Chapter 2
Medicinal Plant: Environment Interaction and Mitigation to Abiotic Stress

Murtaza Abid and M. M. Abid Ali Khan

Abstract Herbal/traditional plant medicine is the most antioxidant-rich category. Abiotic stresses including climatic factors, plant species, extreme temperatures, light intensity, soil and air pollution, drought, flooding, salinity and osmotic changes, and other environmental factors affected both the enzymatic and nonenzymatic antioxidant defense system in plants. The activities of the antioxidant enzymes such as polyphenol oxidase (PPO), catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), phenylalanine ammonia-lyase (PAL), ascorbate peroxidase (APX), and lipoxygenase (LOX) are altered in stressed conditions which lead to the changes in malondialdehyde (MDA), superoxide radical, and hydrogen peroxide content of the cells. The different components of nonenzymatic defense system such as glycine betaine (GB), proline, glutathione (GSH), ascorbic acid (AsA), tocopherols, carotenoids, flavonoids, and phenolic compounds also play a crucial role as they interact at cellular level. A common factor between most stresses is the active production of reactive oxygen species (ROS). They are actively produced and used as signaling molecules by cells in response to most abiotic stresses. Due to the highly reactive nature of ROS, their production and detoxification need to be strictly controlled. Studies on transformed plants expressing increased activities of single enzymes of the antioxidant defense system indicate that it is possible to confer a degree of tolerance to stress by these means. The advent of plant transformation has placed within our grasp the possibility of engineering greater stress tolerance in plants by enhancements of the antioxidant defense system.

Keywords Medical plant · Environment · Interaction · Abiotic stress · Antioxidant defense system

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D. Egamberdieva, A. Tiezzi (eds.), *Medically Important Plant Biomes: Source of Secondary Metabolites*, Microorganisms for Sustainability 15,
https://doi.org/10.1007/978-981-13-9566-6_2

2.1 Introduction

2.1.1 Reactive Oxygen Species and Antioxidant System

Plants have integrated antioxidant systems, which include enzymatic and nonenzymatic antioxidants that are usually effective in blocking harmful effects of ROS. Ethylene biosynthesis and membrane breakdown involving lipid peroxidation seem to involve free radicals. Since plants have less evolved mechanisms of stress avoidance, they require important means of adaptation to changing environmental conditions. A cyanide-insensitive respiratory pathway in chloroplasts competes for electrons with photosynthetic electron transport (Bennoun 1994) and may also reduce oxygen. Furthermore, some important sites, such as the reaction center protein of PSII (DI) and the apoplastic space, appear to have very little protection against oxidative damage (Castillo and Greppin 1988; Luwe et al. 1993). To save themselves from these lethal oxygen intermediates, plant cells and its organelles like chloroplast, mitochondria, and peroxisomes employ antioxidant defense systems. A great deal of research has established that the induction of the cellular antioxidant machinery is important for protection against various stresses (Tuteja 2007; Khan and Singh 2008; Gill et al. 2011; Singh et al. 2008). The components of antioxidant defense system are enzymatic and nonenzymatic antioxidants. Enzymatic antioxidants include SOD, CAT, POD, APX, monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR), and nonenzymatic antioxidants are GSH, AsA (both water soluble), carotenoids and tocopherols (lipid soluble), proline, glycine betaine, flavonoids, and phenols (Gill et al. 2011; Mittler et al. 2004; Singh et al. 2008).

2.1.2 Abiotic Stresses

Plants are often subjected to hostile environmental conditions which cause abiotic stress conditions that play a major role in determining productivity and yields (Boyer 1982) and also the differential ecological distribution of the plants species (Chaves et al. 2003). A significant feature of plant adaptation to abiotic stresses is the activation of multiple responses involving complex gene interactions and crosstalk with many molecular pathways (Basu 2012; Umezawa et al. 2006). Abiotic stresses elicit complex cellular responses that have been elucidated by studying plant abiotic responses at the whole-plant, morphological, physiological, biochemical, cellular, and molecular levels (Grover et al. 2001). The development of stress-tolerant plants either by genetic engineering or through conventional breeding has been done. The elucidation of the different components and molecules playing important role in abiotic stress responses of a broad range of species in both model and crops plant is in progress. Now, efforts are being made to expand our knowledge on plant response to abiotic stresses using holistic system biology approaches,

taking advantage of available high-throughput tools such as transcriptomics, proteomics, and metabolomics. The objective of this chapter is to provide an insight of abiotic stress biology in medicinal plants. In the present chapter, we present some details about the enzymatic and nonenzymatic responses of plants to various abiotic stresses for the better adaptation to face the environmental constraints.

2.1.2.1 Types of Abiotic Stresses

Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant (Taiz and Zeiger 1991). Alternatively, stress could be defined as a significant deviation of the optimal condition of life (Larcher 2003). The effects of the following types of abiotic stresses have been studied in quite detail (Fig. 2.1).

Temperature

Among stile conditions, temperature stress is considered to be one of the most damaging because of the ever-changing components of the environment. Heat stress has several impacts on the life processes of organisms, and plants, in particular, are most affected since they are as sessile and cannot move to more favorable environments.

The changing climate and global warming have made the study related to temperature stress as the major concerns for plant scientists worldwide. High temperature (HT) is now considered to be one of the major abiotic stresses for decreasing crop production and yield (Hasanuzzaman et al. 2012). As there is an optimum temperature limit in every plant species for plant growth and metabolism, there may be devastating effects of temperature on the growth and survival of plants. The US Environmental Protection Agency (EPA) indicates that global temperatures have risen during the last 30 years (EPA Student's Guide to Global Climate Change. www.epa.gov 2011) and also said that the decade from 2000 to 2009 was the hottest period ever recorded. High temperature stress is defined as the rise in temperature beyond a critical threshold for a period of time sufficient to cause irreversible damage to plant growth and development (Wahid 2007). The growth and development of plants involves numerous biochemical reactions, all of which operates at a particular temperature (Zróbek 2012).

Low temperature (LT) or cold stress also affects plant growth and crop productivity and leads to substantial crop losses (Xin and Browse 2000; Sanghera et al. 2011). Chilling stress results from cool temperatures low enough to produce injury without forming ice crystals in the cells, whereas freezing stress results in ice formation within plant tissues. Plants differ in their tolerance to chilling (0–15 °C) and freezing (<0 °C) temperatures. Both chilling and freezing stresses are together termed low temperature or cold stress: the damage due to cold stress can range from chilling injury and freezing injury to suffocation and heaving. In general, plants from temperate climatic regions are considered to be chilling tolerant to variable degrees, and their freezing tolerance can be increased by exposing to cold, but

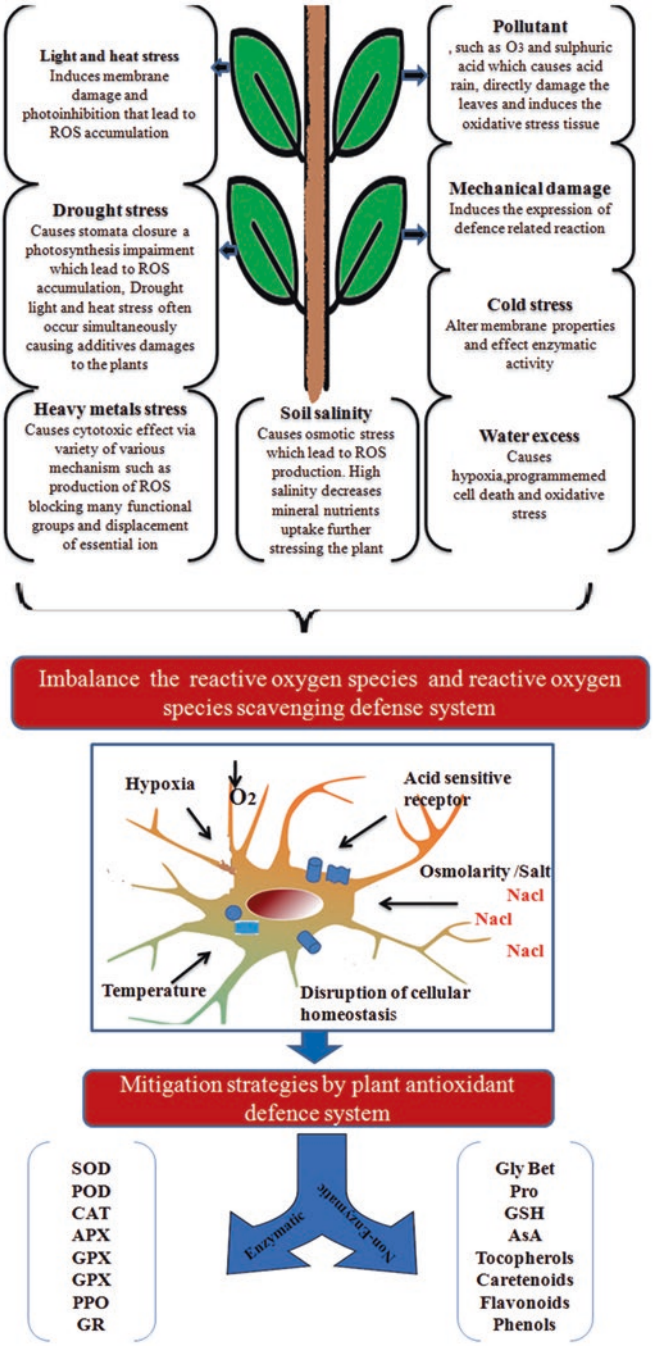

Fig. 2.1 Different types of abiotic stresses and their enzymatic and nonenzymatic responses

non-freezing, temperatures; this process is known as cold acclimation. However, generally, the plants of tropical and subtropical origins are sensitive to chilling stress and lack this mechanism of cold acclimation (Sanghera et al. 2011). Low temperature may affect several aspects of crop growth, viz., survival, cell growth and division, photosynthesis, water transport, growth, and finally crop yield.

The alterations at the cellular level due to HT or LT lead to the excess accumulation of toxic compounds, especially reactive oxygen species (ROS). The end result of ROS accumulation is oxidative stress (Mittler 2002; Yin et al. 2008; Suzuki and Mittler 2006). In response to HT, the reaction catalyzed by ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) results into the production of H_2O_2 due to increases in its oxygenase reactions (Kim and Portis 2004). LT conditions can also inhibit the activity of the Calvin-Benson cycle and create an imbalance between light absorption and light use. Enhanced photosynthetic electron flux to O_2 and over-reduction of the respiratory electron transport chain (ETC) can also result in ROS accumulation during chilling which causes oxidative stress (Hu et al. 2008). Plants have developed a variety of responses to extreme temperatures that reduce injury and ensure the maintenance of cellular homeostasis (Kotak et al. 2007). Researchers have explored the direct link between ROS scavenging and plant stress tolerance under extreme temperature (Suzuki and Mittler 2006). There is a relation between temperature stress tolerance and enhanced activities of enzymes involved in antioxidant systems of plants. Plants cope with the deleterious effects of oxidative damage caused by extreme temperatures by making use of several nonenzymatic and enzymatic antioxidants. Numerous studies on plants have shown that enhancing antioxidant defense confers stress tolerance to either HT or LT stress (Almeselmani et al. 2006, 2009; Nagesh Babu and Devaraj 2008; Huang and Guo 2005).

Water

Water is a universal solvent and 70% of living cell comprises of water. Water stress limits the growth and productivity of crops particularly in arid and semi-arid areas causing heavy economic losses in agriculture. Inoculation of plants with native beneficial microorganisms may increase rough tolerance of plants growing in arid or semi-arid areas (Marulanda et al. 2007). A large number of microorganisms exist in the rhizosphere, and plants select those bacteria which secrete organic compounds through exudates and contribute to their well-being (Bazin et al. 1990). Water (drought) stress impairs electron transport system leading to the formation of activated oxygen (Chandra et al. 1998). Activated oxygen compound such as H_2O_2, O^{-2}, and OH^- may accumulate during water deficit stress and damage the photosynthetic apparatus. Superoxide dismutase (SOD) and ascorbate peroxidase along with the antioxidant ascorbic acid and glutathione prevent oxidative damage in plants (Allen 1995). Oxidative molecules initiate damage in the chloroplast and cause a series of damaging effects including chlorophyll (Chl) degradation, lipid peroxidation, and loss of protein activity (Zhang and Kirkham 1994). In aromatic plants, growth and

essential oil production are influenced by various environmental factors, such as water stress (Burbott and Loomis 1969).

Drought

The response of plants toward drought stress is a complex phenomenon, and it seems to involve the synthesis of polyamines, some new proteins whose function is still not clear (Caplan et al. 1990). Abscisic acid is an important component since it stimulates stomatal guard cells to close, reducing water loss. This process also reduces the availability of CO_2 for photosynthesis, which can lead to the formation of reactive oxygen species from plants, which direct the electrons in the photo system. In tomato, cytosolic Cu/Zn-SOD was induced strongly by drought, while the chloroplastic Cu/Zn-SOD remained unchanged. An increase in glutathione reductase activity was reported in wheat and cotton plants under drought stress (Burke et al. 1985). It was assumed that in addition to removing H_2O_2, NADP was made available that can accept electrons from ferredoxin, thereby minimizing chances of superoxide formation. In drought-tolerant *Hordeum* species, levels of glutathione reductase and ascorbate peroxidase increased, but SOD activity was not examined (Smirnoff and Colombe 1988). Drought-stressed cotton was found to be resistant to paraquat (Burke et al. 1985), which proves the existence of a common protective mechanism against these stresses. Drought-induced changes in lipid peroxidation and the activities of SOD and catalase were compared in two mosses, the drought-tolerant *Tortula ruralis* and the drought-sensitive *Cratoneuron filicinum* (Dhindsa and Matowe 1981). In the presence of stress, the drought-tolerant moss showed lower levels of lipid peroxidation and increased levels of enzymes. The opposite occurred in the sensitive moss. Oxidized glutathione (GSSG) was shown to be a good indicator of drought stress (Dhindsa 1991). Drought-tolerant and intolerant maize inbred were analyzed by Malan et al. (1990), and resistance was found to correlate with Cu/Zn-SOD and glutathione reductase activities. Sairam et al. (1997, 1998) showed that H_2O_2 scavenging systems comprising of ascorbate peroxidase, glutathione reductase, and catalase are more important in conferring tolerance against drought-induced oxidative stress than superoxide dismutase alone.

Flood or Anorexia

Stress on plants imposed by water logging and deeper submergence (flooding) of the soil is one of the major abiotic constraints on growth, species' distribution and agricultural productivity (Jackson 2004), and grain yields (Setter and Waters 2003). Flooding stress also plays a role in adaptive strategies and evolution. A major constraint resulting from excess water is an inadequate supply of oxygen to submerged tissues (Armstrong and Drew 2002) and other changes in the soil that influence plants; levels of the plant hormone ethylene (Smith and Russell 1969; Jackson 1982) and products of anaerobic metabolism by soil microorganisms (e.g., Mn^{2+},

Fe^{2+}, S_2, H_2S, and carboxylic acids) can accumulate (Ponnamperuma 1983; McKee and McKevlin 1993). Moreover, availability of carbon dioxide, light, and oxygen to the shoots is reduced (Jackson and Ram 2003).

Oxidative stress and increased ROS production are an important part of many stress situations, including hypoxia. Post-hypoxic hydrogen peroxide accumulation has been shown in the roots and leaves of *Hordeum vulgare* (Kalashnikov et al. 1994) and in wheat roots (Biemelt et al. 2000). The presence of H_2O_2 in the apoplast and in association with the plasma membrane under hypoxic conditions in four plant species has been shown (Blokhina et al. 2001). Indirect evidence of ROS formation such as TBARS contents (i.e., lipid peroxidation products) under low oxygen have been detected (Yan et al. 1996; Chirkova et al. 1998; Blokhina et al. 1999). Flooding in *Zea mays* resulted in a significant increase in TBARS content, production of superoxide radical and hydrogen peroxide, and membrane permeability in the leaves (Yan et al. 1996). An excessive accumulation of superoxide due to the reduced activity of SOD under flooding stress has also been reported (Yan et al. 1996).

Light

Plants require adequate light in order to grow and survive. Light is the primary source of energy, and plants convert light to chemical energy through photosynthesis. Light is an essential prerequisite for chlorophyll (Chl) biosynthesis and chloroplast development. Light reactions of photosynthesis require light and are essential for the synthesis of carbohydrates.

Intense light has long been known to disrupt metabolic processes in plants, including photosynthesis, glucose assimilation, electron transport chain, and phosphorylation (Egneus et al. 1975). However, excessive exposure to high light intensities can cause considerable damage to plants. The effects of H_2O_2 on gene expression have also been reported to be different when it was induced in response to high light (Golemiec et al. 2014).The flux of ROS generated in cells is activated under high light and is capable of leaving the thylakoid membrane and reaching the cytoplasm or even the nucleus (Fischer et al. 2007), which makes its role as a signaling molecule feasible. In a recent study (Zhao et al. 2011), it was shown that under high temperature treatments, large amounts of $O^{\bullet 2-}$and H_2O_2 were generated and accumulated in cucumber leaves, leading to premature senescence, which is indicated by the changes in protein, lipid peroxidation (LPO), and chlorophyll content. Following high light illumination, $^1O_2$ accumulates and modifies the expression of a group of genes encoding chloroplast proteins, leading to a significant change in chloroplast structure and functional modifications.

Low light induced oxidative stress by modulating the activity of antioxidant enzymes. It has been shown that exogenous H_2O_2 can have a beneficial effect on low light-induced oxidative stress (Zhang et al. 2011). Low light induces oxidative stress (Sielewiesiuk 2002), which increases ROS and causes lipid peroxidation. H_2O_2 pretreatment of cucumber leaves resulted in decreased levels of O^2-,

endogenous H_2O_2, and malonaldehyde by moderating the activities of antioxidant enzymes, thus reducing lipid peroxidation and stress intensity at low light.

Salinity

Salt stress causes oxidative damage as a result of water deficit and triggers generation of reactive oxygen species, which disrupts biological membranes and thus results in either the death of the plant or reduction in productivity. High salt concentration might interfere with the electron transport chain in different organelles and generates ROS such as singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radicals (Elstner 1982; Hernandez et al. 1993, 1995). Excess of ROS triggers phytotoxic reactions such as lipid peroxidation, protein degradation, and DNA mutation (Stewart and Bewley 1980; Fridovich 1986; Davies 1987). Mannitol is accumulated by a wide range of species in response to salinity (Stoop et al. 1996).

pH

Soil pH is one of the important factors determining plant growth as it may affect the availability of nutrients to the plants. Nutrients are most available to plants in the optimum 5.5–7.0 range. In some cases, aluminum (Al) becomes soluble at pH levels below 5.0 becoming toxic to plant growth.

Soil acidification is becoming a very serious environmental problem affecting plant growth and yield since the use of acidic and physiologically acidic nitrogen fertilizers is increased and the ever-increasing environmental pollution causes acid rain (Russell et al. 2006; Zhang et al. 2009; Guo et al. 2010). The pH value of most acidic soil is highly reduced from the 1980s to the 2000s, and the pH is under 4.0 in some highly acidic soils (Guo et al. 2010). Proton toxicity (low pH stress) is considered to be one of the major stresses limiting plant growth in acid soils (Kochian et al. 2005). Low pH levels directly inhibited plant growth via high H activity (Schubert et al. 1990; Koyama et al. 2001). A high concentration of H triggers typical oxidative stress on plants by inducing the accumulation of excess reactive oxygen species (ROS), such as superoxide radicals (O) and hydrogen peroxide (HO) in plant tissues (Shi et al. 2006; Liu et al. 2011). To counteract oxidative damage, plants have evolved complex antioxidant systems including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DR), and antioxidants such as α-tocopherol, ascorbate, and reduced glutathione (Asada 1999; Mittler 2002). Studies have indicated that higher activity levels of antioxidant enzymes may contribute to better H tolerance by increasing the protective capacity against oxidative damage (Liu and Liu 2011; Chen et al. 2013).

Alkaline stress is defined as the presence of alkaline salts (Na_2CO_3 or $NaHCO_3$) in the soil (Paz et al. 2012). It is one of the most critical abiotic stresses which plants face in the era of climate change. A number of researches have shown that alkaline

stress is more hazardous than saline stress because of its additional high pH stress (Campbell and Nishio 2000; Hartung et al. 2002; Chen et al. 2012; Radi et al. 2012). High pH value may lead to reduction in seed germination, damage to the root cell structure, alterations in the nutrient availability, and disorder in nutrient uptake, thus resulting in a decreased yield of agricultural plants (Peng et al. 2008). The effects of alkalinity was studied on maize plants, and it was shown that alkaline-stressed plants showed a decrease in growth parameters, leaf relative water content (LRWC), and the contents of photosynthetic pigments, soluble sugars, total phenols, and potassium ion (K^+), as well as potassium/sodium ion (K^+/Na^+) ratio. By contrast, alkaline stress increased the contents of soluble proteins, total free amino acids, proline, Na^+, and malondialdehyde (MDA), as well as the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in stressed plants (Latef and Tran 2016).

The effects of NaCl and $NaHCO_3$ stresses were investigated in tomato roots. The relative growth rate and respiration of tomato plants were considerably decreased in both NaCl and $NaHCO_3$ treatments, especially under $NaHCO_3$ stress. As the concentration of NaCl and $NaHCO_3$ increased, Na accumulation in roots was increased, while accumulation of N, P, K, Fe, and Mg was less. Among both the treatments, $NaHCO_3$ treatments induced much higher levels of reactive oxygen species (ROS) and lipid peroxidation as well as higher activities of antioxidant enzymes and higher concentrations of ascorbate-glutathione. However, after few days of treatment, $NaHCO_3$ stress led to decreased accumulation of ROS, antioxidant enzyme activities, and ascorbate-glutathione content (Gong et al. 2014).

Pollutants

Atmospheric pollutants such as ozone (O_3) and sulfur dioxide (SO_2) have been shown to generate free radical (Mehlhorn 1990; Pell et al. 1977) and influence the growth of forest. Ozone seems to be a greater threat to plants than SO_2 (Heagle and Annu 1989). Mehlhorn (1990) suggested that the damaging effects of O_3 are due to its oxidizing potential and the consequent formation of free radicals followed by initiation of chain reactions. The O_3 concentration in the intercellular air spaces of leaves is close to zero (Laisk et al. 1989). Thus, ozone is not likely to reach the chloroplast, but it causes pigment bleaching and lipid peroxidation (Heath 1987). Stimulation of synthesis and degradation of the PSII-DI protein occurs in spruce trees following O_3 treatment (Lutz et al. 1957) and a decrease in the activity and quantity of RuBisCO has been found in poplar following exposure to O_3 (Landry and Pell 1993).

SO_2 exposure results in tissue damage and release of stress ethylene from both photosynthetic and non-photosynthetic tissues (Peiser and Yang 1985), and fumigation with SO_2 results into a change in cytoplasmic pH. The concentration of proton in the cytoplasm is one of the most important factors regulating cellular activity. SO_2 causes considerable acidification of the cytoplasm it reacts with water to form sulfurous acid which may then be converted into sulfuric acid (Laisk et al. 1988;

Veljovic-Jovanovic et al. 1993). The oxidation of sulfite to sulfate in the chloroplast also gives rise to the formation of free radicals (Polle et al. 1992). The oxidation of sulfite is initiated by light and is mediated by photosynthetic electron transport. This results in loss of photosynthetic function caused by inhibition of the activity of SH-containing, light activated enzymes of the chloroplast (Shimazaki and Sugahara 1980; Covello et al. 1989).

Radiation

Sunlight contains energetic short wavelength ultraviolet (UV) photons which are highly injurious because of their destructive interactions with amino acids, nucleic acids, or membrane lipids (1). The intensity of UV radiation reaching the earth's surface varies greatly with season, time of day, latitude, ozone layer thickness, altitude, and cloud cover. Sometimes, differential responses are studied in UV-A (400–320 nm) and the UV-B (320–290 nm) regions. In both cases, however, the basic mechanisms of photochemical damage remain similar although different receptor molecules (chromophores) may be involved.

A depletion of ozone layer led to a significant increase in ultraviolet-B (UV-B) radiation (280–320 nm). UV-B can manipulate plant processes either causing direct damage or via different regulatory effects (Rozema et al. 1999; Potters et al. 2009). It can cause either direct injury to DNA leading to mutations which could be heritable or direct or indirect damage to physiological functions of plant (Ormrod and Hale 1995; Lidon 2012). UV-B results in altered plant growth and productivity. UV-B injury also causes membrane changes and protein denaturation.

A wide range of morphological, growth, biochemical, and physiological responses of plant have been reported in the presence of UV-B radiation (Caldwell et al. 1998; Zhang et al. 2009; He et al. 2003). Flavonoids play a major role in protecting plants from UV-B damage (Liang et al. 2006). These flavonoids generally absorb the light in the region of 280–320 nm and thus are capable of acting as a UV filter, thereby protecting the photosynthetic tissues from damage (Siefermann and Harms 1987). Flavonoids stabilize and protect the lipid phase of the thylakoid membrane and are quenchers of the excited triplet state of chlorophyll and singlet oxygen (Agrawal and Rathore 2007). In addition, carotenoids also have antioxidant properties which act as an internal filter against UV-B radiation. Plants scavenge reactive oxygen species by detoxification mechanism produced by enzymatic antioxidant such as catalase, peroxidase, superoxide dismutase, phenylalanine ammonia-lyase, etc. (Moran and Porath 1980).

2.1.3 *Effects of Abiotic Stresses on Antioxidant Status of Medicinal Plants (Table 2.1)*

***Ocimum basilicum* L.** Water stress caused the following physiological and biological changes in basil plants. It resulted in the accumulation of reactive oxygen species in the cell. It resulted in higher antioxidant activity, and the highest concentration of CAT and GPX activity was reported. Increased water stress caused increased chlorophyll content in leaves, whereas APX activity decreased. Inoculation with rhizobacteria could be efficiently used to improve growth, antioxidant status, and photosynthetic pigments in basil under water stress. *Pseudomonades* sp. under water stress considerably improved CAT enzyme activity in the leaves and increased it. Combination of three bacterial species caused the highest GPX and APX activity and chlorophyll content in leaves under water stress (Heidari and Golpayegani 2012).

***Withania somnifera* L.** Iron stress was induced by adding higher quantity of $FeSO_4$. It caused disturbance in balancing of nutrients and induces oxidative stress in plants (root and leaf tissues analyzed for catalase, superoxide dismutase, and guaiacol peroxidase (GPX) have shown, an increase in content with respect to exposure of time) (Rout et al. 2015).

***Camellia sinensis* L.** Drought stress caused increased water loss rate (WLR) and decrease in relative water content (RWC), dry mass, chlorophyll, carotenoid, and total phenolic contents of leaf and antioxidants like ascorbate and glutathione in tea. Leaf antioxidant enzymes SOD, CAT, and GR showed differential activities, whereas there was an increase in reactive oxygen species (ROS) and lipid peroxidation resposible for gradual decrease in POD activities. An increased activity of POD, GR, CAT, and higher phenol content was reported. Drought stress altered antioxidant response with apparent decrease in mineral nutrient (Zn, Ca, Na, Fe, Mg, and K) contents of leaves suggesting that mineral deficiency-mediated drought stress-induced oxidative damage in tea. Tea plants exposed to heavy metals (HM) (e.g., Cd, Cu, Al) also showed reduction in growth and antioxidant responses (Upadhyaya and Panda 2013).

The antioxidant responses to increasing concentrations of copper were investigated in the leaves of two cultivars (TS-462 and TS-520) of tea commonly grown in the Darjeeling hills. Exposure to excess Cu resulted in increased lipid peroxidation, reduced chlorophyll content, higher levels of phenolic compounds, and an increase in peroxidase enzyme levels. Two new peroxidase isozymes (POD1 and POD2) were detected in plants exposed to Cu. TS-520 was found to be more sensitive to increasing concentrations of Cu. Superoxide dismutase activity increased in TS-462 but declined in TS-520 when exposed to higher Cu concentrations. A sharp increase in the activity of ascorbate peroxidase was noticed at the 10 days of exposure in the more tolerant cultivar. On the other hand, catalase levels increased only marginally

Table 2.1 Enzymatic and nonenzymatic response of some medicinal plants toward abiotic stresses

No.	Plant	Stress	Site	Enzymatic effects	Non-enzymatic effects	References
1.	*Ocimum basilicum* L.	Water	Leaf	CAT ↑	–	Heidari and Golpayegani (2012)
				GPX ↑		
				APX ↓		
3.	*Urtica dioica* L.	Pb	Leaf and root	–	–	Gjorgieva et al. (2013)
6.	*Olea europaea* L.	Temperature + radiation	Leaf and root	SOD ↓	Phenols	Sofo et al. (2004)
				CAT ↓		
				APX ↓		
				POD ↓		
				LOX ↓		
7.	*Plantago ovata* Forsk.	Salt	Leaf	SOD ↑	–	Kala (2015)
				CAT ↑		
				POD ↑		
8.	*Plantago maritima*	Salt	Leaf	SOD ↑	–	Sekmen et al. (2007)
				CAT ↑		
				GR ↑		
				APX ↑		
9.	*Plantago media*	Salt		SOD ↓	–	Sekmen et al. (2007)
				CAT ↓		
				GR ↓		
				APX ↑		
10.	*Plantago crassifolia*	Salt	Leaf	–	Proline ↑	Al Hassan et al. (2016)
					GB ↑	
					Sorbitol ↑	
11.	*Plantago coronopus*	Salt	Leaf	–	Proline ↑	Al Hassan et al. (2016)
					GB ↑	
					Sorbitol ↑	
12.	*Plantago major*	Salt	Leaf	–	Proline –	Al Hassan et al. (2016)
					GB ↑	
					Sorbitol ↑	
13.	*Scutellaria baicalensis* L.	Temperature	Leaf	PAL ↓	Baicalin ↓	Yuan et al. (2011)
				CAT ↓	Baicalein ↓	
				SOD ↓		
				POD ↓		

(continued)

Table 2.1 (continued)

No.	Plant	Stress	Site	Enzymatic effects	Non-enzymatic effects	References
		Hg	Leaf and root	SOD_L ↑	$NPSH_L$ ↑	Calgaroto et al. (2010)
				SOD_R ↑	$NPSH_R$ ↑	
				CAT_L ↑	AsA_L ↑	
				CAT_R –	AsA_R ↑	
				APX_L ↑	Carotenoids–	
				APX_R ↓	$Proline_L$ ↑	
					$Proline_R$ ↓	
19.	*Trifolium resupinatum* L.	SO_2	Leaf	SOD	–	Bayat et al. (2014)
				CAT ↑		
				GPX ↑		
20.	*Thymus vulgaris* L.	Drought	Leaf	–	Phenols ↓	Khosh-Khui et al. (2012)
		Temperature + extraction	Leaf	–	–	Hossain et al. (2013)
25.	*Catharanthus roseus* (L.) G. Don.	Salt	Leaf	POD ↓	AsA ↑	Jaleel et al. (2007, 2008) and Amirjani (2015)
				SOD ↓	Glutathione ↑	
				PPO ↓		
				CAT ↑		
				GR ↑		
				POD ↑		

"↑" – increased activity
"↓" – decreased activity

in both the cultivars. Exposure to Cu resulted in accumulation of products of lipid peroxidation in the leaves. The level of thiobarbituric acid reactive substances (TBARS) increased steadily with Cu concentration and time of exposure in both cultivars up to 7 days beyond which the rate of increase declined. Aluminum exposure also caused an increase in SOD activity in cultured tea cells (Ghanati et al. 2005; Saha et al. 2012).

Seedlings of *Camellia sinensis* were grown hydroponically in order to study the effect of fluorine (F) on growth parameters, antioxidant defense system, photosynthesis, and leaf ultrastructure. Fresh and dry mass, chlorophyll (Chl) content, and net photosynthetic rate (PN) decreased with increasing F concentration. Superoxide dismutase activity decreased significantly, and catalase and guaiacol peroxidase activities reached maximum under F stress. Proline, malondialdehyde, and hydrogen peroxide contents increased significantly. These results suggested that antioxidant defense system of leaves did not sufficiently scavenge excessive reactive oxygen species. The cell ultrastructure was not changed under low F stress; however, it was destroyed at high F stress (Li et al. 2011).

Seedlings of *Camellia sinensis* (L.) were studied for the effect of aluminum (Al) on leaf antioxidant defense system and cell ultrastructure. It was seen that

malondialdehyde content decreased at low Al concentration but increased at high Al concentration. Hydrogen peroxide content increased at high Al dose, and no differences were observed at low Al dose. Superoxide dismutase activity remained practically constant at low Al concentration but increased sharply at high Al concentration. Catalase and guaiacol peroxidase activities decreased following an initial increase, reaching their peaks at low Al dose. Ascorbate peroxidase activity increased and glutathione level fluctuated with increasing Al concentrations. Transmission electron microscope analysis of Al-treated leaves showed that although cell ultrastructural integrity was maintained at low concentration of Al, significant membrane damage was observed at high concentration (Li et al. 2011).

A possible connection between the effects of aluminum (Al) on the growth of tea plants and the active oxygen species scavenging system in root tips of intact tea plants and suspension-cultured tea cells was examined. Compared with the control, the activities of superoxide dismutase, catalase, and ascorbate peroxidase increased by Al both in roots of intact plants and cultured cells. The level of lipid peroxidation of membrane, the activity of membrane bound peroxidase, and the content of lignin and cell wall-bound phenols were reduced by the treatment with Al in cultured tea cells (Ghanati et al. 2005).

Olea europaea **L.** The effects of water recovery on the activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, polyphenol oxidase, and lipoxygenase and on malondialdehyde levels were investigated in 2-year-old *Olea europaea* L. (cv. "Coratina") plants grown in environmental conditions characterized by high temperatures and irradiance levels and gradually subjected to a controlled water deficit. After reaching the maximum level of water stress, plants were subjected to a rewatering treatment for 30 days, under both environmental irradiance and semi-shade conditions.

The activities of SOD, CAT, APX, POD and LOX, and MDA levels decreased during the rewatering period in both leaves and roots, and these decrements were faster in plants rewatered in semi-shade conditions (SHP) than in plants under environmental light (NSHP). In contrast, PPO activity increased during rewatering in both leaf and root tissues. Thus, the lower expression of the enzymatic antioxidant system in SHP with respect to NSHP may be due to a reduced need of activated oxygen species removal (Sofo et al. 2004).

Since SOD and APX are the main antioxidant enzymes of chloroplasts (Alscher et al. 2002; Mehlhorn et al. 1996), the more marked reduction of SOD and APX activities in SHP with respect to that in NSHP at the same rewatering level suggested that lower PPFD levels induce a different response of olive tree to oxidative stress because in semi-shade conditions, the need of antioxidant defenses is reduced. Different POD isoforms have a higher affinity for H_2O_2 if compared with CAT but require some phenolic compounds (e.g., guaiacol) as substrates (Mehlhorn et al. 1996; Sofo et al. 2004).

***Plantago* spp.** Activity of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase in leaves of isabgol (*Plantago ovata* Forsk.) was studied under

salt stress. Salt stress caused significant increase in the activity of superoxide dismutase, catalase, and peroxidase in the leaves of isabgol. All the isabgol genotypes responded differently with respect to CAT and POD activity under salt stress. At biochemical level, SOD, CAT, and POD are major antioxidant enzymes associated with scavenging reactive oxygen species (ROS), and SOD is likely to be central in the defense against toxic ROS (Marschner 1995). However, SOD detoxifies superoxide anion free radicals accompanying the formation of H_2O_2 which is very damaging to the chloroplasts, nucleic acids, and proteins and can be eliminated by CAT and POD (Marschner 1995). An increase in the antioxidant enzymes under salt stresses could be indicative of an increased production of ROS and buildup of a protective mechanism to reduce oxidative damage triggered by stress in plants. Catalase in peroxisomes breaks down H_2O_2. Peroxidase in cytosol and chloroplast can perfectly scavenge H_2O_2 (Kala 2015). Increase of peroxidase activity by salt treatment in plants has also been reported by Kahrizi et al. (2012).

Effect of drought and salinity on growth, development, and yield of isabgol HI-5 has been investigated by Surekha (1997) and Varshney and Surekha (2001). Vandana (2003) screened five isabgol genotypes, viz., HI-5, HI-34, HI-96, GI-2, and PB-80, for salt tolerance. Among these genotypes GI-2 and HI-96 were found salt tolerant while PB-80 and HI-5 salt-sensitive on the basis of growth, development, and yield parameters (Kala 2015).

Salt-tolerant *Plantago maritima* and salt-sensitive *Plantago media* were studied for plant growth, relative water content, stomatal conductance, lipid peroxidation, and antioxidant system in relation under salt stress. Reduction in shoot length was higher in *P. media* than in *P. maritima*, and shoot dry weight decreased in *P. media* and did not change in *P. maritima*. There was reduction in RWC and stomatal conductance in *P. media*, whereas no effect was seen on leaf RWC in *P. maritima*, and negligible reduction in stomatal conductance was observed. Activities of superoxide dismutase, catalase, and glutathione reductase decreased in *P. media* with increasing salinity. Ascorbate peroxidase activity in leaves of *P. media* was increased. However, activities of CAT, APX, and GR increased at high concentration of NaCl, while their activities did not change at low concentrations in *P. maritima*. SOD activity in leaves of *P. maritima* increased with increasing salinity. Concomitant with this, four SOD activity bands were identified in leaves of *P. maritima*; two bands only were observed in *P. media*. Peroxidase activity increased under both salt concentrations in *P. maritima* but only at lower concentrations in *P. media*. Confirming this, five POX activity bands were identified in leaves of *P. maritima*, but only two bands were determined in *P. media*. Malondialdehyde levels in the leaves increased under salt stress in *P. media* but showed no change and decreased in *P. maritima* (Sekmen et al. 2007).

Three *Plantago* species, *P. crassifolia* and *P. coronopus* both halophytes and *P. major* (salt-sensitive), were studied for salt stress. It was seen that *P. major* was quite resistant to salt stress on the basis of growth parameters. Salt-treated plants of the three taxa accumulated Na^+ and Cl^- in response to increasing NaCl concentrations to a lesser extent in *P. major* than in the halophytes. In the halophytes, K^+

concentration decreased at moderate salinity levels but increased at high salt conditions, whereas in *P. major* K^+ contents were reduced at NaCl stress.

The content of the common osmolytes in plants – proline (Pro), glycine betaine (GB), and total soluble sugars (TSS) – was evaluated in leaves treatments with salt. Pro contents showed no significant increase in controls, but stronger salt stress conditions induced the accumulation of this osmolyte in *P. crassifolia*; the induction of Pro biosynthesis was even stronger in *P. coronopus*, while no increase over the control was detected in *P. major* under the same conditions. In the case of GB, it accumulated in the leaves of *Plantago* plants to maximum concentrations in *P. crassifolia* and *P. major*, and it did not increase significantly at higher salt concentrations in *P. coronopus*. Total soluble sugar levels decreased moderately in *P. coronopus* and *P. crassifolia* with increasing NaCl concentrations, while no significant change was detected in *P. major*. In the presence of NaCl, sorbitol levels increased in leaves of all tested *Plantago* species in a concentration-dependent manner (Al Hassan et al. 2016).

***Scutellaria baicalensis* L.** *Scutellaria baicalensis* is a traditional Chinese medicinal plant, but increasing average annual temperatures have made plants unsuitable for medicinal use. Two flavones, baicalin and baicalein, are the major active ingredients of *S. baicalensis*. It was demonstrated that protracted heat treatment inhibited the accumulation of baicalin and baicalein as well as the activity of phenylalanine ammonia-lyase (PAL). PAL is involved in the phenylpropanoid pathway, which produces baicalin in the plant.

Heat treatment also affected the activities of the antioxidant enzymes such as catalase, superoxide dismutase, and peroxidase. Cells continued growing during the protracted heat stress. Long-term exposure to high temperatures did not affect *S. baicalensis* cell growth but inhibited flavonoid biosynthesis and reduced the content of baicalin and baicalein. These two compounds play important roles in the balance between ROS and antioxidant enzyme activities in adaptive responses to high heat (Yuan et al. 2011).

***Glycyrrhiza uralensis* L.** *Glycyrrhiza uralensis* seeds were germinated and grown with different concentrations of cadmium acetate, in order to investigate the effects of cadmium on the growth, uptake, SOD, POD, CAT, PPO, and PAL activities in *Glycyrrhiza uralensis* seedlings. Results suggested that increased cadmium concentrations lead to decreased shoot elongation and seedling biomass. SOD activity in the cotyledons, hypocotyls, and radicles increased gradually. POD activity in the cotyledons, hypocotyls, and radicles concentrations increased continuously with rising cadmium concentrations. CAT activity in the cotyledons, hypocotyls, and radicles increased gradually with increasing cadmium concentrations. PPO activity showed significant increases in the cotyledons, hypocotyls, and radicles. A significant change of PAL activity in the cotyledons, hypocotyls, and radicles was observed with increasing cadmium concentrations (Zheng et al. 2010).

***Pfaffia glomerata* L.** The role of the antioxidant enzymes in adaptive responses of the accumulator *P. glomerata* species under cadmium (Cd) stress was studied. The lipid peroxidation rates in leaves and roots were smaller at the start of the experiment for all Cd levels. SOD activity increased in leaves and in roots as Cd levels increased. Cd stress induced an increase in the activity of APX in leaves, whereas in roots APX activity was reduced at high concentration of Cd. At the end of the experiment, CAT activity in leaves was reduced as Cd concentration increased. Nevertheless, the GR and GPX activities increased. In roots, GR activity was reduced (Marques and Soares 2011).

Oxidative stress caused by mercury (Hg) was investigated in *Pfaffia glomerata* plantlets. Accumulation of Hg in tissue increased with increasing concentration of Hg. Root and shoot fresh weight and delta-ALA-D activity were significantly decreased, and chlorophyll and carotenoid concentrations were not affected at high concentration of Hg. H_2O_2 concentration in shoot increased curvilin early with higher level of mercury, whereas lipid peroxidation increased in roots and shoots. SOD activity showed a straight correlation with H_2O_2 concentration, whereas CAT activity increased in shoots. Shoot APX activity was either decreased at low Hg concentration or increased at high Hg concentration. Conversely, root APX activity was only increased at low Hg concentration. In general, AsA, non-protein thiols (NPSH), and proline concentrations increased upon addition of Hg, with the exception of proline in roots, which decreased (Calgaroto et al. 2010).

***Bacopa monnieri* L.** *Bacopa monnieri* L. plants were exposed to cadmium (Cd) stress and analyzed for the accumulation of metal and its influence on various enzymatic and nonenzymatic antioxidants, TBARS, photosynthetic pigments, and protein content. The accumulation of Cd was found to be increased with increasing concentration and time duration, and more Cd was accumulated in the root. TBARS content of the roots and leaves increased with increase in Cd concentration and exposure time. Enhancement in the activities of SOD, APX, and GPX was recorded in stressed roots and leaves of *B. monnieri*. A considerable decrease in CAT activity in Cd-treated *B. monnieri* was seen. There is an increase in the cysteine amino acid and non-protein thiol contents of the roots in *B. monnieri* followed by a decline but in leaves, cysteine and non-protein thiol contents were found to be enhanced at all the Cd concentrations and exposure periods. A significant reduction in the level of ascorbic acid, total chlorophyll, and protein contents was observed. *B. monnieri* was able to combat metal-induced oxidative injury involving a mechanism of activation of various enzymatic and nonenzymatic antioxidants (Singh 2006).

***Salicornia brachiate* L.** The effect of salinity stress was studied on the activities of antioxidant enzymes and polyphenol content in *S. brachiata*. Polyphenol content was found to increase in *S. brachiata* treated with waste water of tannery. PPO activity of *S. brachiata* was increased and then significantly declined under stress. CAT activity of the control and the experimental plants of *S. brachiata* showed an increase initially and then drastically reduced when compared to the control. The results indicate a decline in CAT activity under extreme salinity, which suggests that

CAT appears not to be an efficient scavenger of H_2O_2 in *S. brachiata*. The activity of SOD increased with increasing concentrations of NaCl during the growth period (Santhanakrishnan et al. 2014).

***Zygophyllum* Species** Two *Zygophyllum* species (*Z. album* and *Z. coccineum*) were grown, and the effects of soil heavy metal stress on shoot heavy metal concentrations, lipid peroxidation, antioxidant enzyme activities, and the root plasma membrane (PM) lipid composition were analyzed. Heavy metal concentrations and lipid peroxidation increased in the shoot of both species grown in the polluted area. The activities of ascorbate oxidase (ASO), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), and superoxide dismutase (SOD) were increased, whereas those of catalase (CAT) were decreased in both species under the polluted conditions. PM total lipids, phospholipids, glycolipids, and sterols were decreased in *Z. album* and *Z. coccineum* as a result of the polluted soil. Heavy metal stress increased phosphatidylethanolamine (PE) and decreased phosphatidylinositol (PI) and phosphatidylglycerol (PG), with no significant change in phosphatidylcholine (PC) in the root PM of both species. Phosphatidylserine (PS) decreased in the PM of *Z. album*, whereas it increased in the PM of *Z. coccineum* under the pollution conditions. Heavy metal stress changed the composition and concentration of fatty acids of the root PM, resulting in increased saturated/unsaturated ratio of both species (Morsy et al. 2012).

***Trifolium resupinatum* L.** Different concentrations of SO_2 had a significant effect on Persian clover root weight and antioxidant system. Increasing SO_2 stress decreased root fresh and dry weight and antioxidant capacities (IC_{50}) and increased antioxidant activities (I%) of Persian clover leaves significantly in comparison to the control plants (under 0 ppm) and increased SOD, CAT, and GPX activity. Inoculation of Persian clover plants with native and standard *Rhizobium* increased root weight and did not show a significant effect on antioxidants activity and capacity, but interaction between *Rhizobium* inoculation and SO_2 treatment reduced significantly the stress effects of high concentration of SO_2 on root growth and antioxidants activity and capacity. In fact, level of this change of root growth and antioxidant system under SO_2 pollution stress in inoculated plants was lower than in the non-inoculated plants. As a result, an increase in SO_2 concentration caused a decrease in root weight and increase in antioxidants activity and capacity of Persian clover. Inoculation with *Rhizobium* strains could alleviate the effect of SO_2 pollution on antioxidant system by effects on root growth (Bayat et al. 2014).

***Thymus vulgaris* L.** In this study, a pot experiment was conducted to assess the effect of drought on the antioxidant activity of thyme. The FRAP (ferric reducing antioxidant power) and DPPH (2,2-diphenylpicrylhydrazyl) scavenging assays. Thus results indicated that severe water stress significantly decreased the antioxidant activity of thyme. A higher value of IC_{50} showed a lower antioxidant activity, which indicated that severe water stress significantly decreased the antioxidant activity of thyme. FRAP values showed a trend to reduction by increasing the

irrigation intervals, but this trend was not significant. FRAP value and total phenolic contents of samples were decreased with increase in the duration of irrigation intervals, but the differences were not significant. Control showed higher values and 8-day interval had lower values. The results of the DPPH assay showed that with water deficit conditions, IC50 values of samples were increased significantly. IC50 values were increased from control to severe stress condition (Khosh-Khui et al. 2012).

The effect of temperature and extraction process on the antioxidant activity of various organic crude extracts from the leaves of *Thymus vulgaris* species native to Sultanate of Oman was evaluated. The antioxidant activity of different crude extracts from both extraction methods was measured by DPPH with modification. By Soxhlet extraction method, the activity result found in butanol crude extracts was highest and the lowest in hexane crude extract shown in the following order: butanol>methanol>ethyl acetate extract>chloroform>hexane extract. However, by maceration method, the activity was highest in ethyl acetate and lowest in chloroform: ethyl acetate>methanol extract>butanol>hexane >chloroform (Rahman 2013).

***Elodea* (Egeria) *densa* Planch** *Elodea* plants were incubated in the presence of individual and mixed sulfate salts of Ni, Cd, Cu, Zn, and Mn to study the influence of heavy metals (HM) on shoot growth, structural and functional parameters of the photosynthetic apparatus, lipid peroxidation, enzymatic activities of the antioxidant defense system (superoxide dismutase and catalase), and the content of non-protein (NPSH) and protein thiols (PSH) in leaves.

The accumulation of HM in leaves decreased in a row: Mn>Cu>Cd>Zn>Ni. The largest reduction in chlorophyll content was caused by Mn and Cu, whereas the strongest reduction in carotenoid content was induced by Cu. The presence of Cu produced the largest decrease in the maximal quantum efficiency of photosystem II (PSII) (Fv/Fm). The presence of Cd elevated the content of chlorophyll and carotenoids without altering the photochemical efficiency of PSII; Cd retarded the shoot growth but had no appreciable effect on leaf mesostructure. The addition of the second metal to the growth medium alleviated in most treatments the detrimental action of individual ions owing to the enhanced activities of SOD and catalase and because of the significant increase in the content of NPSH. It is supposed that the observed antagonism of metal ions is related to their competitive interactions restricting the entry of HM into the cell.

The chloroplast dimensions in elodea cells showed no uniform change under the action of HM. The addition of Ni caused a significant reduction of chloroplast volume (more than a 2.5-fold decrease compared to control values). A similar effect was noted under combined application of all metals examined (the reduction by 1.4 times). On the other hand, the long-term exposure (68 days) of plants to Cd or Cd + Ni induced the reliable increase in the chloroplast volume, which was likely caused by the chloroplast swelling. It is not excluded that the presence of Cd resulted in partial destruction of chlorophyll, which was evident from the pale leaf color.

The addition of Ni, Zn, and Mn to the growth medium elevated SOD activity by 20% on the average compared to control values. Under combined application of most metals, SOD activity was substantially higher, especially in the presence of Ni + Cd, Ni + Zn, and Ni + Mn combinations. The heavy metals examined had little influence on CAT activity when applied individually. However, the combined application of two metals enhanced CAT activity by 1.5–2.0 times, with an exception of Mn + Cd and Mn + Cu combinations.

Synthesis of NPSH and PSH is a known means of plant protection against deleterious action of HM. The content of non-protein thiols increased significantly in the presence of individual HM and their combinations. The largest increase was observed in the presence of metal pair Mn + Cu. Non-protein thiols, reduced glutathione (GSH) in particular, play an active role in membrane protection against free radical damage (Maleva et al. 2012).

***Jatropha curcas* L.** In the present study, the effects of aluminum (Al) concentrations on growth, superoxide dismutase, peroxidase, catalase, and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings were investigated. It was seen that with the increasing Al concentrations, the biomass of cotyledons increased initially and then decreased, but the biomass of hypocotyls and radicles decreased gradually. Compared to the control, SOD activity in the cotyledons, hypocotyls, and radicles was all enhanced by Al stress. SOD activity in the hypocotyls increased significantly with increasing Al concentrations. The pattern of SOD isoforms was analyzed by native PAGE, and activity staining revealed that at least four SOD isoenzyme bands in the cotyledons, hypocotyls, and radicles were detected, respectively. Al stress significantly affected the POD activity in the cotyledons showing significant increase. On the activity gels, at least six bands in the cotyledons, hypocotyls, and radicles were observed. POD isoenzyme (II and III) in the cotyledons showed an increase in the staining intensities with the increasing of Al concentration. In the hypocotyls and radicles, the main increase in the staining intensities was isoenzyme IV and III, respectively. Compared to control, CAT activities in hypocotyls and radicles were all increased, while in cotyledons, CAT activities were increased first and then decreased with the increasing Al concentration. Compared to the control, the PAL activities were all increased, but the change trends were different. In the cotyledons and radicles, PAL activities were increased first and then decreased with the increasing Al concentration (Ou-yang et al. 2014).

***Ctenanthe setosa* (Rosc.) Eichler** The relationship of the antioxidant enzyme to drought stress tolerance was studied during leaf rolling in the leaf, petiole, and root of *Ctenanthe setosa*. Chlorophyll and carotenoid content and the chlorophyll stability index decreased in the early period of drought stress but increased in later periods, approaching the control level as leaf rolling increased. Relative water content decreased, while the root/shoot ratio increased during drought stress. LPO measured as MDA content also increased and then declined in the same drought period, contrary to photosynthetic pigment content. SOD activity did not significantly change in leaves. In the petiole and root, however, it decreased in the early drought

period but increased later. GR activity did not significantly change in the leaf and petiole versus the control but increased in root. POD activity increased in the leaf and petiole but decreased in the root. A peroxidase isoenzyme activity band present in the control leaves did not appear in leaves exposed to drought, but in the latter periods, that activity increased. Tolerance of drought stress apparently is closely associated with the antioxidant enzyme system as well as leaf rolling in *C. setosa* (Terzi and Kadioglu 2006).

***Cleome gynandra* L.** The effects of heavy metals on antioxidant defense system were studied in *Cleome gynandra* plants. The decreased value of phenolics with increased concentration of heavy metal copper and cadmium shows that phenol form chelation with metal and thereby reduce the toxicity of the plant during accumulation of the metal. Antioxidant activity was found to be maximum in the plant exposed to control soil, while free radical scavenging activity was reduced much in the plant exposed to heavy metal-contaminated soils. The proline value was considerably increased with increased concentration of copper and cadmium. Superoxide dismutase, catalase, and glutathione were increased significantly in the plant sample exposed to heavy metal-contaminated soils. Among the two metals, cadmium affects the plant to a greater extent than copper (Haribabu and Sudha 2011).

***Catharanthus roseus* (L.) G. Don.** *Catharanthus roseus* (L.) G. Don. was studied for salinity stress, and the ability of triadimefon (TDM), a triazole group of fungicide, to ameliorate the stress was also studied. There was decreased overall growth of this plant and reduced chlorophyll content, protein, and antioxidant enzymes such as POX, SOD, and PPO. The root alkaloid ajmalicine increased under salt treatment. When these stressed plants were treated with TDM, it minimized the injurious effects of NaCl stress by increasing the root and shoot growth, leaf area, dry weight (DW), chlorophyll and protein contents, and the activities of antioxidant enzymes like POD, SOD, and PPO. The quantity of ajmalicine was also increased with the TDM treatment when compared to both control and NaCl-treated plants (Jaleel et al. 2008).

Antioxidant responses were analyzed in *Catharanthus roseus* under salt stress in order to investigate the plant's protective mechanisms against long-term salt-induced oxidative stress. High salinity caused a decrease in reduced glutathione and an enhancement in total ascorbate content and the antioxidant enzyme and ascorbate peroxidase activities. Moreover, salinity induced a significant decline in superoxide dismutase and peroxidase activities. The changes found in catalase activities may be of great importance in the H_2O_2 detoxification mechanism under oxidative stress (Jaleel et al. 2007).

The effect of responses of *Catharanthus roseus* to NaCl stress has been explored. The plants were exposed to different concentrations of salt and the effect of treatment on germination, growth parameter, and antioxidant defense system investigated. Increasing the NaCl concentration reduced germination percentage, and the fresh and dry weights of treated plants also showed a decrease. Ascorbic acid

content increased in the presence of stress, and glutathione concentration showed a significant increase. NaCl caused a significant decrease of SOD activity and enhanced the activities of catalase, peroxidase, and glutathione reductase. The MDA content increased with the increasing concentrations of NaCl. MDA content of samples treated by NaCl also increased (Amirjani 2015).

2.1.4 Medicinal Plants and Their Antioxidant Properties

In addition to providing defense, plants have long been a source of exogenous (i.e., dietary) antioxidants. It is believed that two-thirds of the world's plant species have medicinal importance, and almost all of these have excellent antioxidant potential (Krishnaiah et al. 2011). The in vitro evaluation of antioxidant activity of medicinal plants or their phytochemicals several biochemical tests have been used. In ethanopharmacological and nutraceutical investigations, these assays are done to understand the probable mechanism of action of plant antioxidants (Antolovich et al. 2002) in minimizing the oxidative stress linked pathophysiology of diseases. There are several in vitro assays used to measure and confer antioxidant activity to plants; however, each of these has its own limitations regarding applicability. In these assays, plants are generally assessed for their function as reducing agents, hydrogen donors, singlet oxygen quenchers, or metal chelators, after which they are classified as primary (chain-breaking) and secondary (preventive) antioxidants. Primary antioxidants act by donating a hydrogen atom, while secondary antioxidants function via binding of metal ions capable of catalyzing oxidative processes and scavenging oxygen, absorbing UV radiation, inhibiting enzymes, or decomposing hydroperoxides (Kasote 2013).

Since time immemorial, plants have been a source of food and medicines, either in the form of traditional preparations or as pure active principles (Hegde et al. 2014). Most of the medicinal effects of plants have been attributed to their potent antioxidant activity. It has been suggested that free radicals are involved in the pathology of more than 50 human diseases, including aging (Halliwell 1991). Plants are rich storehouse of secondary metabolites, and the complex diversity of these metabolites makes them fascinating candidates for study. Plant antioxidants such as ascorbic acid and flavonoids have been shown to be the best exogenous antioxidants. Indeed, these compounds not only restrain ROS production by scavenging free radicals but also help boost endogenous antioxidant defenses of the body (Halliwell 2006).

The chemical structure of polyphenols is responsible for its antioxidant potential as they determine the conjugation reactions with methyl, sulfate, or glucuronide groups (Scalbert and Williamson 2000). Flavonoids are the most important and abundant dietary polyphenols, with over 5000 reported to date (Ross and Kasum 2002; Dai and Mumper 2010). In addition to their remarkable antioxidant property, polyphenols have pro-oxidant properties also.

2.2 Conclusion

Plants are exposed to harsh climatic and environmental conditions which lead to stress. Abiotic stresses overproduce ROS in plants which are highly unstable and toxic to the cells and leads to oxidative damage. Manageable amounts of ROS are produced during normal metabolic processes, but excessive amounts damage nucleic acids, lipids, and proteins, causing them to lose their activity. Since plants are sessile, they need to be equipped with excellent antioxidant defense mechanisms to detoxify the harmful effects of ROS. The antioxidant defenses could be either enzymatic (e.g., superoxide dismutase, catalase, peroxidases, and glutathione reductase) or nonenzymatic (e.g., glutathione, glycine betaine, proline, α-tocopherols, phenols, carotenoids, and flavonoids).

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