Assessment of Antioxidant Potential of Plants in Response to Heavy Metals

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Abstract

Heavy metals (HMs) are consequential environmental contaminant, and their prodigious bioaccumulation in the surroundings has become an enigma for all living organisms including plants. Heavy metal has the potential to react with various indispensable cellular components like DNA, protein, and enzymes and in turn induce several stress responses in plants like oxidative stress which is the root cause for the progression of cell death in the plant. Stress responses inflicted by oxidative stress include severe morphological, metabolic, and physiological amendments in plants like DNA strand breakage, defragmentation of proteins, and damage of pho-

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tosynthetic pigment, which may stimulate cell death. In reaction, plants have a range of mechanisms to minimize the heavy metal toxicity. Plants are endowed with antioxidant defense mechanism, which can be divided into two groups such as enzymatic antioxidants and nonenzymatic antioxidants, for instance, SOD, CAT, APX, GPX, GR and AsA, GSH, carotenoids, alkaloids, tocopherols, proline, and phenolic compounds, respectively, that together act as the scavengers for free radicals to mitigate the damaging impacts of heavy metal agglomeration in the cells. These antioxidant potentials could be assessed by different in vivo and in vitro methods such as hydrogen atom transfer and electron transfer through which we can evaluate the ROS detrimental action of antioxidant enzymes. Therefore, the present chapter attempts to provide the contemporary knowledge regarding the metal-influenced antioxidant status in plants and also provides the precise pathway that should follow for the future research in the area of antioxidant potentials.

Keywords
Antioxidant • Oxidative stress • Heavy metal • Detoxification

5.1 Introduction

Being restricted in distribution, plants are inevitably exposed to several environmental factors (abiotic and biotic), which constitute their macro- and microenvironment. Any digression in these factors from the optimum level is harmful and eventually leads to stress in plants (Kumar et al. 2008; Parvaiz and Satyawati 2008; Sharma et al. 2016). Momentous abiotic factors such as heavy metals (HMs) are imperative environmental pollutants, and their toxicity is a problem for environmental grounds (Nagajyoti et al. 2010). Industry and mining have escort to a relocation of heavy metals, which further resulted in a soil and water pollution. Heavy metals that occur in nature are mainly in two forms: essential and nonessential. Crucial HMs, like copper, iron, zinc, or nickel, are micronutrients, causing toxicity when present at higher concentrations, while nonessential heavy metals, like lead, cadmium, and mercury, are not recognized to have any physiological functions (Nowicka et al. 2016). Increased amount of metals in available soil fractions led an increased bioaccumulation in various parts of the plants (Kabata-Pendias 2004), which potentially induces several functional disorders at multiple level in plants, possibly from the oxidative action of metals (Sun et al. 2007; Shamsi et al. 2008; Kafel et al. 2010). Plants are often susceptible both to the shortage and to the glut accessibility of some HM ions as the increased accumulation of several vital HMs induced plausible changes in the plant (Nagajyoti et al. 2010). Zn, Cu, and Pb are acknowledged as prooxidants, and responsible for the production of the ROS at the higher concentration (Ferrat et al. 2003; Fatima and Ahmad 2005; Drążkiewicz et al. 2004; Caregnato et al. 2008). However, as a consequence of higher net production of reactive oxygen species, there occurs a photooxidative disintegration of DNA, proteins, and lipids that eventually causes cell fatality in plants (Tripathy and Oelmüller 2012). In view of the fact that the stimulation of oxidative stress is a significant process of HM lethality (Nagajyoti et al. 2010; Yadav 2010) likewise, the ability to detoxify

ROS is also a significant factor for excessive concentration of metal tolerance. That is why to ensure continued existence, plants have developed proficient antioxidant mechanism that possesses two arms: (i) enzymatic components such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR) and (ii) nonenzymatic antioxidants such as ascorbic acid (AA), reduced glutathione, α-tocopherol, carotenoids, flavonoids, and the osmolyte proline (Das and Roychoudhury 2014). Chromanols and prenylquinones (isoprenoid antioxidants) are thought to involve in response to heavy metal-stimulated stress (Nowicka et al. 2016). This antioxidant system marks the essentiality of ROS detoxification for the cellular existence (Gill et al. 2011; Das and Roychoudhury 2014). Plants are the source of dietary antioxidants; approximately all plants possess antioxidant prospects in retort to generated stress (Krishnaiah et al. 2011; Kasote et al. 2015). The secondary metabolites also participate significantly in therapeutic properties of plants (Abeysinghe et al. 2014). Gill and Tuteja (2010) in their article propounded that the antioxidant resistance mechanism works in recital to manage the rush of uncontrolled oxidation and defend plant cells from oxidative damage through the escaping of free radical. Thus, the efficacy of its antioxidant defenses is very decisive for a plant's resistance to metals (Kafel et al. 2010).

5.2 Occurrence, Accumulation, and Transport of Heavy Metals (HMs)

HMs are characterized as metals with the atomic mass over 20 and the density higher than 5 $g \cdot cm^{-3}$ (Emamverdian et al. 2015). Heavy metals are regarded as trace elements because of their trace concentration (less than 10 ppm) in the plant (Kabata and Pendias 2001; Tchounwou et al. 2012). Most of the HMs are positively charged, nondegradable, and persistent in the environment (Eshagberi 2012). Naturally HMs are present abundantly into the outermost layer of the earth (Tchounwou et al. 2012). High degree of HM pollution can be observed in the surroundings (Hajar et al. 2014) and these heavy metals cause toxicity even at very low concentration (Lenntech Water Treatment and Air Purification 2014; Nagajyoti et al. 2010). Different anthropogenic activities such as industrial, agricultural, domestic medical, and technological uses have led to their extensive allocation in the environment (Tchounwou et al. 2012). HMs include lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium (Cr), iron (Fe), arsenic (As), silver (Ag), and the platinum group elements (Nagajyoti et al. 2010), among which Cd, Cr, Cu, Hg, Pb, and Zn are the major toxic elements present in the environment (Lasat 2000; Tangahu et al. 2011). These contaminations occur through the weathering of rock, volcanic eruptions, and many anthropogenic activities (He et al. 2005). Anthropogenic sources of HMs are the differential industrial activities such as waste from metal processing refineries; contamination from the nuclear power stations; coal and petroleum combustion power plants; wood preservation; waste from the plastic, paper, and textile manufacturing plants; microelectronics; and high-tension electrical lines (Arruti et al. 2010; Tchounwou et al. 2012).

Many varieties of plants successfully absorb hazardous contaminants like Pb, Cd, Cr, As, and an assortment of radionuclides from soils, as they enter into the food web and show progressive bioaccumulation at successive trophic levels. The accessibility of metals is an active process in soil that depends on precise combinations of chemical, biological, and environmental constraints (Peijnenburg and Jager 2003; Hajar et al. 2014). Absorption, movement, and transportation of these metals within the plant tissue are largely reliant on plant species, type of concentrations, and also the oxidation state of HMs (Tangahu et al. 2011). The pH, reduction capability, and soil organic matters (SOM) influence the HMs to exist in ionic form for easy availability to the plant (Fritioff and Greger 2003). The plant performs as "hyperaccumulators" as well as "excluders." Accumulators continue to be present in spite of concerted pollutants in the shoots. The excluders confined pollutant uptake (Sinha et al. 2007). Basic HM tolerance is present in all plant species. Thus, they scamper a compound organization, including absorption, transportation, and chelation; these imperative metals are concerned firmly in homeostasis of essential metal micronutrients. The traits of these HM elements separate the plant kingdom into two categories: hyperaccumulating and non-accumulating plants (Viehweger 2014). "Hyperaccumulator" plants could thrive in toxic environments, require little maintenance, and produce high biomass, whereas non-accumulating plants (typically have a shoot-to-root ratio considerably less than one) can accumulate toxic ion at higher concentration approximately thousands ppm level (Salido et al. 2003; Singh et al. 2015). Hyperaccumulator plants can accommodate heavy metals 1000 times more than excluder plants (Tangahu et al. 2011). Different types of transport mechanism such as intrinsic protein, proton pumps, and co- and antitransporters implicated in ion uptake and transportation, after absorption transportation of these ions into shoots, are desirable (Fernández et al. 2015). Different types of heavy metal transporters such as IRT1, ZnT1, heavy metal ATPase-HMA2, and HMA4 are able to transport Zn, Cu, Cd, Pb, Ni, and Fe (Viehweger 2014). Contaminants are translocate from the root to shoot in the plant by two regulatory mechanism that is evaporation and transpiration (Tangahu et al. 2011).

5.3 Heavy Metal-Induced Oxidative Stress and Antioxidant Potential of Plant System

There are two sorts of metals that reside in the earth's crust that correspond as an imperative micronutrients for plant development such as iron, manganese, zinc, copper, magnesium, molybdenum, and nickel and nonessential elements such as cadmium, antimony, chromium, lead, arsenic, selenium, and mercury. Plants entail them in petite quantities for their growth, metabolism, and development, though the concentration of essential and inessential metals is a significant aspect in the plant development and growth, but their surplus concentration can restrict the plant growth (Zengin and Munzuroglu 2005; Emamverdian et al. 2015; Tripathi et al. 2016). All plant species, either sensitive or tolerant, could tolerate a minimal amount of metal stress. Heavy metals, irrespective of their redox-associated mode of action, are capable of disturbing antioxidant equilibrium in plant cells, inducing ROS, and

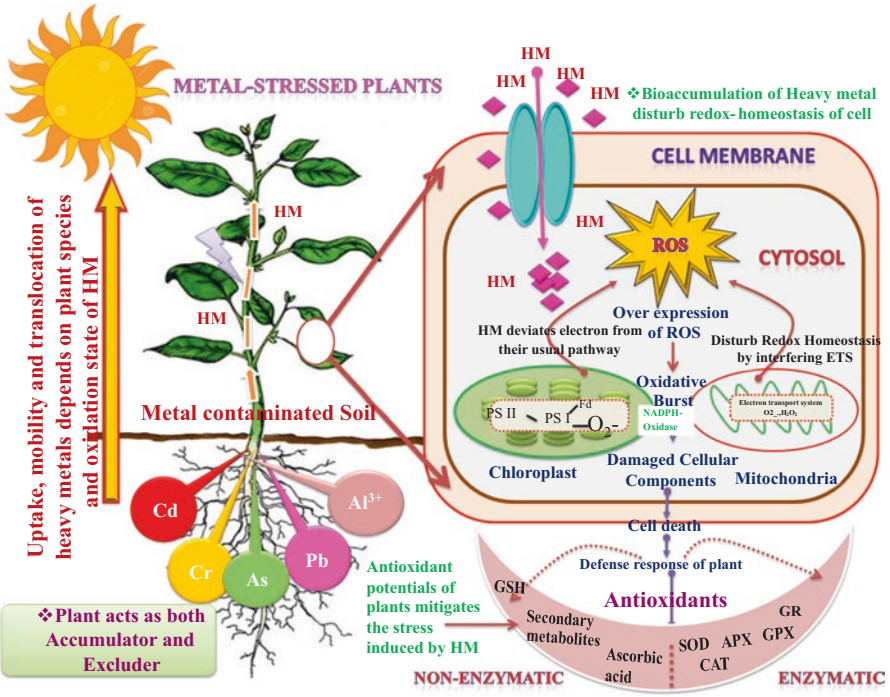

Fig. 5.1 Integrated response of plant toward metal-induced oxidative damage and activation of antioxidant potential of plant

directly reacting with functioning cellular macromolecules and organelles. Likewise, substitution of these crucial cations with the toxic HMs can disturb the equilibrium between cations and enzymatic cofactors (Tangahu et al. 2011). Some redox-active HMs like iron, copper, and chromium can exist in various oxidative states which could produce reactive oxygen species through the Fenton-type reactions and Haber–Weiss cycling, whereas non-redox metals like cadmium, lead, and mercury produce ROS indirectly, mostly by causing depletion of glutathione and through distracting the ETC (electron transport chain) (Pinto et al. 2003; Yadav 2010; Nowicka et al. 2016). However, non-oxido-reducing metals such as zinc and lead induced indirectly oxidative stress as a result of toxicity to metabolic pathways and membrane-coupled ETC (Verma and Dubey 2003; Caregnato et al. 2008). The generation of ROS is a usual process in HM stress treatment. Reactive oxygen species like $O_2^{-\bullet}$, H_2O_2, and $OH^{\bullet}$ are usually produced due to stress; further they bear strong oxidizing activities that can react with different biomolecules (Fig. 5.1).

Plants in contact with several HM ions move the poise of free radical metabolism toward an accommodation of hydrogen peroxide (Mithöfer et al. 2004). Elevated free radical concentrations exert an inhibitory impact on cell molecules like DNA, proteins, and lipids, for instance, nonenzymatic lipid peroxidation, consequently escort to the accommodation of oxidative burst in various cell sites (Schrader and Fahimi 2006). Hg^{2+} ions restrain the functions of antioxidative enzymes particularly

of glutathione reductase and also elevate an ephemeral depletion of GSH (Schützendübel and Polle 2002; Mithöfer et al. 2004). Weihong et al. (2009) through the experiment, studied the effect of HMs like Cd and Zn on plant *Vetiveria zizanioides* and illustrated that Cd and Zn was found involved in plant growth inhibition. The level of antioxidants became enhanced such as SOD, POD and CAT, MDA and proline whereas GSH content and water-soluble proteins decreased as the level of Zn and Cd increased to a toxic level. Plants occupy various innate and extrinsic defense policies for tolerance or detoxification whenever confronted with the stressful circumstance, which occurred through the higher concentrations of HMs (Viehweger 2014; Emamverdian et al. 2015). To study the oxidative stress and antioxidant response under Cu toxicity on nodules of *white lupin* and soybean plant Sánchez-Pardo (2012) did an investigation and revealed that Cu in excess concentration cause severe damages in ultrastructures due to emerged oxidative stress in the White lupin nodules, such damages were reported as the breakage of peribacteroidal membrane with rising numbers of vesicles in the cytosol. While in the nodules of soybean damage appeared in the form of degradation of bacteroidal membrane, and precipitation in vacuoles cells. Although white lupin was proved as more sensitive to Cu stress, the antioxidative effect (total thiol content and APX activity) was found less effective in white lupin than soybean.

5.4 Delineating the Complete Outline of Free Radical Production in Plants

The source of production of reactive oxygen species in plants is mainly the chloroplast, mitochondria, peroxisomes and over and above ER, cell membrane, cell wall, and the apoplast (Das and Roychoudhury 2014). ROS generated in these cell organelles due to stress induced signalling and enzymes like peroxidase, amine oxidase and NADPH oxidase present in cell walls and plasma membrane (Tripathy and Oelmüller 2012). Reactive oxygen species are very fatal which induce a broad injury to protein, DNA, and lipids and disturb the normal cellular pathways (Apel and Hirt 2004).

Furthermore, Gill and Tuteja (2010) have demonstrated that generally the production of ROS in plant tissue occurs mainly in photosystem I and photosystem II of the chloroplast and plasma membrane and also in complex I (ubiquinone) and complex III of the mitochondrial ETC. In a regular physiological activity of the plant, the electron moves from PSI and PSII of the chloroplasts, mitochondrial membrane, ETC, and peroxisome (Kasote et al. 2015). These negative ions (electron) react with molecular oxygen and form superoxide radical ($O_2^{-\bullet}$) (Fig. 5.1; Table 5.1). The superoxide radical is subsequently converted to hydroperoxyl radical ($HO_2^{\bullet}$) and finally to H_2O_2 (Zhao et al. 2005; Kasote et al. 2015). The ROS comprise of highly reactive free radicals (containing unpaired electrons) like $O_2^{-\bullet}$ (superoxide radical) and $OH^{\bullet}$ (hydroxyl radical), the most highly reactive and toxic form of oxygen, and non-radicals (has no unpaired electrons) like H_2O_2 (hydrogen peroxide) and $^1O_2$ (singlet oxygen) (Gill and Tuteja 2010). Environmental

Table 5.1 Effect of different heavy metals on the plant growth and elevating antioxidant potential of different plant spp.

Metals	Plant species	Concentration	Effect	Antioxidant enzymes	References
Co (cobalt)	Duckweed (*Lemna minor*)	1 mM	Impaired function of oxygen-evolving complex, decreases plant growth and biomass, chlorophyll content, starch accumulation, water potential, and transpiration rate	SOD and POD	Begović et al. (2016)
			Increase ROS production		
	Wilczek (*Vigna radiata*)	100–250 mg/kg	Decreased root and shoot length of plant	Catalase, peroxidase, and polyphenol oxidase	Jaleel et al. (2009)
			Inhibition of cell division and elongation		
			Dry weight of root and shoot declined		
	Soybean (*Glycine max*)	250 mg/kg	Decreased root nodule formation and leg hemoglobin content	CAT, SOD, POX, and APX	Jayakumar et al. (2008)
			Increase ROS production		
Pb (lead)	Bean (*Vicia faba*)	5 µm	Decreased nutrient uptake by plant	SOD, GPOX, APX, GR, and CAT	Shahid et al. (2014)
	Thale cress (*Arabidopsis thaliana*)	100 µm	Induces increase of H_2O_2 and lipid hydroperoxide	SOD, CAT, GR, GPX, and POD	Phang et al. (2011)
	Water hyacinths (*Eichhornia crassipes*)	1000 mg/L Pb	Higher accumulation in roots, petiole, and leaf tissue	SOD and CAT	Malar et al. (2014)
			Decreased chlorophyll content	APX and POX	
			MDA content increased in leaf and root tissues		
			Biomass reduction		
			Chlorosis and drying at edges in seedlings		

(continued)

Table 5.1 (continued)

Metals	Plant species	Concentration	Effect	Antioxidant enzymes	References
Cr (chromium)	Pea (*Pisum sativum*)	100 µm	Reduce photosynthetic process and nutrient uptake by plant	SOD and APX	Tripathi et al. (2015)
	Green gram (*Vigna radiata*)	50 µM	Increased lipid peroxidation and H_2O_2 generation	SOD and APX	Shanker et al. (2004)
			Decreased dry weight, root, and shoot		
	Tomato plant (*Lycopersicum esculentum*)	50 mg/L	Higher concentration of Cr accumulated in roots	SOD, CAT, and POX	Mangabeira et al. (2006)
			Yellowing of leaves and complete wilting		
			Root and shoot dry biomass decreased		
Cd (cadmium)	Mung bean (*Vigna radiata*)	1.5 mM	Severe oxidative stress, decreased plant height and root length, and reduced chlorophyll content	SOD,CAT, and GPX	Nahar et al. (2016)
	Mutant tobacco plant (*Nicotiana tabacum*)	500 µm	Increased H_2O_2 and superoxide production, plant growth reduced, and chlorophyll content declined	SOD, proline, and glutathione	Iannone et al. (2015)
	Pea (*Pisum sativum*)	68 µmol	Reduction in plant growth and photosynthetic pigment, cause oxidative injury by enhancing the production of ROS	SOD, CAT, POD, and GR	Agrawal and Mishra (2009)
	Raddish (*Raphanus sativus*)	50 µM	Oxidative stress and accelerated cell senescence in mesophyll area of leaf blade	Phenols, catalase, and glutathione reductase	Vitoria et al. (2001)
Ni (nickel)	Watercress (*Nasturtium officinale*)	25 mg/l	Weak plant growth, chlorosis, metabolic disorder, and ROS production	SOD, APX, and CAT	Duman and Ozturk (2010)
	Wheat (*Triticum aestivum*)	50 µg/l	Growth reduction, decreased chlorophyll content, and increased ROS production	Proline and SOD	Parlak (2016)
	Pigeon pea (*Cajanus cajan*)	1.5 mM	Reduction in seedling growth, decreased dry weight, increased lipid peroxidation, and elevated ROS generation	SOD, POD, and GR	Rao and Sresty (2000)
	Rapeseed (*Brassica napus*)	0.5 mM	Stunted plant growth, brownish roots, chlorosis, and induced ROS generation	APX, GPX, and CAT	Kazemi et al. (2010)

Hg (mercury)	Rattlebush (*Sesbania drummondii*)	100 mg/l	Reduction in biomass, photosynthetic activity declined, and increased oxidative stress	GSH, SOD, APX, and GR	Israr et al. (2006)
	Alfalfa (*Medicago sativa*)	40 µm	Physiological disorder such as stomatal closure, water flow, and trigger oxidative stress	SOD, POD, CAT, APX and GR	Zhou et al. (2008)
	Cucumber seedlings (*Cucumis sativus*)	250 µm	Decreased chlorophyll content and root, shoot, length induced oxidative stress	CAT and APX	Cargnelutti et al. (2006)
	Saltbush (*Atriplex codonocarpa*)	1 mg/l	Increased solute leakage, growth inhibition, and elevated ROS production	Ascorbate and GR	Lomonte et al. (2010)
	Wheat (*Triticum aestivum*)	30 mg/kg	Oxidative damage, reduced enzymatic activity	SOD, CAT, and APX	Li et al. (2013)
Zn (zinc)	Maize plant (*Zea mays*)	50 µM	Inhibited biomass production	SOD, CAT, GPX, and POD	Islam et al. (2014)
			Decreased chlorophyll content		
			Total soluble protein		
			Elevate the ROS production		
	Alfalfa (*Medicago sativa*)	900 µM	Induced production of H_2O_2	SOD, CAT, APX, and GR	Dai et al. (2015)
			Reducing growth, caused leaf chlorosis and nutritional disturbances		
	Vetiver (*Vetiveria zizanioides*)	80 mg/kg	Total biomass decreased, proline content increased, total soluble protein decreased	SOD, POD, and CAT	Weihong et al. (2009)
	Tomato (*Lycopersicon esculentum*)	500 µM	Decreased chlorophyll content, reduced growth and ROS generation	CAT, GST, and APX	Sbartai et al. (2012)

(continued)

Table 5.1 (continued)

Metals	Plant species	Concentration	Effect	Antioxidant enzymes	References
Cu (copper)	Mustard plant (*Brassica juncea*)	150 mg/kg	Reduced growth of plant, activities of nitrate reductase, and carbonic anhydrase. Decreased chlorophyll content and proline increased	CAT, POD, and SOD	Fariduddin et al. (2009)
	Mustard plant (*Brassica junica*)	150 mg/kg	Decreased shoot–root length, biomass, decreased chlorophyll content	CAT,POX, and SOD	Yusuf et al. (2016)
			Reduced activities of RuBisCO and carbonic anhydrase		
	White lupin (*Lupinus albus*)	192 µM	Reduced nitrogen fixation, ROS production, chlorosis, necrosis, and abnormal root morphology	APX and CAT	Sanchez-Pardo et al. (2012)
	Soybean plant (*Glycine max*)				
	Ashwagandha (*Withania somnifera*)	200 µM	Inhibition of cell elongation and division, reduction of biomass, reducing membrane fluidity, and decreased concentration of carotenoid and photosynthetic pigment, ROS generation	APX, MDHAR, DHAR, GST and G-POD	Khatun et al. (2008)
As (arsenic)	Tobacco (*Nicotiana tabacum*)	5 mg/l	Impedes the photosynthesis, reduced essential nutrient content, ROS generation	SOD, POD, and GSH	Han et al. (2015)
	Rice plant (*Oryza sativa*)	50 µM	Hindered plant length and weight, oxidative stress enhanced	SOD, APX, GPX, and CAT	Dixit et al. (2016)
	Watercress (*Nasturtium officinale*)	100 µM	Decreased dry weight of roots and shoots and chlorophyll content of leaves, oxidative damage	CAT,APX, and SOD	Namdjoyan and kermanian (2013)
	Mung bean (*Phaseolus aureus*)	10 µM	Inhibited growth caused physiological disorders such as membrane damage, oxidative damage	SOD, CAT, APX, GR, ASC, GHS	Malik et al. (2012)

fluctuations such as increased salt concentration, low water availability, and elevated HM concentration result in closure of stomata which further leads to inadequate intracellular carbon dioxide level and induced ROS formation which induce rigorous injury in the photosystem (Das and Roychoudhury 2014).

5.5 Why Does All Plant Possess Antioxidant Potential?

Metals cause phytotoxicity when it is transported to the plant from the earth's crust. The most prominent consequence of HMs in plant cells is on the growth productivity (Kumar et al. 2013). HM stress declines the capability of the plant to assimilate carbon and elevate the photosynthetic electron flow toward oxygen from which the formation of $O_2^{-\bullet}$, H_2O_2, and $OH^{\bullet}$ radical increases (Gill and Tuteja 2010). As mentioned above, in plants, ROS are constantly generated chiefly in chloroplasts, mitochondria, and peroxisomes. Therefore, generation and elimination of reactive oxygen species should be regulated by the antioxidative defense system in restricted manner (Apel and Hirt 2004), but in the stress condition, the production of ROS elevates and destructs the whole cell metabolism (Sharma et al. 2012). These destructive properties of ROS generate the complex range of nonenzymatic and enzymatic detoxification device in plants (Apel and Hirt 2004). Antioxidants are reducing agents which restrain the oxidation of other molecules, because oxidation reactions generate free radicals which create cell damage (Sies 1997; Bansal and Kaushal 2014). Plants generate antioxidants like glutathione and ascorbic acid (AA) in the chloroplast, stroma, and cytosol with the help of NADPH (Alscher et al. 1997). These antioxidants interact with numerous cellular molecules and affect the growth productivity and development of the plant by interfering in cell division and cell elongation (Foyer and Noctor 2005). These antioxidants also influence gene expression to elevate the defense mechanism in the plant cell. The key reason for the stimulation of these antioxidant mechanisms might be the genetic structure of plants which have innate capacity to produce phytochemicals to execute their continuous physiological task (Kasote et al. 2015). Plants produce secondary metabolites which also illuminate the reactive oxygen species because these metabolites play a significant role in adjustment of plants against environmental fluctuations (Baier and Dietz 2005). ROS can cause many disorders in the cell by affecting many physiological reactions (Ragavendran et al. 2012). Stress damages the cell by increasing the production of ROS (Rahman 2007). So for the inhibition of these reactive species, detoxification system evolves such as enzymatic and nonenzymatic antioxidant. These systems include catalase, peroxidase, SOD, ascorbic acid tocopherol, GSH, etc. (Prakash and Sharma 2014; Gout et al. 2001) (Fig. 5.1). SOD enzyme scavenges the superoxide radical and forms hydrogen peroxide which is also highly toxic for the cell (Kusvuran 2012). SOD destroys superoxide anion by converting it to peroxide (Cannon et al. 1987). Catalase breaks the H_2O_2 into H_2O and oxygen (Mittler 2002). Polyphenol oxidase is an antioxidant enzyme which scavenges H_2O_2 in chloroplasts and plays a significant function in lignin biosynthesis (Mittler 2002). Ascorbic oxidase regulates the reduced glutathione and NADPH. Vitamin C is a water-soluble antioxidant which scavenges the peroxy radicals (Sies 2007).

5.6 Enzymatic Antioxidant

5.6.1 Superoxide Dismutase (SOD)

SOD is considered as the essential defensive antioxidant against oxygen free radicals. SOD is a metalloenzyme which converts superoxide anion ($O_2^{-\bullet}$) to H_2O_2. SOD has been present in all aerobes that work against toxic oxygen species which are usually produced as the by-products of many biological oxidation reactions (Imlay 2008). Begović et al. (2016)) reported increased concentration of SOD in duckweed (*Lemna minor*) in retort to toxicity of cobalt. SOD is localized in mitochondria, chloroplast, cytosol, and peroxisomes (Mittler 2002), and the amount of SOD escalates in accordance with the level of stress condition. Superoxide is the initial product of the monovalent reduction of oxygen and also the first free radical in the plant cell. SOD catalyzes the dismutation reaction by metal ion like Cu, Mn, and Fe at the active site. Based on metal ion, superoxide dismutase is categorized mainly in three isozymes: Mn-SOD, Fe-SOD, and Cu/Zn-SOD (Mittler 2002). The effect of the Cr toxicity on SOD transcription has been demonstrated on the green gram and black gram resulted in a substantial elevation in the production of ROS due to reduced SOD synthesis (Karuppanapandian et al. 2006; Karuppanapandian and Manoharan 2008).

5.6.2 Catalase

Catalase is the foremost discovered and characterized enzyme, which possesses antioxidant activity, and it is a Fe-containing enzyme present in diverse organisms, including prokaryotes (Zamocky et al. 2008). It consists of polypeptides of 50–70 kDa which are arranged in tetramers and each monomer encloses a heme prosthetic group (Regelsberger et al. 2002). It catalyzes the dismutation reaction of H_2O_2 into H_2O and O_2. Catalase obliterates the H_2O_2 generate in peroxisome by β-oxidation of fatty acids, photorespiration, and purine catabolism (Mittler 2002; Vellosillo et al. 2010) and prevents the diffusion of H_2O_2 from cytosol (Lopez-Huertas et al. 2000). There is elevated level of catalase in a bean (*Vicia faba*) for the destruction of ROS produced due to lead toxicity (Shahid et al. 2014). However during stress condition like salinity, drought, and HMs, the enzyme production is found to be reduced (Karuppanapandian et al. 2006; Karuppanapandian and Manoharan 2008) which limits the plant's tolerance to environmental stress. Li et al. (2013) conducted their experiment on *Triticum aestivum* (wheat plant) with mercury (Hg)-contaminated soil and found the increased intense activity of catalase (CAT) antioxidant enzyme in a wheat plant grown in a highly polluted soil.

5.6.3 Ascorbate Peroxidase and Guaiacol Peroxidase

Ascorbate peroxidase (APX) is a heme peroxidase present in higher eukaryotes (Takeda et al. 1998). In chloroplast and cytosol, the level of H_2O_2 is illuminated by

the APX. It uses ascorbic acid for the breakdown of H_2O_2 and yields water and monodehydroascorbate (Asada 2000). APX isoforms are classified on the basis of subcellular localization, such as chloroplasts, mitochondria, peroxisome, and cytosol (Caverzan et al. 2012). APX activity frequently increases with the function of other enzymes, like CAT, SOD, and GSH reductase (Shigeoka et al. 2002). Sharma et al. (2016) reported in their article about the significant increase in ascorbate peroxidase (APX) activity with chromium (Cr)-stressed *Oryza sativa* (rice) seedling under the influence of EBL (epibrassinolide).

Guaiacol peroxidase (GPX) is a significant member of peroxidase enzyme. GPXs are usually acknowledged as "stress enzymes" and found in the cellular cytoplasm and apoplasm (Sharma et al. 2012). GPX is reported to involve in many processes such as growth of plants and its development. It also takes part in ROS scavenging. GPX is an iron-enclosing protein and oxidizes certain substrates at the expenditure of H_2O_2. It relieves the cell from excess peroxide which generates in stress condition (Sharma et al. 2012). GPX deteriorate indole-3-acetic acid and also play a significant part in the biosynthesis of lignin (Karuppanapandian et al. 2011). GPX scavenges H_2O_2 produced due to stress from the cytosol, vacuole, and cell wall and in the extracellular space (Koji et al. 2009). The study reported on *Arabidopsis thaliana* seedlings exposed to lethal lead (Pb) level represented the increased activity of GPX antioxidant enzyme (Phang et al. 2011). The study on *Avicennia marina* (gray mangrove) relevant to glutathione antioxidant system for the evaluation of HM stress showed the incensement of GPX activity in a dose-dependent fashion in response to accumulated leaf metals (Zn, Cu, or Pb) (Caregnato et al. 2008). Similarly, in another study on *Vicia faba* plant showed the increased activity of APX and GPX in relation to lead stress (Shahid et al. 2014).

5.6.4 Monodehydroascorbate Reductase (MDHAR) and Dehydroascorbate Reductase (DHAR)

MDHAR is a FAD enzyme and important constituent of the glutathione–ascorbate cycle which is the major antioxidant system of plant tissue (Yoon et al. 2004). MDHAR catalyzes the ascorbate production through the MDA radical. Ascorbate is used to detoxify H_2O_2 via APX (Mittler 2002). MDHAR regenerate the ascorbate with the help of NAD(P)H. The monodehydroascorbate reductase functionality has been seen in many cell organelles such as chloroplast, cytosol, mitochondria, glyoxysomes, and peroxisomes (Leterrier et al. 2005).

DHAR is assessed as a chloroplast enzyme and contains thiol group. It plays an active role in the protection against oxidative stress (Noctor and Foyer 1998). DHAR also catalyzes the revival of ascorbic acid. Ascorbate regenerates from the DHA by the thiol enzyme DHAR, but the MDHAR produce more ascorbate than DHAR (Asada 2006; Minkov et al. 1999). DHAR overproduction in tobacco and *Arabidopsis* had been shown under environmental stress (Chen and Gallie 2006; Eltayeb et al. 2007). An investigation on *Raphanus sativus* (radish) to cadmium (Cd) treatment showed increased concentration of antioxidant enzyme MDHAR and

DHAR via the activation of ascorbate–glutathione cycle for the removal of H_2O_2 (Vitória et al. 2001).

5.6.5 Glutathione Reductase (GR)

Glutathione reductase is a flavoprotein present in all organisms (Romero-Puertas et al. 2006). Glutathione reductase (GR) is also recognized as glutathione disulfide reductase (GSR) (Kotapati et al. 2014). Glutathione reductase is a homodimeric and oxidoreductase enzyme which is NADPH dependent. It is an imperative enzyme of the ASH–GSH cycle which scavenges hydrogen peroxide with the united exploit of some antioxidant enzymes such as ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase GSH, and ascorbic acid (Noctor and Foyer 1998; Gutteridge and Halliwell 2000). It plays an essential function in scavenging of ROS by catalyzing reduction of glutathione disulfide to the sulfhydryl form GSH (Zitka et al. 2012). GR is localized mainly in chloroplasts and also little quantity of GR has been found in mitochondria and cytosol (Ding et al. 2012). Agrawal and Mishra (2009) reported increased concentration of glutathione reductase in *Pisum sativum* under cadmium stress.

5.7 Nonenzymatic Antioxidant

5.7.1 Ascorbic Acid

Ascorbic acid (AA) is the plentiful, influential, and water-soluble antioxidant present in chloroplast and all cell organelles including the cell wall. Ascorbic acid takes active role in plant growth and development in stress condition (Sharma et al. 2012). Ascorbate also works as a cofactor for some hydroxylase enzymes like prolyl hydroxylase (Kuiper and Vissers 2013). AA acts to avoid or minimize the harmful effect caused by ROS in plants (Smirnoff 2005; Ahmad et al. 2000). Hg-stimulated oxidative burst in saltbush (*Atriplex codonocarpa*) is found to be decreased by ascorbate (Lomonte et al. 2010). It destroys the several forms of ROS including singlet oxygen, superoxide, and hydroxyl radicals (Padh 1990) and shields the membranes from oxidative damage. Ascorbic acid also maintains α-tocopherol in the reduced state (Traber and Stevens 2011) and indirectly scavenges H_2O_2 through the AsA peroxidase (Chugh et al. 2011). The study on *Phaseolus vulgaris* (bean) seedling with heavy metal (Pb, Cu, Cd, and Hg) showed the significant increase in ascorbic acid content in primary leaves after 10 days of metal exposure (Zengin and Munzuroglu 2005).

5.7.2 Tocopherols

α-Tocopherol is a lipophilic antioxidant and generates only by photosynthetic organism. Tocopherol has a chromanol head group attached to the phytyl tail (Wang and Quinn 2000). Tocopherols are proficient denominator of free radicals (Kiffin et al. 2006). Tocopherols are crucial component of biological membrane and act as antioxidant in higher plants (Kiffin et al. 2006). They protect the chlorophyll membrane by physical quenching and it also undergoes reaction with oxygen (O_2) in chloroplast and shielding the photosynthetic pigment (Igamberdiev et al. 2004). The study conducted on *Arabidopsis thaliana* plant in the existence of Cd and Cu shows marked increase in α-tocopherol (Collin et al. 2008).

5.7.3 Glutathione (GSH)

Glutathione is a thiol tripeptide, a low molecular weight enzyme which presents in cytosol, ER, mitochondria, peroxisomes, vacuoles, and apoplast (Das and Roychoudhury 2014). GSH is the most significant endogenous antioxidant enzyme which is active in the neutralization of ROS directly and also maintains the exogenous antioxidants like ascorbate and tocopherol in their reduced forms (Ahmad et al. 2012). GSH has a high reductive capacity due to nucleophilic character (Halliwell 2006). GSH scavenges H_2O_2, $OH^{\bullet}$, and $O_2^{\bullet -}$ and prevents the reduction of different biomolecules. GSH also act as an imperative function in the regeneration of ascorbic acid (Ahmad et al. 2012). Glutathione occurs in the cell in two states: reduced and oxidized, the reduced form is GSH and oxidized form is GSSG. As reported in an article, conducted on *Pisum sativum* plant, glutathione (GSH) is found to be increased under cadmium (Cd) stress (Metwally et al. 2005).

5.8 Secondary Metabolites

Plants generate an ample of secondary metabolites such as flavonoids, phenolic acids, alkaloids, etc. (Hartmann et al. 1995). These secondary metabolites have no contribution in the photosynthetic mechanism, substrate oxidation, solute transportation, translocation, nutrient absorption, and differentiation (Mazid et al. 2011), but these metabolites play a significant function in ROS disintegration (Fini et al. 2011). These secondary metabolites are also important for plants to survive under stress condition. Their liberation differs from plant to plant and species to species on exposure to stress (Korkina 2007). These metabolites are formed by basic pathways like glycolysis or shikimic acid pathways (Kasote et al. 2015). Phenolics have shown the most prominent antioxidant functionality between all secondary metabolites (Kasote et al. 2015)

5.8.1 Secondary Metabolites as Antioxidant

Plant metabolites are chiefly differentiated into primary and secondary forms. Primary metabolites are those compounds which produced through primary metabolism, like sugars, amino acids, fatty acids, etc. Primary metabolites are indispensable for cell maintenance (Kliebenstein and Osbourn 2012), whereas secondary metabolites are requisite for the normal cell growth and development. Secondary metabolites also take part in the defense system of the plant (Korkina 2007). Secondary metabolites constantly remain in the plant cell. Secondary metabolites also occur in passive and active forms. In passive form, metabolites already exist in tissue, while active forms of secondary metabolites are generated in response to stress (Korkina 2007), and these metabolites are synthesized by basic pathways like glycolysis or shikimic acid pathways (Aharoni et al. 2005).

These secondary metabolites may also be of two types: one is nitrogen containing such as alkaloids containing terpenoid indole alkaloids, tropane alkaloids, and purine alkaloids (Ziegler and Facchini 2008) and the other is nitrogen deficient like terpenoids and phenolics (Kasote et al. 2015). Phenolics have shown the most prominent antioxidant reactivity between all secondary metabolites.

5.8.2 Phenolics

Plant phenolics are chiefly categorized into different groups, such as phenolic acids, flavonoids, lignins, stilbenes, and tannins (Myburgh 2014). Phenolic compounds usually have more than one aromatic ring with hydroxyl groups. The antioxidant capability of phenolics elevated with increase in hydroxyl group numbers and its conjugation with the side chain of aromatic rings (Flora 2009). Between all these phenolics, flavonoids are the chief active plant's secondary metabolite and act as an antioxidant under stress condition (Hernández et al. 2009). Posmyk et al. (2009) have observed increased level of phenolic compound in red cabbage seedling exposed to copper. Flavonoids occur broadly within the plant tissue and are usually found in leaves, floral parts, and pollens. Flavonoids generally concentrate in the plant vacuole as glycosides. Flavonoids act as a secondary ROS scavenger and get activated on the loss of photosynthetic system, because of the more excitation energy (Fini et al. 2011). Flavonoids perform as an ROS scavenger in the plant tissue by neutralizing the free radicals before they injured the cell (Løvdal et al. 2010).

Flavonoids are also capable to modify peroxidation reaction by altering the lipid packing arrangement (Sharma et al. 2012). They stabilize membranes by diminishing membrane fluidity. Most of the plant root exudates elevate the amount of phenolics on exposure to heavy metals (Winkel-Shirley 2002). Many flavonoid biosynthetic genes are activated under stress conditions. In many stress conditions like wounding, drought, metal toxicity, and nutrient deficiency, it has been seen that flavonoid concentration increases in response to these stresses (Winkel-Shirley 2002). Anthocyanins, a derivative of flavonoids, gather in the vacuoles and possess an antioxidative capability (Kähkönen and Heinonen 2003), but its location

prevents them to contact directly with ROS generation sites. However, its level is found to be increased under Cd stress (Mobin and Khan 2007). Keilig and Ludwig-Müller (2009) propounded in their article about the potential role of flavonoids with response to cadmium (Cd) in tolerant *Arabidopsis thaliana* seedling.

5.8.3 Terpenoids

Terpenoids are a huge class of secondary metabolites containing more than 40,0000 different compounds (Aharoni et al. 2005), ranging in structure from linear to polycyclic. Terpenoids are organic compounds derived from the isoprene unit which also have an antioxidative role in plants (Grassmann et al. 2002). Based on the different compositions, it is classified into monoterpenes, diterpenes, triterpenes, and tetraterpenes (Rabi and Bishayee 2009). Monoterpenes, sesquiterpenes, and diterpenes are acquired remarkable antioxidant activity in different in vitro analyses (Baratta et al. 1998). Tetraterpenes possess strong antioxidant activity within both in vivo and in vitro studies (Palozza and Krinsky 1992; Kasote et al. 2015).

5.8.4 Alkaloids

Alkaloids are nitrogen-containing most plentiful secondary metabolites present at 10–15 % concentration, in nearly all plant tissues (Schardl et al. 2006). Alkaloids are heterocyclic compounds containing negatively charged nitrogen due to which it possesses antioxidant properties. Caffeine obtained from the *Thea sinensis* leaves and *Coffea arabica* also shows antioxidant activity. Alkaloids are frequently accommodated in the plant tissue in response to several stresses (Ali and Alqurainy 2006). Several alkaloids have been established as effective inhibitors of $^1O_2$ such as indole alkaloids like strychnine and brucine that have a basic nitrogen atom in a rigid, cage-like conformation. These alkaloids are physical quenchers and not smashed chemically by the course of quenching. Thus, in principle, they could destroy singlet oxygen. Srivastava and Srivastava (2010) reported in his article about the increased alkaloid content in the root of *Catharanthus roseus* in response to cadmium and nickel stress.

5.8.5 Carotenoids

Carotenoids are lipid-soluble molecules and beta carotene is the main precursor of vitamin A. Carotenoids defend the plant from oxidative stress (Britton et al. 2009). Carotenoids are present in photosynthetic organisms as a light-harvesting pigment, expanding the light spectrum range, which utilize in the photosynthetic mechanism. Carotenoids also quench the $^1O_2$ within the photosynthetic machinery (Li et al. 2012). They absorb light in the region from 450 to 570 nm and pass the confined energy to chlorophyll pigment and also serve as an antioxidant scavenging

superoxide anion produced by quenching of the triplet state of the chlorophyll molecules (Young and Lowe 2001). Andrianos et al. (2016) described the increased concentration of carotenoids in *Solanum tuberosum* and *Daucus carota* cultivated in a greenhouse and irrigated with a water solution including different concentrations of chromium and nickel.

5.9 In Vitro and In Vivo Strategies for ROS and Plant Antioxidant Potential Measurement

There is a rising curiosity among the scientific world and ingenuities with regard to the measurement of ROS and antioxidant prospective in plant tissue. In plant tissue, reactive oxygen species detect mainly with the help of histochemical method. Because of their highly reactive nature and extremely short lifetimes, the studies of free radical generation in plants are very difficult. The quantitative biochemical analysis does not make available exact information for the localization of reactive oxygen species in plants (Cheeseman 2006). The histochemical localization of ROS provides the opportunity to identify the specific sites of their in situ production that greatly helps to detect the distribution and accommodation of reactive oxygen species in the cell. Histochemical revealing of ROS is mainly done by the use of 3,3′-diaminobenzidine (DAB) and nitro blue tetrazolium (NBT) stain (Kuźniak et al. 2014). ROS detection could also be done by the use of fluorescent probes which is the simplest, greatest, and accessible method. Dihydroethidium (DHE), MitoSOX Red, and 5-(and 6)-chloromethyl-2,7-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) are used to measure superoxide anion and hydrogen peroxide production in the cell (Fryer et al. 2003), while for the evaluation of antioxidant, a credible number of methods are available through which an easy evaluation could be carried for the measuring of reactive oxygen species scavenging activity. Approximately 19 in vitro and 10 in vivo methods are applied for the measurement of antioxidant ability (Alam et al. 2013).

There are copious in vitro assays that are available to fully elucidate the antioxidant behavior of plants conversely, and every method has its own margins concerning its applicability. In vitro methods are usually used to confirm the antioxidant ability of the plant particularly on the basis of certain reaction like reduction, quenching, or metal chelation, and on that basis they are further classified as primary and secondary antioxidants (Kasote et al. 2015) (Fig. 5.2; Table 5.2).

The primary antioxidant works by donating a proton, whereas secondary metabolites work by binding of metal ion which is able to catalyze oxidative reactions and UV absorbance and impeding hydroperoxide activities (Kasote et al. 2015). The efficiency of antioxidant mechanism mainly depends on bond dissociation energy and ionization potential (Karadag et al. 2009). Based on the inactivation mechanism involved, a basic classification of antioxidant assays falls under two categories:

(i) Hydrogen atom transfer (HAT)-based assays
(ii) Electron transfer (ET)-based assays

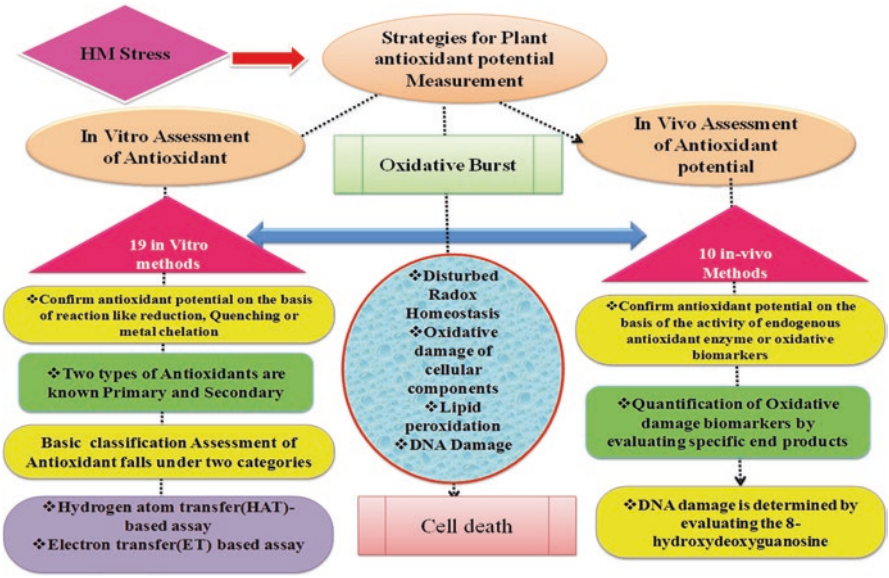

Fig. 5.2 Theoretical model illustrating the most probable strategy adopted by plant against antioxidant potential measurement. Model representing the overall strategies adopted for the measurement of antioxidant potentials that generally falls under two categories: in vitro assessment method (hydrogen atom transfer (HAT)-based assays and electron transfer (ET)-based assays) and in vivo assessment method

The HAT-based methods are used to determine the potential of an antioxidant to destroy the ROS and in the formation of stable compound. Antioxidant potential evaluation primarily depends on the competition kinetics. HAT assay reaction is fast and completed in minutes and the reactions are pH dependent.

HAT-based assays include oxygen radical absorbance capacity (ORAC) method, lipid peroxidation inhibition capacity (LPIC) assay, total radical trapping antioxidant parameter (TRAP), inhibited oxygen uptake (IOC), crocin bleaching nitric oxide radical inhibition activity, hydroxyl radical scavenging activity by p-NDA (p-butrisidunethyl aniline), scavenging of H_2O_2 radicals, ABTS radical scavenging method, and scavenging of superoxide radical formation by alkaline (SASA) (Badarinath et al. 2010).

ET-based methods calculate the potential of an antioxidant. The color of oxidant gets changed on the reduction (Fig. 5.2). The extent of color change is interconnected to the concentration of antioxidants in the sample. Electron transfer reactions are usually slow and require longer times to attain a final point, so antioxidant potential calculations are mainly based on percent decline in the product rather than kinetics. ET reactions depend upon the pH (Prior et al. 2005) (Table 5.2).

ET-based assay includes Trolox equivalent antioxidant capacity (TEAC) decolorization, ferric reducing antioxidant power (FRAP), DPPH free radical scavenging assay, copper (II) reduction capacity total phenols by Folin–Ciocalteu, and

Table 5.2 Assessment of antioxidant potential in plants

In vitro assay	Methods	References
β-carotene or crocin bleaching assay	HAT	Ordoudi and Tsimidou (2006)
ORAC (oxygen radical absorbance capacity)	HAT	Haytowitz and Bhagwat (2010)
IOU (inhibited oxygen uptake)	HAT	Filippenko et al. (2009)
LPIC (lipid peroxidation inhibiting capacity) assay	HAT	Shalaby and Shanab (2013)
TRAP (total radical trapping antioxidant parameter)	HAT	Sies (2007)
Copper reduction assay	ET	Campos et al. (2009)
FRAP (ferric reducing antioxidant power) assay	ET	Ou et al. (2002)
Total phenolic content assay by Folin–Ciocalteu reagent	ET	Ainsworth and Gillespie (2007)
TEAC (Trolox equivalent antioxidant capacity)	ET	Gliszczyńska-Świgło (2006)
DMPD (N,N-dimethyl-p-phenylenediamine) assay	ET	Çekiç et al. (2015)
ABTS [(2,2'-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid)] assay	HAT and ET	Johnston et al. (2006)
DPPH (2,2-diphenyl-1-picrylhydrazyl) assay	HAT and ET	Ozgen et al. (2006)
SASA (scavenging of superoxide radical formation by alkaline)	HAT	Badarinath et al. (2010)

N,N-dimethyl-p-phenylenediamine (DMPD) assay (Badarinath et al. 2010), whereas in the in vivo assay, plants' antioxidant potential is usually examined on the basis of the reactivity of endogenous antioxidant enzymes or oxidative biomarkers prior and subsequent stimulation of oxidative stress (Kasote et al. 2015). In these techniques the action of antioxidant enzyme like superoxide dismutase, catalase, GPX, and GR is directly estimated. While several other techniques are made by the evaluation of oxidative damage biomarkers and definite yield formed by the interaction of ROS and biologically significant macromolecules like DNA, lipids, and protein-like DNA, damage is determined by evaluating the 8-hydroxydeoxyguanosine (Kasote et al. 2015) (Table 5.2).

5.10 Conclusion and Future Outlook

The planet's inhabitants are burgeoning exponentially and stretching the earth's limited resources; as the population is increasing, food consumption follows the same upward trend (FAO 2009). Based on the UN report (2015), the world population reached 7.3 billion as of mid-2015, whereas the Indian population reached 1.3 billion (World Population Prospects: The 2015 Revision. New York: United Nations. 2015). Population detonation by diverse human activities upshot the quantity of

waste production and pollution is on the rise. The environmental collision of various activities affects abiotic and biotic factors, such as water quality, soil and sediment quality, air quality, noise, and vibration generated beyond the permissible limits and various types of waste generated. Among them, heavy metal pollution is the major pollution. Besides natural source, anthropogenic activities such as flawed disposal of waste from different industries (nanoparticles manufacturing factories, smelters, power plants, electroplating, and mines), conflagration by-product, and automobile discharges are the major sources of HM pollution. HMs in limited quantity are significant for the healthy growth of plants, but their accumulation in productive soil in excess leads to phytotoxicity which declines the physical and biochemical activities, germination and growth retardation, structural breakage, and reduced yield.

In these contexts, plants produce and accommodate numerous enzymatic and nonenzymatic antioxidants like AA, glutathione, and phenolics. In response to heavy metal stress, plants trigger increased ROS level through the Fenton-type reactions and Haber–Weiss cycling. These ROS species scavenge by the erection of enzymes and nonenzymatic antioxidants. Significant scientific information has been gathered in the form of plant redox biology and the antioxidant resistance device possessed by it. Therefore, it becomes a prerequisite to delineate the different activities that are generating heavy metal saddle on the environment. However, this chapter, though, covers largely the discernible detrimental impacts induced by HMs in plants with the integrated response adopted by plants toward metal stress, particularly in the form of antioxidant ability and also assessment strategies adopted toward the measurement of antioxidant ability; further research is still required for cultivating plant species with improved antioxidant potentials that could be able to feed the ever-growing world population. Furthermore, there is a need to produce such transgenic plant varieties or genetically modified (GM) plants that have the potential to resist against the weed, pest, diseases, soil salinity, and also heavy metal-induced phytotoxicity.

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