ can give rise to more ROS, which greatly increases its cytotoxicity. In the presence of transition metals, such as $Fe^2+$ or $Cu^2+$, the extremely reactive hydroxyl radical can be formed in the Fenton reaction (Shigeoka and et al, 2002). $H_2O_2$ plays a dual role in plants: at low concentrations, it acts as a signal molecule involved in acclimatory signaling triggering tolerance to various biotic and abiotic stresses and, at high concentrations, it leads to PCD (Quan, Zhang, and Shi, 2008). $H_2O_2$ has also been shown to act as a key regulator in a broad range of physiological processes, such as senescence (Peng, Ou, and Liu, 2005), photorespiration and photosynthesis (Noctor, and Foyer, 1998), stomatal movement (Bright and et al, 2006), cell cycle (Mittler and et al, 2004) and growth and development (Foreman and et al, 2003). $H_2O_2$ is starting to be accepted as a second messenger for signals generated by means of ROS because of its relatively long life and high permeability across membranes (Quan, Zhang and Shi, 2008). Detoxification of hydrogen peroxide ($H_2O_2$) in plants is essential for cell protection and cell signaling (Neill and et al, 2002). In an interesting study the response of pre-treated citrus roots with $H_2O_2$ (10mM for 8 h) or sodium nitroprusside (SNP; 100 mM for 48 h) was investigated to know the antioxidant defense responses in citrus leaves grown in the absence or presence of 150mM NaCl for 16d (Tanoua and et al, 2009).

1.3. Singlet oxygen

Singlet oxygen, an electronically excited species of $O_2$, is also very toxic. Singlet oxygen ($O_2^+$) is another form of reactive oxygen but here there is no addition of an extra electron to molecular oxygen; rather, an electron is elevated to a higher energy orbital, thereby freeing oxygen from its spin-restricted state. This removal of spin restriction causes singlet oxygen to react rapidly with organic molecules, potentially causing damage (Op den Camp, and et al, 2003). Its significance has been realized due to the development of methods for its generation, free from other contaminants as well as its detection (Halliwell and Gutteridge, 2000). In plants, singlet oxygen can be formed by photoexcitation of chlorophyll and its reaction with oxygen, which can indeed result in lipid damage. Normally, efficient photoprotective agents such as carotenoids quench the excited state of chlorophyll, thus preventing the formation of singlet oxygen. However, recent work has indicated that singlet oxygen can also act as a signaling molecule, mediating the expression of a number of genes (Op den Camp, and et al, 2003). Insufficient energy dissipation during photosynthesis can lead to formation of chlorophyll (Chl) triplet state. The Chl triplet state can react with $O_2^+$ to give the very reactive $O_2^+$. It has been found that the formation of $O_2^+$ during photosynthesis has a powerful damaging effect on PSI and PSII as well as on the whole photosynthetic machinery. Further, various abiotic stresses such as salinity, drought etc. lead to closing of stomata and resulted low intercellular $CO_2$ concentration in the chloroplast favours the formation of $O_2^+$ (Hatz and et al, 2007).

In an interesting study it has been found that a photosensitizer in bacteria can generate $O_2^+$ upon exposure to light, which leads to the oxidation of proteins or lipids and ultimately bacteria death. (Maisch and et al, 2007). Recently, it has been reported that in optimal growth conditions $O_2^+$ was responsible for more than 80% of the nonenzymatic LPO in Arabidopsis leaf tissues (riantaphyllides and et al, 2008). Further, other study showed that, in Arabidopsis mutants favouring $O_2^+$...
production, photooxidative stress led to a dramatic increase of LPO that preceded cell death (Tryptaphyllides and et al, 2008). The life time of \( ^1O_2 \) within the cell is probably 3 \( \mu s \) or less (Hackbarth and et al, 2010). A fraction of \( ^1O_2 \) has been shown to be able to diffuse over considerable distances of several hundred nanometers (nm). Singlet oxygen can last for 4 \( \mu s \) in water and 100 \( \mu s \) in a nonpolar environment. \( ^1O_2 \) reacts with most of the biological molecules at near diffusion-controlled rates (Foyer and Harbinson, 1994). It directly oxidizes protein, unsaturated fatty acids, and DNA (Wagner and et al, 2004). It causes nucleic acid modification through selective reaction with deoxyguanosine (Kasai, 1997). It is thought to be the most important species responsible for light-induced loss of photosystem II (PSII) activity which may trigger cell death (Krieger-Liszkay and et al, 2008). \( ^1O_2 \) can be quenched by \( \beta \)-carotene, \( \alpha \)-tocopherol or can react with the D1 protein of photosystem II as target (Krieger-Liszkay, 2005). In a study, the impact of \( ^1O_2 \) (produced by Rose Bengal, a photosensitizer) on the ATP hydrolysis and ATP-driven proton translocation activity of CF1Cfo was investigated and found that both activities were reduced dramatically within 1 min of exposure. They also showed that oxidized thylakoid ATP synthase was more susceptible to Singlet oxygen than CF1Cfo in its reduced state (Buchert and Forreiter, 2010). Chilling increases photoinhibition and hence increases the production of singlet oxygen by photosystem II and it also favours enhanced lipid hydroperoxidation that leads to membrane damage (Yoshimura and et al, 2004). Because of the short half-life of singlet oxygen and superoxide, it is likely that these ROS are not having a direct effect on nuclear gene expression, but rather that these molecules interact with components that are more closely associated with their site of origin from within chloroplasts, such as membrane lipids (Op den Camp and et al, 2003).

1.4. Hydroxyl radicals
OH is the most reactive among all ROS. It has a single unpaired electron, thus, it can react with oxygen in triplet ground state. In the presence of suitable transitional metals, especially Fe, OH can also be produced from \( O_2^- \) and \( H_2O_2 \) at neutral pH and ambient temperatures by the iron-catalyzed, \( O_2^- \)-driven Fenton reaction (Fig. 2).

\[
H_2O_2 + O_2^- \xrightarrow{Fe^{2+},Fe^{3+}} OH^- + O_2 + OH^+
\]

**Fig. 2.** Produce Hydroxyl radicals from \( O_2^- \) and \( H_2O_2 \) by the iron-catalyzed

OH interacts with all biological molecules and causes subsequent cellular damages such as lipid peroxidation, protein damage, and membrane destruction (Foyer and et al, 2007). Because cells have no enzymatic mechanism to eliminate OH, its excess production can eventually lead to cell death (Pinto and et al, 2003, Vranova and et al, 2002). Under illumination, formation of OH by the Fenton reaction at the active site of the enzyme RbcL leads to its fragmentation in chloroplast lysates (Ishida and et al, 1997, Luo and et al, 2002). The oxidation of organic substrates by OH may proceed by two possible reactions, either by addition of OH to organic molecules or due to abstraction of a hydrogen atom from it. Because of short lifetime and the strongly positive redox potential (close to +2V) of “free” OH, its sites of reaction are close to its point of formation (Eltsner, 1982). In this context, organic oxygen radicals such as alkoxy, peroxy, semiquinones, reduced hydrogen peroxide, and hydrogen peroxide-electron donor complexes (crypto-O2H), as well as metallo-oxygen complexes, have been proposed as the ultimately active species besides destructive free OH (Eltsner, 1987).

2. Sources of ROS in plant cells
The mechanism by which ROS is generated in aerobic organisms is poorly understood. ROS are produced in both stressed and unstressed condition at several cells organelles such as chloroplast, mitochondria plasma membranes, peroxisomes, apoplast, endoplasmic reticulum, and cell walls, with a highly oxidizing metabolic activity or with intense rate of electron flow are a major source of ROS in plant cells. The ability of phototrophs to convert light into biological energy is critical for life on Earth and therefore photosynthesizing organisms are especially at the risk of oxidative damage, because of their bioenergetic lifestyle and the abundance of the photosensitizers and polyunsaturated fatty acids (PUFA) in the chloroplast envelope. This situation leads to oxidative stress.

The appearance of \( O_3 \) in the atmosphere enabled respiratory metabolism and efficient energy generation systems which use \( O_3 \) as final electron acceptor, lead to the formation of ROS in cells (Temple and et al, 2005). In light the chloroplasts and peroxisomes are the main source of ROS generation (Foyer and et al, 1997). In the darkness the mitochondria appear to be the main ROS producers. It has been estimated that 1-5% of the \( O_3 \) consumption of isolated mitochondria results in ROS production (Moller, 2001). Since chloroplasts, mitochondria and peroxisomes are the main ROS producers in a plant cell, the majority of the ROS-scavenging enzymes are located in these organelles. This includes the major cellular isoforms of the superoxide dismutases, ascorbate peroxidases and catalases, but also glutathione peroxidases, and peroxiredoxins.

Thus besides the classical use of inhibitors or the isolation of mutants, manipulation of the antioxidant enzyme levels in specific cellular compartments by genetic tools can influence the accumulation of specific ROS in given cellular compartments (Pnueli and et al, 2003, Rizhsky and et al, 2003, Rizhsky and et al, 2004).
2.1. Chloroplasts
In higher plants and algae, photosynthesis takes place in chloroplasts, which contain a highly organized thylakoid membrane system that harbours all components of the light-capturing photosynthetic apparatus and provides all structural properties for optimal light harvesting (Pfannschmidt, 2003). There are three types of oxygen-consuming processes closely associated with photosynthesis: (a) the oxygenase reaction of ribulose-1,5 bisphosphate carboxylase-oxygenase (Rubisco), (b) direct reduction of molecular oxygen by photosystem I (PSI) electron transport, and (c) chlororespiration (Keller and et al., 1998). Oxygen generated in the chloroplasts during photosynthesis can accept electrons passing through the photosystems, thus results in the formation of O$_2^\cdot$- Therefore, the presence of ROS producing centers such as triplet Chl, ETC in PSI and PSII make chloroplasts a major site of ROS production. In chloroplasts, various forms of ROS are generated from several locations. Chl associated with the electron transport chain (ETC) is the primary source of O$_2^\cdot$, which may also arise as a byproduct of LP, which is catalyzed by lipoxygenase (Asada, 2006, Foyer and Noctor, 2009). O$_2^\cdot$ is a natural byproduct of photosynthesis, mainly formed at PS II even under low-light conditions (Buchert, and Forreiter, 2010). ETCs in PSI and PSII are the main sources of ROS in chloroplasts. Production of ROS by these sources is enhanced in plants by conditions limiting CO$_2$ fixation, such as drought, salt, and temperature stresses, as well as by the combination of these conditions with high-light stress. Under normal conditions, the electron flow from the excited PS centers to NADP which is reduced to NADPH with the accompanying to aerobic respiration (Rhoads and et al., 1997). However, ROS production in mitochondria takes place under normal respiratory conditions but can be enhanced in response to various biotic and abiotic stress conditions. Complex I and III of mitochondrial ETC are the very well known sites of O$_2^\cdot$- production. O$_2^\cdot$- is the primary ROS formed by monovalent reduction in the ETC. In aqueous solution, O$_2^\cdot$- is moderately reactive, but this O$_2^\cdot$ can further reduced by SOD dismutation to H$_2$O$_2$ (Halliwell, 1977). It has been estimated that about 1-5% of mitochondrial O$_2$ consumption leads to H$_2$O$_2$ production (Leegood, and Walker, 1982). This H$_2$O$_2$ can react with reduced Fe$^{2+}$ and Cu$^{+}$ to produce highly toxic OH and these uncharged OH can penetrate membranes and leave the mitochondrion (Luo and et al, 2002, Ishida and et al, 1997). Several enzymes present in mitochondrial matrix can produce ROS. Some of them produce ROS directly, for example aconitase, whereas some others like I-galactono-γ lactone dehydrogenase (GAL), are able to feed electrons to ETC (Rasmusson, Geisler and Moller, 2008).

Flavonoids that are present in plants in high concentrations in the cytoplasm and isoprene that is mostly synthesized in the chloroplasts could also function as O$_2^\cdot$, quenchers (Del Rio and et al, 2002, Hu, 2007). The water soluble chlorophyll binding protein (WSCP) binds to free Chl molecules as well as to its biosynthetic intermediates and does not allow them to get hotoactivated to produce O$_2^\cdot$. It acts as a physical barrier between free Chl molecules and molecular oxygen (Schmidt and Fufezan, 2003).

2.2. Mitochondria
Plant mitochondria as “energy factories” are believed to be a major site of ROS production (Rasmusson and et al, 2004). It differs significantly from their animal counterparts, with specific ETC components and functions in processes such as photosynthesis. The cellular environment of plant mitochondria is also distinctive because of the presence of photosynthesis, which creates an O$_2$ and carbohydrate (sucrose, glucose and fructose) rich environment (Noctor and et al, 2006). The mitochondrial ETC harbours electrons with sufficient free energy to directly reduce O$_2$ which is considered the unavoidable primary source of mitochondrial ROS generation, a necessary accompaniment to aerobic respiration (Rhoads and et al., 2006). However, ROS production in mitochondria takes place under normal respiratory conditions but can be enhanced in response to various biotic and abiotic stress conditions. Complex I and III of mitochondrial ETC are the very well known sites of O$_2^\cdot$- production. O$_2^\cdot$- is the primary ROS formed by monovalent reduction in the ETC. In aqueous solution, O$_2^\cdot$- is moderately reactive, but this O$_2^\cdot$ can further reduced by SOD dismutation to H$_2$O$_2$ (Halliwell, 1977). It has been estimated that about 1-5% of mitochondrial O$_2$ consumption leads to H$_2$O$_2$ production (Leegood, and Walker, 1982). This H$_2$O$_2$ can react with reduced Fe$^{2+}$ and Cu$^{+}$ to produce highly toxic OH and these uncharged OH can penetrate membranes and leave the mitochondrion (Luo and et al, 2002, Ishida and et al, 1997). Several enzymes present in mitochondrial matrix can produce ROS. Some of them produce ROS directly, for example aconitase, whereas some others like I-galactono-γ lactone dehydrogenase (GAL), are able to feed electrons to ETC (Rasmusson, Geisler and Moller, 2008).
component system involved in a series of oxidation-reduction reactions between redox couples or pairs; transfer of electrons from a suitable donor (reductant) to a suitable electron acceptor (oxidant). These oxidation-reduction reactions involve either the transport of electrons only as in the case of the cytochromes, or electrons and protons together, as occurs between NADH and FAD. The part of the electron-transport chain that actually uses O$_2$ is the terminal oxidase enzyme, cytochrome oxidase (Halliwell, 1999). Cytochrome oxidase releases no detectable oxygen radicals into free solution. However, during the transfer of electrons through earlier components of the transport chain a few electrons do leak out directly on to O$_2$, resulting in the generation of O$_2^-$. It follows that damage to mitochondrial organization that severely affects the smooth flow of electrons through the electron-transport chain could favor leakage of electrons and increase O$_2^-$ production (Halliwell, 1999). The supposition that mitochondria are the principal source of ROS during oxidative tissue injury derives from the observations that isolated mitochondria produce O$_2^-$ through either auto-oxidation of the flavin component of complex I (NADH hydrogenase), and/or by auto-oxidation of the ubisemiquinone at complex III. In an interesting work microscopic observations were done to monitor in vivo the behaviour of mitochondria, as well as the production and localization of ROS during protoplast PCD induced by UV-C (Gao and et al, 2008). It was noted that UV-C exposure induces quick appearance of ROS in the protoplasts, which was restricted in chloroplasts and the mitochondria. It was suggested that the mitochondrial transmembrane potential loss and the changes in distribution and mobility of mitochondria, as well as the production of ROS play important roles during UV-induced plant PCD. A Nicotiana sylvestris mitochondrial mutant was used to study the role of plant mitochondria in the regulation of cellular redox homeostasis and stress resistance (Gao and et al, 2008) and it was noted that the cytoplasmic male-sterile mutant (CMSII) impaired in complex I function and displayed enhanced nonphosphorylating rotenone-insensitive [NAD(P)H dehydrogenases] and cyanide-insensitive (alternative oxidase) respiration which was not associated with increased oxidative stress. The loss of complex I function reveals effective antioxidant crosstalk and acclimation between the mitochondria and other organelles to maintain whole cell redox balance. This reorchestration of the cellular antioxidative system was associated with higher tolerance to ozone and tobacco mosaic virus (Dutilleul and et al, 2003).

To investigate the effect of ROS on plant mitochondria, Pastore et al. used the ROS producing system consisting of xanthine plus xanthine oxidase on the rate of membrane potential (DJ) generation due to either succinate or NADH addition to durum wheat mitochondria and showed that the early ROS production inhibits the succinate dependent, but not the NADH dependent, DJ generation and O$_2$ uptake. It was found that early generation of ROS can affect plant mitochondria by impairing metabolite transport, thus preventing further substrate oxidation, DJ generation and consequent large-scale ROS production (Pastore and et al, 2002). Using a proteomic approach, Sweetlove et al. demonstrated that the expression of various mitochondrial proteins was up- or down-regulated following exposure to H$_2$O$_2$. The up-regulated proteins included antioxidant defence proteins such as peroxiredoxins and protein disulphide isomerase, whereas those associated with the TCA cycle were down-regulated. However, H$_2$O$_2$ can affect the proteome not just by increasing or decreasing the levels of proteins indirectly, but also by direct modification of protein activities, as described earlier. A detailed analysis of the protein profile of various organelles following exposure to different ROS is required, to decode specific signaling pathways in response to various stimuli (Sweetlove and et al, 2002).

2.3. Other sources of ROS generation in plants

Other important sources of ROS production in plants that have received little attention are detoxification reactions catalysed by cytochrome P450 in cytoplasm and endoplasmic reticulum (Dybing and et al, 1976). Organic substrate, RH, reacts first with Cyt P450 and then is reduced by a flavoprotein to form a radical intermediate (Cyt P450R−). Triplet oxygen can readily react with this radical intermediate as each has one unpaired electron. This oxygenated complex (Cyt P450-ROO−) may be reduced by cytochrome b or occasionally the complexes may decompose releasing O$_2^-$ (Mittler, 2002). Cell walls are also regarded as active sites for ROS production. Role of cell-wall-associated peroxidase in H$_2$O$_2$ generation has been shown. In horseradish, peroxidase associated with isolated cell walls catalyzes the formation of H$_2$O$_2$ in the presence of NADH. The reaction is stimulated by various monophenols, especially of coniferyl alcohol. ROS are also generated at plasma membrane level or extracellularly in apoplast in plants. Malate dehydrogenase was found to be the sole candidate for providing NADH (Gross, 1977).

Cell-wall-located enzymes have been proved to be responsible for apoplastic ROS production (Foyer and Noctor, 2005, Maisch and et al, 2007). The cell-wall-associated oxalate oxidase, also known as germin, releases H$_2$O$_2$ and CO$_2$ from oxalic acid (Hu, 2007). pH dependent cell-wall peroxidases are activated by alkaline pH, which, in the presence of a reductant produces H$_2$O$_2$ (Bolwell and Woftastek, 1997). This enzyme was reported to be involved in apoplastic hydrogen peroxide accumulation during interactions between different cereals species and fungi (Lane, 2002). Amine oxidase-like enzymes may contribute to defense responses
occurring in the apoplast following biotic stress, mainly through \( \text{H}_2\text{O}_2 \) production. Amine oxidases catalyze the oxidative deamination of polyamines (i.e., putrescine, spermine, and spermidine) using FAD as a cofactor (Cona and et al, 2006). Heyno and coworkers, based on their study, concluded that apoplastic OH generation depends fully, or for the most part, on peroxidase localized in the cell wall (Heyno and et al, 2011). Peroxisomes are probably the major sites of intracellular \( \text{H}_2\text{O}_2 \) production, as a result of their essentially oxidative type of metabolism (Del Rio and et al, 2006). The main metabolic processes responsible for the generation of \( \text{H}_2\text{O}_2 \) in different types of peroxisomes are the glycolate oxidase reaction, the fatty acid β-oxidation, the enzymatic reaction of flavon oxidases, and the disproportionation of \( \text{O}_2^- \) radicals (Baker and Graham, 2002). During photorespiration, the oxidation of glycolate by glycolate oxidase in peroxisomes accounts for the majority of \( \text{H}_2\text{O}_2 \) production (Noctor and et al, 2002). Like mitochondria and chloroplasts, peroxisomes also produce \( \text{O}_2^- \) as a consequence of their normal metabolism.

### 3. ROS Detoxification

In the presence of transition metal ions hydrogen peroxide may be reduced to hydroxyl radicals by superoxide. Superoxide and hydrogen peroxide are much less reactive than OH. The main risk for a cell that produces the two former reactive oxygen intermediates may be posed by the intermediates’ interaction, leading to the generation of highly reactive hydroxyl radicals. Because there are no known scavengers of hydroxyl radicals, the only way to avoid oxidative damage through this radical is to control the reactions that lead to its generation. Thus, cells had to evolve sophisticated strategies to keep the concentrations of superoxide, hydrogen peroxide, and transition metals such as Fe and Cu under tight control. Scavenging or detoxification of excess ROS is achieved by an efficient antioxidative system comprising of the nonenzymic as well as enzymic antioxidants (Noctor and Foyer, 1998). Enzymatic antioxidants include SOD, CAT, APX, MDHAR, DHAR and GR and non-enzymatic antioxidants are GSH, AA (both water soluble), carotenoids and tocopherols (lipid soluble) (Mittler and et al, 2004, Gill and et al, 2011, Dalton and et al, 1999). Maintenance of a high antioxidant capacity to scavenge the toxic ROS has been linked to increased tolerance of plants to environmental stresses (Chen and et al, 2010, Zaefyzadeh and et al, 2009).

#### 3.1. Nonenzymatic ROS Scavenging

Nonenzymatic antioxidants include the major cellular redox buffers ascorbate and glutathione (GSH), as well as tocopherol, flavonoids, alkaloids, and carotenoids. Ascorbic acid is the most abundant, water soluble and powerful antioxidant acts to prevent or in minimizing the damage caused by ROS in plants (Athar and et al, 2008). It occurs in all plant tissues, usually being higher in photosynthetic cells and meristems (and some fruits). Its concentration is reported to be highest in mature leaves with fully developed chloroplast and highest chlorophyll. It has been reported that ASH mostly remain available in reduced form in leaves and chloroplast under normal physiological conditions (Smirnoff, 2000). About 30 to 40% of the total ascorbate is in the chloroplast and stromal concentrations as high as 50 mM have been reported (Foyer and Noctor, 2005). In plants, mitochondrion play central role in the metabolism of ASH. Plant mitochondria are not only synthesise ASH by L-galactono-γ-lactone dehydrogenase but also take part in the regeneration of ASH from its oxidised forms (Szarka and et al, 2007). ASH is considered as a most powerful ROS scavenger because of its ability to donate electrons in a number of enzymatic and non-enzymatic reactions. It can provide protection to membranes by directly scavenge the \( \text{O}_2^- \) and OH and by regenerate a-tocopherol from tocopheroxyl radical. Mutants with altered GSH content (Creissen and et al, 1999) or decreased ascorbic acid levels (Conklin and Williams, 1996) are hypersensitive to stress. Whereas GSH is oxidized by ROS forming oxidized glutathione (GSSG), ascorbate is oxidized to monodehydroascorbate (MDA) and dehydroascorbate (DHA). Through the ascorbate-glutathione cycle, GSSG, MDA, and DHA can be reduced reforming GSH and ascorbate. In response to chilling, heat shock, pathogen attack, and drought stress, plants increase the activity of GSH biosynthetic enzymes (Vanacker and et al, 2000, Vernoux and et al, 2002) and GSH levels (Noctor and et al, 2002). A high ratio of reduced ascorbate and GSH is essential for ROS scavenging in cells. Reduced states of the antioxidants are maintained by glutathione reductase (GR), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR), using NADPH as reducing power (Tsugane and et al, 1999, Asada and Takahashi, 1987). In addition, the overall balance among different antioxidants must be tightly controlled. The importance of this balance is evident when cells with enhanced glutathione biosynthesis in chloroplasts show oxidative stress damage, possibly due to changes of the overall redox state of chloroplasts (Creissen and et al, 1999). Little is known about flavonoids and carotenoids in ROS detoxification in plants. However, overexpression of β-carotene hydroxylase in Arabidopsis leads to increased amounts of xanthophyll in chloroplasts and results in enhanced tolerance towards oxidative stress induced in high light (Davison and et al, 2002).

#### 3.2. Enzymatic ROS Scavenging

Various workers have reported increased activities of many enzymes of the antioxidant defense system in plants to combat oxidative stress induced by various environmental stresses. Enzymatic ROS scavenging mechanisms in plants include superoxide dismutase (SOD), Catalases (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX). Metalloenzyme SOD is the
most effective intracellular enzymatic antioxidant which is ubiquitous in all aerobic organisms and in all subcellular compartments prone to ROS mediated oxidative stress. It is well established that various environmental stresses often lead to the increased generation of ROS, where, SOD has been proposed to be important in plant stress tolerance and provide the first line of defense against the toxic effects of elevated levels of ROS, dismutating superoxide to H₂O₂, APX, GPX, and CAT subsequently detoxify H₂O₂.

The SODs remove O₂⁻ by catalyzing its dismutation, one O₂⁻ being reduced to H₂O₂ and another oxidized to O₂. It removes O₂⁻ and hence decreases the risk of OH⁻ formation via the metal catalyzed Habere Weiss-type reaction. This reaction has a 10,000 fold faster rate than spontaneous dismutation. SODs are classified by their metal cofactors into three known types: the copper/zinc (Cu/Zn-SOD), the manganese (Mn-SOD) and the iron (Fe-SOD), which are localized in different cellular compartments (Mittler, 2002). The activity of SOD isoforms can be detected by negative staining and identified on the basis of their sensitivity to KCN and H₂O₂. The Mn-SOD is resistant to both inhibitors; Cu/Zn-SOD is sensitive to both inhibitors whereas; Fe-SOD is resistant to KCN and sensitive to H₂O₂. The subcellular distribution of these isoforms is also distinctive. The Mn-SOD is found in the mitochondria of eukaryotic cells and in peroxisomes, some Cu/Zn-SOD isoforms are found in the cytosolic fractions, and also in chloroplasts of higher plants (Del Rio and et al, 2002). The Fe-SOD isoforms, often not detected in plants (Ferreira and et al2002) are usually associated with the chloroplast compartment when present (Alschier and et al, 2002). The prokaryotic Mn-SOD and Fe-SOD, and the eukaryotic Cu/Zn-SOD enzymes are dimers, whereas Mn-SOD of mitochondria are tetramers. In eukaryotic cells the intracellular concentration of O₂⁻ is tightly regulated by Cu/Zn SOD and Mn SOD. The Cu/Zn SOD enzyme, found in virtually all eukaryotic cells, has a relative molecular mass of about 32 kD and contains two protein subunits each of which bears an active site containing one Cu and one Zn cation. Primarily located in the cytosol, but also present in lysosomes, nucleus, and the mitochondrial intermembranous space, Cu/Zn SOD catalyzes the dismutation of O₂⁻ to H₂O₂ and O₂.

\[
O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \text{ (ground-state)}
\]

Mn SOD. First isolated from Escherichia coli, Mn-SOD in eukaryotic cells is expressed mainly in the mitochondria. Mn SOD is not inhibited by cyanide or diethyldithiocarbamate, has a relative mass of 40 kD, is destroyed by treatment with chloroform plus ethanol and contains manganese at its active site. Despite these differences Mn SOD essentially catalyzes the same reaction as Cu/Zn SOD (Halliwell and et al, 2000). All forms of SOD are nuclear-encoded and targeted to their respective subcellular compartments by an amino terminal targeting sequence. Several forms of SOD have been cloned from a variety of plants (Scandalios, 1990). dismutation of O₂⁻ generates H₂O₂ which is usually removed in cells by two types of enzymes, catalase and peroxidases. Catalase directly catalyzes the decomposition of H₂O₂ to ground state O₂ and is unique in that H₂O₂ serves as both a donor and acceptor; while the peroxidases remove H₂O₂ by using it to oxidize other substrates, such as NADH and glutathione (GSH).

3.2.1.Catalase. (CAT).
Catalase consists of four subunits, each of which contains a ferric heme group bound to its active site. Catalase activity in cells is largely found in subcellular organelles bound to a single membrane and known as peroxisomes. The catalase reaction is essentially a dismutation reaction similar to SOD; one H₂O₂ is reduced to H₂O and the other is oxidized to O₂. CAT has one of the highest turnover rates for all enzymes: one molecule of CAT can convert 2.6 million molecules of H₂O₂ to H₂O and O₂ per minute. CAT is important in the removal of H₂O₂ generated in peroxisomes by oxidases involved in b-oxidation of fatty acids, photosynthesis and purine catabolism. The CAT isoforms have been studied extensively in higher plants (Polidoros and Scandalios, 1999).

3.2.2.Glutathione peroxidase. (GPX)
GPXs are a large family of diverse isoforms that like APX, detoxifies H₂O₂ to H₂O, but uses GSH directly as a reducing agent and organic and lipid hydroperoxides, therefore help plant cells from oxidative stress (Noctor and et al, 2002). The GPX cycle is closed by regeneration of GSH from GSSG by GR. Unlike most organisms, plants have multiple genes encoding SOD and APX. GPX consists of four protein subunits, each of which contains one atom of the element selenium at its active site (Halliwell, 1999). GPX removes H₂O₂ by coupling its reduction to H₂O, with the oxidation (GSSG) of GSH. Most cells contain substantial activities of both GPX and catalase. Usually, subcellular compartmentalization influences H₂O₂ removal. H₂O₂ produced by peroxisomal enzymes such as glycolate oxidase and urate oxidase is largely disposed of by catalase, whereas H₂O₂ arising from the mitochondria, the endoplasmic reticulum or by the action of cytosolic Cu/Zn SOD is acted upon by GPX.

3.2.3.Ascorbate peroxidase (APX)
APX is thought to play the most essential role in scavenging ROS and protecting cells in higher plants, algae, euglena and other organisms. APX is involved in scavenging of H₂O₂ in water-water and ASH-GSH cycles and utilizes ASH as the electron donor. The APX family consists of at least five different isoforms including thylakoid (tAPX) and glyoxisome membrane forms (gmAPX), as well as chloroplast stromal soluble form
like fluidity, transport, loss of enzyme activity, protein damage to biomolecules such as lipids, proteins and DNA.

Pathogens, and so forth. Enhanced level of ROS can cause damage to biomolecules such as lipids, proteins and DNA. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, DNA damage, and so forth ultimately resulting in cell death.

4.1. Lipid peroxidation

When ROS level reaches above threshold, enhanced lipid peroxidation takes place in both cellular and organelar membranes, which, in turn, affect normal cellular functioning. The peroxidation of lipids is considered as the most damaging process known to occur in every living organism. Membrane damage is sometimes taken as a single parameter to determine the level of lipid destruction under various stresses. Now, it has been recognized that during LPO, products are formed from polyunsaturated precursors that include small hydrocarbon fragments such as ketones, MDA, etc and compounds related to them (Garg and Manchanda, 2009). When ROS level reaches above threshold, enhanced lipid peroxidation takes place in both cellular and organelar membranes, which, in turn, affect normal cellular functioning. Lipid peroxidation aggravates the oxidative stress through production of lipid-derived radicals that themselves can react with and damage proteins and DNA. The level of lipid peroxidation has been widely used as an indicator of ROS mediated damage to cell membranes under stressful conditions. Increased peroxidation (degradation) of lipids has been reported in plants growing under environmental stresses. Increase in lipid peroxidation under these stresses parallels with increased production of ROS. Malondialdehyde (MDA) is one of the final products of peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage (Halliwell and Gutteridge, 2000). Two common sites of ROS attack on the phospholipid molecules are the unsaturated (double) bond between two carbon atoms and the ester linkage between glycerol and the fatty acid. The polyunsaturated fatty acids (PUFAs) present in membrane phospholipids are particularly sensitive to attack by ROS. A single OH can result in peroxidation of many polyunsaturated fatty acids because the reactions involved in this process are part of a cyclic chain reaction.

4.2. Protein oxidation

The attack of ROS on proteins may cause modification of proteins in a variety of ways, some are direct and others indirect. Direct modification involves modulation of a protein’s activity through nitrosylation, carbonylation, disulfide bond formation, and glutationylation. Proteins can be modified indirectly by conjugation with breakdown products of fatty acid peroxidation (Yamauchi and et al, 2008).

Thiol groups and sulphur containing amino acids are very susceptible sites for attack by ROS. Activated oxygen can abstract an H atom from cysteine residues to form a thyl radical that will cross-link to second thyl radical to form disulphide bridge. Several metals, including Cd, Pb, and Hg have been shown to cause the depletion of protein

(sAPX), cytosolic form (cAPX) (Noctor and Foyer, 1998). APX has a higher affinity for H2O2 (mM range) than CAT and POD (mM range) and it may have a more crucial role in the management of ROS during stress. In contrast to CAT, APX requires an ascorbate and GSH regeneration system, the ascorbate-glutathione cycle. Detoxifying H2O2 to H2O by APX occurs by oxidation of ascorbate to MDA which can be regenerated by MDA reductase (MDAR) using NAD(P)H as reducing equivalents. MDA can spontaneously dismutate into dehydroascorbate. Ascorbate regeneration is mediated by dehydro-ascorbate reductase (DHAR) driven by the oxidation of GSH to GSSG. Finally, glutathione reductase (GR) can regenerate GSH from GSSG using NAD(P)H as a reducing agent. Different isoforms are specifically targeted to chloroplasts, mitochondria, peroxisomes, as well as to the cytosol and apoplast (Asada and Takahashi, 1987). Whereas GPX is cytosolic, CAT is located mainly in peroxisomes. Specific roles for antioxidant enzymes have been explored via transgenic approaches. Over expression of tobacco chloroplast SOD to chloroplasts did not alter tolerance toward oxidative stress, which suggests that other antioxidant mechanisms might be limiting. However, expression of a pea chloroplast SOD in tobacco increased resistance to methylviologen–induced membrane damage (Allen, 1995).

CAT is indispensable for oxidative stress tolerance because transgenic tobacco plants with suppressed CAT have enhanced ROS levels in response to both abiotic and biotic stresses (Willekens and et al, 1997). The extent of oxidative stress in a cell is determined by the amounts of superoxide, H2O2, and hydroxyl radicals. Therefore, the balance of SOD, APX, and CAT activities will be crucial for suppressing toxic ROS levels in a cell. Changing the balance of scavenging enzymes will induce compensatory mechanisms. For example, when CAT activity was reduced in plants, scavenging enzymes such as APX and GPX were upregulated. Unexpected effects can also occur. When compared to plants with suppressed CAT, plants lacking both APX and CAT were less sensitive to oxidative stress (Rizhsky and et al, 2002).

Because photosynthetic activity of these plants was decreased, reduction in APX and CAT might result in suppression of ROS production via chloroplasts.

4. ROS and Oxidative Damage to Biomolecules

Production and removal of ROS must be strictly controlled in order to avoid oxidative stress. When the level of ROS exceeds the defense mechanisms, a cell is said to be in a state of “oxidative stress”. However, the equilibrium between production and scavenging of ROS is perturbed under a number of stressful conditions such as salinity, drought, high light, toxicity due to metals, pathogens, and so forth. Enhanced level of ROS can cause damage to biomolecules such as lipids, proteins and DNA. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, DNA damage, and so forth ultimately resulting in cell death.

(sAPX), cytosolic form (cAPX) (Noctor and Foyer, 1998). APX has a higher affinity for H2O2 (mM range) than CAT and POD (mM range) and it may have a more crucial role in the management of ROS during stress. In contrast to CAT, APX requires an ascorbate and GSH regeneration system, the ascorbate-glutathione cycle. Detoxifying H2O2 to H2O by APX occurs by oxidation of ascorbate to MDA which can be regenerated by MDA reductase (MDAR) using NAD(P)H as reducing equivalents. MDA can spontaneously dismutate into dehydroascorbate. Ascorbate regeneration is mediated by dehydro-ascorbate reductase (DHAR) driven by the oxidation of GSH to GSSG. Finally, glutathione reductase (GR) can regenerate GSH from GSSG using NAD(P)H as a reducing agent. Different isoforms are specifically targeted to chloroplasts, mitochondria, peroxisomes, as well as to the cytosol and apoplast (Asada and Takahashi, 1987). Whereas GPX is cytosolic, CAT is located mainly in peroxisomes. Specific roles for antioxidant enzymes have been explored via transgenic approaches. Over expression of tobacco chloroplast SOD to chloroplasts did not alter tolerance toward oxidative stress, which suggests that other antioxidant mechanisms might be limiting. However, expression of a pea chloroplast SOD in tobacco increased resistance to methylviologen–induced membrane damage (Allen, 1995).

CAT is indispensable for oxidative stress tolerance because transgenic tobacco plants with suppressed CAT have enhanced ROS levels in response to both abiotic and biotic stresses (Willekens and et al, 1997). The extent of oxidative stress in a cell is determined by the amounts of superoxide, H2O2, and hydroxyl radicals. Therefore, the balance of SOD, APX, and CAT activities will be crucial for suppressing toxic ROS levels in a cell. Changing the balance of scavenging enzymes will induce compensatory mechanisms. For example, when CAT activity was reduced in plants, scavenging enzymes such as APX and GPX were upregulated. Unexpected effects can also occur. When compared to plants with suppressed CAT, plants lacking both APX and CAT were less sensitive to oxidative stress (Rizhsky and et al, 2002).

Because photosynthetic activity of these plants was decreased, reduction in APX and CAT might result in suppression of ROS production via chloroplasts.

4. ROS and Oxidative Damage to Biomolecules

Production and removal of ROS must be strictly controlled in order to avoid oxidative stress. When the level of ROS exceeds the defense mechanisms, a cell is said to be in a state of “oxidative stress”. However, the equilibrium between production and scavenging of ROS is perturbed under a number of stressful conditions such as salinity, drought, high light, toxicity due to metals, pathogens, and so forth. Enhanced level of ROS can cause damage to biomolecules such as lipids, proteins and DNA. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, DNA damage, and so forth ultimately resulting in cell death.

4.1. Lipid peroxidation

When ROS level reaches above threshold, enhanced lipid peroxidation takes place in both cellular and organelar membranes, which, in turn, affect normal cellular functioning. The peroxidation of lipids is considered as the most damaging process known to occur in every living organism. Membrane damage is sometimes taken as a single parameter to determine the level of lipid destruction under various stresses. Now, it has been recognized that during LPO, products are formed from polyunsaturated precursors that include small hydrocarbon fragments such as ketones, MDA, etc and compounds related to them (Garg and Manchanda, 2009). When ROS level reaches above threshold, enhanced lipid peroxidation takes place in both cellular and organelar membranes, which, in turn, affect normal cellular functioning. Lipid peroxidation aggravates the oxidative stress through production of lipid-derived radicals that themselves can react with and damage proteins and DNA. The level of lipid peroxidation has been widely used as an indicator of ROS mediated damage to cell membranes under stressful conditions. Increased peroxidation (degradation) of lipids has been reported in plants growing under environmental stresses. Increase in lipid peroxidation under these stresses parallels with increased production of ROS. Malondialdehyde (MDA) is one of the final products of peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage (Halliwell and Gutteridge, 2000). Two common sites of ROS attack on the phospholipid molecules are the unsaturated (double) bond between two carbon atoms and the ester linkage between glycerol and the fatty acid. The polyunsaturated fatty acids (PUFAs) present in membrane phospholipids are particularly sensitive to attack by ROS. A single OH can result in peroxidation of many polyunsaturated fatty acids because the reactions involved in this process are part of a cyclic chain reaction.

4.2. Protein oxidation

The attack of ROS on proteins may cause modification of proteins in a variety of ways, some are direct and others indirect. Direct modification involves modulation of a protein’s activity through nitrosylation, carbonylation, disulphide bond formation, and glutationylation. Proteins can be modified indirectly by conjugation with breakdown products of fatty acid peroxidation (Yamauchi and et al, 2008).

Thiol groups and sulphur containing amino acids are very susceptible sites for attack by ROS. Activated oxygen can abstract an H atom from cysteine residues to form a thyl radical that will cross-link to second thyl radical to form disulphide bridge. Several metals, including Cd, Pb, and Hg have been shown to cause the depletion of protein
4.3. DNA damage

ROS are a major source of DNA damage (Imlay and Linn, 1988). ROS can cause oxidative damages to nuclear, mitochondrial, and chloroplastic DNA. DNA is cell’s genetic material and any damage to the DNA can result in changes in the encoded proteins, which may lead to malfunctions or complete inactivation of the encoded proteins. Oxidative attack on DNA results in deoxyribose oxidation, strand breakage, removal of nucleotides, variety of modifications in the organic bases of the nucleotides, and DNA-protein crosslinks. Further, changes in the nucleotides of one strand can result in the mismatches with the nucleotides in the other strand, yielding subsequent mutations. Enhanced DNA degradation has been observed in plants exposed to various environmental stresses such as salinity (Liu and et al, 2000), and metal toxicity (Meriga and et al, 2004). Both the sugar and base moieties of DNA are susceptible to oxidation by ROS. Oxidative attack to DNA bases generally involves OH addition to double bonds, while sugar damage mainly results from hydrogen abstraction from deoxyribose (Dizdaroglu, 1993). The hydroxyl radical is known to react with all purine and pyrimidine bases and, also, the deoxyribose backbone (Halliwell and Gutteridge, 2000). OH generates various products from the DNA bases which mainly include C-8 hydroxylation of guanine to form 8-oxo-7,8 dehydro-2'-deoxyguanosine, hydroxymethyl urea, urea, thymine glycol, thymine and adenine ring-opened, and saturated products (Tsuboi and et al, 1998). 8-Hydroxyguanine is the most commonly observed product. 0$_2^-$ only reacts with guanine, whereas H$_2$O$_2$ and O$_2^-$ do not react with bases at all (Halliwell and Aruoma, 1991). ROS-induced DNA damages include various mutagenic alterations as well. For example, mutation arising from selective modification of G:C sites, especially, indicates oxidative attack on DNA by ROS. ROS attack DNA bases indirectly through reactive products generated by ROS attack to other macromolecules such as lipid (Fink and et al, 1997). ROS attack to DNA sugars leads to single-strand breaks. ROS abstract hydrogen atom from the C4$_-$ position of deoxyribose, leading to generation of a deoxyribose radical that further reacts to produce DNA strand breakage (Evans and et al, 2004). Under physiological conditions, neither H$_2$O$_2$ alone nor O$_2^-$ can cause in vitro strand breakage. Therefore, it was concluded that the toxicity associated with these ROS in vivo is most likely the result of Fenton reaction. When OH attacks on either DNA or proteins associated with it, DNA protein crosslinks are formed (Oleinick and et al, 1987). DNA protein crosslinks cannot be readily repaired and may be lethal if replication or transcription proceeds repair. Mitochondrial and chloroplastic DNA are more susceptible to oxidative damage than nuclear DNA due to the lack of protective protein, histones, and close locations to the ROS producing systems in the former (Richter, 1992). Even though repair system exists for damaged DNA, excessive changes caused by ROS lead to permanent damage to the DNA with potentially
detrimental effects for the cell.

5. The role of ROS in signaling
ROS generation in cellular compartments such as the mitochondria or chloroplasts results in changes of the nuclear transcriptome, indicating that information must be transmitted from these organelles to the nucleus, but the identity of the transmitting signal remains unknown. Three principal modes of action indicate how ROS could affect gene expression. ROS sensors could be activated to induce signaling cascades that ultimately impinge on gene expression. Alternatively, components of signaling pathways could be directly oxidized by ROS. Finally, ROS might change gene expression by targeting and modifying the activity of transcription factors.

5.1. ROS Sensing by Histidine Kinases
In prokaryotes and fungi two-component signaling systems function as redox sensors (Quinn and et al., 2002, Whistler and et al., 1998). In prokaryotes, two-component signaling systems usually consist of a histidine kinase that senses the signal and a response regulator that functions as a transcription factor. The transmembrane sensory kinase functions through its capacity to auto phosphorylate a histidine residue in response to the presence or absence of an external stimulus. The phosphoryl group is subsequently transferred from the histidine to an aspartate residue in the response regulator. The induced conformational change in the response regulator alters its DNA binding affinity and thus promotes gene expression of certain promoters. Also in budding and fission yeast, histidine kinases of two-component signaling systems can function as sensors of oxidative stress (Singh, 2000). In contrast to animals, plants contain a range of two-component histidine kinases (Hwang and et al., 2002). Whether some of these proteins can function as ROS sensors is currently under investigation.

5.2. ROS Activation of Mitogen-Activated Protein Kinase (MAPK)
Although histidine kinases are part of two-component signal transduction systems in prokaryotes that act on their own, in fungi and plants these sensors are integrated into more complex pathways. The yeast Sln1 kinase transfers its phosphoryl group via the intermediary component Ypd1 to its final destination in the response regulator Ssk1. Stress inhibits autophosphorylation of Sln1 and hence the nonphosphorylated form of Ssk1 accumulates and activates the Hog1 mitogen-activated protein kinase (MAPK) cascade (Gustin and et al., 1998). Sequence analyses of the rice and Arabidopsis genomes reveal an extraordinary complexity in MAPK signaling components that comprise more than 100 MAPK, MAPKK, and MAPKKK genes in these plants. MAPK signaling modules are involved in eliciting responses to various stresses, to hormones, and during cytokinesis.

H$_2$O$_2$ activates several MAPKs. In Arabidopsis, H$_2$O$_2$ activates the MAPKs, MPK3, and MPK6 via MAPKKK ANP1. Overexpression of ANP1 in transgenic plants resulted in increased tolerance to heat shock, freezing, and salt stress (Kristensen and et al., 2004). H$_2$O$_2$ also increases expression of the Arabidopsis nucleotide diphosphate (NDP) kinase 2 (Moon and et al., 2003). Overexpression of AtNDPK2 reduced accumulation of H$_2$O$_2$ and enhanced tolerance to multiple stresses including cold, salt, and oxidative stress. The effect of NDPK2 might be mediated by the MAPKs, MPK3, and MPK6 because NDPK2 can interact and activate the MAPKs. These data suggest a scenario in which various stresses induce ROS generation that in turn activate MAPK signaling cascades. Although neither the mechanism of activation nor the downstream targets of the MAPK pathways are yet known, ROS-induced activation of MAPKs appears to be central for mediating cellular responses to multiple stresses.

5.3. ROS Inhibition of Protein Phosphatases
Because H$_2$O$_2$ is a mild oxidant that can oxidize thiol residues, it has been speculated that H$_2$O$_2$ is sensed via modification of thiol groups in certain proteins. Recent work has identified human protein tyrosine phosphatase PTP1B to be modified by H$_2$O$_2$ at the active site cysteine (van Montfort and et al., 2003). Inactivation of PTP1B by H$_2$O$_2$ is reversible and can be brought about by incubation with glutathione. A similar regulation likely occurs in plants because PTP1, an Arabidopsis PTP that can inactivate the Arabidopsis MPK6, can be inactivated by H$_2$O$_2$ (Gupta and Luan, 2003). Also, phosphatases involved in abscisic acid (ABA) signaling within guard cells have been identified whose in vitro activity was modulated reversibly by H$_2$O$_2$ (Meinhard and et al., 2002).

5.4. ROS Activation of Transcription Factors
Comparing the mechanisms for ROS-induced gene expression in prokaryotes, fungi, and plants may reveal common mechanisms (Georgiou, 2002). In E. coli, the transcription factor OxyR is of paramount importance in oxidative stress signaling (Zheng and et al., 1998). In budding yeast, Yap1 plays a similar role. Budding yeast mutants deficient in Yap1 reveal that most ROS-induced genes depend on this transcription factor. OxyR and Yap1 are redox-sensitive transcription factors and modulate gene expression in response to oxidative stress. ROS regulates the activity of the transcription factors through covalent modification of cysteine thiol groups in OxyR and Yap1. Different types of ROS react with different cysteinyl residues and can give rise to differently modified products, possibly explaining how ROS species can induce different sets of genes via the same transcription factor (Delaunay and et al., 2000). One major difference between OxyR and Yap1 is that the yeast transcription factor is not sensing ROS directly but through the activity of Gpx3, which acts as a hydroperoxidase and peroxiredoxin. The higher degree
of complexity in yeast reflects the increased flexibility of eukaryote signaling systems. Accordingly, it is not surprising that additional regulation of redox-sensitive transcription factors was established in fission yeast (Takeda and et al, 1995). Similar to yeast, plants have evolved a MAPK pathway and several protein phosphatases for ROS signaling. Although no redox-sensitive transcription factor has yet been identified in plants, it is likely that such transcription factors exist. Gene expression in response to oxidative stress seems to be coordinated via the interaction of transcription factors with specific oxidative stress-sensitive cis-elements in the promoters of these genes. There is evidence that oxidative stress responsive cis-elements exist in yeasts, animals, and plants. Work in budding and fission yeast has shown that homologs of the mammalian ATP and AP-1 transcription factors function as key mediators of diverse stress signals binding to conserved cis-regions of stress-inducible promoters (Gasch and et al, 2000, Chen and et al, 2003). Microarray analysis of H$_2$O$_2$-induced gene expression in Arabidopsis indicates potential H2O2-responsive cis-elements in genes regulated by H$_2$O$_2$ (Desikan and et al, 2001). One of these elements, the as-1 promoter element, also has high homology with the redox-sensitive mammalian AP-1 cis-element (Karim and et al, 1997). However, recent analysis of transgenic plants indicates that ROS other than H$_2$O$_2$ activate this as-1 element (Garreton and et al, 2002). Further analysis will reveal whether similarity exists among plant, animal, and fungal regulatory cis-elements of ROS-responsive genes.

5.5. ROS as signals for gene expression
Transcriptome analysis with full genome chips has revolutionized our knowledge regarding gene expression. Oxidative stress affects approximately 10% of the yeast transcriptome (Causton and et al, 2001). Exposure of yeast cells to various stresses including H$_2$O$_2$ defines a large set of genes denoted as common environmental stress response (CESR). CESR-induced genes play a role in carbohydrate metabolism, ROS detoxification, protein folding and degradation, organellar function, and metabolite transport. CESR-repressed genes are involved in energy consumption and growth, RNA processing, transcription, translation, and ribosome and nucleotide biosynthesis (Causton and et al, 2001).

In plants, ROS-induced genes have been identified for receptor kinase (Desikan and et al, 2000), annexin (Moseykio and et al, 2002), and peroxisome biogenesis (Desikan and et al, 2000). Recent approaches using cDNA profiling and DNA microarrays have analyzed large-scale gene expression in response to ROS. Following exposure of Arabidopsis cells to H$_2$O$_2$, a total of 175 genes (i.e., 1–2% of the 11,000 genes on the microarray) showed changes in expression levels (Desikan and et al, 2001). Of the 113 induced genes, several encoded for proteins with antioxidant functions or were associated with defense responses or other stresses. Still others coded for proteins with signaling functions. Exposing a plant to sublethal doses of one stress that results in protection from lethal doses of the same stress at a later time is termed stress acclimation. Global changes in gene expression were analyzed in tobacco plants that were treated with superoxide-generating methyl viologen after pretreatment with a sublethal doses (Vranova and et al, 2002). Approximately 2% of the tobacco genes were altered in their expression in acclimated leaves. Genes with predicted protective or detoxifying functions and signal transduction were upregulated in acclimated leaves, implying a variety of cellular responses during acclimation tolerance. The effects of oxidative stress on the Arabidopsis mitochondrial proteome have been analyzed (Sweetlove and et al, 2002). Whereas two classes of antioxidant defense proteins, peroxiredoxins, and protein disulphide isomerase accumulated in response to oxidative stress, proteins associated with the TCA cycle were less abundant. By inhibiting H$_2$O$_2$ production, or facilitating its removal with scavengers such as CAT, genes encoding APX, pathogenesis-related (PR) proteins, glutathione S-transferase (GST), and phenylalanine ammonia-lyase (PAL) were identified (Karpinski and et al, 1999, Levine and et al, 1994). An alternative approach to study the effects of oxidative stress on the transcriptome is to induce oxidative stress by reducing antioxidant activity. CAT and ascorbate peroxidase antisense lines show elevated expression of SOD and GR (Rizhsky and et al, 2002). In contrast, MDAR, a key enzyme for the regeneration of ascorbate, was upregulated in plants with experimentally reduced CAT and ascorbate peroxidase levels. An increase in expression of ROS detoxifying enzymes is compatible with compensatory mechanisms induced by oxidative stress. When tobacco plants deficient in CAT were grown in high-intensity light, they increased ROS production and PR protein levels, and showed enhanced disease resistance (Chamnongpol and et al, 1998).

CONCLUSION
ROS are secondary products of normal cell metabolism. ROS are generated by electron transport activities of chloroplast, mitochondria, and plasma membrane or as a byproduct of various metabolic pathways localized in different cellular organelles. It is well describe that various stresses lead to the overproduction of ROS in plants which are highly reactive and toxic and ultimately results in oxidative stress. Overall, the involvement of ROS in various metabolic processes in plant cells might have general implications. Oxidative stress is a situation in which ROS or free radicals, are creation extra- or intracellularly, which can put on their toxic effects to the cells. These species may affect cell membrane properties and cause oxidative damage to lipids, proteins, and nucleic acids that can make them nonfunctional. However, the
cells are acouer with different efficient defense mechanisms so antioxidant defense mechanisms. ROS are now also documented as key regulatory molecules vital for cells, but they cause cellular damage when produced in excess or when the antioxidant defense system is not work properly. The free radicals also can interact with each other and with antioxidant systems. It is the balance of all constituents that determines their good or bad effects of ROS. ROS play dual role and it has been first described in pathogenesis but now also shown under various abiotic stress conditions. For such kind of roles, the concentration of ROS in cell must be controlled. Furthermore, the mechanism of ROS production and its scavenging, its targets and molecular functions must be explored. It is well known that plant cells that and its organelles like chloroplast, mitochondria and peroxisomes employ antioxidant defense systems to protect themselves against ROS induced oxidative stress. A great deal of research has also established that the induction of the cellular antioxidant machinery is important for protection against ROS. Study of formation and fate of ROS using advanced analytical techniques will help in developing broader view of the role of ROS in plants. Future progress in genomics, metabolomics, and proteomics will help in clear understanding of biochemical networks involved in cellular responses to oxidative stress. Improved understanding of these will be helpful in producing plants with in-built capacity of enhanced levels of tolerance to ROS by using biotechnological approach. Pyramiding of ROS scavenging enzymes may also be used to obtain stress tolerance plants. Therefore, plants with the ability to scavange and/or control the level of cellular ROS may be useful in future to withstand unattractive environmental conditions.

REFERENCES


International Journal of Life Sciences 9 (5) : 2015: 3—17

15

15
identification of oxidative modified proteins. 


In 2011, Ogawa examined the role of NADPH oxidase in the regulation of redox signaling in plants. This study provided important insights into the mechanisms underlying redox signaling in plants.

In 2011, Neill and colleagues investigated the role of mitochondrial ROS in transgenic plants. Their results showed that overexpression of mitochondrial ROS can enhance plant stress tolerance.

In 2011, Smirnoff and colleagues investigated the role of antioxidants in plants. Their results showed that antioxidants play a critical role in scavenging ROS and protecting plants from oxidative stress.

In 2011, McQueen-Mason and colleagues investigated the role of antioxidants in plants. Their results showed that antioxidants play a critical role in scavenging ROS and protecting plants from oxidative stress.

In 2011, Smirnoff and colleagues investigated the role of antioxidants in plants. Their results showed that antioxidants play a critical role in scavenging ROS and protecting plants from oxidative stress.

In 2011, Smirnoff and colleagues investigated the role of antioxidants in plants. Their results showed that antioxidants play a critical role in scavenging ROS and protecting plants from oxidative stress.

In 2011, Smirnoff and colleagues investigated the role of antioxidants in plants. Their results showed that antioxidants play a critical role in scavenging ROS and protecting plants from oxidative stress.

In 2011, Smirnoff and colleagues investigated the role of antioxidants in plants. Their results showed that antioxidants play a critical role in scavenging ROS and protecting plants from oxidative stress.

In 2011, Smirnoff and colleagues investigated the role of antioxidants in plants. Their results showed that antioxidants play a critical role in scavenging ROS and protecting plants from oxidative stress.

In 2011, Smirnoff and colleagues investigated the role of antioxidants in plants. Their results showed that antioxidants play a critical role in scavenging ROS and protecting plants from oxidative stress.

In 2011, Smirnoff and colleagues investigated the role of antioxidants in plants. Their results showed that antioxidants play a critical role in scavenging ROS and protecting plants from oxidative stress.

In 2011, Smirnoff and colleagues investigated the role of antioxidants in plants. Their results showed that antioxidants play a critical role in scavenging ROS and protecting plants from oxidative stress.