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Review

Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants

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ABSTRACT

Various abiotic stresses lead to the overproduction of reactive oxygen species (ROS) in plants which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress. The ROS comprises both free radical ($O_2^{\bullet -}$, superoxide radicals; $OH^{\bullet}$, hydroxyl radical; $HO_2^{\bullet}$, perhydroxy radical and $RO^{\bullet}$, alkoxy radicals) and non-radical (molecular) forms (H_2O_2, hydrogen peroxide and $^1O_2$, singlet oxygen). In chloroplasts, photosystem I and II (PSI and PSII) are the major sites for the production of $^1O_2$ and $O_2^{\bullet -}$. In mitochondria, complex I, ubiquinone and complex III of electron transport chain (ETC) are the major sites for the generation of $O_2^{\bullet -}$. The antioxidant defense machinery protects plants against oxidative stress damages. Plants possess very efficient enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaicol peroxidase, GOPX and glutathione-*S*- transferase, GST) and non-enzymatic (ascorbic acid, ASH; glutathione, GSH; phenolic compounds, alkaloids, non-protein amino acids and α-tocopherols) antioxidant defense systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging of ROS. ROS also influence the expression of a number of genes and therefore control the many processes like growth, cell cycle, programmed cell death (PCD), abiotic stress responses, pathogen defense, systemic signaling and development. In this review, we describe the biochemistry of ROS and their production sites, and ROS scavenging antioxidant defense machinery.

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1. Introduction

About ~2.7 billion years ago molecular oxygen was introduced in our environment by the O_2-evolving photosynthetic organisms and ROS have been the uninvited companions of aerobic life [1]. The O_2 molecule is a free radical, as it has two impaired electrons that have the same spin quantum number. This spin restriction makes O_2 prefer to accept its electrons one at a time, leading to the generation of the so called ROS, which can damage the cells. ROS are also produced continuously as byproducts of various metabolic pathways that are localized in different cellular compartments such as chloroplast, mitochondria and peroxisomes [2,3]. 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. Oxygen generated in the chloroplasts during photosynthesis can accept electrons passing through the photosystems, thus forming $O_2^{\bullet -}$. Under steady state conditions, the ROS molecules are scavenged by various antioxidative defense mechanisms [5]. The equilibrium between the production and the scavenging of ROS may be perturbed by various biotic and abiotic stress factors such as salinity, UV radiation, drought, heavy metals, temperature extremes, nutrient deficiency, air pollution, herbicides and pathogen attacks. These disturbances in equilibrium lead to sudden increase in intracellular levels of ROS which can cause significant damage to cell structures (Fig. 1) and it has been estimated that 1–2% of O_2 consumption leads to the formation of ROS in plant tissues [6]. Through a variety of reactions, $O_2^{\bullet -}$ leads to the formation of H_2O_2,

Abbreviations: $RO^{\bullet}$, alkoxy radicals; APX, ascorbate peroxidase; ASH, ascorbate; CAT, catalase; Cd, cadmium; DTNB, 5,50-Dithiobis(2-nitrobenzoic acid); GPOX, guaiacol peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; $OH^{\bullet}$, hydroxyl radical; $HO_2^{\bullet}$, perhydroxy radical; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; NBT, nitroblue tetrazolium; NPT, non-protein thiols; PCD, programmed cell death; ROS, reactive oxygen species; $^1O_2$, singlet oxygen; SOD, superoxide dismutase; $O_2^{\bullet -}$, superoxide radicals; TBA, thiobarbituric acid; TFs, Transcription factors.

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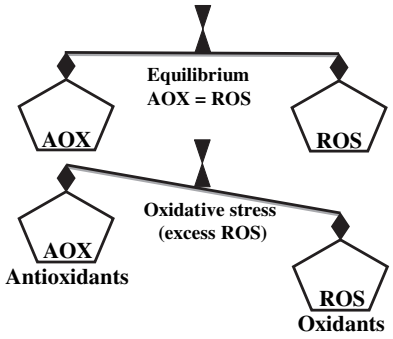


Fig. 1. Equilibrium between AOX and ROS.

OH• and other ROS. The ROS comprising $O_2^{\bullet -}$, H_2O_2, $^1O_2$, $HO_2^{\bullet -}$, OH•, ROOH, ROO•, and RO• are highly reactive and toxic and causes damage to proteins, lipids, carbohydrates, DNA which ultimately results in cell death (Fig. 2). Accumulation of ROS as a result of various environmental stresses is a major cause of loss of crop productivity worldwide [7–13]. ROS affect many cellular functions by damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation (LPO) [5]. 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 [14]. ROS can damage cells as well as initiate responses such as new gene expression. The cell response evoked is strongly dependent on several factors. The subcellular location for formation of an ROS may be especially important for a highly reactive ROS, because it diffuses only a very short distance before reacting with a cellular molecule. Stress-induced ROS accumulation is counteracted by enzymatic antioxidant systems that include a variety of scavengers, such as SOD, APX, GPX, GST, and CAT and non-enzymatic low molecular metabolites, such as ASH, GSH, α-tocopherol, carotenoids and flavonoids [13,15]. In addition, proline can now be added to an elite list of non-enzymatic antioxidants that microbes, animals, and plants need to counteract the inhibitory effects of ROS [16]. Plant stress tolerance may therefore be improved by the enhancement of *in vivo* levels of antioxidant enzymes. The above said antioxidants found in almost all cellular compartments, demonstrating the importance of ROS detoxification for cellular survival [13]. Now, it has also been shown that ROS influence the expression of a number of genes and signal transduction pathways which suggest that cells have evolved strategies to use ROS as biological stimuli and signals that activate and control various genetic stress-response programs [17]. Recently, it has become apparent that plants actively produce ROS which may control many different physiological processes such as biotic and abiotic stress-response, pathogen defense and systemic signaling. Here we have covered the chemistry of ROS and their production sites and ROS scavenging antioxidant defense machinery.

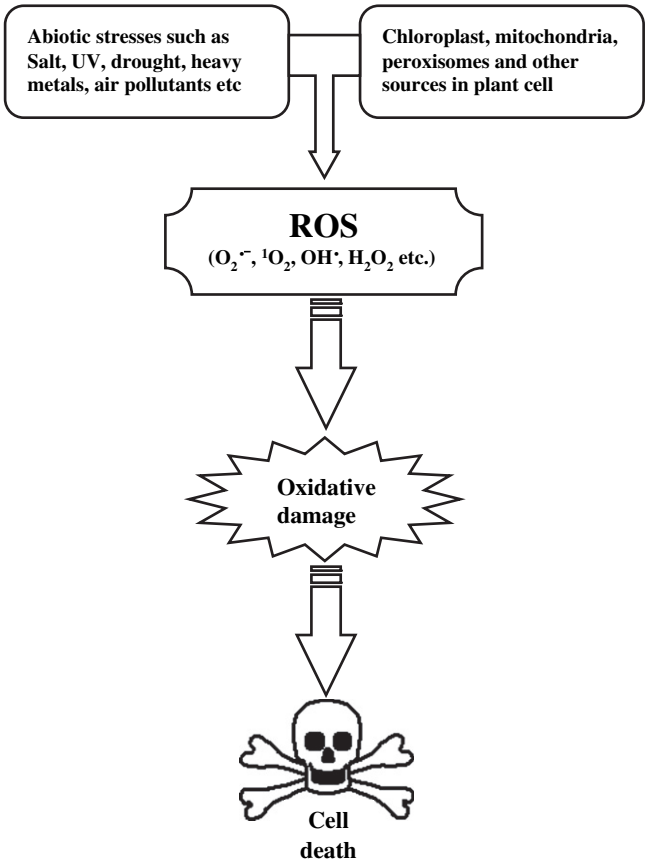


Fig. 2. Abiotic stress induced ROS production and cell death.

2. ROS chemistry

It is well established that organelles such as chloroplast, mitochondria or peroxisomes 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_2 in the atmosphere enabled respiratory metabolism and efficient energy generation systems which use O_2 as final electron acceptor, lead to the formation of ROS in cells [18]. Although, atmospheric oxygen is relatively non-reactive, it can give rise to ROS which include $O_2^{\bullet -}$, H_2O_2, OH• and $^1O_2$ [19] (Fig. 3). It has been estimated that 1–2% of O_2 consumed by plants is sidetracked to produce ROS in various subcellular loci [6]. The single electron reduction of O_2 results in the generation of the $O_2^{\bullet -}$. At low pH, dismutation of $O_2^{\bullet -}$ is unavoidable, with one $O_2^{\bullet -}$ giving up its added electron to another $O_2^{\bullet -}$, and then with protonation resulting in the generation of H_2O_2. Furthermore, $O_2^{\bullet -}$ can be protonated to form the HO_2•. Additionally, in the presence of transition metals such as copper and iron, further reactions take place, e.g. through the Haber–Weiss mechanism or the Fenton reaction to give up OH•, which is the most reactive chemical species in the biological world. $O_2^{\bullet -}$ can also react with another very influential signaling free radical species, NO• to give up peroxynitrite ($OONO^-$). $^1O_2$ is another form of ROS but here there is no addition of an extra electron to O_2; rather an electron is elevated to a higher energy orbital, thereby freeing oxygen from its spin-restricted state. $^1O_2$ can be formed by photoexcitation of chlorophyll and its reaction with O_2.

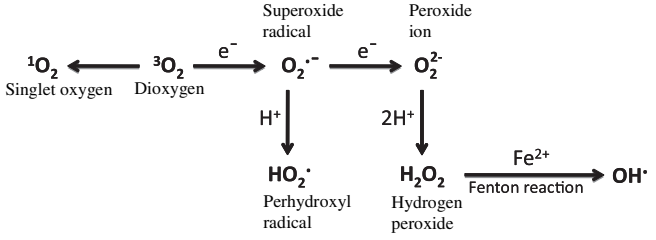


Fig. 3. Generation of ROS by energy transfer.

2.1. Superoxide radicals ($O_2^{\bullet -}$)

It has been well established that ROS appear continuously during photosynthesis in the chloroplasts by partial reduction of O_2 molecules or energy transfer to them. The major site of $O_2^{\bullet -}$ production is the thylakoid membrane-bound primary electron acceptor of PSI. The production of ROS is an inevitable consequence of aerobic respiration. When the terminal oxidases-cytochrome c oxidase and the alternative oxidase-react with O_2, four electrons are transferred and H_2O is released. However, occasionally O_2 can react with other ETC components. Here, only one electron is transferred, and the result is the $O_2^{\bullet -}$, a moderately reactive ROS with approximately 2–4 μs of half-life. It has been noted that $O_2^{\bullet -}$ is usually the first ROS to be generated. In plant tissues about 1–2% of O_2 consumption leads to the generation of $O_2^{\bullet -}$ [20]. The $O_2^{\bullet -}$ is produced upon reduction of O_2 during electron transport along the noncyclic pathway in the ETC of chloroplasts and other compartments of the plant cell. Reduction of O_2 to the $O_2^{\bullet -}$ can occur in the ETC at the level of PS I. The generation of $O_2^{\bullet -}$ may trigger the formation of more reactive ROS like OH•, and more possibly $^1O_2$ [1,21], each of which may cause peroxidation to membrane lipids and cellular weakening. It has been noted that $O_2^{\bullet -}$ can undergo protonation to give up − a strong oxidizing agent, $HO_2^{\bullet}$ in negatively charged membrane surfaces, which directly attack the PUFA [22]. Furthermore, $O_2^{\bullet -}$ can also donate an electron to iron (Fe^{3+}) to yield a reduced form of iron (Fe^{2+}) which can then reduce H_2O_2, produced as a result of SOD led dismutation of $O_2^{\bullet -}$ to OH•. The reactions through which $O_2^{\bullet -}$, H_2O_2 and iron rapidly generate OH• is called the Haber–Weiss reaction, whereas the final step which involves the oxidation of Fe^{2+} by H_2O_2 is referred to as the Fenton's reaction:

$$O_2^{\bullet -} + Fe^{3+} \rightarrow {}^1O_2 + Fe^{2+}$$

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2Fe^{3+}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet} \quad \text{(Fenton reaction)}$$

Scarpeci et al. [23] studied the methyl viologen (MV, $O_2^{\bullet -}$ propagator in the light) induced generation of $O_2^{\bullet -}$ in *Arabidopsis thaliana* chloroplasts during active photosynthesis and suggests that $O_2^{\bullet -}$ generated in photosynthetically active chloroplasts leads to the activation of genes involved in signalling pathways. 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 $O_2^{\bullet -}$ by SOD and low-molecular-weight antioxidant amarathine 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 [24].

2.2. Singlet oxygen ($^1O_2$)

Singlet oxygen, O_2 ($^1\Delta_g$) or $^1O_2$ is the first excited electronic state of O_2 and, is an unusual ROS because it is not related to electron transfer to O_2. Insufficient energy dissipation during photosynthesis can lead to formation of chlorophyll (Chl) triplet state. The Chl triplet state can react with $^3O_2$ to give up the very reactive $^1O_2$. It has been found that the formation of $^1O_2$ 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 $^1O_2$. The life time of $^1O_2$ in a cell has been measured to be approximately 3 μs [25] and in this time, a fraction of $^1O_2$ may be able to diffuse over considerable distances of several hundred nanometers (nm). It has been found that $^1O_2$ can last for nearly 4 μs in H_2O and 100 μs in polar solvent. $^1O_2$, an oxidizing agent for a wide range of biological molecules and can react with proteins, pigments, nucleic acids and lipids, and is thought to be the most important species responsible for light induced loss of PSII activity which may trigger call death [26,27]. In an interesting study it has been found that a photosensitizer in bacteria can generate $^1O_2$ upon exposure to light, which leads to the oxidation of proteins or lipids and ultimately bacteria death. [28]. Recently, it has been reported that in optimal growth conditions $^1O_2$ was responsible for more than 80% of the nonenzymatic LPO in Arabidopsis leaf tissues [29]. Further, this study showed that, in Arabidopsis mutants favouring $^1O_2$ production, photooxidative stress led to a dramatic increase of LPO that preceded cell death [29].

It is well established that $^1O_2$ is efficiently quenched by β–carotene, tocopherol or plastoquinone and if not, $^1O_2$ can activate the upregulation of genes, involved in the molecular defense responses against photooxdative stress [27]. 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 $^1O_2$ than CF1CFo in its reduced state [30]. Furthermore, gene expression and growth of phototrophic bacterium *Rhodobacter sphaeroides* was monitored in the presence of $^1O_2$ and it was noted that this bacterium mounts a transcriptional response to $^1O_2$ that requires the alternative σ factor, σ^E [31]. The global gene expression analysis identified ≈180 genes (≈60 operons) whose RNA levels increased ≥3-fold in cells with increased σ^E activity. It was predicted that gene products encoded by four newly identified σ^E-dependent operons are involved in stress-response and thus protect the cells from $^1O_2$ damage, or the conservation of energy [31]. op den Camp et al. [32] studied the role of $^1O_2$ in the induction of gene expression and used the conditional fluorescent (*flu*) mutant of Arabidopsis that accumulates the photosensitizer protochlorophyllide (Pchlide) in chloroplast in the dark. It was noted that after the excitation of Pchlide by light, $^1O_2$ is generated in the plastid and is involved in activating distinct groups of early stress-response genes that are different from those activated by $O_2^{\bullet -}/H_2O_2$. It was suggested that $^1O_2$ does not act primarily as a toxin but rather as a signal that activates several stress-response pathways [32].

Recently, other sources of $^1O_2$ production have also been reported in plants. It has been noted that plants trigger the production of antimicrobial secondary metabolites (phytoalexins) as a mechanism of resistance in plant-pathogen interactions [33]. The occurrence of phenalenone chromophores in phytoalexins of plants originally nonphototoxic which suggests that these plants respond to pathogen attacks by biosynthesizing $^1O_2$ photosensitizers. Furthermore, some species constitutively produce different types of secondary metabolite with photosensitizing properties that make use of $^1O_2$ to increase their efficacy as antimicrobial agents [33].

2.3. Hydrogen peroxide (H_2O_2)

The univalent reduction of $O_2^{\bullet -}$ produces H_2O_2. H_2O_2 is moderately reactive and has relatively long half-life (1 ms) whereas, other ROS such as $O_2^{\bullet -}$, OH• and $^1O_2$, have much shorter half-life (2–4 μs) [6]. It has been well established that excess of H_2O_2 in the plant cells leads to the occurrence of oxidative stress. H_2O_2 may inactivate enzymes by oxidizing their thiol groups. Tewari et al. [34] noted significantly high accumulation of H_2O_2 in the middle portion of trichomes in Cu-deficient leaves of *Morus alba* cv. Kanva 2 in

comparison with the plants grown under Cu-excess. Furthermore, the activities of various antioxidant enzymes such as SOD, CAT, APX and GR increased in both Cu-deficient and Cu-excess plants. It was suggested that Cu deficiency aggravates oxidative stress condition through excessive ROS production which disturbed the redox couple, whereas, excess of Cu damaged the roots, accelerated the rate of senescence in the older leaves, induced antioxidant responses and disturbed the cellular redox environment in the young leaves of mulberry plants [34]. Cd induced decrease in Ca lead to Cu/Zn SOD down-regulation which resulted in the over-production of the H_2O_2 and $O_2^{\bullet -}$ in *Pisum sativum* [35]. Chloroplast is one of the source of ROS because of its photoactive nature. It has been found that chlorophyllase 1 (encoded by *AtCLH1*) of *A. thaliana* showed quick induction after tissue damage caused by *Erwinia carotovora* (bacterial necrotroph) or by *Alternaria brassicicola* (fungul necrotroph). It was found that *E. carotovora* under high light resulted in elevated levels of H_2O_2 in *AtCLH1* silenced plants. Interestingly, downregulation of *AtCLH1* resulted in increased susceptibility to *A. brassicicola*, resistance to which requires jasmonate signaling [36].

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 [37]. H_2O_2 has also been shown to act as a key regulator in a broad range of physiological processes, such as senescence [38], photorespiration and photosynthesis [39], stomatal movement [40], cell cycle [15] and growth and development [41]. 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 [37]. In an interesting study the response of pre-treated citrus roots with H_2O_2 (10 mM 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 150 mM NaCl for 16d [42]. It was noted that H_2O_2 and SNP increased the activities of leaf SOD, CAT, APX and GR along with the induction of related-isoform(s) under non-NaCl-stress conditions. Salinity induced reduction in the ASH redox state was partially prevented by H_2O_2 and SNP pre-treatments, on the other side the GSH redox state was increased by SNP under normal and NaCl-stress conditions. Moreover, NaCl-dependent protein oxidation was totally reversed by pre-treatments with H_2O_2 and SNP [42].

2.4. Hydroxyl radicals ($OH^{\bullet}$)

Particularly, $OH^{\bullet}$, are among the most highly reactive ROS known. In the presence of suitable transitional metals, especially Fe, $OH^{\bullet}$ can also be produced from $O_2^{\bullet -}$ and H_2O_2 at neutral pH and ambient temperatures by the iron-catalyzed, $O_2^{\bullet -}$-driven Fenton reaction.

$$H_2O_2 + O_2^{\bullet -} \xrightarrow{Fe^{2+},Fe^{3+}} OH^- + O_2 + OH^{\bullet}$$

These $OH^{\bullet}$ are thought to be largely responsible for mediating oxygen toxicity *in vivo*. $OH^{\bullet}$ can potentially react with all biological molecules like DNA, proteins, lipids, and almost any constituent of cells and due to the absence of any enzymatic mechanism for the elimination of this highly reactive ROS, excess production of $OH^{\bullet}$ ultimately leads to cell death [43].

3. ROS production in different organelles

Photosynthesizing plants are especially at the risk of oxidative damage, because of their oxygenic conditions and the abundance of the photosensitizers and PUFA in the chloroplast envelope. In light the chloroplasts and peroxisomes are the main source of ROS generation [44]. In the darkness the mitochondria appear to be the main ROS producers. It has been estimated that 1–5% of the O_2 consumption of isolated mitochondria results in ROS production [45].

3.1. Mitochondria

Plant mitochondria as "energy factories" are believed to be a major site of ROS production such as H_2O_2 as well as the ROS targets [46]. It differs significantly from their animal counterparts, with specific ETC components and functions in processes such as photorespiration. 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 [47]. 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 [48]. 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 $O_2^{\bullet -}$ production. In aqueous solution, $O_2^{\bullet -}$ is moderately reactive, but this $O_2^{\bullet -}$ can further reduced by SOD dismutation to H_2O_2 [37,45,49,50]. It has been estimated that about 1–5% of mitochondrial O_2 consumption leads to H_2O_2 production [45]. This H_2O_2 can react with reduced Fe^{2+} and Cu^+ to produce highly toxic $OH^{\bullet}$, and these uncharged $OH^{\bullet}$ can penetrate membranes and leave the mitochondrion [48,50,51]. Peroxidation of mitochondrial membrane PUFA is initiated by the abstraction of a hydrogen atom by ROS, especially by $OH^{\bullet}$. This leads to the formation of cytotoxic lipid aldehydes, alkenals, and hydroxyalkenals, such as the much-studied 4-hydroxy-2-nonenal and malondialdehyde (MDA). Once formed, LPO products can cause cellular damage by reacting with proteins, other lipids, and nucleic acids. Key oxylipins and smaller, lipid-derived reactive electrophile species may also be produced from LPO [52]. 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 [53]. 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 [53].

It is well known that abiotic stresses strongly affect the plant cell bioenergetics. Plant mitochondria may control ROS generation by means of energy-dissipating systems. Therefore, mitochondria may play a central role in cell adaptation to abiotic stresses, which are known to induce oxidative stress at cellular level. It has been found that the energy-dissipating systems of durum wheat mitochondria diminish mitochondrial ROS production. ROS induced activation of the ATP-sensitive plant mitochondrial potassium channel (PmitoKATP) and the plant uncoupling protein (PUCP) has been found in the mitochondria of control and hyper osmotic stressed seedlings, in turn, dissipate the mitochondrial membrane potential and reduce the chances of large-scale ROS production [54]. To investigate the effect of ROS on plant mitochondria, Pastore et al. [55] used the ROS producing system consisting of xanthine plus xanthine oxidase on the rate of membrane potential ($\Delta\Psi$) 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, $\Delta\Psi$ 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,

$\Delta\Psi$ generation and consequent large-scale ROS production [55]. A *Nicotiana sylvestris* mitochondrial mutant was used to study the role of plant mitochondria in the regulation of cellular redox homeostasis and stress resistance [56] 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* [56].

3.2. 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 [4]. Oxygen generated in the chloroplasts during photosynthesis can accept electrons passing through the photosystems, thus results in the formation of $O_2^{\bullet -}$. Therefore, the presence of ROS producing centres such as triplet Chl, ETC in PSI and PSII make chloroplasts a major site of ROS ($O_2^{\bullet -}$, $^1O_2$ and H_2O_2) production. Various abiotic stresses such as excess light, drought, salt stress and CO_2 limiting conditions enhance the production of ROS in chloroplasts. Normally, the electron flow from the excited photosystem centers is directed to $NADP^+$, which is reduced to NADPH. It then enters the Calvin cycle and reduces the final electron acceptor, CO_2. In situations of overloading of the ETC, a part of the electron flow is diverted from ferredoxin to O_2, reducing it to $O_2^{\bullet -}$ *via* Mehler reaction [57,58]. Later studies have revealed that the acceptor side of ETC in PSII also provides sides (QA, QB) with electron leakage to O_2 producing $O_2^{\bullet -}$ [59]. $^1O_2$ is a natural byproduct of photosynthesis, mainly formed at PS II even under low-light conditions [30]. On the external, "stromal" membrane surface, $O_2^{\bullet -}$ is spontaneously dismutated to H_2O_2 by CuZn-SOD [59]. Therefore, chloroplasts are also a major source for ROS production.

Recent researches have linked chloroplast-produced ROS with the hypersensitive response [60]. Chloroplast-produced ROS have been shown to be capable of transmitting the spread of wound-induced PCD through maize tissue [61]. The expression of animal anti-apoptotic *Bcl-2* family members in transgenic tobacco has revealed the involvement of chloroplast in oxidative stress-induced PCD [62]. It has been shown that in *A. thaliana* cell suspension cultures the cells contain well-developed, functional chloroplasts when grown in the light, but not when grown in the dark and can be used as model systems to study PCD. In a study treatment with antioxidant of light-grown cultures resulted in increased apoptotic like-PCD induction which suggests the involvement of chloroplast-produced ROS apoptotic like-PCD regulation. It has been suggested that chloroplasts can play a significant role in apoptotic like-PCD regulation [63]. Chilling induced accumulation of ROS in cucumber resulted in decreased net photosynthetic rate and cytochrome respiratory pathway. Meanwhile, chilling resulted in an enhancement of the protective mechanisms such as thermal dissipation, alternative respiratory pathway, and ROS scavenging mechanisms (SODs and APXs) in chloroplasts and mitochondria [64]. It has also been found that plum pox virus (PPV) infection produced an alteration in the pea chloroplast ultrastructure, giving rise to dilated thylakoids, an increase in the number of plastoglobuli and a decreased amount of starch content. PPV infection also effect PSII by decreasing the amount of Rubisco, oxygen-evolving enhancer and PSII stability factor proteins. Finally it was found that the symptoms observed in pea leaves could be due to an imbalance in antioxidant systems as well as to an increased generation of ROS in chloroplasts, induced probably by a disturbance of the electron transport chain, suggesting that chloroplasts can be a source of oxidative stress during viral disease development [65].

3.3. Peroxisomes

Peroxisomes are small, usually spherical microbodies bounded by a single lipid bilayer membrane. Peroxisomes are subcellular organelles with an essentially oxidative type of metabolism and are probably the major sites of intracellular ROS production. Like mitochondria and chloroplasts, peroxisomes produce $O_2^{\bullet -}$ radicals as a consequence of their normal metabolism. Two sites of $O_2^{\bullet -}$ generation are established in peroxisomes [66]. First one is in the organelle matrix, where xanthine oxidase (XOD) catalyzes the oxidation of xanthine and hypoxanthine to uric acid [67]. Second site is in the peroxisome membranes dependent on NAD(P)H where a small ETC is composed of a flavoprotein NADH and cytochrome b, and here $O_2^{\bullet -}$ is produced by the peroxisome ETC. MDHAR participates in $O_2^{\bullet -}$ production by peroxisome membranes [66]. The main metabolic processes responsible for the generation of H_2O_2 in different types of peroxisomes are the photorespiratory glycolate oxidase reaction, the fatty acid β-oxidation, the enzymatic reaction of flavin oxidases, and the disproportionation of $O_2^{\bullet -}$ radicals [2,66,68]. Recently, it has been demonstrated that NO• radicals are also produced in peroxisomes. Plant peroxisomes also play a significant role in photomorphogenesis degradation of branched amino acids, biosynthesis of the plant hormones jasmonic acid and auxin, and the production and glycine betaine [69]. The existence of regulatory proteins like heat shock proteins, kinases, and phosphatases has also been suggested in peroxisomes [70,71].

In one hand increased production of H_2O_2 and $O_2^{\bullet -}$ in the peroxisomes lead to oxidative damage and possibly cell death [66] but on the other hand it has also been shown that small levels of H_2O_2 and $O_2^{\bullet -}$ work as signal molecules which mediate pathogen-induced PCD in plants [72,73]. Therefore, it has been suggested that peroxisomes should be considered as cellular compartments with the capacity to generate and release important signal molecules such as $O_2^{\bullet -}$, H_2O_2 and NO• into the cytosol, which can contribute to a more integrated communication system among cell compartments [67]. Rodriguez-Serrano et al. [35] studied the peroxisome movement in *Arabidopsis* line expressing the GFP-SKL peptide targeted to peroxisomes and observed peroxisome-associated fluorescence in all plant tissues. Furthermore, it was also noted that the plants treated with 100 μM $CdCl_2$ produced a significant increase in speed, which was dependent on endogenous ROS and Ca^{2+} but was not related to actin cytoskeleton modifications [35].

3.4. 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 [74]. ROS are also generated at plasma membrane level or extracellularly in apoplast in plants. pH-dependent cell wall-peroxidases, germin-like oxalate oxidases and amine oxidases have been proposed as a source of H_2O_2 in apoplast of plant cells [75]. pH dependent cell-wall peroxidases are activated by alkaline pH, which, in the presence of a reductant produces H_2O_2. Alkalization of apoplast upon elicitor recognition precedes the oxidative burst and production of H_2O_2 by a pH-dependent cell wall peroxidase

has been proposed as an alternative way of ROS production during biotic stress [75].

4. ROS and cell biochemistry

4.1. Lipid peroxidation (LPO)

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 [76]. Some of these compounds react with thiobarbituric acid (TBA) to form coloured products called thiobarbituric acid reactive substances (TBARS) [77]. LPO, in both cellular and organelle membranes, takes place when above-threshold ROS levels are reached, thereby not only directly affecting normal cellular functioning, but also aggravating the oxidative stress through production of lipid-derived radicals [78]. The overall process of LPO involved three distinct stages: initiation, progression and termination steps. Initiation step involves transition metal complexes, especially those of Fe and Cu. However, $O_2^{\bullet -}$ and H_2O_2 are capable of initiating the reactions but as OH• is sufficiently reactive, the initiation of LPO in a membrane is initiated by the abstraction of a hydrogen atom, in an unsaturated fatty acyl chain of a polyunsaturated fatty acid (PUFA) residue, mainly by OH•. In an aerobic environment, oxygen will add to the fatty acid at the carbon-centered lipid radical to give rise to a ROO•. Once initiated, ROO• can further propagate the peroxidation chain reaction by abstracting a hydrogen atom from adjacent PUFA side chains. The resulting lipid hydroperoxide can easily decompose into several reactive species including: lipid alkoxyl radicals, aldehydes (malonyldialdehyde), alkanes, lipid epoxides, and alcohols [79,80]. A single initiation event thus has the potential to generate multiple peroxide molecules by a chain reaction. The overall effects of LPO are to decrease membrane fluidity; make it easier for phospholipids to exchange between the two halves of the bilayer; increase the leakiness of the membrane to substances that do not normally cross it other than through specific channels and damage membrane proteins, inactivating receptors, enzymes, and ion channels.

Initiation step

$$\underset{\text{(Lipid)}}{RH} + OH^{\bullet} \rightarrow R^{\bullet} + H_2O \quad \underset{\text{(Lipid Alkyl radical)}}{}$$

Propagation step

$$R^{\bullet} + O_2 \rightarrow \underset{\text{(Lipid Peroxy radical)}}{ROO^{\bullet}}$$

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$$

$$ROOH \rightarrow RO^{\bullet} \quad \text{Epoxides, hydroperoxides, glycol, aldehydes}$$

Termination step

$$R^{\bullet} + R^{\bullet} \rightarrow \underset{\text{(Fatty acid dimer)}}{R + R}$$

$$R^{\bullet} + ROO^{\bullet} \rightarrow \underset{\text{(Peroxide bridged dimer)}}{ROOR}$$

$$ROO^{\bullet} + ROO^{\bullet} \rightarrow \underset{\text{(Peroxide bridged dimer)}}{ROOR + O_2}$$

It has been found that the PUFAs (linoleic acid (18:2) and linolenic acid (18:3)) are particularly susceptible to attack to $^1O_2$ and HO•, giving rise to complex mixtures of lipid hydroperoxides. Increased PUFA peroxidation decreases the fluidity of the membrane, increases leakiness and causes secondary damage to membrane proteins [81]. Several aldehydes such as 4-hydroxy-2-nonenal (HNE) and MDA, as well as hydroxyl and keto fatty acids, are formed as a result of PUFA peroxidation (Fig. 4). The aldehyde breakdown products can form conjugates with DNA and proteins. Aldehydes formed in the mitochondria may be involved in causing cytoplasmic male sterility in maize because a restorer gene in this species encodes a mitochondrial aldehyde dehydrogenase [81].

It has also been noted that plants exposed to various abiotic stresses exhibit an increase in LPO due to the generation of ROS. Treatment with Cd significantly increased the accumulation of lipid peroxides in different plants [82–87]. Khan and Panda [88] studied the cultivar response of *Oryza sativa* under salt stress and found that it increased the LPO in both cvs. of rice but its level was higher in Begunbitchi than Lunishree and they correlated the higher free radicals scavenging capacity and more efficient protection mechanism of Lunishree against salt stress with lower level of LPO in comparison to Begunbitchi. Kukreja et al. [89] noted marked increase in LPO in *Cicer arietinum* roots under salinity stress. Similar increase in MDA content has also been noted in *C. arietinum* L. cv. Gokce [90]. It has also been reported that water stress increased the LPO, membrane injury index, H_2O_2 and OH• production in leaves of stressed *Phaleolus vulgaris* plants [91]. Liu et al. [92] reported that transgenic tobacco plants overexpressing *VTE1* (catalyzes the penultimate step of tocopherol synthesis) from *Arabidopsis* exposed to drought conditions showed decreased LPO, electrolyte leakage and H_2O_2 content in comparison to wild type plants. Simova-Stoilova et al. [93] reported that the weakening of membrane integrity and

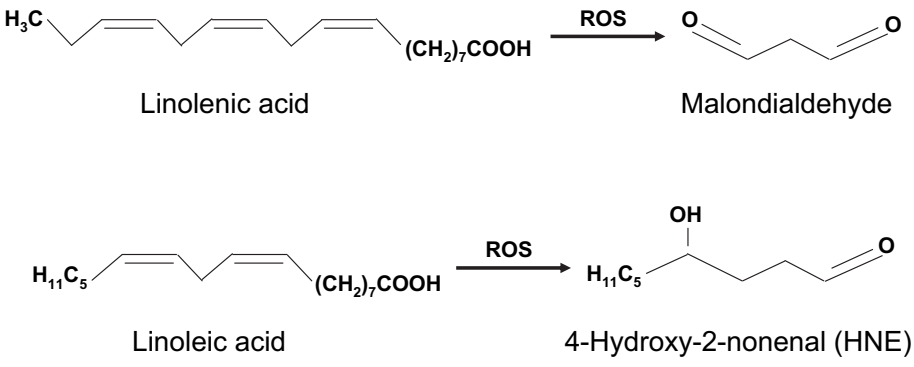


Fig. 4. PUFA oxidation.

oxidative damage to lipids were more pronounced in the sensitive varieties under field drought conditions in wheat plants. Pan et al. [94] also reported increase in MDA content in liquorice seedlings (*Glycyrrhiza uralensis* Fisch) under salt and drought stress. Agarwal [95] reported that UV B irradiated *Cassia auriculata* L. seedlings showed oxidative damage with an increase in MDA and H_2O_2 content. The response of five cherry tomato varieties to oxidative stress under moderate water deficit was investigated [96]. It was suggested that LPO as a important determinant physiological process in selecting tomato plants tolerant to water stress [96]. Recently, the radish *PHGPx* gene introduced into a yeast *PHGPx*-deletion mutant was reported to significantly rescue the growth of the recombinant cell exposed to linolenic acid, indicating a similar role to the yeast *PHGPx3* gene in protection of membrane against LPO [97].

4.2. Protein oxidation

Protein oxidation is defined as covalent modification of a protein induced by ROS or byproducts of oxidative stress. Most types of protein oxidations are essentially irreversible, whereas, a few involving sulfur-containing amino acids are reversible [98]. Protein carbonylation is widely used marker of protein oxidation [81,99]. The oxidation of a number of protein amino acids particularly Arg, His, Lys, Pro, Thr and Trp give free carbonyl groups which may inhibit or alter their activities and increase susceptibility towards proteolytic attack [81]. Protein carbonylation may occur due to direct oxidation of amino acid side chains (e.g. proline and arginine to γ-glutamyl semialdehyde, lysine to amino adipic semialdehyde, and threonine to aminoketobutyrate) [100] (Fig. 5). Whatever the location of ROS synthesis and action, ROS are likely to target proteins that contain sulfur-containing amino acids and thiol groups. Cys and Met are quite reactive especially with $^1O_2$ and OH•. Activated oxygen can abstract an H atom from cysteine residues to form a thiyl radical that will cross-link to a second thiyl radical to form disulphide bridges [101]. Alternatively, oxygen can add onto a methionine residue to form methionine sulphoxide derivatives [102].

It has been found that various stresses lead to the carbonylation of proteins in tissues. Carbonylation of storage proteins has been noted in dry *Arabidopsis* seeds but carbonylation of a number of other proteins increased strongly during seed germination [99]. Bartoli et al. [103] found that protein carbonylation was higher in the mitochondria than in chloroplasts and peroxisomes in wheat leaves which suggest that the mitochondria are more susceptible to oxidative damage. A number of carbonylated proteins in a soluble fraction from green rice leaf mitochondria have been identified [104]. Cd^{2+} treatment raised the carbonylation level from 4 to 5.6 nmol/mg protein in pea plants [105]. The carbonylation level increases from 6.9 to 16.3 nmol/mg of peroxisomal protein as a result of Cd^{2+} treatment of the intact plant that can be due to the higher local ROS concentration in the peroxisomes [105]. Proteins can be damaged in oxidative conditions by their reactions with LPO products, such as 4-hydroxy-2-nonenal (HNE).

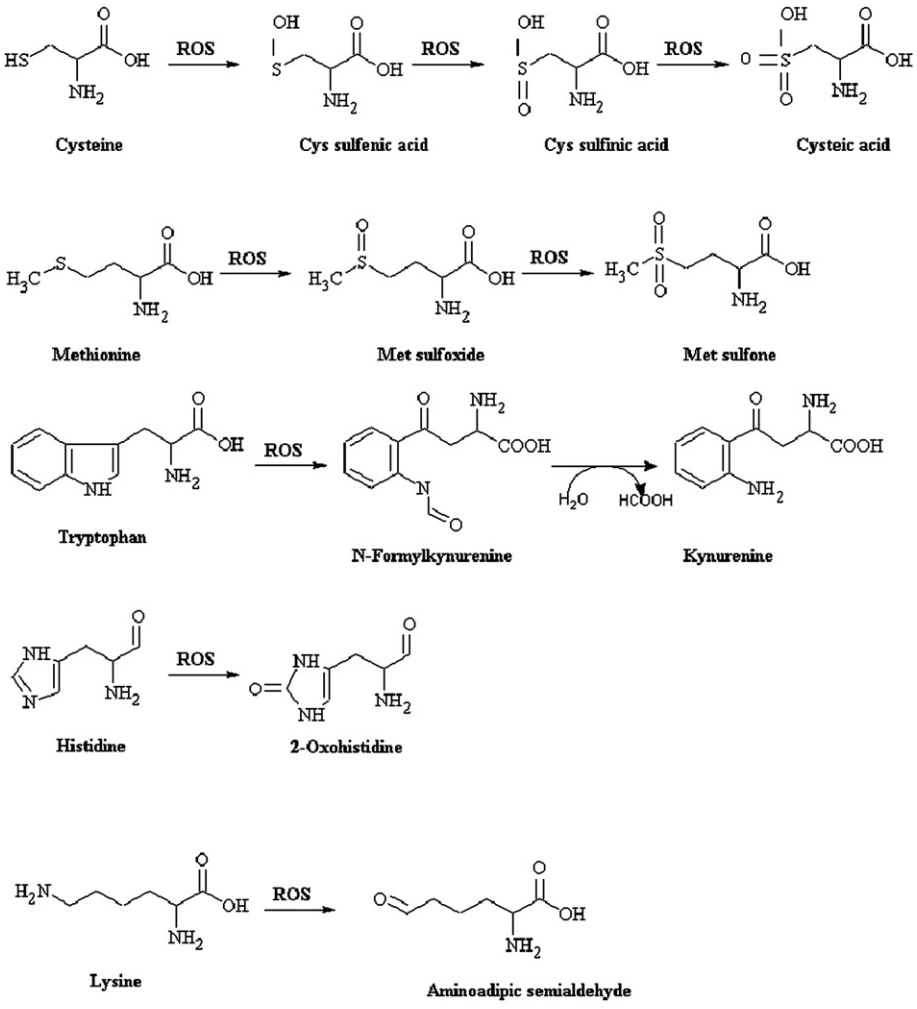


Fig. 5. Protein oxidation.

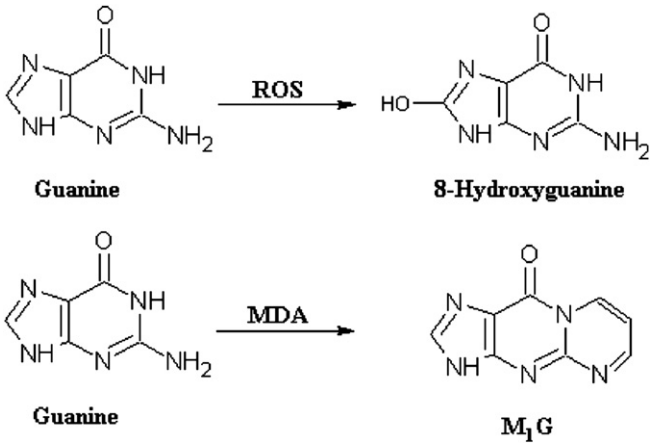


Fig. 6. DNA oxidation.

Treatment of mitochondria with 4-hydroxy-2-nonenal (HNE) or paraquat (which causes superoxide formation in chloroplasts and mitochondria) or cold or drought treatment of plants leads to formation of a covalent HNE-derived adduct of the lipoic acid moiety of several mitochondrial enzymes, including Gly decarboxylase (an enzyme in the photorespiratory pathway), 2-oxoglutarate dehydrogenase (a TCA cycle enzyme), and pyruvate decarboxylase [106,107].

4.3. DNA damage

Though the plant genome is very stable but its DNA might get damaged due to the exposure to biotic and abiotic stress factors which might damage the DNA, and thereby exerts genotoxic stress [108]. Endogenously generated damage to DNA is known as "spontaneous DNA damage" which is produced by reactive metabolites ($OH^{\bullet}$, $O_2^{\bullet -}$ and $NO^{\bullet}$). High levels of ROS can cause damage to cell structures, nucleic acids, lipids and proteins [109]. It has been reported that $OH^{\bullet}$ is the most reactive and cause damage to all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone [110], $^1O_2$ primarily attacks guanine, and H_2O_2 and $O_2^{\bullet -}$ don't react at all [111] (Fig. 6). ROS is capable of inducing damage to almost all cellular macromolecules including DNA which includes base deletion, pyrimidine dimers, cross-links, strand breaks and base modification, such as alkylation and oxidation [112,113]. DNA damage results in various physiological effects, such as reduced protein synthesis, cell membrane destruction and damage to photosynthetic proteins, which affects growth and development of the whole organism [114]. DNA damage can result either in arrest or induction of transcription, induction of signal transduction pathways, replication errors, cell membrane destruction and genomic instability [114,115]. The major type of DNA damage caused by exposure to UV-B is the formation of dimers between adjacent pyrimidines, i.e., UV photoproducts consist primarily of cyclobutane pyrimidine dimmers (CPDs) and 6-4PPs dimers [108]. LPO and DNA damage individually have been considered major determinants of seed viability loss. Recently, it has been shown that LPO in leaves and roots of *Vicia faba* increased with the addition of arsenate, indicated oxidative stress [116]. A number of mechanisms are available for repairing DNA damage both in the nucleus and in the mitochondria. These include direct reversal of the damage, replacement of the base, and replacement of the whole nucleotide [108,112].

5. ROS scavenging antioxidant defense mechanism

Exposure of plants to unfavourable environmental conditions such as temperature extremes, heavy metals, drought, water availability, air pollutants, nutrient deficiency, or salt stress can increase the production of ROS e.g., $^1O_2$, $O_2^{\bullet -}$, H_2O_2 and $OH^{\bullet}$. To protect themselves against these toxic oxygen intermediates, plant cells and its organelles like chloroplast, mitochondria and peroxisomes employ antioxidant defense systems. A great deal of research has established that the induction of the cellular antioxidant machinery is important for protection against various stresses [10,12,13,117] (Fig. 7). The components of antioxidant defence

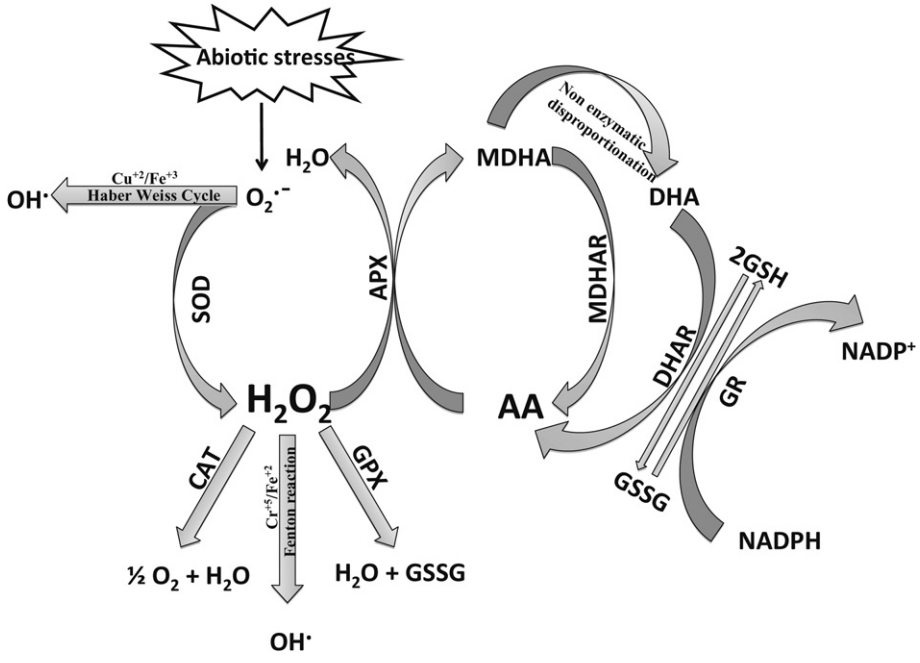


Fig. 7. ROS and antioxidants defense mechanism.

system are enzymatic and non-enzymatic antioxidants. 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) [13,15,117].

5.1. ROS scavenging enzymatic antioxidants

5.1.1. Superoxide dismutase (SOD)

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. The SODs remove $O_2^{\bullet -}$ by catalyzing its dismutation, one $O_2^{\bullet -}$ being reduced to H_2O_2 and another oxidized to O_2 (Table 1). It removes $O_2^{\bullet -}$ and hence decreases the risk of $OH^{\bullet}$ formation *via* the metal catalyzed Haber–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 [7]. In *A. thaliana* genome, three FeSOD genes (*FSD1*, *FSD2* and *FSD3*), three Cu/ZnSOD genes (*CSD1*, *CSD2* and *CSD3*), and one MnSOD gene (*MSD1*) have been reported [118]. The activity of SOD isozymes can be detected by negative staining and identified on the basis of their sensitivity to KCN and H_2O_2. 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 H_2O_2. The subcellular distribution of these isozymes is also distinctive. The Mn-SOD is found in the mitochondria of eukaryotic cells and in peroxisomes [119]; some Cu/Zn-SOD isozymes are found in the cytosolic fractions, and also in chloroplasts of higher plants [66]. The Fe-SOD isozymes, often not detected in plants [120] are usually associated with the chloroplast compartment when present [121] (Table 2). The prokaryotic Mn-SOD and Fe-SOD, and the eukaryotic Cu/Zn-SOD enzymes are dimers, whereas Mn-SOD of mitochondria are tetramers. Peroxisomes and glyoxysomes of *Citrillus vulgaris* have been shown to contain both Cu/Zn- and Mn-SOD activity [122], but there are no reports of extracellular SOD enzymes in plants. 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 [123]. The upregulation of SODs is implicated in combating oxidative stress caused due to biotic and abiotic stress and have a critical role in the survival of plants under stresses environment. Significant increase in SOD activity under salt stress has been observed in various plants *viz.* mulberry [124], *C. arietinum* [89] and *Lycopersicon esculentum* [125]. Eyidogan and Oz [90] noted three SOD activity bands (MnSOD, FeSOD and Cu/ZnSOD) in *C. arietinum* under salt stress. Furthermore, significant increase in the activities of Cu/ZnSOD and MnSOD isozymes under salt stress was observed. Pan et al. [94] studied the effect of salt and drought stress on *Glycyrrhiza uralensis* Fisch and found significantly increased SOD activity but an additional MnSOD isoenzyme was detected under only salt stress. Moreover, increased SOD activity has also been detected following Cd treatment in *Hordeum vulgare* [126], *A. thaliana* [127], *O. sativa* [128], *Triticum aestivum* [129], *Brassica juncea* [82]; *Vigna mungo* [87], *C. arietinum* [130]. Increase in SOD activity following drought stress was noted in three cultivars of *P. vulgaris* [91], *Alternanthera philoxeroides* [131] and *O. sativa* [132]. Wang and Li [133] studied the effect water stress on the activities of total leaf SOD and chloroplast SOD in *Trifolium repens* L. and reported significant higher increase in SOD activity under water stress. Simonovicova et al. [134] reported increase in SOD activity in *H. vulgare* L. cv. Alfor root tips under Al stress at 72 h. Yang et al. [135] showed the combined effect of drought and low light in *Picea asperata* Mast. seedlings grown at two watering regimes i.e., well-watered, 100% of field capacity and drought, 30% of field capacity and light availabilities (HL, 100% of full sunlight and low light, 15% of full sunlight) and found that under high light condition, drought significantly increased the SOD activity in comparison to low light. In an interesting study Rossa et al. [136] studied the light regulation of SOD in red alga *Gracilariopsis tenuifrons* and they found that the blue light wavelength exerted a greater induction of SOD activity than other specific wavelengths. Agarwal [95] reported that UV B (7.5 and 15.0 kJ m^{-2}) irradiation showed significant increase in SOD activity in *C. auriculata* L. seedlings. Li et al. [137] reported significant increase in SOD activity in two cultivars of *Brassica compestris* under Cu stress. A general induction in SOD activity in *Anabaena doliolum* under NaCl and Cu^{2+} stress has also been reported [138].

There have been many reports of the production of abiotic stress tolerant transgenic plants overexpressing different SODs (Table 3). Transgenic rice plants overexpressing *OsMT1a* demonstrated enhanced drought tolerance [139]. Protoplasts with Mn-SOD overexpression showed less oxidative damage, higher H_2O_2 content and significant increase in SOD and GR activities under photooxidative stress [140]. Overexpression of a Mn-SOD in transgenic *Arabidopsis* plants also showed increased salt tolerance [141]. Furthermore, they showed that Mn-SOD activity as well as the activities of Cu/Zn-SOD, Fe-SOD, CAT and POD was significantly higher in transgenic *Arabidopsis* plants than control [141]. Cu/Zn-SOD overexpressing transgenic tobacco plants showed multiple stress tolerance [142]. Overexpression of Mn-SOD in transformed *L. esculentum* plants also showed enhanced tolerance

Table 2
Different SODs, their location in cell organelles.

SOD isozymes	Location	Resistant to	Sensitive to
Fe-SOD	Chloroplast	KCN	H_2O_2
Mn-SOD	Mitochondria and Peroxisomes	KCN and H_2O_2	–
Cu/Zn-SOD	Chloroplast and Cytosol	–	H_2O_2 and KCN

Table 1
Major ROS scavenging antioxidant enzymes.

Enzymatic antioxidants	Enzyme code	Reactions catalyzed
Superoxide dismutase (SOD)	EC 1.15.1.1	$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow 2H_2O_2 + O_2$
Catalase (CAT)	EC 1.11.1.6	$H_2O_2 \rightarrow H_2O + {}^1/_2O_2$
Ascorbate peroxidase (APX)	EC 1.11.1.11	$H_2O_2 + AA \rightarrow 2H_2O + DHA$
Guaicol peroxidase (GPX)	EC 1.11.1.7	$H_2O_2 + GSH \rightarrow H_2O + GSSG$
Monodehydroascorbate reductase (MDHAR)	EC 1.6.5.4	$MDHA + NAD(P)H \rightarrow AA + NAD(P)^+$
Dehydroascorbate reductase (DHAR)	EC 1.8.5.1	$DHA + 2GSH \rightarrow AA + GSSG$
Glutathione reductase (GR)	EC 1.6.4.2	$GSSG + NAD(P)H \rightarrow 2GSH + NAD(P)^+$

Table 3
ROS scavenging enzymatic and non-enzymatic antioxidants and their role in transgenic plants for abiotic stress tolerance.

Gene	Source	Target transgenic	Response in transgenic plants	Reference
Superoxide dismutase (SOD)				
Cu/Zn SOD	*Oryza sativa* L.	*Nicotiana tabacum*	Enhanced tolerance to salt, water, PEG stresses and enhancement in chloroplast antioxidant system	[142]
Cu/Zn SOD	*Avicennia marina*	*Oryza sativa* Pusa Basmati-1	Transgenic plants were more tolerant to MV mediated oxidative stress, salinity stress and drought stress	[275]
Mn SOD	*Nicotiana plumbaginifolia*	*Triticum aestivum* cv. Oasis protoplast	Photooxidative stress tolerance, lower oxidative damage, higher H_2O_2 and significant increase in SOD and GR activities	[140]
MnSOD	*Tamarix androssowii*	*Populus davidiana* x *P. bolleana*	Salt tolerance, 8-23 fold increase in relative weight gains of the transgenic plants and increase in SOD activity	[276]
Mn SOD	*Arabidopsis*	*Arabidopsis* ecotype Columbia	Salt tolerance, Increased Mn-SOD, Cu/Zn-SOD, Fe-SOD, CAT and POD under salt stress	[141]
Mn SOD + APX	*Nicotiana tabacum*	*Festuca arundinacea* Schreb. cv. Kentucky-31	MV, H_2O_2, and Cu, Cd and As tolerance, Low TBARS, ion leakage and chlorophyll degradation and increase in DOS and APX activity	[144]
Mn SOD + CAT	*Escherichia coli*	*Brassica campestris* L. ssp. pekinensis cv. Tropical Pride)	Resistance to SO_2, increase in the activities of SOD, CAT, GR and APX	[161]
MnSOD + FeSOD	*Nicotiana plumbaginifolia and Arabidopsis thaliana*	*Medicago sativa* L.	Mild water stress tolerance with high photosynthetic activity	[277]
Catalase (CAT)				
CAT	*Triticum aestivum* L.	*Oryza sativa* L. cv. Yuukara or Matsumae	Low temprature stress tolerance due to effective detoxification of H_2O_2 by CAT	[278]
CAT3	*Brassica juncea*	*Nicotiana tabacum*	Cd stress tolerance, better seedling growth and longer roots	[159]
katE	*Escherichia coli*	*Nicotiana tabacum* 'Xanthi'	katE transgene increased the resistance of the chloroplast's translational machinery to salt stress by scavenging hydrogen peroxide	[279]
Ascorbate peroxidase (APX)				
cAPX	*Pisum sativum*	*Lycopersicon esculentum* cv. Zhongshu No. 5	Enhanced tolerance to UV-B, heat, drought and chilling stresses, increase in APX activity	[280,281]
APX3	*Arabidopsis thaliana*	*Nicotiana tabacum* cv. Xanthi	Water deficit tolerance with higher photosynthesis	[282]
APX1	*Hordeum vulgare* L.	*Arabidopsis thaliana*	Salt tolerance due to higher APX, SOD, CAT and GR and low H_2O_2 and MDA content	[283]
swpa4	*Ipomoea batatas*	*Nicotiana tabacum*	Resistance to various stresses MV, H_2O_2, NaCl, Mannitol and to *P. parasitica nicotianae. swpa4* function as a positive defense signal in the H_2O_2-regulated stress response and transgenic plants showed 50-fold higher POD specific activity	[284]
Glutathione reductase (GR)				
GR	*Escherichia coli*	*Triticum aestivum*, cv. Oasis protoplast	Higher GSH content and GSH/GSH + GSSG ratio than control, no increase in SOD and GR activities	[140]
GR	*Arabidopsis thaliana* ecotype Columbia	*Gossypium hirsutum* L. cv. Coker 312	Chilling stress tolerance and photoprotection	[285]
GR		*Gossypium hirsutum* L.	No oxidative stress tolerance	[286]
GR	*Arabidopsis thaliana* ecotype Columbia	*Gossypium hirsutum* L. cv. Coker 312	No chilling stress tolerance	[287]
Monodehydroascorbate reductase (MDAR)				
MDAR1	*Arabidopsis thaliana* ecotype Columbia	*Nicotiana tabacum*	Ozone, salt and PEG stress tolerance due to higher MDAR activity and higher level of reduced AsA	[184]
Dehydroascorbate reductase (DHAR)				
DHAR	*Arabidopsis thaliana*	*Nicotiana tabacum*	Drought and salt tolerance with higher DHAR activity and reduced AsA content	[184]
DHAR	*Arabidopsis thaliana*	*Nicotiana tabacum*	Ozone and drought tolerance with higher DHAR activity and reduced AsA content	[187]
DHAR	*Oryza sativa*	*Arabidopsis thaliana* L. (ecotype Wassilewskija)	Salt tolerance due to slight increase in DHAR activity and total ascorbate	[186]
DHAR	*Human*	*Nicotiana tabacum* cv. Xanthi	Tolerance to MV, H_2O_2, low temperature and NaCl stress	[288]
Glutathione *S*-transferase (GST)				
parB	*Nicotiana tabacum*	*Arabidopsis thaliana* ecotype Landsberg erecta	No whole-plant salt resistance despite antioxidant activity	[289]
Nt107	*Nicotiana tabacum*	*Gossypium hirsutum* L.	No tolerance to salinity, chilling, or herbicides and no increase in antioxidant activity	[199]
GST	*Suaeda salsa*	*Oryza sativa* cv. Zhonghua No.11	Salt and paraquat stress tolerance due to GST, CAT and SOD activity	[290]
NtPox parB	*Nicotiana tabacum*	*Arabidopsis thaliana*	Protect against Al toxicity and oxidative stress	[291]
NtPox parB AtPox	*Nicotiana tabacum* L. and *Arabidopsis*	*Arabidopsis ecotype Landsberg*	Protect against Al toxicity and oxidative stress	[292]
GST + GPX	*Nicotiana tabacum*	*Nicotiana tabacum* L. cv. Xanthi NN	Increased thermal or salt-stress tolerance due to glutathione and ascorbate content	[197]
Glutathione peroxidase (GPX)				
GPX	*Chlamydomonas*	*Nicotiana tabacum cv. Xanthi*	Tolerant to MV under moderate light intensity, chilling stress under high light intensity or salt stress due to low MDA and high photosynthesis and antioxidative system	[293]

Table 3 (*continued*)

Gene	Source	Target transgenic	Response in transgenic plants	Reference
GPX-2	*Synechocystis PCC 6803*	*Arabidopsis thaliana*	Tolerance to H_2O_2, Fe ions, MV, chilling, high salinity or drought stresses	[294]
Proline P5CS (Δ^1-Pyrroline-5-carboxylate-synthetase)				
P5CS	*Vigna aconitifolia* L.	*Triticum aestivum L. cv. CD200126*	Drought tolerance due protection mechanisms against oxidative stress	[248]
P5CS	*Vigna aconitifolia* L.	*Saccharum spp. variety RB855156*	Drought tolerance by proline accumulation in transgenic which acts as a component of antioxidative defense system rather than as an osmotic adjustment mediator	[295]
P5CS	*Arabidopsis thaliana* L. and *Oryza sativa* L.	*Petunia hybrida cv. Mitchell*	Drought tolerance and high proline	[296]
Proline P5CR (Δ^1-pyrroline-5-carboxylate reductase)				
P5CR	*Triticum aestivum*	*Arabidopsis thaliana* L.	Salt tolerance	[297]
P5CR	*Arabidopsis thaliana*	*Glycine max* L. Merr. cv. Ibis	Drought stress tolerance	[298]
O-acetylhomoserine-oacetylserine (OAH-OAS) sulfhydrylase				
Met25	Yeast	*Linum ussitatissimum* cv. Linola	Increased cysteine and methionine biosynthesis resulted in significant increase in glutathione and thus protection against *Fusarium* Infection	[299]

against salt stress [143]. Further, the combined expression of Cu/Zn-SOD and APX in transgenic *Festuca arundinacea* plants led to increased tolerance to MV, H_2O_2, Cu, Cd and As [144]. Myouga et al. [145] studied the role of Fe-SODs in early chloroplast development in *A. thaliana* and found that *Arabidopsis* have three types of Fe-SODs but only FSD2 and FSD3 play essential roles in early chloroplast development. It was concluded that heteromeric *FSD2* and *FSD3* act as ROS scavengers in the maintenance of early chloroplast development by protecting the chloroplast nucleoids from ROS [145].

5.1.2. Catalases (CAT)

CATs are tetrameric heme containing enzymes with the potential to directly dismutate H_2O_2 into H_2O and O_2 (Table 1) and is indispensable for ROS detoxification during stressed conditions [76]. CAT has one of the highest turnover rates for all enzymes: one molecule of CAT can convert ≈6 million molecules of H_2O_2 to H_2O and O_2 per minute. CAT is important in the removal of H_2O_2 generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration and purine catabolism. The CAT isozymes have been studied extensively in higher plants [146] e.g. 2 in *H. vulgare* [147], 4 in *Helianthus annuus* cotyledons [148] and as many as 12 isozymes in *Brassica* [149]. Maize has 3 isoforms (*CAT1*, *CAT2* and *CAT3*), found on separate chromosomes and are differentially expressed and independently regulated [123]. *CAT1* and *CAT2* are localised in peroxisomes and the cytosol, whereas, *CAT3* is mitochondrial. CAT isozymes have been shown to be regulated temporally and spatially and may respond differentially to light [150,151]. The *Escherichia coli* CAT encoded by the *katE* gene overexpressed in *O. sativa* conferred tolerance to transgenic rice plants under salt stress [152]. It has also been reported that apart from reaction with H_2O_2, CAT also react with some hydroperoxides such as methyl hydrogen peroxide (MeOOH) [153]. The variable response of CAT activity has been observed under metal stress. Its activity declined in *Glycine max* [154], *Phragmites australis* [155], *Capsicum annuum* [156] and *A. thaliana* [157] whereas, its activity increased in *O. sativa* [128], *B. juncea* [82], *T. aestivum* [129], *C. arietinum* [130] and *V. mungo* roots [87] under Cd stress. Hso and Kao [158] reported that pretreatment of rice seedlings with H_2O_2 under non-heat shock conditions resulted in an increase in CAT activity and protected rice seedlings from subsequent Cd stress. Eyidogan and Oz [90] reported a significant increase in CAT activity in *C. arietinum* leaves under salt treatment. Similarly, increase in CAT activity in *C. arietinum* roots following salinity stress was noted by Kukreja et al. [89]. Srivastava et al. [138] reported a decrease in CAT activity in *A. doliolum* under NaCl and Cu^{2+} stress. Simova-Stoilova et al. [93] reported increased CAT activity in wheat under drought stress but it was higher especially in sensitive varieties. In another study, Sharma and Dubey [132] reported a decrease in CAT activity in rice seedlings following drought stress. It has also been reported that high ligh condition increased the CAT activity in *P. asperata* under drought stress [135]. The UV-B stress also led to significant increase in CAT activity in *C. auriculata* seedlings [95]. Contrarily, Pan et al. [94] studied the combined effect of Salt and drought stress and found that it decreases the CAT activity in *Glycyrrhiza uralensis* seedlings.

Azpilicueta et al. [148] reported that incubation of *H. annuus* leaf discs with 300 and 500 μM $CdCl_2$ under light conditions increased *CATA3* transcript level but this transcript was not induced by Cd in etiolated plants. Moreover, in roots of the transgenic CAT-deficient tobacco lines (*CAT 1AS*), the DNA damage induced by Cd was higher than in wild type tobacco roots [159]. Transgenic rice plants overexpressing *OsMT1a* showed increase in CAT activity and thus enhanced tolerance to drought [139]. A CAT gene from *B. juncea* (*BjCAT3*) was cloned and up-regulated in tobacco under Cd. CAT activity of transgenic plants was approximately two-fold higher than that of WT which was correlated with enhanced tolerance under Cd stress [160]. In a study the maize Cu/ZnSOD and/or CAT genes were targeted to the chloroplasts of *Brassica campestris* L. ssp. pekinensis cv. Tropical Pride and it was noted that exposure of SOD + CAT *B. campestris* plants to 400 ppb SO_2 showed enhanced tolerance than WT [161]. Further it was reported that enhancement of either SOD or CAT activity individually had only a minor effect on 400 ng ml^{-1} SO_2 tolerance in *B. campestris* transformed with *E. coli* SOD and CAT genes. It was noted that the co-transformed strains that overexpressed both SOD and CAT showed high resistance to SO_2 [162]. There have been many reports on CAT producing abiotic stress tolerant transgenic plants (Table 3).

5.1.3. 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 H_2O_2 in water-water and ASH-GSH cycles and utilizes ASH as the electron donor (Table 1). 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 (sAPX), cytosolic form (cAPX) [39]. APX has a higher affinity for H_2O_2 (μM

range) than CAT and POD (mM range) and it may have a more crucial role in the management of ROS during stress. Enhanced expression of APX in plants has been demonstrated during different stress conditions. Increased leaf APX activity under Cd stress has been reported in *Ceratophyllum demersum* [163], *B. juncea* [82], *T. aestivum* [129] and *V. mungo* [87]. Hso and Kao [158] reported that pretreatment of *O. sativa* seedlings with H_2O_2 under non-heat shock conditions resulted in an increase in APX activity and protected rice seedlings from subsequent Cd stress. Enhanced activity of APX was also found in salt stressed *A. doliolum* [138]. Significant increase in APX activity was noted under water stress in three cultivars of *P. vulgaris* [91] and *P. asperata* [135]. Sharma and Dubey [132] found that mild drought stressed plants had higher chloroplastic-APX activity than control grown plants but the activity declined at the higher level of drought stress. Pekker et al. [164] studied the expression of cAPX in leaves of de-rooted bean plants in response to iron overload and found that cAPX expression (mRNA and protein) was rapidly induced in response to iron overload. The findings of Koussevitzky et al. [165] suggest that cytosolic *APX1* plays a key role in protection of plants to a combination of drought and heat stress. Simonovicova et al. [134] also reported increase in APX activity in *H. vulgare* L. cv. Alfor root tips under Al stress at 72 h.

It has also been noted that overexpression of APX in *Nicotiana tabacum* chloroplasts enhanced plant tolerance to salt and water deficit [142]. Yang et al. [139] correlated the enhanced tolerance of *OsMT1a* overexpessing transgenic rice plants to drought stress with the increase in APX activity. In a study the expression patterns of APX were analysed in roots of etiolated *O. sativa* seedlings under NaCl stress and the mRNA levels for two cytosolic (*OsAPX1* and *OsAPX2*), two peroxisomal (*OsAPX3* and *OsAPX4*), and four chloroplastic (*OsAPX5*, *OsAPX6*, *OsAPX7*, and *OsAPX8*) were quantified in rice genome. It was noted that 150 mM and 200 mM NaCl increased the expression of *OsAPX8* and the activities of APX, but there was not any effect on the expression of *OsAPX1, OsAPX2*, *OsAPX3*, *OsAPX4*, *OsAPX5*, *OsAPX6*, and *OsAPX7* in rice roots [166]. Transgenic *Arabidopsis* plants over-expressing *OsAPXa* or *OsAPXb* exhibited increased salt tolerance. It was found that the overproduction of *OsAPXb* enhanced and maintained APX activity to a much higher extent than *OsAPXa* in transgenic plants under different NaCl concentrations [167]. Overexpression of *C. annuum* APX-like 1 gene (*CAPOA1*) in transgenic tobacco plants exhibited increased tolerance to oxidative stress (MV-mediated), and also enhanced resistance to the oomycete pathogen, *Phytophthora nicotianae*. However, the transgenic plants were not found to be resistant to the bacterial pathogen, *Pseudomonas syringae* pv. tabaci, but showed weak resistance to *Ralstonia solanacearum*. It was suggested that the overproduction of APX increased the POD activity which strengthen the ROS scavenging system and leads to oxidative stress tolerance and oomycete pathogen resistance [168]. Overexpression of APX in transgenic plants conferred abiotic stress tolerance (Table 3).

5.1.4. Guaiacol peroxidase (GPOX)

APX can be distinguished from plant-isolated guaiacol peroxidase (GPOX) in terms of differences in sequences and physiological functions. GPOX decomposes indole-3-acetic acid (IAA) and has a role in the biosynthesis of lignin and defence against biotic stresses by consuming H_2O_2. GPOX prefers aromatic electron donors such as guaiacol and pyragallol usually oxidizing ascorbate at the rate of around 1% that of guaiacol [169]. The activity of GPOX varies considerably depending upon plant species and stresses condotion. It increased in Cd-exposed plants of *T. aestivum* [170], *A. thaliana* [157] and *C. demersum* [163]. Radotic et al. [171] noted an initial increase in GPOX activity in spruce needles subjected to Cd stress and subsequent Cd-treatments caused a decline in the activity. A concomitant increase in GPOX activity in both the leaf and root tissues of *Vigna radiate* [172], *O. sativa* [173] has also been reported under salinity stress.

5.1.5. Glutathione reductase (GR)

GR is a flavo-protein oxidoreductase, found in both prokaryotes and eukaryotes [174]. It is a potential enzyme of the ASH-GSH cycle and plays an essential role in defense system against ROS by sustaining the reduced status of GSH. It is localized predominantly in chloroplasts, but small amount of this enzyme has also been found in mitochondria and cytosol [175,176]. GR catalyzes the reduction of GSH, a molecule involved in many metabolic regulatory and antioxidative processes in plants where GR catalyses the NADPH dependent reaction of disulphide bond of GSSG and is thus important for maintaining the GSH pool [177,178] (Fig. 8). Actually, GSSG consists of two GSH linked by a disulphide bridge which can be converted back to GSH by GR. GR is involved in defence against oxidative stress, whereas, GSH plays an important role within the cell system, which includes participation in the ASH-GSH cycle, maintenance of the sulfhydryl (−SH) group and a substrate for GSTs [177]. GR and GSH play a crucial role in determining the tolerance of a plant under various stresses [178]. GR activity found to be increased in the presence of Cd in *C. annuum* [156], *A. thaliana* [127], *V. mungo* [87], *T. aestivum* [129] and *B. juncea* [82]. Eyidogan and Oz [90] reported increased GR activity in the leaf tissue of *C. arietinum* L. cv. Gokce under salt stress, Whereas, Kukreja et al. [89] noted increased GR activity in *C. arietinum* roots following salt stress. Srivastava et al. [138] reported decline in GR activity in *A. doliolum* under Cu^{2+} stress but it increased under salt stress. Sharma and Dubey [132] noted a significant increase in GR activity in drought stressed *O. sativa* seedlings. Under high light condition drought increased the GR activity in *P. asperata* Mast. seedlings but no prominently drought-induced differences in GR activities were observed in low light seedlings [135].

Bashir et al. [179] studied the expression patterns and enzyme activities of GR in graminaceous plants under Fe-sufficient and Fe-deficient conditions by isolating cDNA clones for chloroplastic GR (*HvGR1*) and cytosolic GR (*HvGR2*) from barley. Both proteins showed *in vitro* GR activity, and the specific activity for *HvGR1* was 3 times higher than *HvGR2*. The expression patterns of *GR1* and *GR2* in rice, wheat, barley, and maize was examined by northern blot analysis and upregulation of *HvGR1*, *HvGR2*, and *TaGR2* was found in response to Fe-deficient conditions than Fe-sufficient [140]. Overexpression of a eukaryotic GR from *B. campestris* (*BcGR*) and *E. coli* GR (*EcGR*) was studied in *E. coli* in pET-28a. It was found that *BcGR* overproducing *E. coli* showed better growth and survival rate than the control but far better growth was noted in *E. coli* strain transformed with the inducible *EcGR* in the presence of paraquat, SA and Cd [180]. In an interesting study, transgenic *N. tabacum* with

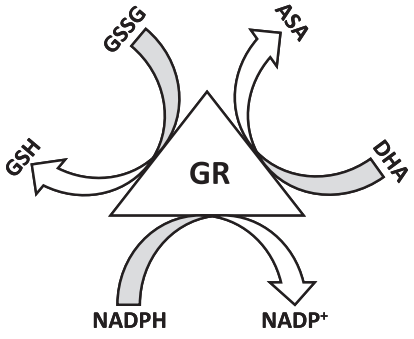


Fig. 8. Glutathione reductase and cellular redox.

30–70% less GR activity were used to find out the possible mechanism of GR against oxidative stress. Transgenic plants with less GR activity showed enhanced sensitivity to oxidative stress. It was suggested that GR plays an important role in the regeneration of GSH and thus protects against oxidative stress also by maintaining the ASH pool [181]. Transgenic plants that produce GR have been found to be abiotic stress tolerant (Table 3).

5.1.6. Monodehydroascorbate reductase (MDHAR)

MDHAR is a flavin adenin dinucleotide (FAD) enzyme that is present as chloroplastic and cytosolic isozymes. MDHAR exhibits a high specificity for monodehydro asorbate (MDHA) as the electron acceptor, preferring NADH rather than NADPH as the electron donor. Asada [169] studied the multi-step reduction of FAD in detail. The first step is the reduction of the enzyme-FAD to form a charge transfer complex. The reduced enzyme donates electrons successively to MDHA, producing two molecules of ascorbate *via* a semiquinone form [E-FAD-NADP(P)$^+$]. It is well established that the disproportionation by photoreduced ferrodoxin (redFd) in the thylakoids is of great importance. Since redFd can reduce MDHA more effectively than NADP$^+$, MDHAR cannot participate in the reduction of MDHA in the thylakoidal scavenging system. Therefore, MDHAR only function in the presence of NAD(P)H, whereas, redFd not [169]. Accompanying APX, MDHAR is also located in peroxisomes and mitochondria, where, it scavange H_2O_2 [66]. Schutzendubel et al. [182] have noted enhanced MDHAR activity in Cd-exposed *Pinus sylvestris* and a declined MDHAR activity in Cd-exposed poplar hybrids (*Populus* × *Canescens*). Sharma and Dubey [132] reported that the activities of enzymes involved in regeneration of ASH i.e., MDHAR, DHAR and GR were higher in drought stressed rice seedlings. It has also been reported that the increase in MDAR activity contribute towards chilling tolerance in tomato fruit [183]. Overexpression of MDAR in transgenic tobacco increased the tolerance against salt and osmotic stresses [184].

5.1.7. Dehydroascorbate reductase (DHAR)

DHAR regenerate ASH from the oxidized state and regulates the cellular ASH redox state which is crucial for tolerance to various abiotic stresses leads to the production of ROS. It has also been found that DHAR overexpression also enhance plant tolerance against various abiotic stresses (Table 3). In a study, under Al stress, the role of MDAR or DHAR in ASH regeneration has been studied in transgenic tobacco plants overexpressing *A. thaliana* cytosolic DHAR (*DHAR-OX*) or MDAR (*MDAR-OX*). It was found that *DHAR-OX* transgenic plants showed higher levels of ASH with or without Al, whereas, *MDAR-OX* plants only showed higher ASH level in the absence of Al in comparison to WT. Significantly higher levels of ASH and APX in *DHAR-OX* plants showed better tolerance under Al stress but not *MDAR-OX* plants. It is clear that plants overexpressing DHAR showed tolerance to Al stress by maintaining high ASH level [185]. It has also been noted that the overexpression of DHAR in tobacco protected the plants against ozone toxicity [186]. Overexpression of DHAR increased salt tolerance in *Arabidopsis* [187] and drought and ozone stress tolerance in tobacco [188].

5.1.8. Glutathione S-transferases (GST)

The plant glutathione transferases, formerly known as glutathione *S*-transferases (GST, EC 2.5.1.18) are a large and diverse group of enzymes which catalyse the conjugation of electrophilic xenobiotic substrates with the tripeptide glutathione (GSH; γ-glu–cys–gly). Plant GSTs are known to function in herbicide detoxification, hormone homeostasis, vacuolar sequestration of anthocyanin, tyrosine metabolism, hydroxyperoxide detoxification, regulation of apoptosis and in plant responses to biotic and abiotic stresses [189]. Noctor et al. [190] reported that GSTs have the potential to remove cytotoix or genotoxic compounds, which can react or damage the DNA, RNA and proteins. In fact, GSTs can reduce peroxides with the help of GSH and produce scavengers of cytotoxic and genotoxic compounds. Plant GST gene families are large and highly diverse with 25 members reported in soybean, 42 in maize and 54 in Arabidopsis [191,192]. These are generally cytoplasmic proteins, but microsomal, plastidic, nuclear and apoplastic isoforms has also been reported [193]. GSTs are very abundant proteins in some cases representing more than 1% of soluble proteins in plant cells [194]. An increased GST activity was found in leaves and roots of Cd-exposed *P. sativum* plants [195] and in roots of *O. sativa* and *Phragmites australis* plants [155,196]. Gapinska et al. [125] noted increased GST activity in *L. esculentum* roots under salinity stress. In an experiment drought tolerant (M35-1) and drought sensitive (SPV-839) sorghum varieties were subjected to 150 mM NaCl for 72 h and M35-1 exhibited efficient H_2O_2 scavenging mechanisms with significantly higher activities of GST and CAT [197].

It has also been found that GST overexpression also enhance plant tolerance to various abiotic stresses (Table 3). Transgenic tobacco seedlings overexpressing GST and GPX showed enhanced seedling growth under stressed environment. Additionally, significant increase in MDHAR activity, GSH and ASH content along with GST and GPX has also been noted in transgenic GST/GPX expressing (GST+) seedlings than WT. These results indicate that overexpression of GST/GPX in transgenic tobacco seedlings provides increased GSH-dependent peroxide scavenging and alterations in GSH and ASH metabolism that lead to reduced oxidative damage [198]. The induction of *Osgstu3* and *osgtu4*, encode tau class GSTs was reported under various abiotic stress conditions in the roots of rice seedlings [196]. Transgenic tobacco plants overexpressing *Gst-cr1* showed significant increase in the activities of GST and GPX which strengthen the antioxidant defense of transgenic plants to resist the oxidative stress. [199]. GST *Nt107* expressing transgenic *Gossypium hirsutum* lines were used to investigate the tolerance potential under various stresses like chilling, salinity, and herbicides and it was noted that transgenic seedlings exhibited ten-fold and five-fold higher GST activity under control and salt stress conditions, respectively [200]. Transgenic tobacco plants overexpressing *Prosopis juliflora* GST (*PjGSTU1*) survived better than control plants under 15% PEG stress. Further, GFP fusion studies revealed the presence of *PjGSTU1* in the chloroplast of transgenic plants which was correlated with its role in ROS removal [201]. Contrarily, it has also been reported that GST acts as a negative regulator of defense response. A GST gene was amplified from cDNA of *N. tabacum* roots infected with *Phytophthora parasitica* var. Nicotianae and it was cloned in an RNAi vector and reduced expression of the gene was detected by RT-PCR. It was noted that GST silenced plants showed increased resistance to *P. parasitica* infection than control which clearly indicate its role as a negative regulator of defense response [202].

5.1.9. Glutathione peroxidase (GPX)

GPXs (EC 1.11.1.9) are a large family of diverse isozymes that use GSH to reduce H_2O_2 and organic and lipid hydroperoxides, and therefore help plant cells from oxidative stress [190]. Millar et al. [203] identified a family of seven related proteins in cytosol, chloroplast, mitochondria and endoplasmic reticulum, named *AtGPX1-AtGPX7* in Arabidopsis. Stress increases GPX activity in cultivars of *C. annuum* plants [156] but decreases in roots and causes no significant change in the leaves of Cd-exposed *P. sativum* plants [195]. Recently, Yang et al. [97] introduced the radish phospholipid hydroperoxide GPX gene (*RsPHGPx*) into a yeast *PHGPx*-deletion mutant and found that it significantly rescue the growth of the recombinant cell exposed to linolenic acid, indicating a similar role to the yeast *PHGPx3* gene (*ScPHGPx3*) in protection of membrane

against LPO. It has also been reported that *PHGPx* mRNA levels show increase in plant tissues under salt stress [204], heavy metal stress [205], oxidative stress [205,206] and mechanical stimulation [207]. Gapinska et al. [125] reported that 150 mM NaCl stress significantly increased the GPX activity in *L. esculentum* Mill. cv "Perkoz" roots.

Leisinger et al. [208] reported the upregulation of a GPX homologous gene (*Gpxh* gene) in *Chlamydomonas reinhardtii* following oxidative stress. It was noted that *Gpxh* gene showed strong induction by the $^1O_2$ -generating photosensitizers neutral red, methylene blue and rose Bengal. It was also noted that Gpxh showed transcriptionally up-regulation by $^1O_2$ photosensitizers when *Gpxh* promoter fusions with the arylsulfatase reporter gene [208]. It was noted that GPX activity in transgenic *G. hirsutum* seedlings was 30-60% higher under normal conditions, but was not different than GPX activity in WT seedlings under salt stress conditions [200]. Overexpression of GPX has been found to enhance abiotic stress tolerance in transgenic plants (Table 3).

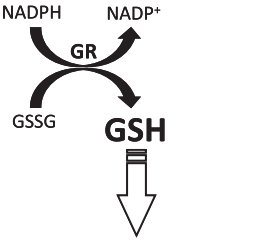
5.2. Non-enzymatic antioxidants

5.2.1. Ascorbic acid (Vitamin C)

Ascorbic acid is the most abundant, powerful and water soluble antioxidant acts to prevent or in minimizing the damage caused by ROS in plants [209,210]. 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 [211]. About 30 to 40% of the total ascorbate is in the chloroplast and stromal concentrations as high as 50 mM have been reported [5]. In plants, mitochondrion play central role in the metabolism of ASH. Plant mitochondria are not only synthesize ASH by L-galactono-γ-lactone dehydrogenase but also take part in the regeneration of ASH from its oxidised forms [212]. The regeneration of ASH is extremely important because fully oxidized dehydroascorbic acid has a short half-life and would be lost unless it is reduced back. 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 $O_2^{\bullet -}$ and OH• and by regenerate α-tocopherol from tocopheroxyl radical. In chloroplast, ASH acts as a cofactor of violaxantin de-epoxidase thus sustaining dissipation of excess excitation energy [211]. In addition to the importance of ASH in the ASH-GSH cycle, it also play important role in preserving the activities of enzymes that contain prosthetic transition metal ions [39]. The ASH redox system consists of L-ascorbic acid, MDHA and DHA. Both oxidized forms of ASH are relatively unstable in aqueous environments while DHA can be chemically reduced by GSH to ASH [213]. Evidence to support the actual role of DHAR, GSH and GR in maintaining the foliar ASH pool has been observed in transformed plants overexpressing GR [214]. *N. tabacum* and *Populus* × *Canescens* plants have higher foliar ASH contents and improved tolerance to oxidative stress [214,215]. Demirevska-Kepova et al. [216] reported that the content of oxidized ascorbate increased during Cd exposure in *H. vulgare* plants. Yang et al. [135] reported that high light condition and drought significantly increased the ASH content in *P. asperata* seedlings. Agarwal [95] reported that the ASH and DHA content as well as the GSH/GSSG content and GSH:GSSG was significantly increased by the UV-B stress in *C. auriculata* seedlings. Contrarily, a decrease in the ASH in the roots and nodules of *Glycine max* under Cd stress has also been observed [154]. Cd also decreases the ASH content in *Cucumis sativus* chloroplast and in the leaves of *A. thaliana* and *P. sativum* [127,217,218,300], respectively, whereas, it remained unaffected in *Populus* × *Canescens* roots [218,300].

5.2.2. Glutathione (GSH)

Tripeptide glutathione (γglu-cys-gly; GSH is one of the crucial metabolites in plants which is considered as most important intracellular defense against ROS induced oxidative damage. It occurs abundantly in reduced form (GSH) in plant tissues and is localized in all cell compartments like cytosol, endoplasmic reticulum, vacuole, mitochondria, chloroplasts, peroxisomes as well as in apoplast [219,220] and plays a central role in several physiological processes, including regulation of sulfate transport, signal transduction, conjugation of metabolites, detoxification of xenobiotics [221] and the expression of stress-responsive genes [222] (Fig. 9). It is well established that GSH also plays important role in several growth and development related events in plants, including cell differentiation, cell death and senescence, pathogen resistance and enzymatic regulation [223]. The synthesis of glutathione occurs in two ATP-dependent steps. First, glutamate-cysteine ligase (GCL) catalyzes formation of γ-glutamylcysteine from Cys and Glu which is thought to be the rate limiting step of the pathway. Second, glutathione synthetase (GS) adds Gly to γ-glutamylcysteine to yield GSH. As synthesized, GSH provides a substrate for multiple cellular reactions that yield GSSG (i.e., two glutathione molecules linked by a disulfide bond). The balance between the GSH and GSSG is a central component in maintaining cellular redox state [5]. GSH is necessary to maintain the normal reduced state of cells so as to counteract the inhibitory effects of ROS induced oxidative stress [224]. It is a potential scavenger of $^1O_2$, H_2O_2 [39,225] and most dangerous ROS like OH• [226]. Additionally, GSH plays a key role in the antioxidative defense system by regenerating another potential water soluble antioxidant like ASH, *via* the ASH-GSH cycle [213]. It has been reported that when the intensity of a stress increases, GSH concentrations usually decline and redox state becomes more oxidized, leading to deterioration of the system [227]. GSH is a precursor of PCs, which plays important role in controlling cellular heavy metal concentration. The role of GSH in the antioxidant defence system provides a strong basis for its use as a stress marker. However, the concentration of cellular GSH has a major effect on its antioxidant function and it varies considerably under abiotic stresses. Furthermore, strong evidence has indicated that an elevated GSH concentration is correlated with the ability of plants to withstand metal-induced oxidative stress. It has been found that enhanced antioxidant activity in the leaves and chloroplast of *Phragmites australis* Trin. (cav.) ex Steudel was associated with a large pool of GSH which resulted in protecting the activity of many photosynthetic enzymes against the thiophilic bursting of Cd [228]. Increased concentration of GSH has been observed with the increasing Cd concentration in *P. sativum* [229], *Sedum alfredii* [230]



- Abiotic stress tolerance
- Detoxification of xenobiotics
- Redox balance of the cell
- Protection of thiol groups
- Regulating the expression of stress defense genes
- Signaling for sulfur metabolism

Fig. 9. Glutathione and plant metabolism.

and *V. mungo* [231]. Srivastava et al. [138] reported an appreciable decline in GR activity and GSH pool under Cu stress and significantly higher increase under salt stress. Sumithra et al. [232] reported that the activities of ROS scavenging enzymes and GSH concentration were found to be higher in the leaves of Pusa Bold than in CO4 cvs. of *Vigna radiata*, whereas, GSSG concentration was found to be higher in the leaves of CO4 compared to those in Pusa Bold which indicates that Pusa Bold has efficient antioxidative characteristics which could provide better protection against oxidative damage in leaves under salt-stressed conditions. Agarwal [95] reported that GSH/GSSG content and GSH:GSSG were significantly increased by the UV-B stress in *C. auriculata* seedlings. Xiang et al. [221] observed that plants with low levels of GSH were highly sensitive to even low levels of Cd^{2+} due to limited PC synthesis.

GSH is particularly important in plant chloroplasts because it helps to protect the photosynthetic apparatus from oxidative damage. Overexpression of a chloroplast-targeted γ-glutamylcysteine synthetase (γ-ECS) in transgenic tobacco plants resulted in three times increase in GSH level. [233]. Paradoxically, increased GSH biosynthetic capacity in the chloroplast resulted in greatly enhanced oxidative stress, which was manifested as light intensity–dependent chlorosis or necrosis. Such phenotype was associated with foliar pools of GSH and γ-glutamylcysteine being in a more oxidized state. Furthermore, the manipulation of both the content and redox state of the foliar thiol pools were achieved using hybrid transgenic plants with enhanced GSH or GR activity in addition to elevated levels of γ-ECS. It has been suggested that γ-ECS–transformed plants suffered continuous oxidative damage caused by a failure of the redox-sensing process in the chloroplast [233].

5.2.3. Proline (Pro)

Other than as an osmolyte, now Pro is considered as a potent antioxidant and potential inhibitor of PCD. Therefore, Pro can now be regarded as nonenzymatic antioxidants that microbes, animals, and plants require to mitigate the adverse effects of ROS [16]. The synthesis of L-Pro from L-glutamic acid *via* Δ^1-pyrroline-5-carboxylate (P5C) is catalyzed by the activities of the enzymes Δ^1-pyrroline-5-carboxylate synthetase (*P5CS*) and Δ^1-pyrroline-5-carboxylate reductase (*P5CR*) in plants [234]. On the other hand, mitochondrial enzymes Pro dehydrogenase (oxidase) (ProDH) and P5C dehydrogenase (P5CDH) metabolize L-Pro into L-Glu *via* P5C. It is well documented that following salt, drought and metal stress there is a dramatic accumulation of Pro may be due to increased synthesis or decreased degradation. Free Pro has been proposed to act as an osmoprotectant, a protein stabilizer, a metal chelator, an inhibitor of LPO, and $OH^{\bullet}$ and $^1O_2$ scavenger [235,236]. Sorbitol, mannitol, myo-inositol and Pro has been tested for $OH^{\cdot}$ scavenging capacity and it has been found that Pro appeared as effective scavenger of $OH^{\bullet}$ [237]. Therefore, Pro is not only an important molecule in redox signaling, but also an effective quencher of ROS formed under salt, metal and dehydration stress conditions in all plants, including algae [238]. In an interesting study Chen and Dickman [16] reported that addition of Pro to DARas mutant cells effectively quenched ROS levels and prevented cell death. Furthermore, Pro also protected WT *C. trifolii* cells from UV light, salt, heat and H_2O_2 stress. It has also been noted that Pro also protected the yeast cells from herbicide MV. It was suggested that the ability of Pro to scavenge ROS and ability to inhibit ROS-mediated apoptosis can be a important function in response to cellular stress. Increased accumulation of Pro has been correlated with improved tolerance to various abiotic stresses especially salt and drought. Enhanced synthesis of Pro under drought or salt stress has been implicated as a mechanism to alleviate cytoplasmic acidosis and maintain $NADP^+$:NADPH at values compatible with metabolism [239]. An additional advantage of the refilling of $NADP^+$ supply by Pro synthesis may be to support redox cycling, which is especially important in plant antioxidant defense mechanisms during stress [240]. It has also been noted that salt stress increased the accumulation of Pro in the leaves of two rice cultivars differing in salt tolerance [241]. Several lines of evidence suggested the important role for Pro synthesis in potentiating pentose-phosphate pathway activity [239] because this pathway is an important component of antioxidative defense mechanisms, which need NADPH to maintain GSH and ASH in the reduced state. Siripornadulsil et al. [242] reported that the measurements of reduced/oxidized GSH ratios and analyses of levels of MDA indicate that free Pro levels are correlated with the GSH redox state and MDA levels in heavy metal-treated algae. These results suggest that the free Pro acts as an antioxidant in Cd-stressed cells [242]. Gajewska and Sklodowska [243] studied the effect of Ni on pea plants and found that stimulation of GST activity and accumulation of Pro in the tissues rather than antioxidative enzymes are involved in response of pea plants to Ni stress.

Transgenic tobacco cells (silenced at their tobacco Pro dehydrogenase (*NtProDH*) gene) accumulated more Pro than WT cells and showed enhanced osmotolerance [244]. It was noted that Pro ameliorated the inhibition of growth of BY-2 cells under salt stress. BY-2 cells showed significant decrease in the activities of SOD, CAT and POD under salt stress but exogenously applied Pro alleviated the reduction in CAT and POD activities under salt stress. [245]. The potato transgenic plants overexpressing *P5CS* cDNA from *A. thaliana* showed significant increase in Pro levels under salt stress and showed less altered tuber yield and weight in comparison to control plants [246]. Su and Wu [247] reported that both constitutive expression and stress-inducible expression of the *P5CS* cDNA in transgenic *O. sativa* have led to the accumulation of *P5CS* mRNA and Pro which resulted in higher salt and water deficiency stress tolerance. Overexpression of *Vigna aconitifolia P5CS* cDNA under the control of a stress-induced promoter complex-AIPC resulted in enhanced Pro accumulation under water deficit. It has also been found that overexpression of Pro biosynthetic pathway genes enhance the abiotic stress tolerance in transgenic plants (Table 3).

5.2.4. α-Tocopherols (Vitamin E)

Tocopherols, a lipid soluble antioxidant are considered as potential scavengers of ROS and lipid radicals [249]. Tocopherols are considered as a major antioxidant in biomembranes, where they play both antioxidant and non-antioxidant functions. Tocopherols are considered general antioxidants for protection of membrane stability, including quenching or scavenging ROS like $^1O_2$. Tocopherols are localized in plants in the thylakoid membrane of chloroplasts. Out of four isomers of tocopherols (α-, β-, γ-, δ-) found in plants, α-tocopherol has the highest antioxidative activity due to the presence of three methyl groups in its molecular structure [250]. It is synthesized from γ-tocopherol in chloroplasts by γ-tocopherolmethyltransferase (γ-TMT; VTE4). A high level of α-tocopherol has been found in the leaves of many plant species including Arabidopsis but these are low in γ-tocopherol. It has been found that nitration of γ-tocopherol considered to be an important mechanism for the regulation and detoxification of NOx in animal tissues. In plants, *in vivo* 5-nitro-γ-tocopherol (5-NγT) was also identified in leaves of the Arabidopsis mutant line (*vte4*). Reduced NOx concentration has been found in the leaves of *vte4* mutant than *vte1* and WT. Germinating seeds of *Brassica napus*, *N. tabacum* and *A. thaliana* also showed the presence of 5-NγT. It can be said that γ-tocopherol or 5-NγT prolongs early development by reducing NOx concentration [251]. Tocopherols has been shown to prevent the chain propagation step in lipid autooxidation which makes it an effective free radical trap. Additionally, it has been estimated that

one molecule of α-tocopherol can scavenge up to 120 $^1O_2$ molecules by resonance energy transfer [252]. Recently, it has been found that oxidative stress activates the expression of genes responsible for the synthesis of tocopherols in higher plants [253]. Increased levels of α-tocopherol and ASH have been found in tomato following trizole treatment which may help in protecting membranes from oxidative damage and thus chilling tolerance in tomato plants [254]. Increase in tocopherol during water stress in plants has also been reported by many workers [253,254]. Srivastava et al. [138] reported a general induction in α-tocopherol content in *A. doliolum* under NaCl and Cu^{2+} stress. Trebst et al. [255] reported that blocking of tocopherol biosynthesis at the 4-hydroxyphenylpyruvate dioxygenase by the herbicide pyrazolynate resulted in quick disappearance of α-tocopherol in high light, as well as of PS II activity and the D1 protein. Therefore, tocopherols are essential to keep photosynthesis active [255]. Bergmuller et al. [256] reported increased levels of α-tocopherol and γ-tocopherol in WT and γ-tocopherol in *vte4-1* following high light, high temperature, cold treatment induced oxidative stress. Interestingly no difference in chlorophyll content and photosynthetic quantum yield was observed in WT and *vte4-1* which suggests that γ-tocopherol replaced α-tocopherol in *vte4-1* to protect photosynthetic apparatus against oxidative stress. Giacomelli et al. [257] reported that eight genotypes of Arabidopsis showed increased concentrations of α-tocopherol, ASH and GSH in response to high light (1,000 μmol photons m^{-2} s^{-1}), whereas, GSH content was higher in ASH deficient *vtc2* genotypes in response to control light (120 μmol photons m^{-2} s^{-1}) in comparison to others. Tocopherol cyclase (VTE1, encoded by *VTE1* gene) catalyzes the penultimate step of tocopherol synthesis [92]. Overexpression of *VTE1* from *Arabidopsis* in transgenic tobacco plants showed decreased LPO, electrolyte leakage and H_2O_2 content than WT following drought [92].

5.2.5. Carotenoids (Car)

Plants have evolved several mechanisms for getting rid of excess energy in photosynthetic membranes, some of which involve isoprenoid compounds. In all photosynthetic organisms, the carotenoids β-carotene and zeaxanthin and tocopherols serve an important photoprotective role, either by dissipating excess excitation energy as heat or by scavenging ROS and suppressing LPO. Car are pigments that are found in plants and microorganisms. There are over 600 carotenoids occurring in nature. Car a lipid soluble antioxidant plays a multitude of functions in plant metabolism including oxidative stress tolerance. Car carry out three major functions in plants. First, they absorb light at wavelength between 400 and 550 nm and transfer it to the Chl (an accessory light-harvesting role) [258]. Second, they protect the photosynthetic apparatus by quenching a triplet sensitizer (Chl^3), $^1O_2$ and other harmful free radicals which are naturally formed during photosynthesis (an antioxidant function) [259]. Third, they are important for the PSI assembly and the stability of light harvesting complex proteins as well as thylakoid membrane stabilization (a structural role) [13,260]. Car within the photosynthetic apparatus are known to quench $^1O_2$ [259], but the photosynthetic growth of cells lacking Car suggests there are other mechanisms to protect cells from $^1O_2$ damage. Rai et al. [261] and Singh et al. [87] reported decreased Car and Chl contents in *Phyllanthus amarus* and *V. mungo* plants with increasing Cd concentration, respectively. Car content of *H. vulgare* seedlings decreased under Cd-stress [216]. An increase in Car content was also reported following Cd stress [262]. It has been considered that some isoprenoids (including several carotenoids and tocopherols) play an effective role in photoprotection [263]. Furthermore, it has been proved that monoterpene improved thermotolerance at elevated temperatures and that monoterpene had a protecting role against oxidative stress [264].

5.2.6. Flavonoids

Flavonoids occur widely in the plant kingdom, and are commonly found in leaves, floral parts, and pollens. Flavonoids usually accumulate in the plant vacuole as glycosides, but they also occur as exudates on the surface of leaves and other aerial plant parts. Flavonoid concentration in plant cells is often over 1 mM [265]. Flavonoid scan be classified into flavonols, flavones, isoflavones, and anthocyanins based on their structure. Flavonoids are suggested to have many functions like flowers, fruits, and seed pigmentation, protection against UV light; defence against phytopathogens (pathogenic microorganisms, insects, animals); role in plant fertility and germination of pollen and; acting as signal molecules in plant-microbe interactions [266]. Flavonoids are among the most bioactive plant secondary metabolites. Most flavonoids outperform well-known antioxidants, such as ASH and α-tocopherol [202]. Flavinoids serve as ROS scavengers by locating and neutralizing radicals before they damage the cell thus important for plants under adverse environmental conditions [267]. Flavonoids function by virtue of the number and arrangement of their hydroxyl groups attaches to ring structures. Their ability to act as antioxidants depends on the reduction potentials of their radicals and accessibility of the radicals. Flavonoids and other phenolic compounds absorb UV light, and plants able to synthesize these compounds were more tolerant to high UV irradiation than mutants impaired in the flavonoid pathway [268]. Many flavonoid biosynthetic genes are induced under stress conditions. It has been found that there is considerable increase in flavonoid levels following biotic and abiotic stresses, such as wounding, drought, metal toxicity and nutrient deprivation [269]. Production of flavonoids in response to UV-B, cold and drought were reported earlier. Mutant plants (*tt4*, deficient in chalcone synthase, and *tt5*, deficient in chalcone isomerase), unable to accumulate flavonoids were found to be more sensitive to UV light [270]. Flavonols are among the most abundant flavonoids in plants and it has also been reported that flavonols accumulate in their glycosylated form after an inductive light treatment and absorb UV-B light in the 280–320 nm region and therefore regarded as effective UV filters [271]. In a study to investigate the protective role of flavonoids against UV-B, two isolines of the Clark cultivar (the standard line with moderate levels of flavonoids and the magenta line with reduced flavonoids) were grown in the field with or without natural levels of UV-B and it was found that high levels of flavonoids lead to a reduction in UV-B sensitivity at the proteomic level [272]. It has also been found that flavonoid pathway play important role for better tolerance under nitrogen deficiency in *A. thaliana* [273]. Flavonoids are also involved in the resistance to pathogens and in acting as feeding deterrents [274].

6. Conclusions

It is well documented that various abiotic 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 condition in which ROS or free radicals, are generated extra- or intra-cellularly, which can exert their toxic effects to the cells. These species may affect cell membrane properties and cause oxidative damage to nucleic acids, lipids and proteins that may make them nonfunctional. However, the cells are equipped with excellent antioxidant defense mechanisms to detoxify the harmful effects of ROS, The antioxidant defenses could be either non-enzymatic (e.g. glutathione, praline, α-tocopherols, carotenoids and flavonoids) or enzymatic (e.g. superoxide dismutase, catalase glutathione peroxidase and glutathione reductase). ROS are now also considered as key regulatory

molecules vital for cells, but they cause cellular damage when produced in excess or when the antioxidant defense system is not properly functioning. 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 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. Overexpression of ROS scavenging enzymes like isoforms of SOD (Mn-SOD, Cu/Zn-SOD, Fe-SOD), CAT, APX, GR, DHAR, GST and GPX resulted in abiotic stress tolerance in various crop plants due to efficient ROS scavenging capacity. Pyramiding of ROS scavenging enzymes may also be used to obtain abiotic 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 harsh environmental conditions.

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