Chapter 3
Heavy Metal Toxicity and Antioxidative Response in Plants: An Overview

Shumailah Ishtiyaq, Harsh Kumar, Mayank Varun, Bhumesh Kumar, and Manoj S. Paul

Abstract Environmental pollution caused by heavy metals has received worldwide attention. The intractable and pertinacious nature of heavy metals leads to severe threat to environment, and affects the life of both plants and animals, causing serious diseases in humans. Heavy metal toxicity in plants cause imbalances in the redox metabolism that leads to oxidative damage which is characterized by enhanced production of reactive oxygen species (ROS). To minimize the deleterious consequences of ROS, plants in general have developed biological detoxification and defense mechanisms that protect the cellular components from being oxidized. Antioxidative defense activity of plants is composed of enzymatic scavengers such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and guaiacol peroxidase (GPX) and non-enzymatic components like ascorbic acid (AA), α-tocopherol, carotenoids, flavonoids and proline that plays the most crucial and effective role in detoxifying ROS and the changes in their activity is often used to predict metal tolerance. In this chapter, the current state of knowledge about the role of ROS, defense mechanisms and adaptation strategies of plants with special reference to antioxidative defense system to alleviate heavy metal toxicity is discussed. Recent researches have thrown ample lights on how enzymatic and non-enzymatic machinery of plants can protect, regulate and integrate cell responses to heavy metal stress.

Keywords Antioxidant defense · Oxidative stress · Reactive oxygen species · Secondary metabolism · Metal chelation · Glutathione · Phytoremediation

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3.1 Introduction

Early on, metal pollution only affected the small portion of the population which was in close proximity of the metal source. However, at the turn of the nineteenth century, the start of the industrial revolution resulted in a greater demand for products such as coal, iron and steel. Pollutants were no longer restricted to small areas, but instead, were distributed over a wide area, by means of air and water. This has caused deleterious effects to the ecosystem and human health. Today, many soils throughout the world have been known to be polluted with heavy metals.

Heavy metals include arsenic (As), silver (Ag), lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), chromium (Cr), copper (Cu) iron (Fe), etc. Heavy metals are better described as ‘trace metals’—metals found at low concentrations, usually less than one ppm or less, in a specified source, e.g. soil, plant tissue or groundwater (Phillips 1981). Heavy metals are not considered as a pollutant if persist in low concentrations. They occur naturally in the environment due to their presence in bedrocks. Some heavy metals such as zinc and copper are also essential micronutrients for living organisms. The term ‘*heavy metal pollution*’ refers to heavy metal levels that are relatively higher than the normal background concentration; mere presence of the metal is insufficient evidence of pollution (Alloway and Ayres 1997).

Heavy metal concentrations in soil range from less than 1 mg kg^{-1} to over 1000 mg kg^{-1} (Adriano 2003). They are present in the earth’s crust naturally in different minerals at different concentration and many of these metals are essential for cells (e.g. Cu, Fe, Mn, and Zn) (Marschner 1995). The world-wide emissions of metals to the atmosphere by natural sources is estimated as: Ni: 26, Pb: 19, Cu: 19, As: 7.8, Zn: 4, Cd: 1.0, Se: 0.4, (thousand tonnes $year^{-1}$). Whereas, from anthropogenic sources: Pb: 450, Zn: 320, Ni: 47, Cu: 56, As: 24, Cd: 7.5, Se: 1.1 (thousand tonnes $year^{-1}$) (Valavanidis and Vlachogianni 2010). It is obvious from these numbers that Pb, Zn, Ni and Cu are the most important metal pollutants generated by anthropogenic activities.

3.2 Heavy Metals and Their Impact on Plants

3.2.1 Cadmium

Cadmium is commercially used in paint pigments, glass making, electroplating, photography, dying, stabilizer, and in batteries. It is classified as a probable human carcinogen (group B1) by the USEPA and toxic to organisms. In the past, there have been examples of marked Cd contamination in areas where food has been grown. This was particularly so for rice crops in Japan where Cd concentrations from 200 to 2000 ppb were found (Elinder 1985). In general, soils which have been historically contaminated with Cd from industrial operations are now no longer used for agricultural purposes. The accumulation of Cd in water and soil has caused major environmental and human health concern. It is a biologically non-essential metal, toxic to both plant and human beings (Shukla et al. 2007).

Plants upon exposure to Cd show various symptoms such as chlorosis, growth inhibition, burning of root tips, and finally death (Kahle 1993). Cd affects stomatal function, water transport and cell wall elasticity (Baszynskiet al. 1980). Baryla et al. (2001) reported an increase in the stomatal resistance of plants that were treated with Cd. Inhibition of photosynthesis is another toxic effect of Cd, which is brought about by reduced stomatal conductance in response to metal toxicity and sensitivity of photosystem II to high Cd concentration. Cd may affect PS II on both the oxidising (donor) and reducing (acceptor) side (Haag-Kerwer et al. 1999). Rubisco activity in the Calvin cycle is inhibited by high Cd. The clearest symptom of Cd phytotoxicity is leaf chlorosis. High Cd concentration in the plant induces increased respiration and activities of the tricarboxylic acid cycle as well as other pathways of carbohydrate utilisation (Arisi et al. 2000).

Hyperaccumulation of Cd is a rare phenomenon in higher plants. *Thlaspi caerulescens* has been identified as Cd hyperaccumulator (Baker et al. 2000). Ecotypes of *T. caerulescens* accumulate up to 12,500 mg kg^{-1} Cd dry weight, without showing signs of toxicity (Peer et al. 2003). Cd hyperaccumulation in *Arabidopsis hallerii* has also been reported (Cosio et al. 2004). Vetiver grass could be used to remediate Cd-polluted soil, since it accumulated 218 g Cd ha^{-1} at a soil Cd concentration of 0.33 mg Cd kg^{-1} (Chen et al. 2000). Several plant species have been identified which are capable of accumulating and tolerating moderate to high levels of Cd in their tissues such as *Solanum lycopersicum*, *Chenopodium murale*, *Poa annua, Calotropis procera*, and *Abutilon indicum* (Varun et al. 2012, 2015; D'Souza et al. 2013).

3.2.2 Arsenic

Arsenic (As) is a naturally occurring metalloid, commonly used in pesticides and wood preservatives. In India, As contamination of groundwater has resulted in widespread well contamination and possess serious health risk. Frequent use of As contaminated water in irrigation resulting in As poisoning of humans and other animals through dietary intake (McArthur et al. 2001). Similar contamination is also been reported from various regions with As in subsoils worldwide.

Arsenite [AsO_2^- or As(III)] and arsenate [AsO_4^{-3} or As(V)] are the dominant inorganic arsenic moieties found in terrestrial plants and both forms are phytotoxic. Arsenate is predominantly found in aerobic soils. Formation of ADP-As complexes instead of ATP leads to cell death. Arsenate is often designated as more phytotoxic of the two arsenic from but the relative toxicities are species-specific (Wang et al. 2002).

Non-accumulators have a phytotoxic threshold at approximately 5–100 mg kg^{-1}As dry weight (wt.). Fern pteriodophyte, *Pteris vittata* can accumulate As in contaminated as well as non-contaminated soils suggesting that hyperaccumulation is a constitutive trait (Wang et al. 2002). *Holcus lanatus* can accumulate upto 560 mg kg^{-1} As, and *Pteris vittata* can accumulate up to 27,000 mg kg^{-1} As dry wt., with phytotoxic symptoms appearing around 10,000 mg kg^{-1}As dry wt. (Gumaelius et al. 2004). Ferns like *Pteris cretica, P. longifolia*, and *P. umbrosa* can hyperaccumulate As (Wei and Chen 2006). Another fern, *Pityrogramma calomelanos* has also been discovered as an As hyperaccumulator, it showed great potential in phytoremediation of As contaminated soils (Francesconi et al. 2002).

3.2.3 Mercury

Over the past few decades, several thousand tons of Hg have been released to the environment by human activity (Ebinghaus et al. 1999). Once released into the atmosphere it can be exchanged between soil and water by wind erosion, degassing of mineralized rock formations, and volcanic eruptions. Use of elemental Hg-Au amalgamation in mining is a common practice, which results in significant Hg contamination in the surrounding environment. Organomercurials like methyl-Hg and phenylmercuric acetate are the most toxic forms of Hg followed by ionic Hg(II), while elemental Hg(0) is the least toxic form.

Mercury is a persistent environmental pollutant with bioaccumulation ability in fish, animals, and human beings (Chang et al. 2009). The mechanism and extent of Hg toxicity depend strongly on the type of compound and the redox state of mercury. Terrestrial plants are generally insensitive to the harmful effects of Hg. However, it is known to affect photosynthesis and oxidative metabolism by interfering with electron transport in chloroplasts and mitochondria. Mercury also inhibits the activity of aquaporins and reduces plant water uptake (Sas-Nowosielska et al. 2008).

Hg is toxic to plants, and to date Hg hyperaccumulating plants have not been identified. However, Hg hyperaccumulating *Amanita muscaria* (mushroom) has been identified and can accumulate 96–1900 ng g^{-1} dry wt. (Falandysz et al. 2003). Most of the phytoremediation studies focused on converting the organomercurials to Hg(0), which is volatile and is released into the atmosphere. Transgenic poplar and cottonwood trees expressing *merA* and/or *merB* could be used as phytoremediators which do not require harvesting or replanting each season (Che et al. 2003). Plants growing on Hg-polluted soils show a reduced growth and vitality (Tomiyasu et al. 2017). Specific Hg-adapted metallophytes do not seem to exist. However, 13 plant species accumulated Hg out of 87 examined from a highly contaminated waste area originating from a chemical plant in Italy (Massa et al. 2010). *Polygonum aviculare* was identified as the best accumulator; however, its slow growth and low productivity prevents it to be used for phytoextraction purposes.

3.2.4 Chromium

Chromium (Cr) with its great economic importance in industrial world is a major metal pollutant of the environment. It is used in industries for chrome plating, wood preservation, textile dyeing and pigmentation, manufacturing pulp and paper, and tanning (Sharma and Adholeya 2011). Leather tanning industries produce large amounts of organic and chemical pollutants, especially, chromium. This Cr containing untreated effluent and sludge discharged by tanneries possess a serious threat to the environment and human health. Chromium is present in many oxidation states [Cr(II) to Cr(VI)], the most common forms are Cr(0, III, and VI). World Health Agency and EPA has determined Cr(IV) as a carcinogen. It is considered to be 1000 times more toxic than Cr(III). Cr(VI) contamination in the soil and groundwater has been reported in various regions of California, USA (EPA 2004).

Cr is a toxic and non-essential element to plants. The toxic effects of Cr are primarily dependent on the metal speciation, which determines its uptake, translocation and accumulation (Shankar et al. 2005). The two stable forms of Cr i.e. Cr(III) and Cr(VI) are taken up by the plants by different mechanisms. Barcelo et al. (1986) reported an inhibition of micronutrients like P, K, Zn, Cu and Fe translocation within bean plant parts was observed when treated with Cr in growth matrix. Sujatha and Gupta (1996) reported that tannery effluent irrigation caused micronutrient deficiencies in several agricultural crops. Cr induced chlorosis was also observed in rye grass (Ottabbong 1989). Cr compounds are highly toxic to plants and are detrimental to their growth and development (Shankar et al. 2005).

Davies et al. (2002) reported that while some plants are not affected by low Cr concentration (3.8×10^{-4} μM Kg^{-1}), however it is toxic to most of the higher plants at 100 μM Kg^{-1} dry weight. Zhu et al. (1999) reported that *Eichhornia crassipes* was a good accumulator of Cr and Cd. Pulford et al. (2001) investigated Zn and Cr content in different tree species and found Cr to be accumulated mainly in roots, whereas Zn was translocated to shoots. Zavoda et al. (2001) found that *Helianthus annuus* and *Brassica juncea* accumulates significant Cr in roots.

3.2.5 Copper

Copper (Cu) is not poisonous in its metallic state but some of its salts are poisonous. Cu is an essential element and enzyme co-factor for oxidases (cytochrome c oxidase, superoxide dismutase) and tyrosinases. Cu contamination in soil usually results from mining, pesticides, and industrial wastes. It deposits in the liver and disrupts the liver's activity thus adversely affect the nervous system, reproductive system, adrenal function, development of the child, etc. (Badiye et al. 2013).

Cu contributes to several physiological processes in plants including photosynthesis, respiration, carbohydrate distribution, nitrogen and cell wall metabolism, seed production and disease resistance (Kabata-Pendias 2001). Leaf chlorosis is an initial symptom of Cu toxicity (Zhu and Alva 1993). Mitchell et al. (1988) reported leaf chlorosis in *Banksia ericifolia*, *Casuarina distyla* and *Eucalyptus eximia* grown at elevated Cu. At higher Cu toxicity, leaves become wilted before eventually becoming necrotic. Copper toxicity can be associated with a purpling of foliage but this is not apparent in all species (O'Sullivan et al. 1997).

Copper toxicity has a significant effect on root-shoot growth and biomass of the plants (Minnich et al. 1987). In a study conducted by Patterson and Olson (1983) the germination of six tree species was found less sensitive to Cu than subsequent root elongation. *Citrus paradisi* x *Poncirus trifoliata* seedlings exposed to excess Cu produce few new roots and have a thickened tap root (Zhu and Alva 1993). Most Cu-tolerant plants are excluders, and no confirmed Cu accumulators have been identified to date. Liao and Chang (2004) identified an aquatic weed; *Eichhornia crassipes* can accumulate 21.62 kg Cu ha^{-1}, and could be used for phytoremediation Cu contaminated waste water. *Elsholtzia haichowensis* has been identified as a Cu tolerant and accumulating plant species in mining areas (Yang et al. 2002).

3.2.6 Zinc

Zinc (Zn) is considered as one of the most important micronutrients for animals and plants but on the other hand is toxic if it exceeds the sufficient level (Hambidge and Krebs 2007). About 70% of the world's Zn originates from mining, while the remaining 30% comes from recycling secondary zinc. Farm manures also have high concentrations of Zn, which make them a promising amendment for Zn-deficient soils. Zinc is an essential element for living organisms and is toxic to living organisms only at very high concentrations (McIntyre 2003). It is an essential component for protein production in all terrestrial life and is the second metal only to iron in terms of abundance in living organisms.

The first symptom of Zn toxicity exhibited by most plants is a general chlorosis of the younger leaves (Fontes and Cox 1995). This chlorosis can further progress to reddening due to anthocyanin production in younger leaves. Plants exhibiting Zn toxicity have smaller leaves than their control plants (Ren et al. 1993). Zinc stressed *Glycine max* exhibit vertically oriented leaves (Fontes and Cox 1995). In severe cases, plants may exhibit necrotic lesions on leaves and eventually entire leaf death. In roots, Zn toxicity is apparent as a reduction in the growth of the main root, fewer and shorter lateral roots and a yellowing of roots (Ren et al. 1993). Khurana and Chatterjee (2001) reported a reduction in biomass, seed number, seed weight and soluble proteins in sunflower (*Helianthus annuus*) plants grown in Zn-laden soil.

The first Zn hyperaccumulator identified was *Thlaspi caerulescens*. It can accumulate 25,000–30,000 μg g^{-1} Zn, although *T. caerulescens* can accumulate a maximum dry weight of 40,000 ug g^{-1} Zn in its shoots (Pence et al. 2000). *Arabidopsis halleri* has also been found to increase in its shoot Zn concentration from 300 ug g^{-1} dry wt at 1 μM Zn to 32,000 ug g^{-1} at 1000 μM Zn without phytotoxicity (Zhao et al. 2000).

3.2.7 Lead

Lead (Pb) has been commonly used for thousands of years because of its widespread occurrence, easy to extract and easy to work with. Pb is the most significant toxin of the heavy metals. It accumulates primarily in the surface soil layer, and its concentration decreases with the soil depth. It may exist in the atmosphere as dust, fumes, mist and vapours and in soil as mineral. Soils along roadsides are particularly lead rich because of pollution from vehicles burning leaded gasoline (Eick et al. 1999). Important sources of lead pollution are geological weathering, industrial processing of ore and minerals, leaching of metals from solid wastes. Once it is introduced into the growth matrix, it is very difficult to remove Pb.

A high Pb level in soil induces abnormal morphology in many plant species. It causes irregular radial thickening in pea roots, cell wall of the endodermis, and lignifications of cortical parenchyma (Paivoke 1983). Lead also induces proliferation effects in the repair process of vascular plants. A significant amount of lead in the plant causes reduction in plant growth (Salim et al. 1995). Lead toxicity can cause plasma membrane alteration in plants because Pb^{2+} is physiologically similar to Ca^{2+} (Srivastava and Gupta 1996). Elevated Pb interferes with chlorophyll formation and the normal metabolism of Fe (Kacabova and Natr 1986). High concentration of Pb has been linked to poor seed germination, high stomatal resistance, inhibited CO_2 uptake and low photosynthetic rate (Poskuta et al. 1987). Lead induced inhibition of seed germination has been reported in *Hordeum vulgare, Elsholtzia argyi, Spartina alterniflora, Pinus halepenis, Oryza sativa* and *Zea mays* (Islam et al. 2007; Senger et al. 2009).

Certain plants (mostly, belonging to the Brassicaceae, Euphorbiaceae, Asteraceae, Lamiaceae, and Scrophulariaceae families) have been identified which have the potential to uptake Pb (USEPA 2000). Many plants have a strategy of Pb exclusion as *Thlaspi praecox,* which hyperaccumulates Cd and Zn but exclude Pb (Vogel-Mikus et al. 2005), however, several plant species can hyperaccumulate Pb. *Sesbania drummondii*, *Brassica* species and *Pelarogonium* can accumulate significant Pb content in roots (Blaylock et al. 1997; Sahi et al. 2002), without showing any symptoms of toxicity.

3.2.8 Nickel

Nickel (Ni) is an essential element that can be toxic and possibly carcinogenic in high concentrations only. It is found in different concentrations in all soil types of diverse climatic regions. The normal range of Ni in soil is 2–750 ppm, with a critical soil concentration at 100 ppm (Gardea-Torresdey et al. 2005). Exposure to Ni compounds causes irreversible damage to the nervous system, cardiovascular system, lungs and gastrointestinal tract (Axtell et al. 2003). Nickel has been classified among the essential micronutrients and remains associated with some metalloenzymes, but Ni is toxic at elevated concentrations in plants (Srivastava et al. 2005).

Toxic symptoms of Ni include yellowing, necrosis, chlorosis, deformed and stunted growth and ROS generation (Halliwell and Gutteridge 1999).

Serpentine and ultramafic soils are naturally occurring regions of high Ni concentrations characterized by unique Ni-tolerant flora. The majority of Ni hyperaccumulators have been collected from these soils. *Alyssum lesbiacum* and *Thlaspi goesingense* are both Ni hyperaccumulating plants in the Brassicaceae family. In *Alyssum* genus alone, 48 different species have been discovered containing between 1000 and 30,000 µg g^{-1} Ni in leaf (Baker and Brooks 1989; Kerkeb and Kramer 2003). Several Ni phytoextraction using hyperaccumulators has been patented (Chaney et al. 1999).

3.3 Reactive Oxygen Species

Anthropogenic activities and natural tragedies such as pollution, drought, salinity, UV radiation, temperature extremes and heavy metals generate different kinds of stresses that adversely affect the biological systems and cellular metabolic processes such as respiration and photosynthesis which unavoidably lead to the production of reactive oxygen species (ROS). ROS are highly reactive component which includes free radicals such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), as well as non-radical molecules like hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). ROS production takes place by a sequenced reduction of molecular oxygen (O_2) under high energy subjection or electron-transfer reactions (del Río et al. 2006; Blokhina and Fagerstedt 2010; Heyno et al. 2011). The O_2 molecule with two impaired electrons and same spin quantum number act as a toxic and strong oxidizing agent. This spin orientation makes O_2 susceptible to accept electrons one at a time that ultimately leads to the generation of the so-called ROS, which generate oxidative stress on cellular compartments. Several metabolic pathways, localized in different cellular compartments such as chloroplast, mitochondria and peroxisomes also contribute to the ROS production continuously as a byproduct of biochemical reactions (Navrot et al. 2007). Under normal conditions, these oxidants are scavenged by various antioxidative defense mechanisms and maintain a cellular equilibrium (Heyno et al. 2011) but heavy metal load can disturb this balance that eventually leads to a rapid increase in intracellular production of ROS which is significantly destructive for biomolecules and different compartments of the cell (Fig. 3.1). It has been reported that 1–2% of O_2 consumption leads to the formation of ROS in plant tissues (Bhattacharjee 2005).

High concentration ROS are immensely harmful for plants and when the level of ROS exceeds the threshold level, a cell is said to be in a state of ‘oxidative stress’. Oxidative stress is essentially a regulated process and the destiny of plant cell is determined by its capacity to maintain equilibrium between the oxidative and antioxidative state. ROS affect many cellular functions by damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation (Foyer and Noctor 2005). Changes in the concentration of ROS determine its function, like at low concentra-

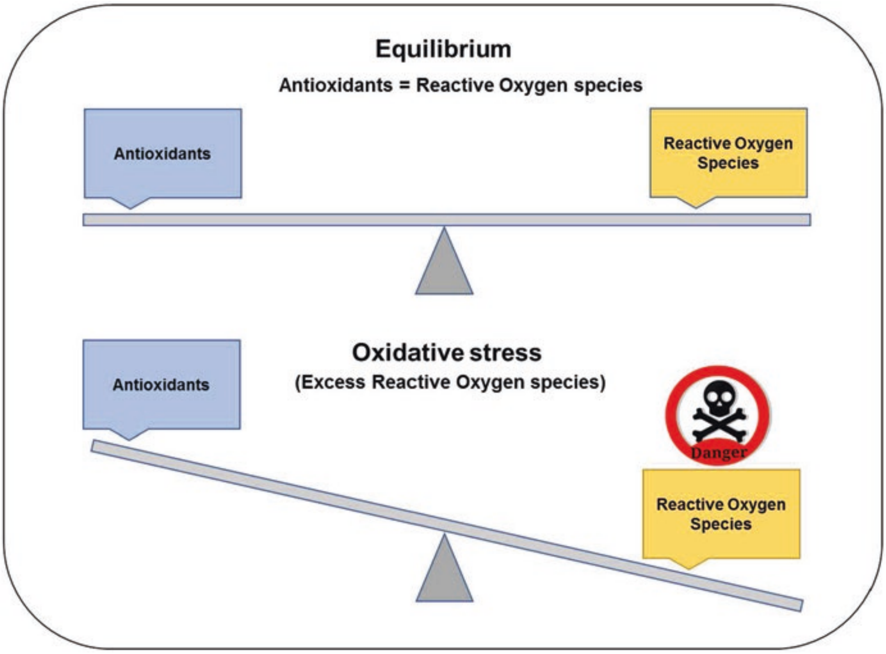

Fig. 3.1 Antioxidants and ROS behaviour under equilibrium and oxidative stress

tion it acts as a signalling factor that trigger the defense machinery of the cell while at high concentration it becomes detrimental for the cell (Gratao et al. 2005).

3.4 Types of ROS

ROS has been well known for playing a dual role as both harmful and beneficial species which is categorised by its intracellular concentration. The most common ROS include singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl ions ($OH^{\bullet}$). Although, atmospheric oxygen is relatively non-reactive in its ground state as it has two unpaired electrons with parallel spin which makes it paramagnetic and, therefore does not participate in reactions with organic molecules until gets activated (Fig. 3.2), but when the cell is exposed to excess excitation energy reversion of one of its unpaired electron takes place that contribute to the emergence of singlet state with two electrons in different spins. This reaction controls the spin orientation of electrons and thereby $^1O_2$ undergoes divalent reduction with concurrent shifting of two electrons. In the process of ROS formation several intermediates are formed as shown in Fig. 3.3.

Under heavy metal stress, highly reactive singlet oxygen ($^1O_2$) can be produced via reaction between oxygen and the chlorophyll triplet state. The chlorophyll trip-

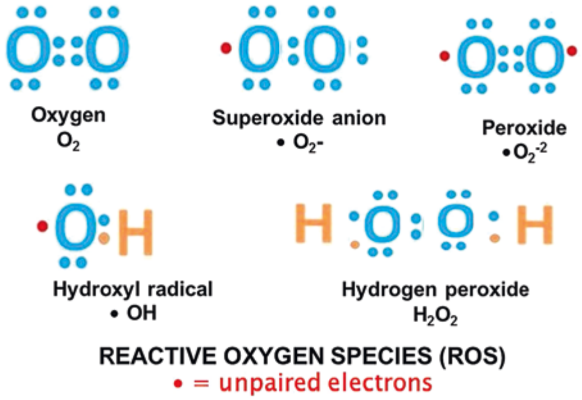

Fig. 3.2 Different types of ROS showing number of unpaired electrons

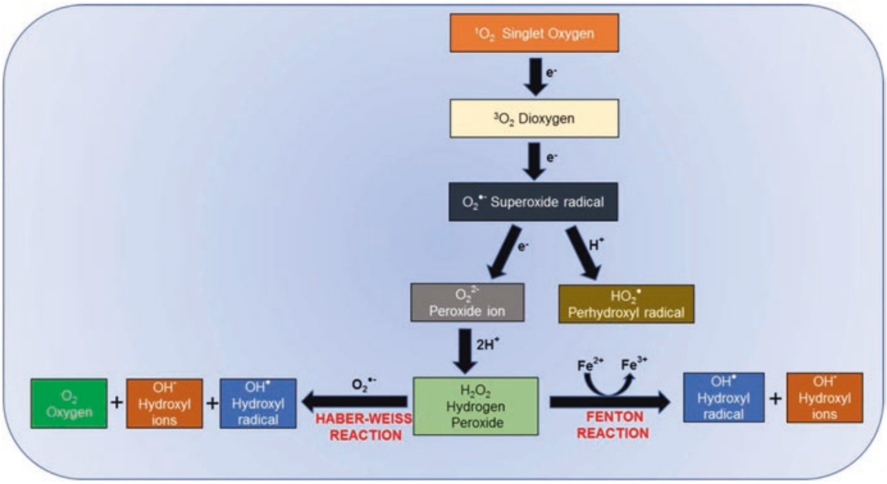

Fig. 3.3 Schematic representation of generation of different reactive oxygen species by energy transfer in plants

let state can react with $^3O_2$ to give up the highly reactive $^1O_2$ (Krieger 2005). This formation of photosynthetic singlet oxygen may damage the photosynthetic machinery of plants i.e., PSI and PSII (Skovsen et al. 2005). $^1O_2$, an oxidizing agent can damage the molecules like 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 cell death (Scandalios 2005). $^1O_2$ can be quenched by β-carotene, α-tocopherol or can react with the D1 protein of photosystem II as a target. Therefore, Singlet oxygen plays an important role in providing protection against photooxidative stress (Halliwell 2006).

The formation of superoxide radical ($O_2^{\bullet -}$) in chloroplast is mainly formed in the thylakoid localized PSI during non-cyclic electron transport chain (ETC), as well as other cellular compartments. It is generally considered to be the first ROS generated under stressful environment. It causes membrane lipid peroxidation by transformation into $OH^{\bullet}$ and $^1O_2$ which are more reactive and toxic. The $O_2^{\bullet -}$ is synthesised through reduction of O_2 during electron transport of chloroplasts and other compartments of the plant cell. $O_2^{\bullet -}$ is a nucleophilic reactant with both oxidizing and reducing properties that can be generated by electrons flow in PS I which accelerate the production of other highly harmful radicals like $OH^{\bullet}$, and more possibly $^1O_2$ (Halliwell 2006).

During photooxidation reactions, NADPH oxidase as well as xanthine oxidase (XOD) also contributes to H_2O_2 production in plants. H_2O_2 is moderately reactive and is relatively long-lived molecule with a half-life of 1 ms (Allen et al. 1997). H_2O_2 has no unpaired electrons, unlike other oxygen radicals; it can cross membrane via aquaporins and consequently can cause oxidative damage far from the site of its formation. It may inactivate enzyme by oxidizing their thiol group, such as Calvin cycle, Cu/Zn-SOD, and Fe-SOD.

Hydroxyl radical is one of the most dangerous radical as it has a single unpaired electron, thus it can react with oxygen in triplet ground state. Hydroxyl radical has the capability to damage different cellular components such as lipid peroxidation (LPO), protein damage and protein destruction (Foyer et al. 1997). Excess formation of $OH^{\cdot}$ can lead to cell death because the cells have no enzymatic mechanism to eliminate OH.

3.5 Sites of Production of ROS

ROS are continuously and predominantly produced at several locations in chloroplasts, mitochondria, peroxisomes, plasma membrane, endoplasmic reticulum and cell wall. But when ROS is formed by the inevitable leakage of electrons and a number of adverse abiotic stress factors such as light, drought, low temperature and heavy metal stress, it may cause significant damage to plants cell structures, whereas at low concentrations, it acts as secondary messenger for the plant.

3.5.1 Mitochondria

Mitochondria is known as "energy factory" and it is a major site for ROS production (Navrot et al. 2007). Plant mitochondria differ from animal counterparts by being involved in photorespiration. The cellular environment of plant mitochondria is also distinctive because of the presence of photosynthesis, which creates O_2 and carbohydrate rich environment (Noctor et al. 2006). When plants are exposed to heavy metal toxicity, distribution and transport of metal ions to apoplast followed by cytosolar uptake causes ROS generation due to their redox-active nature (Sharma and Dietz 2009). The Major components of mtETC responsible for ROS production is complex I and complex III. Mitochondrial matrix contains several enzymes which produce ROS but some of them produce ROS directly like aconitase and other like 1-galactono-ɣ-lactone dehydrogenase (GAL) which indirectly feeds electrons to ETC (Rasmusson et al. 2008). $O_2^{\bullet-}$ is the primary ROS formed by monovalent reduction in the ETC. In aqueous solution, $O_2^{\bullet-}$ is moderately reactive, but this $O_2^{\bullet-}$ is converted to H_2O_2 by the Mn-SOD and the APX (Sharma et al. 2012). Abiotic stresses like heavy metals plays a major role as they affect the plant cell bioenergetics and influence the tight coupling of ETC and ATP generation by reducing the electron carriers like ubiquinone thus ultimately generate oxidants (Blokhina and Fagerstedt 2010). Transition metals such as Cu, Fe and Zn are crucial for a proper functioning of several enzymes involved in the TCA cycle, electron transport, synthesis of ATP and antioxidative defense (Nouet et al. 2011; Tan et al. 2010). Findings of several studies focusing on plant metal stress responses point toward the emergence of oxidative stress and mitochondrial dysfunction as determinants in metal-induced cytotoxicity. In several plant species, metal stress enhances mitochondrial ROS generation mainly by affecting respiratory gas exchange rates (Losch 2004).

3.5.2 *Chloroplast*

Chloroplast is an important cell organelle which plays an important role as photosynthesis takes place here. It is a highly complex cell organelle which contains organised thylakoid membrane system that harbours all components of the light-capturing photosynthetic apparatus. PSI and PSII are the main sources of ROS in Chloroplasts (Fig. 3.4). ROS production by these sources is enhanced in plants by abiotic factors such as drought, salinity, temperature extremes as well as combination of these conditions with high light stress (Fannschmidt 2003). Normally, the electron flow from the excited centre to PS centre and ultimately NADP which is then reduced to NADPH and enters the Calvin cycle and reduce the final electron acceptor, CO_2. Due to decreased NADP supply resulting from stress conditions, there is a leakage of electron from ferredoxin to O_2, reducing it to $O_2^{\bullet-}$ (Elstner 1991).

$$2O_2 + 2Fd_{red} \rightarrow 2O_2^{\bullet-} + 2Fd_{ox}$$

PSI and PSII plays an important role in the production of ROS. The site of leakage of electrons from the ETC of PSI is 2Fe-2S and 4Fe-4S clusters. PSII acceptor side of ETC contains QA and QB which is responsible for the generation of $O_2^{\bullet-}$ (Cleland and Grace 1999). PS II contains a reaction centre P_{680} from which the electrons get excited. PS II is responsible for the generation of $^1O_2$, which occurs in two ways, firstly, when abiotic stress upsets the delicate balance between light harvesting and energy utilisation which leads to the formation of triplet Chl ($^3Chl^*$) which on reacting with dioxygen ($3O_2$) liberates $^1O_2$(Karruppanapandian et al.

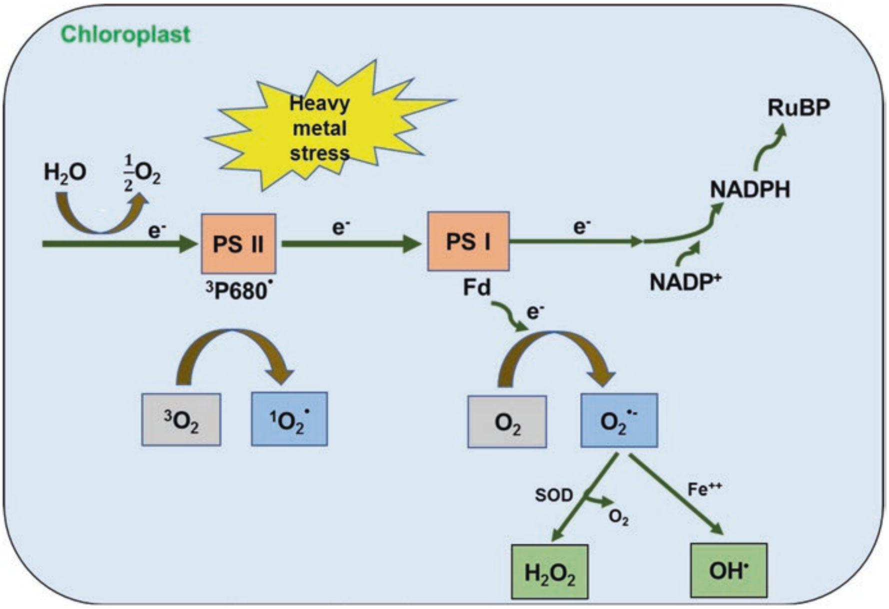

Fig. 3.4 Schematic representation of generation of ROS within chloroplast under heavy metal stress

2011). Secondly, when ETC is over reduced the light harvesting at PS II to generates $^1O_2$ (Asada 2006) and causes peroxidation of membrane lipids, damage membrane proteins and lead to cell death (Triantaphylids et al. 2008)

3.5.3 *Peroxisomes*

Peroxisomes are small, dynamic and spherical organelles bounded by a single lipid bilayer membrane and are the major site of intracellular H_2O_2 production due to integral oxidative metabolism (Palma et al. 2009). The major function of peroxisomes is breakdown of very long chain fatty acids through beta-oxidation. There are two sites for the production of superoxide radical, first site is in the matrix of peroxisomes where xanthine oxidase (XOD) catalyses the oxidation of xanthine and hypoxanthine to uric acid which generates $O_2^{•-}$ and the second one is in the peroxisome membrane dependent on NADPH where a small ETC is composed of NADH and Cyt b and contribute to $O_2^{•-}$ production. Peroxisomes also play an important role in photomorphogenesis degradation of branched amino acids, biosynthesis of plant hormones like auxin and production of glycine betaine (Corpas et al. 2001). Peroxisomes also maintain a redox balance to counteract the oxidative stress. In peroxisomes, three integral peroxisomal membrane polypeptides (PMPs) having different molecular masses of 18, 29 and 32 kDa were involved in the superoxide production. The NADH acts as the electron donor of 18 and 32 kDa, whereas the

29 kDa acts as the electron donor of NADPH to reduce cytochrome c. Therefore, peroxisomes should be considered as important signalling molecules such as $O_2^{\bullet-}$, H_2O_2 and $NO^{\cdot}$.

3.6 Heavy Metal Induced Antioxidative Defense System in Plants

Under normal physiological conditions, harmful oxidizing metabolites are produced at an inexpensive level and an appropriate equilibrium exist between the generation and scavenging of ROS that maintain the redox potential of the cell. Exposure of plants to unfavourable environmental conditions such as temperature extremes, heavy metals, drought, water availability, air pollutants, nutrient deficiency, salinity stress perturbed this balance between the production and quenching of ROS, giving rise to rapid increases in intracellular ROS levels (Noctor et al. 2002; Sharma et al. 2010) which can induce oxidative damage to lipids, proteins, and nucleic acids (Fig. 3.5).

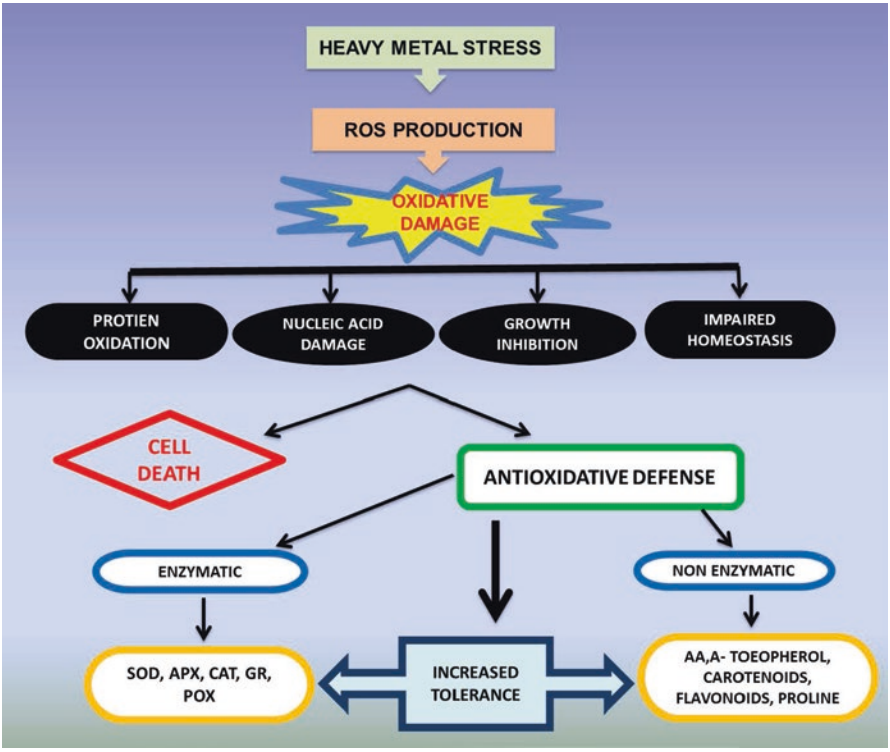

Fig. 3.5 Overview of antioxidant defense mechanism of plants under heavy metal stress

Table 3.1 Enzymatic antioxidants, their function and cellular location

Enzymatic antioxidants	Subcellular location	Enzyme code	Reaction catalysed
Superoxide dismutase (SOD)	Peroxisomes, mitochondria, cytosol, and chloroplast	1.15.1.1	$O^{\bullet-} + 2O_2^{\bullet-} + 2H^+ \rightarrow 2H_2O_2 + O_2$
Catalase (CAT)	Peroxisome and mitochondria	1.11.1.6	$2H_2O_2 \rightarrow O_2 + 2H_2O$
Ascorbate peroxidase (APX)	Peroxisomes, mitochondria, cytosol, and chloroplast	1.11.1.11	$H_2O_2 + AA \rightarrow 2H_2O + DHA$
Glutathione reductase (GR)	Mitochondria, cytoplasm, and chloroplast	1.6.4.2	GSSG + NADPH → 2GSH + NADP+
Guaiacol peroxidase (GPX)	Mitochondria, cytoplasm, chloroplast, and ER	1.11.1.7	$H_2O_2 + DHA \rightarrow 2H_2O + GSSG$

The available form of heavy metals for plants uptake are found as soluble components in the soil solution or those solubilised by root exudates (Blaylock and Huang 2000). Plants require certain heavy metals for their growth and upkeep, but excessive amounts of these metals can become toxic to plants and at the same time the ability of plants to accumulate essential metals equally enables them to acquire other nonessential metals (Djingova and Kuleff 2000). As metals are non-biodegradable, they adversely affect the plant both directly and indirectly. When heavy metal concentrations within the plant exceed the optimal level it start showing some direct toxic effects like inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress (Jadia and Fulekar 1999). To protect the cellular components from oxidative damage by heavy metal contamination, plants have developed many strategies. Among them antioxidative defense mechanism is the most effective fortification to shield the plant cell (Fig. 3.5). Plants possess very efficient scavenging systems for ROS that protect them from destructive oxidative reactions. These defenses are not restricted to the intracellular compartment, but are also found in the apoplast to a limited extent. Antioxidants are molecules that protect the biomolecules of the cell from being oxidized by free radical reactions thus reduce or obstruct the process of cellular damage. Though protective measures of plants are different from species to species, the presence of antioxidative defense is universal for all that includes both enzymatic and non-enzymatic forms (Tables 3.1 and 3.2).

3.6.1 Enzymatic Antioxidants

3.6.1.1 Superoxide Dismutase

Metalloenzyme superoxide dismutase (SOD) is the most effective intracellular enzymatic antioxidant that plays central role in defense against oxidative damage is found in all aerobic organisms and is susceptible to ROS mediated oxidative damage. The enzyme SOD belongs to the group of metalloenzymes and catalyzes the dismutation of $O_2^{\bullet-}$ to O_2 and H_2O_2.

Table 3.2 Non-enzymatic antioxidants, cellular location and their functions

Non-enzymatic antioxidants	Sub-cellular location	Function
Ascorbic acid	Peroxisomes, mitochondria, cytosol, chloroplast, vacuole, and apoplast	Detoxifies H_2O_2 via action of APX
Reduced glutathione	Cytosol, chloroplast, mitochondria, peroxisome, vacuole, and apoplast	Acts as a detoxifying co-substrate for enzymes like peroxidases, GR and glutathione-S- transferase
α -Tocopherol	Mostly in membranes	Protects against and detoxifies products of membrane lipid peroxidation
Carotenoids	Chloroplasts and other non-green plastids	Quenches excess energy from the photosystems, LHCs
Flavonoids	Vacuole	Direct scavengers of H_2O_2 and $^1O_2$ and $OH^•$
Proline	Mitochondria, cytosol, and chloroplast	Efficient scavenger of $OH^•$ and $^1O_2$ and protect cell from lipid peroxidation

Three isozymes of SOD (Fig. 3.6) copper/zinc SOD (Cu/Zn-SOD), manganese SOD (Mn-SOD), and iron SOD (Fe-SOD) are reported in plants (Racchi et al. 2001). All forms of SOD are nuclear encoded with an amino terminal targeting sequence that positioned them to their respective subcellular compartments (Bowler et al. 1992). Mn-SOD is localized in mitochondria, whereas Fe- SOD is localized in chloroplasts (Jackson et al. 1978). Cu/Zn-SOD is present in three isoforms, which are found in the cytosol, chloroplast, and peroxisome and mitochondria (del Río et al. 2006). Eukaryotic Cu/Zn-SOD is a dimer and cyanide sensitive whereas the other two (Mn-SOD and Fe-SOD) are cyanide insensitive and may be found as dimer or tetramers forms (del Río et al. 2006). SOD activity has been reported to increase in plants exposed to various environmental stresses, including drought and metal toxicity (Table 3.3). Increased activity of SOD is often associated with increased tolerance of the plant against heavy metal stress. Overproduction of SOD has been reported to result in enhanced oxidative stress tolerance in plants (Gupta et al. 1993). KCN and H_2O_2 are used for the identification and sensitivity of SOD isozymes while their activity can be detected by negative staining. 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.

3.6.1.2 Catalase

Under heavy metal stress catalase (CAT), a tetrameric heme containing enzyme is an indispensable detoxifier of ROS as it possess the potential to directly dismutase H_2O_2 into H_2O and O_2. It has high affinity for H_2O_2, but lesser specificity for organic peroxides (R-O-O-R). It has a very high turnover rate (one molecule of CAT can convert ≈6 million molecules of H_2O_2 to H_2O and O_2 per minute) and it does not require any reducing equivalent like other antioxidant enzymes. CAT plays an

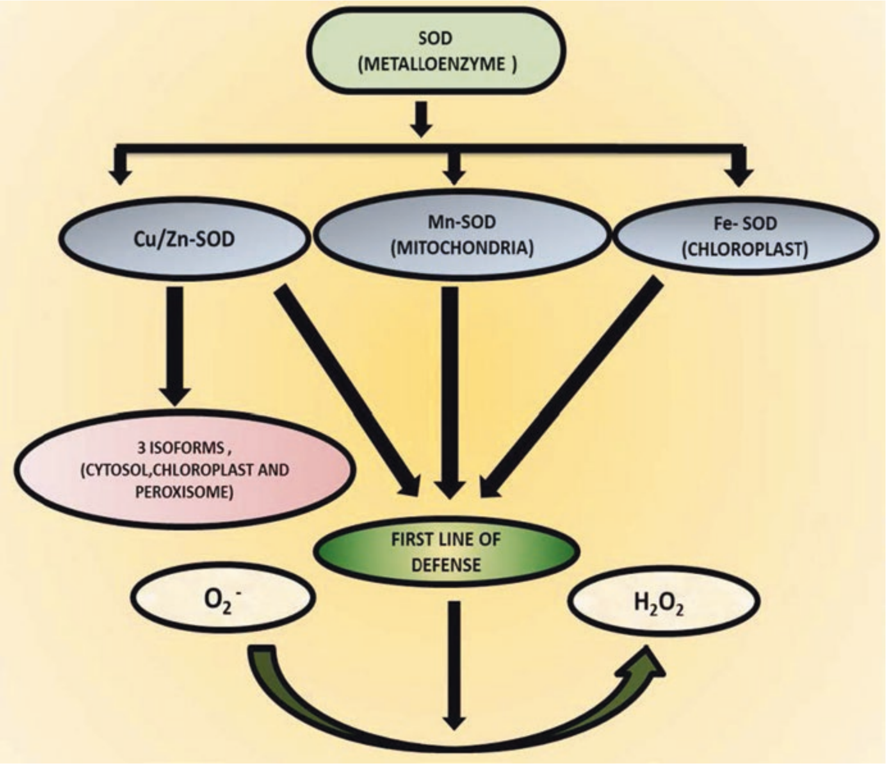

Fig. 3.6 Isozymes of SOD with their sub-cellular location and reaction catalysed

important role in the removal of H_2O_2 produced by oxidases in peroxisome due to β-oxidation of fatty acids, photorespiration and purine catabolism (Garg and Manchanda 2009). Catalase has been shown to depict variable responses under heavy metal stress (Table 3.4).

3.6.1.3 Ascorbate Peroxidase

Ascorbate Peroxidase (APX) is a fundamental constituent of the Ascorbate-Glutathione (ASC-GSH) cycle, and is thought to play an extremely important role in scavenging ROS and protecting cells in higher plants, algae, euglena and other organisms. APX efficiently scavenges H_2O_2 in the cytosol and chloroplast exactly in the manner as CAT detoxifies it in peroxisome. The APX reduces H_2O_2 to H_2O and DHA (dehydroascorbate), using Ascorbic acid (AA) as a reducing agent (Fig. 3.7).

$$H_2O_2 + AA \rightarrow 2H_2O + DHA$$

Table 3.3 Impact of heavy metals on SOD activity

S.N.	Heavy metal	Plant species	Effect on SOD activity	References
1.	Cd	*Triticum aestivum*	Increased	Khan et al. (2007)
2.	As	*Pteris cretica*	Increased	Xie et al. (2009)
3.	As	*Pteris ensiformis*	Increased	Xie et al. (2009)
4.	Cd, Cu, Hg	*Oryza spp.*	Inhibited	Cai-lin et al. (2003)
5.	Pb, Cd	*Solanum nigrum, Brassica juncea*	Increased	Yang et al. (2010)
6.	Pb, Cd	*Zea mays, Raphanus sativus*	Unchanged	Yang et al. (2010)
7.	Cd	*Hordeum vulgare*	Increased	Guo et al. (2004)
8.	Cd	*Arabidopsis thaliana*	Increased	Skorzynska et al. (2004)
9.	Cd	*Oryza sativa*	Increased	Hsu and Kao (2004)
10.	Cr	*Leersia hexandra*	Decreased	Yan et al. (2008)
11.	Cd	*Brassica juncea*	Increased	Mobin and Khan (2007)
12.	Cd	*Vigna mungo*	Increased	Singh et al. (2008)
13.	Cd	*Cicer arietinum*	Increased	Hasan et al. (2008)
14.	Cu	*Brassica campestris*	Increased	Li et al. (2009)

Table 3.4 Changes in catalase activity in response to heavy metals

S.N.	Heavy metal	Plant species	Effect on CAT activity	References
1.	Cd	*Oryza sativa*	Increased	Hsu and Kao (2004)
2.	Cd	*Arabidopsis thaliana*	Declined	Cho and Seo (2005)
3.	Cu	*Anabaena doliolum*	Decreased	Srivastava et al. (2005)
4.	Cd	*Cicer arietinum*	Increased	Hasan et al. (2008)
5.	Pb	*Pteris vittata*	First increased then declined	Zhu and Xia (2012)
6.	Cd	*Brassica juncea, Vigna mungo*	Increased	Mobin and Khan (2007) and Singh et al. (2008)
7.	Mn	*Polygonum pubescens*	First increased then declined	Yan et al. (2011)
8.	Cd	*Glycine max*	Decreased	Balestrasse et al. (2001)
9.	Hg, Cu, Cd	*Oryza sativa*	Fluctuation	Cai-lin et al. (2003)
10.	Cd	*Phragmites australis, Capsicum annuum*	Decreased	Pietrini et al. (2003) and Leon et al. (2002)
11.	Cd	*Triticum aestivum*	Increased	Khan et al. (2007)

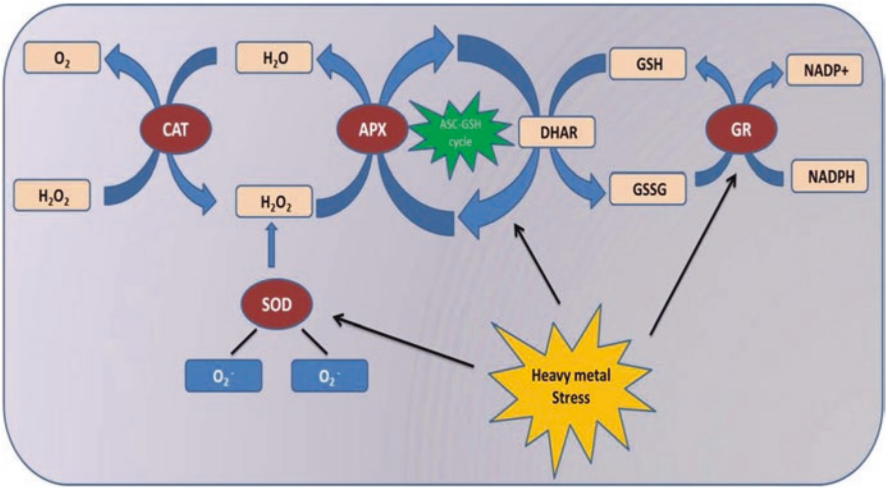

Fig. 3.7 Enzymatic antioxidant regulation for heavy metal-induced oxidative stress tolerance

Based on locations, viz., cytosolic, mitochondrial, peroxisomal, and chloroplastid (stromal and thylakoidal) and types of amino acid, the APX family is made up of five isomers (Sharma and Dubey 2004). APX has a higher affinity for H_2O_2 than CAT and POD (peroxidase) and it may have a more crucial role in the management of ROS during stress. Aravind and Prasad (2003) demonstrated increased leaf APX activity under Cd stress in *Ceratophyllum demersum*. Enhanced activity of APX was also reported by a number of researchers in *Brassica juncea, Triticum aestivum* and *Vigna mungo* (Mobin and Khan 2007; Khan et al. 2007; Singh et al. 2008). Hsu and Kao (2004) reported that under non-heat shock conditions pretreatment of *Oryza sativa* seedlings with H_2O_2 resulted in an increase in APX activity and protect rice seedlings from subsequent Cd stress. Pekker et al. (2002) studied the expression of APX under iron overload in leaves of de-rooted bean plants and found a rapidly induced expression of APX (mRNA and protein) in response to iron surcharge.

3.6.1.4 Glutathione Reductase

Glutathione reductase (GR) is a flavoprotein oxidoreductase which reduces GSSG (oxidized form of glutathione) to GSH (reduced form of glutathione) by using NADPH as a reductant. Reduced glutathione (GSH) is used up to regenerate AA from Monodehydroascorbate (MDHA) and dehydroascorbate (DHA), and as a result is converted to its oxidized form (GSSG) (Fig. 3.8). GR, a crucial enzyme of ASC-GSH cycle, maintain a high cellular GSH/GSSG ratio by catalyzing the formation of a disulfide bond in glutathione disulfide. It is primarily found in

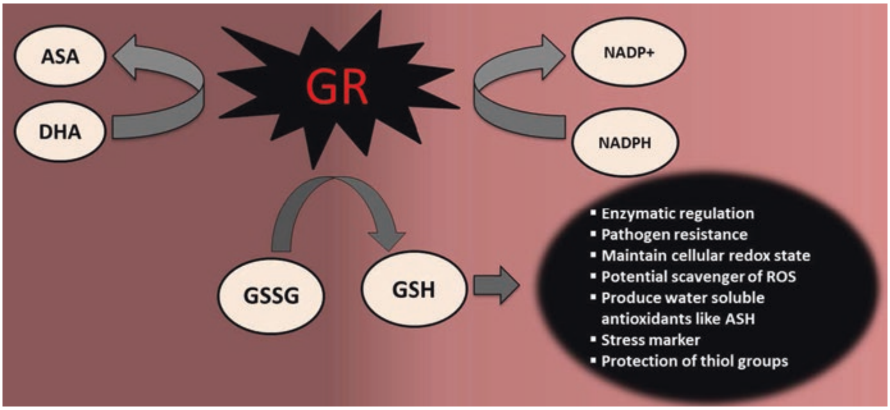

Fig. 3.8 Cellular redox maintained by GR with its primary functions to protect the cells against heavy metal stress

chloroplasts with low amounts occurring in the mitochondria and cytosol. GSH is a low molecular weight compound which reacts with the detrimental ROS members and act as a reductant to prevent thiol groups from being oxidized. GR activity found to be increased in the presence of Cd in *Triticum aestivum, Capsicum annuum*, *Arabidopsis thaliana*, *Vigna mungo, Brassica juncea* (Skorzynska et al. 2004; Khan et al. 2007; Mobin and Khan 2007; Singh et al. 2008).

3.6.1.5 Guaiacol Peroxidase

Guaiacol peroxidase (GPOX) is a heme containing enzyme composed of 40–50 kDa monomers; that eliminates excess H_2O_2 both during normal metabolism as well as during stress. APX can be distinguished from plant-isolated guaiacol peroxidase (GPOX) in terms of differences in sequences and physiological functions. GPOX degrade indole-3-acetic acid (IAA) and plays a vital role in the biosynthesis of lignin as well as defense against biotic stress by consuming H_2O_2. GPOX prefers aromatic electron donors such as guaiacol and pyragallol (Asada 1999). The activity of GPOX varies considerably depending upon plant species and conditions of stress. In most of the studies, GPOX activity was found to be enhanced in response to heavy metal stress (Arvind and Prasad 2003; Cho and Seo 2005). While Radotic et al. (2000) noted an initial increase in GPOX activity in spruce needles subjected to Cd stress and subsequent Cd-treatments caused a decline in the activity.

3.6.2 Non-enzymatic Antioxidants

The other half of the antioxidant machinery is comprises of non-enzymatic antioxidants like ascorbic acid, glutathione, α-tocopherol, carotenoids, phenolics, flavonoids, and amino acid cum osmolyte proline. They also play essential role in plant growth and development by influencing cellular processes like mitosis, cell elongation, senescence and cell death.

3.6.2.1 Glutathione

Tripeptide glutathione (glu-cys-gly; GSH) a critical metabolite of plants is considered to be the most important non-enzymatic intracellular defense against ROS induced oxidative damage. It is localized in all cell compartments like cytosol, chloroplast, endoplasmic reticulum, vacuole, peroxisomes, mitochondria, as well as in apoplast and plays a crucial role in numerous physiological processes, including detoxification of xenobiotics, regulation of sulphate transport, expression of stress-responsive genes, signal transduction and conjugation of metabolites. It occurs abundantly in reduced form (GSH) in plant tissues and maintains the normal reducing environment of cells so as to restrain the inhibitory effects of ROS induced oxidative stress (Harinasut et al. 2003).

Enhanced antioxidant activity in the leaves and chloroplast of *Phragmites australis* was studied by Pietrini et al. (2003). He found its association with a large pool of GSH which resulted in protecting the activity of many photosynthetic enzymes under Cd stress. Increased concentration of GSH has also been observed with the increasing Cd concentration in *Pisum sativum* by Metwally et al. (2005), *Sedum alfredii* by Sun et al. (2007) and *Vigna mungo* by Molina et al. (2008). While Srivastava et al. (2005) reported a significant decline in GR activity and GSH pool under Cu stress.

3.6.2.2 Ascorbic Acid

Ascorbic acid (AA) is the most broadly studied and abundantly found antioxidant compound which is considered to be the most substantial component of a wide range of enzymatic and non-enzymatic reactions as it donates its free electron to them. A minor concentration of AA is being generated by D-galacturonic acid while the remaining considerable amount is produced by Smirnoff-Wheeler pathway in mitochondria, catalyzed by L-galactano-γ-lactone dehydrogenase. AA is the first line of non-enzymatic defense against ROS as 90% of its concentration is found in cytosol and apoplast (Barnes et al. 2002). Demirevska-Kepova et al. (2006) reported that the content of oxidized ascorbate increased during Cd exposure in *Hordeum vulgare* plants. Contrarily, a decrease in the ASH in the roots and nodules of *Glycine max* under Cd stress has also been observed (Balestrasse et al. 2001). Cd also decreases the ASH content in *Cucumis sativus* chloroplast and in the leaves of *Arabidopsis thaliana* and *Pisum sativum* (Skorzynska et al. 2004; Romero et al. 2007) whereas, it remained unaffected in *Populus Canescens* roots (Romero et al. 2007).

3.6.2.3 α -Tocopherol

Vitamin E is an important natural antioxidant of plant leaves, found in the chloroplast envelope, thylakoid membranes and plastoglobuli. Its most common and biologically active form is α-tocopherol. Due to the presence of three methyl groups in its molecular structure, α-tocopherol has the highest antioxidative activity among the four isomers of tocopherols (α, β, γ, δ) found in plants (Kamal and Appelqvist 1996). It is a potent scavenger of photosynthesis-derived reactive oxygen species (mainly $^1O_2$ and $OH^\cdot$), which check the propagation of lipid peroxidation by deactivating lipid peroxyl radicals in thylakoid membranes. Level of α-tocopherol changes differentially in response to environmental limitations (drought, heavy metals, salinity), intensity of stress and sensitivity to stress. It has been observed that increased production of α-tocopherol results from altered expression, degradation and regeneration of genes that regulate different biochemical pathways, contribute to plant stress tolerance, while decreased levels favor enhanced production of ROS and oxidative damage. Srivastava et al. (2005) reported a general induction in α-tocopherol content in *Anabaena doliolum* under NaCl and Cu stress. Shuangxia and Daniell (2014), observed that under heavy metal stress, α-tocopherol content in the TMT leaves increased up to 7.5-fold, twice higher than in the wild type.

3.6.2.4 Carotenoids, Flavonoids, Proline and Phenolic Compounds

Carotenoids are the members of lipophilic antioxidant family which are found in the plastids of photosynthetic and non-photosynthetic plant tissues and in microorganisms. Carotenoids unveil their antioxidative activity by protecting the photosynthetic machinery in different ways;

(i) Reacts with lipid peroxidation products to end the chain reactions,
(ii) Prevent the formation of $^1O_2$ as it reacts with $^3Chl^*$ and excited chlorophyll (Chl^*),
(iii) Scavenge $^1O_2$ and generate heat as a by-product, and
(iv) Dissolve the excess excitation energy, via the xanthophyll cycle.

When excess excitation energy damage the photosynthetic apparatus of plants, flavonoids functions as a secondary ROS scavenging system (Fini et al. 2011). They also have a role in scavenging $^1O_2$ and alleviate the damages caused to the outer envelope of the chloroplast membrane (Agati et al. 2012). Proline, an osmolyte is also regarded as a non-enzymatic antioxidant to prevent the damaging effects of different ROS members. Proline is synthesized using glutamic acid as a substrate, via a pyrroline 5-carboxylate (P5C) intermediate. It is well documented that under heavy metal stress there is a dramatic accumulation of proline in plants. It has been known to act as an osmo-protectant, a metal chelator, a protein stabilizer, an inhibitor of lipid peroxidation, and $OH^{\bullet}$ and $^{1}O_2$ scavenger (Trovato et al. 2008).

Phenolics are secondary metabolites (flavonoids, tannins, hydroxycinnamate esters, and lignin) which possess antioxidant properties. Janas et al. (2009) observed that ROS could serve as a common signal for accumulation to Cu^{2+} stress and cause accumulation of total phenolic compounds in dark grown lentil roots. Dursun et al. (2016) reported that the application of Cd, Cu, and Pb increased the total phenolics in all treatments of *Zea mays*.

3.7 Conclusion

It is well documented that almost all plants exhibit a fundamental metal tolerance when exposed to heavy metals. Some species are even capable of hyperaccumulation of heavy metals by employing different tolerance mechanisms as compared to non-accumulating ones. Overall, under normal physiological metabolism, production and scavenging of reactive oxygen species is in dynamic equilibrium state and the involvement of ROS in various metabolic processes might have general implications but when plants are exposed to heavy metal stress they experience overproduction of ROS which adversely affect the plant and ultimately results in oxidative damage. Oxidative stress is a condition in which intra and extracellular compartments of the cells produce considerable amount of ROS or free radicals, which damage the integrity of cells by its toxic effects like membrane lipid peroxidation, protein cross linking, loss of enzyme activity and ion transport, destruction of nucleic acids, lipids and proteins that ultimately leads to its death. However, the cells are equipped with magnificent antioxidative defense system for the fortification of harmful effects of ROS. This chapter gives an insight into how both arms of the antioxidant machinery; either being enzymatic or non-enzymatic, work in synchronicity to diminish the damaging effects of ROS and develop tolerance against heavy metal stress conditions. Although remarkable progress has been achieved in recent years, there are still equivocation and gaps in our understanding of how heavy metals induced the antioxidative defense system of plants. Explanation of the mechanism of plant resistance to heavy metals, and further exploration of key factors controlling heavy metal damage and resistance are the focus of future research, in order to provide important basis for the plant and heavy metal relationship.

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