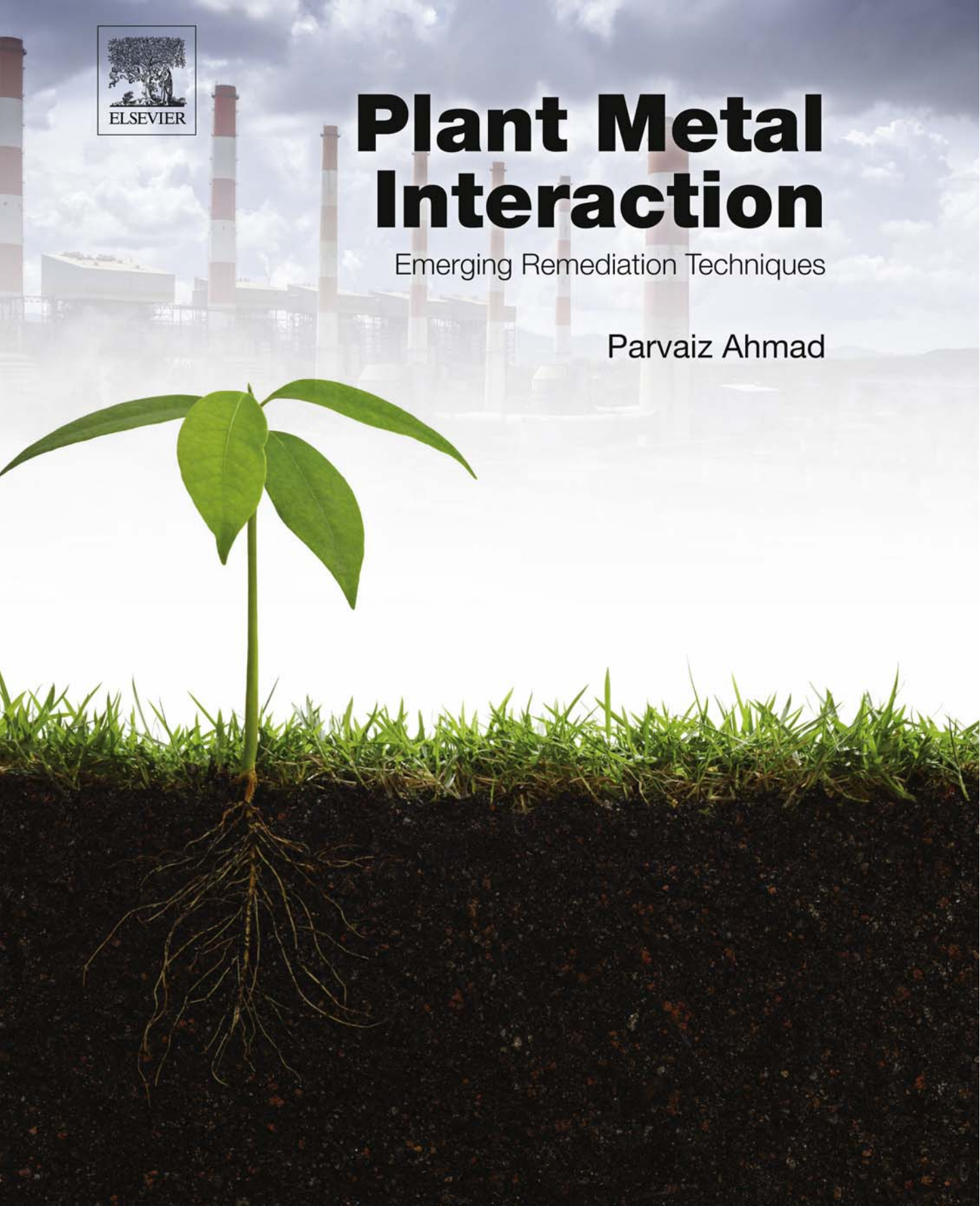

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Plant Metal Interaction
Emerging Remediation Techniques
Parvaiz Ahmad

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Emerging Remediation Techniques

Edited by

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CHAPTER

COPPER STRESS AND RESPONSES IN PLANTS

2

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1. INTRODUCTION

Copper (Cu) is an abundant essential micronutrient element in various rocks and minerals and is required for a variety of metabolic processes in both prokaryotes and eukaryotes (Sun et al., 2014). Much of copper contains enzymes functioning as oxygen carriers (hemocyanin) or redox catalyzer (cytochrome oxidase, nitrate reductase) (Whitacre, 2011; Ferreira et al., 2015). Copper is a transition

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metal with three valence states: Cu°, Cu^{+1}, and Cu^{+2}. In addition, it has been classified as a heavy metal because of its the heavier density (5 g/cm^3) (Singh et al., 2011).

Cu, however, is an essential micronutrient for normal plant metabolism and plays a role in several physiological processes such as photosynthesis, respiration, carbohydrate distribution, and protein metabolism. However, excess Cu can disturb normal development by adversely affecting biochemical reactions and physiological processes in plants (Guzel and Terzi, 2013). We will discuss the effects of copper on plants and their responses to copper stress in this chapter and address how plants cope with Cu stress and what kinds of changes occur in plant morphology, physiology, and metabolism.

2. EXCESS COPPER IS TOXIC TO PLANTS

Chlorosis in leaves is one of the common initial symptoms of copper toxicity (Verma and Bhatia, 2014). The other is stunted root growth, which includes poor development, reduced branching, thickening, and dark coloration (Nair and Chung, 2015). Chlorosis usually causes cream- or white-colored spots or lesions (Lee et al., 1996). Increased copper toxicity results in forming necrotic areas in leaf tips and edges (Taylor and Foy, 1985). Leaves can wilt under excess copper before necrotic areas are formed (Yau et al., 1991).

Cu^{+} and Cu^{2+} ions block photosynthetic electron transport by inhibiting primary quinone acceptor QA, and the secondary quinone acceptor QB thereby maximal quantum yield of photosystem II (PSII) photochemistry is reduced (Zlobin et al., 2014). Excess heavy metals often change membrane permeability by causing leakage of K^{+} and other important ions. It has been reported that Cu^{2+} inhibited in vitro H^{+} transport through the plasmalemma in cucumber roots; however, the transport was induced in corn roots (Barker and Pilbeam, 2014).

3. EFFECT OF COPPER STRESS ON PLANT GROWTH

Excess Cu can affect important physiological processes in plants and cause problems in plant growth and development. Cu taken from the soil must be transported, distributed, and compartmentalized within different tissues and organelles for healthy plant growth and development (Habiba et al., 2015). On the other hand, excessive Cu is characterized by a reduced plant biomass, leaf chlorosis, inhibited root growth, bronzing, and necrosis. The effect of Cu toxicity is largely on root growth and morphology, which has particular importance for the whole plant. Because water and nutrients enter plants by the roots, any defect or malformation of the roots creates problems for plant growth and development. Copper tends to accumulate in the root tissue with little translocated to the shoots (Marschner, 2011). Retarded root growth of *Festuca arudinacea* and *Lolium perenne* plants was observed after 7 days of copper application (Zhao et al., 2010). Kolbert et al. (2012) reported that long-term excess copper leads to inhibition of stem and root development of *Arabidopsis thaliana*. Cell elongation, division, and expansion were also affected. Altered auxin homeostasis in both organs was found to be a cause of the growth alterations because of elevated nitric oxide (NO) production that inhibited PIN1-mediated auxin transport.

Liu et al. (2014) reported that a continuous decrease in maize root activities, and physiological disorders were observed during increased copper stress, which resulted in root cell membrane damage. Intracellular ion and organic substance leaked after Cu^{2+} entered root cells.

Excess copper inhibits leaf expansion, cell elongation, and cell division (Maksymiec, 1997; Panou-Filotheou and Bosabalidis, 2004). Long exposure to copper stress causes folding up of the leaf blade, increased numbers of hair on the lower surface of leaf blades, increased numbers of stomata and decreased stomata sizes, and reduced volume of mesophyll intercellular spaces. All these changes in the leaf anatomy and morphology are disorders from exposure of roots to copper stress that causes decreased transpiration (Panou-Filotheou et al., 2001).

Leaf chlorosis is a typical symptom of plants subjected to high soil Cu concentrations (Miotto et al., 2014). During leaf chlorosis, some morphological and physiological alterations take place in the chloroplasts. Mesophyll chloroplasts in Cu-treated oregano plants are reduced in volume and number (Panou-Filotheou et al., 2001). This observation is in accordance with those of other plants under Cu stress, such as rice (Lidon and Henriques, 1993), beans (Maksymiec et al., 1994), and wheat (Quartacci et al., 2000). Sanchez-Pardo et al. (2014) reported that under copper stress, the structure of the thylakoids in white lupin and soybean showed changes. Disintegration of chloroplast membranes, degradation of grana stacking, swelling of the thylakoids, and increased numbers of plastoglobuli were also observed in soybean plants.

4. EFFECTS OF COPPER STRESS ON PHOTOSYNTHESIS

Increasing levels of copper in the environment affects photosynthesis adversely. The main effects of copper on photosynthesis are related to changes in pigment compositions and ultrastructure of chloroplast, decreased net photosynthesis rate, reduced ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) efficiency, and inhibition of electron transport and PSII activities (Aggarwal et al., 2012).

4.1 ULTRASTRUCTURAL CHANGES IN CHLOROPLASTS

Excess Cu adversely affects architecture, lipid, and pigment composition of thylakoid membranes and causes decreased photochemical activity of PSII (Maksymiec et al., 1995; Xu et al., 2013). Swollen thylakoids and an increase in the number of plastoglobuli have been observed in soybean plants (Sanchez-Pardo et al., 2014). Similar effects have been reported in spinach and wheat leaves after Cu application (Eleftheriou and Karataglis, 1989). The leaves of *Theobroma cacao* seedlings exposed to higher levels of Cu showed damaging effects on the thylakoid and chloroplast membranes. Absence of starch grains and swelling of chloroplast double membranes were also reported (Souza et al., 2014).

One of structural components of thylakoid membranes, acyl lipid levels, is reduced by copper stress. Significant decreases in monogalactosyl diacylglycerol (MGDG), sulfoquinovosyl diacylglycerol, phosphatidyl glycerol, and phosphatidyl choline (PC) have been observed in runner bean plants subjected to copper stress (Maksymiec et al., 1994). The content of digalactosyl diacylglycerols, MGDG, and phosphatidyl glycerol in chloroplasts of the *Hydrilla verticillata* plant increased after an hour of copper exposure (100 μM $Cu(NO_3)_2$). The content of all the lipids except phosphatidic acid and PC have decreased after 3 h of exposure (Rozentsvet et al., 2012). Decrease in acyl lipid content has been shown to result in impaired photosynthesis. Reductions in phosphatidyl glycerol and PC content under excess copper have disturbed grana stacking (Krupa, 1988). Changes in chloroplast ultrastructure are associated with decreased MGDG content. Its low level disturbs organization of PSII and thus PSII activity is reduced (Murata et al., 1990). Fatty acid unsaturation and lipid peroxidation because of copper stress reduces MGDG content, which result in inhibition of PSII (Quartacci et al.,

2000). Lipid peroxidation shows to disturbing lipid–protein–pigment complexes of chloroplasts (Panda and Panda, 2009). Declines in chlorophyll and carotenoid contents has been observed in *Elsholtzia splendens* plants subjected to excess copper for 8 weeks (Peng et al., 2013). Malec et al. (2009) reported that hydrogen peroxide, superoxide, and hydroxyl radicals, which are formed by Cu toxicity, induces degradation of chlorophyll in Cu-treated plants.

Carotenoids protect chlorophylls from peroxidation (Dall'Osto et al., 2012). When carotenoid levels are decreased by Cu treatment, this gives rise to an increase in chlorophyll destruction. The synthesis of 5-aminolevulinic acid and protochlorophyllide reductase activity are believed to be inhibited by copper (van Assche and Clijsters, 1990). Iron and magnesium deficiency induced by excess Cu inhibits synthesis of protochlorophyllide and phytoene synthesis; therefore, chlorophyll and carotenoid contents are decreased (Vijayarengan and Jose, 2014).

4.2 PHOTOSYSTEM II

The role of Cu in PSII-mediated electron transport is known (Puig, 2014). Cu has been determined in PSII-enriched fraction of spinach chloroplasts (Anderson et al., 1964). Sibbald and Green (1987) reported that about 75% of Cu in PSII preparations from barley and spinach was bound to the major antenna complex of PSII (LHCII). Cu is also involved in the water-splitting system (Orzechowska et al., 2008). On the other hand, high concentrations of copper are toxic to PSII. Extensive in vitro studies have shown that PSII is very susceptible to Cu toxicity (Baron et al., 1995). However, the precise location of the Cu binding site on PSII and the underlying mechanisms of copper inhibition are still the subject of debate. Different sites of copper action in PSII are suggested in the literature. Cu^{2+} ions inhibit both the donor and the acceptor sides of PSII, although the oxidizing side of PSII is the most sensitive site of Cu inhibition (Rouillon et al., 2006) at a reversible inhibition on PSII primary electron carrier donor Tyr_Z or on oxygen evolving complex (Burda et al., 2003). Copper ions also affect PSII electron transport on acceptor side. At higher copper concentrations, target sites of Cu^{2+} are primary quinone acceptor Q_A, pheophytin-Q_A-Fe region, and nonheme iron and secondary quinone acceptor Q_B (Mohanty et al., 1989). On the other hand, oxygen evolution is found to be induced by very low equimolar Cu^{2+}/PSII proportions (Burda et al., 2002). Apart from the oxygen-evolving complex, the level of the photoreduced cyt b_{559} is reduced and its rate of photoreduction is decreased by copper stress (Yruela et al., 1996).

4.3 RuBisCo ACTIVITY

The most abundant protein in plants is RuBisCo (Raven, 2013). RuBisCo is an enzyme that catalyzes the carbon dioxide fixation reaction in photosynthesis forming phosphoglycerate with the reaction of ribulose-1,5-bisphosphate and carbon dioxide, and also catalyzes the photorespiration forming the phosphoglycolate and phosphoglycerate with the reaction to O_2 (Parry et al., 2012). Declined RuBisCo activity has been determined in plants grown with high levels of Cu (Schafer et al., 1992). Carboxylase and oxygenase activities of RuBisCo are inhibited by Cu toxicity (Lidon and Henriques, 1991). This effect appears to be due to a metal-induced interaction with essential cysteine residues of the enzyme (Siborova, 1988). Similarly, the concentration of RuBisCo reaction sites and its carbamylation state decreased considerably in response to Cu stress in *Spartina densiflora* (Mateos-Naranjo et al., 2015). This was most likely related to the reduced concentration of the enzyme instead of its activation status.

Adversely, RuBisCo activity is increased in copper-stressed tobacco plants, whereas RuBisCo content is decreased (Son et al., 2014). Simova-Stoilova et al. (2002) have reported that RuBisCo content is reduced in barley leaves subjected to toxic copper concentrations. This reduction is probably from oxidative stress resulting from forming of reactive oxygen species (ROS) by copper toxicity.

Copper stress also causes accumulation of osmoprotectants. Proline is a well-known and studied amino acid under copper stress. Further section will handle changes in proline metabolism in plants exposed to copper.

5. PROLINE METABOLISM

Plants exposed to excess copper have been shown to accumulate proline in their tissues (Ku et al., 2012). Accumulation of proline is an adaptive response of plants against stresses. Proline is believed to be regulatory or signal molecule activating some physiological and molecular responses (Szabados and Savoure, 2010). Mechanism of proline accumulation is related to increased synthesis, decreased catabolism, or increased degradation of proteins (Kavi Kishor et al., 2005). Proline is synthesized in higher plants from glutamate via reactions, which are catalyzed by pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase. Proline can also be formed by transamination of ornithine into proline-5-carboxylate by ornithine amino transferase. Proline catabolism in plant cells is occurred by proline dehydrogenase (PDH) and pyrroline-5-carboxylate dehydrogenase (Monteoliva et al., 2014).

Increased activity of P5CS and proline accumulation has been reported in copper-stressed tobacco plants grown in pots (Ku et al., 2012). In addition, higher pyrroline-5-carboxylate reductase activity in detached rice leaves exposed to Cu has been observed (Chen et al., 2001). The stimulation of P5CS activity in *Triticum aestivum* plants under excess copper has been reported to be from activation of the P5CS gene (Tripathi et al., 2013). Proline accumulation in *T. aestivum* plants under copper toxicity is mostly from contribution of glutamate-based proline synthesis, because the relatively slower activity of ornithine amino transferase in comparison to P5CS and pyrroline-5-carboxylate reductase activities has been determined in *T. aestivum* leaves under copper excess. The contribution of proline catabolism to proline accumulation in the copper-treated plants has also been shown by some studies assessing PDH activity. Inhibition of PDH activity in the Cu-treated *T. aestivum* plants has been reported (Tripathi et al., 2013). The decreased proline catabolism is suggested to contribute to proline accumulation in various plants (Mani et al., 2002). Similarly, Wen et al. (2013) also reported that hydrogen peroxide (H_2O_2) pretreatment enhanced proline accumulation in sweet corn seedlings under copper stress. The increase in the proline content was related to induction of both ornithine and glutamate pathways and reduced PDH pathway. Copper stress-induced increase in proline and NO levels in *Chlamydomonas reinhardtii* indicated that this intracellular NO generation was involved in Cu-induced proline accumulation and signaling based on increased activity of P5CS and upregulated expression of P5CS gene in Cu-treated algae after sodium nitroprusside (SNP) treatment (Rejeb et al., 2014). Proline is also known to be an ROS scavenger like antioxidant enzymes and nonproteinic antioxidants (ascorbic acid (AsA) and glutathione (GSH)). A well-known result of copper stress is the formation of ROS. Plants are equipped with an antioxidant defense system to cope with ROS. This part of the chapter will discuss the effects of copper on an antioxidant system.

6. ANTIOXIDANT SYSTEM

Copper in excess causes generation of ROS such as superoxide radical ($O^{\cdot -}$), H_2O_2, singlet oxygen ($^1O_2$), and hydroxyl radicals (OH) (Tie et al., 2012; Liu et al., 2015). Antioxidant enzymes such as SOD, peroxidase (POX), and catalase (CAT) are involved in the scavenging of ROS. The SOD catalyzes the dismutation of superoxide to H_2O_2 and molecular oxygen. CAT dismutases H_2O_2 into H_2O and O_2, whereas POX decomposes H_2O_2 by oxidation of cosubstrates, such as phenolic compounds and or antioxidants (Halliwell and Gutteridge, 2007). SOD and POX activities have been studied in copper-stressed turf grass cultivars *Festuca arudinacea* and *Lolium perenne* plants (Zhao et al., 2010). SOD activity is induced by increasing copper concentration in *F. arudinacea* roots. POX activity has increased at low copper levels, however, in *L. perenne,* these enzymes are not induced under copper excess. Authors suggest that tolerance of different turf grass cultivars to copper stress may depend on enhanced antioxidant capacity. Recent studies report that an increase in SOD and ascorbate peroxidase (APX) activities in rice (Thounaojam et al., 2012) and jack bean (Andrade et al., 2010) plants. Induced CAT activity is reported in *Spirodela polyrhiza* (Upadhyay and Panda 2010), *Atriplex halimus* (Brahim and Mohamed, 2011), and jack bean plants (Andrade et al., 2010) under copper stress. Similarly, enhanced levels of gene expressions of CAT (*RsCat*) and SOD (*MnSOD*) in *Withania somnifera* under excess Cu levels have been determined (Rout and Sahoo, 2013). Fidalgo et al. (2013) could not find any significant changes in level of antioxidant gene transcripts in *Solanum nigrum* plants under excess copper levels for 28 days. They concluded that this happened because Cu levels used were below the maximum limit that the plants could tolerate. Long-time exposure to copper results in inhibition of antioxidant enzyme activities. A reduction in SOD, CAT, and APX activities has been determined in *Solanum lycopersicon* plants subjected to prolonged copper treatment (Chamseddine et al., 2009).

AsA and GSH are the most important antioxidant metabolites reacting directly with different ROS and participating in the AsA–GSH cycle (Foyer and Noctor, 2011). It has been reported that the AsA–GSH cycle is involved in response to excess Cu (Yruela, 2005). AsA and GSH levels in maize leaves significantly increased under low copper concentration, then decreased as copper concentrations increased (Tie et al., 2012). Similar results have been reported by Wang et al. (2011) in maize under excess copper levels. Mostofa et al. (2014) found that exogenous SNP and GSH successfully decreased Cu-induced ROS and malondialdehyde levels, providing strong evidence that SNP and GSH substantially protects rice seedlings against oxidative stress by inducing antioxidant enzyme activities.

7. SIGNAL TRANSDUCTION IN RESPONSE TO COPPER

Plant cells possess a complex signal transduction network that is activated whenever any stress is sensed by the receptors at the plasma membrane (DalCorso et al., 2010). When the cells recognize the metal, common signaling pathway elements such as calcium fluxes and ROS, are used. Then, stress-related proteins and signaling molecules are synthetized, and specific metal-response genes that confront the stress are activated transcriptionally (Maksymiec, 2007). Here we discuss signal transduction pathways that respond to copper stress in plants.

7.1 SIGNAL PERCEPTION

A signal perception starts a signal transduction pathway after recognition of heavy metals by the cells. To date, much less knowledge is available about a primary recognition for instance by a receptor. Being a central controller in Cu homeostasis, *Chlamydomonas reinhardtii* copper response regulator Crr1 and its *Arabidopsis* homolog SQUAMOSA promoter-binding protein-like 7 (SPL7) have drawn attention in recent years (Garcia-Molina et al., 2014). As indicated previously, Cu is an essential micronutrient for plants that is necessary for photosynthesis, oxidative stress responses, cell wall lignification, and hormone signaling, etc. (Puig et al., 2007). On the other hand, cellular components such as lipids, proteins, or nucleic acids are damaged because of ROS generation because the plants are subjected to excess free Cu ions (Halliwell and Gutteridge, 1984). To keep Cu levels within a proper range, plants are equipped with a homeostatic network. During Cu deficiency in *Arabidopsis*, SPL7 directly binds to GTAC motif-containing Cu response elements located in the promoter regions of Cu-responsive genes (Yamasaki et al., 2009). Cu-metalloreductases FRO4/5 and Cu transport proteins (COPT)1/2/6 are promoted (Bernal et al., 2012), then SPL7 reconstitutes gene expression to use and redistribute Cu within the plant. Thus, translation of Cu-requiring proteins such as superoxide dismutases and laccases are repressed and Cu is delivered to plastocyanin efficiently (Yamasaki et al., 2009). Just after signal perception, plant hormone signaling pathways are activated. We will review hormone signals and their roles in copper stress responses of plants in forthcoming section.

7.2 STRESS SIGNAL TRANSDUCTION BY PLANT HORMONES

Plants synthesize variety of hormones such as abscisic acid (ABA), brassinosteroids, melatonin, or indole acetic acid (IAA) as they are subjected to copper stress (Vázquez et al., 2013). These plant hormones are related to metal-induced signaling and defense reactions. During copper exposure, an increased shoot emergence and plant biomass production has been observed in mustard plants, which are treated with 24-epi-brassinolide (EBL; 10^{-7}, 10^{-9}, and 10^{-11} M) for 8 h (Sharma and Bhardwaj, 2007). In addition, EBL has blocked Cu uptake and accumulation in the mustard seedlings.

Radish seedlings generate copper resistance after 10^{-7} M EBL treatment on seeds. In addition, root and shoot growth increases (Choudhary et al., 2010). Endogenous IAA and ABA concentrations increase in these EBL-treated radish seedlings. Antioxidant enzyme activities, proline, AsA, total phenol, and phytochelatin contents are enhanced; thus, oxidative stress is relieved. Antioxidant capacity and free radical scavenging activity of EBL-treated radish seedlings have also been studied (Choudhary et al., 2011). EBL treatment improves seedling growth, antioxidant levels, and enzyme (GPOX, CAT, SOD, and GR) activities and free radical scavenging capacities and reduces membrane damage in seedlings.

The role of the combined application of homobrassinolide and H_2O_2 to the leaves of mung bean plants subjected to copper stress has been studied (Fariduddin et al., 2014). The exogenously applied homobrassinolide and/or H_2O_2 alleviate copper toxicity in mung bean and suggest their role in maintaining copper homeostasis in these plants.

In addition, putrescine biosynthesis is enhanced under Cu-stressed wheat leaves (Groppa et al., 2007). The enhancement in putrescine concentration is due to increased activities of ornithine decarboxylase and arginine decarboxylase enzymes. Chen et al. (2013) reported that exogenous

putrescine acted as a chemical messenger, induced several antioxidant enzymes (CAT, APX, and POD) and scavenged H_2O_2 in *Populus cathayana* which was exposed to copper stress.

Coapplication of EBR and Spd is very effective in detoxifying Cu in plants (Choudhary et al., 2012). The combined application of EBL and spermidine on radish seedlings affects gene expressions of phosphatidic acid synthesis enzymes, IAA and ABA metabolism; thus, Cu stress tolerance in radish seedlings is enhanced. Munzuroglu et al. (2008) have recorded significant increases in the ABA content of wheat seeds subjected to copper toxicity. In another study, Zengin and Kirbag (2007) have recorded that copper stress increases the content of the ABA in sunflower seedlings. Increased ABA decreases transpiration; thus, the transport of copper ions from roots to shoot is hindered. Similarly, Ku et al. (2012) have reported that the increase of ABA in tobacco leaves, which is induced by Cu, reduce water transport and downregulate metal absorption efficiency.

Melatonin is one of the hormones that accumulates during stress. Melatonin has roles in multiple plant developmental processes and various stress responses (Tan et al., 2012). Posmyk et al. (2008) have reported that exogenous melatonin application improved copper stress in red cabbage seedlings. Pea plants treated with melatonin survive under copper toxicity compared with nontreated plants (Tan et al., 2007).

Auxin controlling primary root elongation increases formation of lateral roots and the number of root hairs affecting plant responses to metal stresses (Yuan et al., 2013). Copper stress inhibits primary root elongation. Cu-mediated auxin redistribution is responsible for the inhibition of primary root elongation that is mediated by PIN1, but not PIN2 or AUX1 (Yuan et al., 2013). During copper stress, a reduction of the auxin responsive promoter DR5 gene is observed at *A. thaliana* primary root meristems, which are exposed to high copper concentrations. However, DR5 accumulation at just above root apex might cause the formation of lateral roots. This might also be involved in the formation of root hairs in the area adjacent to the Cu^{2+}-treated root tips of *A. thaliana* (Lequeux et al., 2010).

Plants synthesize ethylene, depending on the intensity of heavy metal stresses (Schellingen et al., 2014). Mertens et al. (1999) have reported an increasing ethylene production in 7-day-old *A. thaliana* plants exposed to 25–500 μM Cu for up to 6 h. However, Lequeux et al. (2010) have not observed any effect of 24-h copper stress (50 μM) on ethylene production in *A. thaliana* plants. They suggested that ethylene is not involved in the long-term root architecture reorganization.

In addition, increased ethylene production in 4-week-old wheat and sunflower leaves after 14 h of exposure to 1 mM of Cu has been reported by Groppa et al. (2003). Exposure to high copper concentrations (50–400 mM) for a short period (24 h) differently induces ethylene production in various plant parts of *A. thaliana* (Arteca and Arteca, 2007). Hego et al. (2014) have reported that some proteins involved in ethylene biosynthesis, for example 1-aminocyclopropane-1-carboxylate synthase, ETO-like protein, and S-adenosylmethionine synthetase (SAMS) are overexpressed in *Agrostis capillaris* roots, especially at 5 μM Cu and 1 μM Cu exposures.

Gene expressions of 1-aminocyclopropane-1-carboxylate synthase (ACS) that catalysis production of 1-aminocyclopropane-1-carboxylic acid from S-adenosyl-L-methionine and 1-aminocyclopropane-1-carboxylate oxidase that catalysis conversion of 1-aminocyclopropane-1-carboxylate synthase to ethylene have been studied in *Brassica oleracea* seedlings exposed to copper stress. Expressions of the two important enzymes of ethylene biosynthesis, ACS1 and 1-aminocyclopropane-1-carboxylate oxidase 1, are induced by 2.5 mM of $CuCl_2$ treatment. Therefore copper ions are shown to be a very effective inducer of ethylene biosynthesis (Jakubowicz et al., 2010). Ethylene, together with other plant hormones, may contribute to growth inhibition and malformations of root architecture during

Cu stress. These ethylene-regulated responses are mediated by both ROS accumulation and/or ROS signaling. Plants have antioxidant barriers against ROS attacks. An antioxidant system is known to be induced by ethylene under different heavy metal stresses such as Ni and Zn (Khan and Khan, 2014); however, there is no study about the effects of ethylene on the antioxidant enzymes of copper-stressed plants (Iqbal et al., 2013).

7.3 THE ROLES OF REACTIVE OXYGEN SPECIES

Production of ROS is one of the major consequences of copper accumulation in plants. In addition to causing oxidative stress, ROS can also serve as a signal molecule for plants to survive copper stress. H_2O_2 levels increase in response to Cu and cadmium (Cd) treatment in *A. thaliana* (Maksymiec and Krupa, 2006). This increase in H_2O_2 accumulation changes the redox status of the cell and induces the production of antioxidants and the activation of antioxidant mechanisms. H_2O_2 is known as a signal for APX induction. H_2O_2 induced messenger RNA (mRNA) for cytosolic APX in callus cultures of rice embryos (Morita et al., 1999). In tomato plants under copper excess, Wang et al. (2010) suggested that copper stress induced H_2O_2 and NO generation and that antioxidant enzyme activities were induced by NO to alleviate H_2O_2 overaccumulation. Hence, tolerance to copper stress in tomato plants was developed. Many recent studies have indicated that exogenous NO mitigated effects of toxic Cu (Zhang et al., 2009). Furthermore, exogenous NO was described as an effective substance for alleviation of Cu toxicity in many plants, such as rice, tomato and barley seedlings, and wheat seeds (Yu et al., 2005; Cui et al., 2010; Hu et al., 2015). Various mechanisms were reported to contribute to NO-induced increase of Cu tolerance. For instance, alleviation Cu toxicity was determined to be related to exogenous NO-induced antioxidant system and increased adenosine triphosphatase (ATPase) activities (Zhang et al., 2009). Tewari et al. (2008) reported that NO alleviates Cu toxicity by decreasing oxidative stress in the adventitious roots of *Panax ginseng*. Dong et al. (2014) demonstrated that in the hydroponics experiment, the low concentrations (100 μM) of NO provided the resistance toward Cu and had an ameliorating effect on Cu-stressed ryegrass. Wang et al. (2014) showed that the addition of SNP, a donor of exogenous NO, significantly alleviated photosynthetic inhibition in Cu-stressed tomatoes by recovering photosynthetic pigment content and chlorophyll a fluorescence and the improvement of net photosynthetic rate, stomatal conductance, and transpiration rate.

Mitogen-activated protein kinases (MAPKs) can perceive the changes of ROS content in signal transmission for a range of stresses and the distinct MAPK pathways involve specifically to particular metal ions.

7.4 THE MAPK CASCADE

Plants contain specific metal sensors detecting changes in status of metals (deficiency or excess) and trigger signaling cascades that activate the appropriate responses. Signal transduction pathways in plants about metal stress responses have not been identified yet. MAPKs have been shown to activate in *Medicago sativa* seedlings exposed to toxic copper concentrations (Jonak et al., 2004). MAPK gene (*OsMAPK2*) expression in rice under excess copper has been studied (Hung et al., 2005). Copper treatment induces MAPK signaling in rice (Yeh et al., 2007). In that study, Cu^{2+}-induced 42-kDa MBP kinase activities have been determined in rice roots. Accumulation of *OsMAPK2* was found to be related with ROS, extracellular calcium ions, and cantharidin-sensitive protein

phosphatase(s)–mediated signaling cascades. Smeets et al. (2013) reported that oxidative signal-inducible kinase1 was required for the upregulation of antioxidant enzymes such as catalase in leaves and Fe-superoxide dismutase in *A. thaliana* roots exposed to Cu. These processes are involved in the MEKK1-MKK2-WRKY25 cascade.

In brief, MAPKs activate genes required for stress adaptation. Genes for metal transporters and synthesis of chelating compounds are activated by MAPKs.

7.4.1 Chelating Compounds

Copper in plant tissues is kept in a narrow physiological range for homeostatic equilibrium. In the case of copper toxicity, plants trap free Cu by increasing amount of chelating compounds such as chaperones, metallothioneins, and phytochelatins (PCs) (Ritter et al., 2014).

Cu chaperones are from Cu-binding domains. They have roles in detoxifying oxygen radicals and Cu trafficking and detoxification. Antioxidant protein 1 (ATX1) and ATX1-like Cu chaperone (CCH) are two Cu chaperones in *A. thaliana*. mRNA levels of *Arabidopsis CCH* were reported to decrease in response to high concentrations of copper (Mira et al., 2001). Phenotypes of *atx1* mutant and the *cchatx1* double mutant were characterized in plants under excess copper and deficiency (Shin et al., 2012). The shoot and root growth of *atx1* and *cchatx1* mutants were found to be very sensitive to excess Cu. On the other hand, *atx1* and *cchatx1* were also reported to be sensitive to Cu deficiency. It was determined that Cu tolerance and accumulation increased growth was promoted in plants over-expressing ATX1.

Arabidopsis contains the CCH for SOD (CCS), delivering Cu to Cu/zinc-containing superoxide dismutase (ZnSOD). In *Arabidopsis ccs* mutants, no measurable SOD activity was detected in chloroplast and cytosol. This suggests that CCS is necessary for SOD activity. In addition, Fe superoxide dismutase and Cu/ZnSOD activity were not observed in *ccs* mutants grown on media containing high Cu concentrations. However, no difference in phenotype and photosynthetic activities between mutant and wild type was reported. This indicated that lack of Cu/ZnSOD was not vitally important for photosynthesis (Cohu et al., 2009). In addition, *Glycine max* CCS and cuprozinc superoxide dismutase were investigated in photosynthetic cell suspensions under copper stress (Sagasti et al., 2014). Both genes were upregulated by copper stress. Furthermore, *GmCCS* was found to be present in the stroma only, but the *Glycine max* CCS and cuprozinc superoxide dismutase was present in both stroma and thylakoids despite the general idea that the SOD enzymes are typically soluble stroma proteins. The presence of the SOD enzyme on the surface of the thylakoid membranes is reasonable considering that the superoxide radical (O_2^-) is preferentially formed at the acceptor side of PSI.

Metallothioneins are Cys-rich metal chelators that play a role in many cellular processes, including the regulation of cell growth and proliferation, DNA damage repair, and scavenging of ROS (Cherian and Kang, 2006). We summarize here the importance of metallothioneins for plants subjected to copper stress. Phenotypic differences in the root of *Arabidopsis mt1a* mutants, *MT1a-RNAi*, and *mt1a-mt2b* double mutant under excess copper were reported. Decreased accumulation of copper in the roots of these mutants suggested that MT1a was an important player in copper accumulation of the roots (Grennan, 2011). Two MT genes, *BcMT1* and *BcMT2*, isolated from *Brassica campestris* were expressed in *A. thaliana*. Transgenic *Arabidopsis* plants with a reduction in ROS production developed tolerance to copper excess (Lv et al., 2013). Transgenic tobacco plant overexpressing an MT gene, *EhMT1*, from a copper accumulator plant *Elsholtzia haichowensis* developed a tolerance to copper stress. Increased Cu accumulation and POX activities and reduced H_2O_2 level were reported in

EhMT1-overexpressing plants as compared with wild type (Xia et al., 2012). *Pisum sativum* metallothionein A1 gene ($PsMT_{A1}$) expressed in white poplar enhanced tolerance to excess copper through sequestration of Cu ions in roots and leaves and by scavenging of ROS (Turchi et al., 2012).

Ectopic expression of MT2 gene from *Iris lactea* var. *Chinensis*, which is a copper-tolerant species, in *A. thaliana* increased tolerance to Cu stress by reducing H_2O_2 production in the transgenic plants. Root lengths of two transgenic seedlings were also induced more than that of the wild type after treatment with 50 and 100 μM Cu (Gu et al., 2015). Similarly, increased Cu tolerances and rooting efficiency were observed in transgenic *Salix matsudana* overexpressing the *Tamarix hispida ThMT3* gene (Yang et al., 2015).

Similar to metallothioneins (PCs) are polymers of g-Glu-Cys units with the terminal glycine and can bind heavy metals by thiolate coordination (Emamverdian et al., 2015). PCs are known to be induced by copper stress (Boojar and Tavakoli, 2010). Copper stress also induces a stable Cu-phytochelatin complex that has roles in detoxification of copper. However, Lee and Kang (2005) hypothesized that PCs were not a primer factor in copper detoxification because there was no difference in copper sensitivity between *Arabidopsis* mutants (*cad1*-3, which had no PCs) and wild type. It was also demonstrated that *AtPC1*-overexpressing *Arabidopsis* plants were not copper tolerant. However, *A. thaliana* developed copper tolerance when a fission yeast (*Schizosaccharomyces pombe*) phytochelatin–Cd transporter SpHMT1 was introduced into the plant (Huang et al., 2012). Beladi et al. (2011) claimed that an increased phytochelatin synthesis in Sainfoin (*Onobrychis vicifolia*) plants caused less damage in DNA by decreasing the formation of oxygen-free radicals. Fidalgo et al. (2013) showed that the production of PCs was enhanced in roots of *Solanum nigrum* when the plant was exposed to Cu. This resulted in the immobilization of excess Cu in the root and its preclusion from moving toward the shoot. All these data suggest that PCs are of importance for copper detoxification by sequestration Cu ions. Also, copper transport is as important as the sequestration for copper tolerance. There has been very rapid progress in understanding of Cu transport into and within cells in plants. Various copper transporters involved in intracellular copper homeostasis have been identified in plants (Yuan et al., 2011). Here we discuss copper transporters and their roles in responses of plants under copper stress.

7.4.2 Copper Transporters

7.4.2.1 COPT Copper Transporters

Six COPT family transporter proteins (COPT1-6) were identified in *Arabidopsis thaliana*. COPT proteins are responsible for Cu uptake and accumulation (Puig, 2014). *Arabidopsis* COPT1 is the first COPT family member to be identified and characterized (Sancenon et al., 2004). Root apex, stomata, and pollen grains are where COPT1 is mostly expressed (Sancenon et al., 2004). COPT-1 is responsible for copper acquisition in roots and pollen development (Andrés-Colás et al., 2010). Furthermore, COPT-1 was proven to have role in Cu acquisition by studies on *copt-1* lack of mutants, which exhibit decreased root growth under copper deficiency (Yamasaki et al., 2009). Reduction in plant growth and changes in the morphology of leaf, increased accumulation of copper, and sensitivity to excess copper were reported in *A. thaliana* overexpressing COPT1 (Andrés-Colás et al., 2010).

COPT2 mRNA is increased by SPL7 under Cu deficiency (Yamasaki et al., 2009). COPT2 is a cell-surface Cu transporter expressed in roots, young leaves, cotyledons, apical meristem, trichomes, and anthers (Perea-García et al., 2013). COPT2 functions in Cu acquisition and the cross-talk

between iron deficiency responses and low-phosphate signaling (Perea-García et al., 2013). COPT5 is a protein that is located on vacuolar tonoplast and expressed in roots, leaves, cotyledons, and reproductive organs (Garcia-Molina et al., 2011). Under copper scarcity, impaired photosynthesis, chlorosis, and root defects are observed in *copt5* knockout mutants, even though COPT5 transcript levels are not affected by Cu concentration in media (Klaumann et al., 2011). Increased Cu concentration in roots and decreased accumulation of Cu in siliques and seeds of *copt5* knockout mutants are because of reduced vacuolar Cu export. Therefore it is postulated that COPT5 roles in strongly in Cu distribution from the root to reproductive tissues (Klaumann et al., 2011). COPT6 is described as a new member of the COPT family transporters (Peñarrubia et al., 2010). COPT6 overexpression and *copt6* T-DNA insertion mutant studies in both yeast cells and *Arabidopsis* under copper deprivation show that COPT6 responsible for Cu accumulation and distribution to leaves and seeds (Jung et al., 2012).

7.4.2.2 P_{1B}-Type Heavy Metal ATPases

P_{1B}-type heavy metal ATPases transport potentially toxic metals such as Cu^{+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} across cell membranes (Palmgren and Axelsen, 1998; Mattle et al., 2015). Eight members of the P_{1B}-type ATPase subfamily, also known as HMA transporters (AtHMA1–AtHMA8), are encoded by the *Arabidopsis* genome (Yruela, 2009). It has been shown that Cu regulates the expression of four members of the HMA transporters AtHMA5–AtHMA8. AtHMA5 is described as pump that is induced by excess copper and removes Cu from symplast for xylem loading (Andrés-Colás et al., 2006). The responsive to antagonist 1/HMA7 delivers Cu to ethylene receptor and important for normal vegetative development. It has no role in copper resistance of plants (Binder et al., 2010). AtHMA6/PAA1 and AtHMA8/PAA2 are responsible for delivering Cu to plastocyanin (Pilon, 2011). PAA2 works in tandem with PAA1/HMA6, which is located in the inner chloroplast envelope. To maintain adequate photosynthesis under Cu deprivation, plants prioritize the transfer of Cu to plastocyanin. The level of PAA2 increase under Cu-deficient conditions to ensure deviating Cu to plastocyanin. Its level decreases as Cu becomes available. This is proven by using *pc2* mutants, which have a low level of PAA2. This also shows that plastocyanin has a regulatory effect on the level of PAA2 (Tapken et al., 2012).

8. CONCLUSION AND FUTURE PROSPECTS

Even though a lot of work exists to understand the copper stress and detoxification in plants, our knowledge is still limited. We do not know exactly how plants perceive copper. Which signal molecules play a role in the perception of copper by cells and how the genes that function in response to copper stress are regulated protect this uncertainty. On the other hand, many chelating compounds, chaperones, and metal transporters that may have functions in copper stress responses are determined by rapidly emerging sequencing techniques, transcriptomic, metabolomic, and proteomic approaches. Signal transduction in plants under copper stress is versatile. Diversity of hormones such as ABA, IAA, ethylene, and ROS (H_2O_2, NO) affect transcript factors controlling gene expression. However, our understanding of regulatory and cross-talk mechanisms is still rudimentary. Hyperaccumulator plants are best candidates to learn the regulatory and cross-talk mechanisms. These plants accumulate many different proteins under copper stress as well as heavy metals. Dissecting the genes coding these proteins from the hyperaccumulators opens up a way to understand the copper homeostasis network, crop tolerance, and phytoremediation.

REFERENCES

Aggarwal, A., Sharma, I., Tripathi, B.N., Munjal, A.K., Baunthiyal, M., Sharma, V., 2012. Metal toxicity and photosynthesis. In: Itoh, S., Mohanty, P., Guruprasad, K.N. (Eds.), Photosynthesis: Overviews on Recent Progress and Future Perspectives. International Publishing House Limited, India.

Anderson, J.M., Boardman, N.K., David, D.J., 1964. Trace metal composition of fractions obtained by digitonin fragmentation of spinach chloroplasts. Biochem. Biophys. Res. Commun. 17, 685–690.

Andrade, S.A.L., Gratao, P.L., Azevedo, R.A., Silveira, A.P.D., Schiavinato, M.A., Mazzafera, P., 2010. Biochemical and physiological changes in jack bean under mycorrhizal symbiosis growing in soil with increasing Cu concentrations. Environ. Exp. Bot. 68, 198–207.

Andrés-Colás, N., Sancenon, V., Rodriguez-Navarro, S., Mayo, S., Thiele, D.J., Ecker, J.R., Puig, S., Penarrubia, L., 2006. The *Arabidopsis* heavy metal P-type ATPase HMA5 interacts with metallochaperones and functions in copper detoxification of roots. Plant J. 45, 225–236.

Andrés-Colás, N., Perea-Garcia, A., Puig, S., Peñarrubia, L., 2010. Deregulated copper transport affects *Arabidopsis* development especially in the absence of environmental cycles. Plant Physiol. 153, 170–184.

Arteca, R.N., Arteca, J.M., 2007. Heavy-metal-induced ethylene production in *Arabidopsis thaliana*. J. Plant Physiol. 164, 1480–1488.

Barker, A.V., Pilbeam, D.J., 2014. Handbook of Plant Nutrition. CRC Press, p. 632.

Baron, M., Arellano, J.B., Gorge, J.L., 1995. Copper and photosystem II: a controversial relationship. Physiol. Plant 94, 174–180.

Beladi, M., Kashani, A., Habibi, D., Paknejad, F., Golshan, M., 2011. Uptake and effects of lead and copper on three plan species in contaminated soils: role of phytochelatin. Afr. J. Agric. Res. 6, 3483–3492.

Bernal, M., Casero, D., Singh, V., Wilson, G.T., Grande, A., Yang, H., Dodani, S.C., Pellegrini, M., Huijser, P., Connolly, E.L., Merchant, S.S., Krämer, U., 2012. Transcriptome sequencing identifies *SPL7*-regulated copper acquisition genes *FRO4/FRO5* and the copper dependence of iron homeostasis in *Arabidopsis*. Plant Cell 24, 738–761.

Binder, B.M., Rodriguez, I.F., Bleecker, A.B., 2010. The copper transporter RAN1 is essential for biogenesis of ethylene receptors in *Arabidopsis*. J. Biol. Chem. 285, 37263–37270.

Boojar, M.M.A., Tavakoli, Z., 2010. Role of antioxidant enzyme responses and phytochelatins in tolerance strategies of *Alhagi camelorum* Fisch growing on copper mine. Acta Bot. Croat. 69, 107–121.

Brahim, L., Mohamed, M., 2011. Effects of copper stress on antioxidative enzymes, chlorophyll and protein content in *Atriplex halimus*. Afr. J. Biotechnol. 50, 10143–10148.

Burda, K., Kruk, J., Strzalka, K., Schmid, G.H., 2002. Stimulation of oxygen evolution in Photosystem II by copper(II) ions. Z. Naturforsch. 57, 853–857.

Burda, K., Kruk, J., Schmid, G.H., Strzalka, K., 2003. Inhibition of oxygen evolution in Photosystem II by Cu(II) ions is associated with oxidation of cytochrome b_{559}. Biochem. J. 371, 597–601.

Chamseddine, M., Wided, B.A., Guy, H., Marie-Edith, C., Fatma, J., 2009. Cadmium and copper induction of oxidative stress and antioxidative response in tomato (*Solanum lycopersicon*) leaves. Plant Growth Regul. 57, 89–99.

Chen, C.T., Chen, L.M., Lin, C.C., Kao, C.H., 2001. Regulation of proline accumulation in detached rice leaves exposed to excess copper. Plant Sci. 160, 283–290.

Chen, L., Wang, L., Chen, F., Korpelainen, H., Li, C., 2013. The effects of exogenous putrescine on sex-specific responses of *Populus cathayana* to copper stress. Ecotox Environ. Safe 97, 94–102.

Cherian, M.G., Kang, Y.J., 2006. Metallothionein and liver cell regeneration. Exp. Biol. Med. (Maywood) 231, 138–144.

Choudhary, S.P., Bhardwaj, R., Gupta, B.D., Dutt, P., Gupta, R.K., Biondi, S., Kanwar, M., 2010. Epibrassinolide induces changes in indole-3-acetic acid, abscisic acid and polyamine concentrations and enhances antioxidant potential of radish seedlings under copper stress. Physiol. Plant. 140, 280–296.

Choudhary, S.P., Oral, H.V., Bhardwaj, R., Yu, J.Q., Lam-Son, P., Tran, L.S.P., 2012. Interaction of brassinosteroids and polyamines enhances copper stress tolerance in *Raphanus sativus*. J. Exp. Bot. 63, 5659–5675.

Choudhary, S.P., Bhardwaj, R., Gupta, B.D., Dutt, P., Gupta, R.K., Mukesh, K., Biondi, S., 2011. Enhancing effects of 24-epibrassinolide and putrescine on the antioxidant capacity and free radical scavenging activity of *Raphanus sativus* seedlings under Cu ion stress. Acta Physiol. Plant 33, 1319–1333.

Cohu, C.M., Abdel-Ghany, S.E., Gogolin Reynolds, K.A., Onofrio, A.M., Bodecker, J.R., Kimbrel, J.A., Niyogi, K.K., Pilon, M., 2009. Copper delivery by the copper chaperone for chloroplast and cytosolic copper/zinc-superoxide dismutases: regulation and unexpected phenotypes in an *Arabidopsis* mutant. Mol. Plant 2, 1336–1350.

Cui, X.M., Zhang, Y.K., Wu, X.B., Liu, C.S., 2010. The investigation of the alleviated effect of copper toxicity by exogenous nitric oxide in tomato plants. Plant Soil Environ. 56, 274–281.

DalCorso, G., Farinati, S., Furini, A., 2010. Regulatory networks of cadmium stress in plants. Plant Signal Behav. 5, 663–667.

Dall'Osto, L., Holt, N.E., Kaligotla, S., Fuciman, M., Cazzaniga, S., Carbonera, D., Frank, H.A., Alric, J., Bassi, R., 2012. Zeaxanthin protects plant photosynthesis by modulating chlorophyll triplet yield in specific light-harvesting antenna subunits. J. Biol. Chem. 287, 41820–41834.

Dong, Y., Xu, L., Wang, Q., Fan, Z., Kong, J., Bai, X., 2014. Effects of exogenous nitric oxide on photosynthesis, antioxidative ability, and mineral element contents of perennial ryegrass under copper stress. J. Plant Interact. 9, 402–411.

Eleftheriou, E.P., Karataglis, S., 1989. Ultrastructural and morphological characteristics of cultivated wheat growing on copper-polluted fields. Botanica Acta 102, 134–140.

Emamverdian, A., Ding, Y., Mokhberdoran, F., Xie, Y., 2015. Heavy metal stress and some mechanisms of plant defense response. Sci. World J. 2015. http://dx.doi.org/10.1155/2015/756120.

Fariduddin, Q., Khan, T.A., Yusuf, M., 2014. Hydrogen peroxide mediated tolerance to copper stress in the presence of 28-homobrassinolide in *Vigna radiata*. Acta Physiol. Plant. 36, 2767–2778.

Ferreira, P.A.A., Ceretta, C.A., Soriani, H.H., Tiecher, T.L., Soares, C.R.F.S., Rossato, L.V., Nicoloso, F.T., Brunetto, G., Paranhos, J.T., Cornejo, P., 2015. *Rhizophagus clarus* and phosphate alter the physiological responses of *Crotalaria juncea* cultivated in soil with a high Cu level. Appl. Soil Ecol. 91, 37–47.

Fidalgo, F., Azenha, M., Silva, A.F., de Sousa, A., Santiago, A., Ferraz, P., Teixeira, J., 2013. Copper-induced stress in *Solanum nigrum* L. and antioxidant defense system responses. Food Energ. Sec. 2, 70–80.

Foyer, C.H., Noctor, G., 2011. Ascorbate and glutathione: the heart of the redox hub. Plant Physiol. 155, 12–18.

Garcia-Molina, A., Andrés-Colás, N., Perea-Garcia, A., Del Valle-Tascon, S., Peñarrubia, L., Puig, S., 2011. The intracellular *Arabidopsis* COPT5 transport protein is required for photosynthetic electron transport under severe copper deficiency. Plant J. 65, 848–860.

Garcia-Molina, A., Xing, S., Huijser, P., 2014. Functional characterisation of *Arabidopsis* SPL7 conserved protein domains suggests novel regulatory mechanisms in the Cu deficiency response. BMC Plant Biol. 14, 231.

Grennan, A.K., 2011. Metallothioneins, a diverse protein family. Plant Physiol. 155, 1750–1751.

Groppa, M.D., Ianuzzo, M.P., Tomaro, M.L., Benavides, M.P., 2007. Polyamine metabolism in sunflower plants under long-term cadmium or copper stress. Amino Acid 32, 265–275.

Groppa, M.D., Benavides, M.P., Tomaro, M.L., 2003. Polyamine metabolism in sunflower and wheat leaf discs under cadmium or copper stress. Plant Sci. 164, 293–299.

Gu, C.S., Liu, L.Q., Deng, Y.M., Zhu, X.D., Huang, S.Z., Lu, X.-Q., 2015. The heterologous expression of the *Iris lactea* var. *chinensis* Type2 metallothionein *IlMT2b* gene enhances copper tolerance in *Arabidopsis thaliana*. Bull. Environ. Contam. Toxicol. 94, 247–253.

Guzel, S., Terzi, R., 2013. Exogenous hydrogen peroxide increases dry matter production, mineral content and level of osmotic solutes in young maize leaves and alleviates deleterious effects of copper stress. Bot. Stud. 54, 26.

Habiba, U., Ali, S., Farid, M., Shakoor, M.B., Rizwan, M., Ibrahim, M., Abbasi, G.H., Hayat, T., Ali, B., 2015. EDTA enhanced plant growth, antioxidant defense system, and phytoextraction of copper by *Brassica napus* L. Environ. Sci. Pollut. Res. Int. 22, 1534–1544. http://dx.doi.org/10.1007/s11356-014-3431-5.

Halliwell, B., Gutteridge, J.M.C., 2007. Free Radicals in Biology and Medicine. Oxford University Press, NewYork, p. 888.

Halliwell, B., Gutteridge, J.M., 1984. Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. Lancet 1, 1396–1397.

Hego, E., Bes, C.M., Bedon, F., Palagi, P.M., Chaumeil, P., Barré, A., Claverol, S., Dupuy, J.W., Bonneu, M., Lalanne, C., Plomion, C., Mench, M., 2014. Differential accumulation of soluble proteins in roots of metallicolous and nonmetallicolous populations of *Agrostis capillaris* L. exposed to Cu. Proteomics 14, 1746–1758.

Hu, Y., You, J., Liang, X., 2015. Nitrate reductase-mediated nitric oxide production is involved in copper tolerance in shoots of hulless barley. Plant Cell Rep. 34, 367–379.

Huang, J., Zhang, Y., Peng, J.S., Zhong, C., Yi, H.Y., Ow, D.W., Gong, J.M., 2012. Fission yeast HMT1 lowers seed cadmium through phytochelatin-dependent vacuolar sequestration in *Arabidopsis*. Plant Physiol. 158, 1779–1788.

Hung, W.C., Huang, D.D., Yeh, C.M., Huang, H.J., 2005. Reactive oxygen species, calcium and serine/threonine phosphatase are required for copper-induced MAP kinase gene, *OsMAPK2*, expression in rice. Plant Growth Regul. 45, 233–241.

Iqbal, N., Trivellini, A., Masood, A., Ferrante, A., Khan, N.A., 2013. Current understanding on ethylene signalling in plants: the influence of nutrient availability. Plant Physiol. Biochem. 73, 128–138.

Jakubowicz, M., Galganska, H., Nowak, W., Sadowski, J., 2010. Exogenously induced expression of ethylene biosynthesis, ethylene perception, phospholipase D, and Rboh-oxidase genes in broccoli seedlings. J. Exp. Bot. 61, 3475–3491.

Jonak, C., Nakagami, H., Hirt, H., 2004. Heavy metal stress. Activation of distinct mitogen-activated protein kinase pathways by copper and cadmium. Plant Physiol. 136, 3276–3283.

Jung, H., Gayomba, S.R., Rutzke, M.A., Craft, E., Kochian, L.V., Vatamaniuk, O.K., 2012. COPT6 is a plasma membrane transporterthat functions in copper homeostasis in *Arabidopsis* and is a novel target of SQUAMOSA promoter-binding protein-like 7. J. Biol. Chem. 287, 33252–33267.

Kavi Kishor, P.B., Sangam, S., Amrutha, R.N., Sri Laxmi, P., Naidu, K.R., Rao, K.R.S.S., Rao, S., Reddy, K.J., Theriappan, P., Sreenivasulu, N., 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Curr. Sci. 88, 424–438.

Khan, M.I., Khan, N.A., 2014. Ethylene reverses photosynthetic inhibition by nickel and zinc in mustard through changes in PS II activity, photosynthetic nitrogen use efficiency, and antioxidant metabolism. Protoplasma 251, 1007–1019.

Klaumann, S., Nickolaus, S.D., Furst, S.H., Starck, S., Schneider, S., Ekkehard Neuhaus, H., Trentmann, O., 2011. The tonoplast copper transporter COPT5 acts as an exporter and is required for interorganal location of copper in *Arabidopsis thaliana*. New Phytol. 192, 393–404.

Kolbert, Z., Peto, A., Lehotai, N., Feigl, G., Erdei, L., 2012. Long-term copper (Cu_2^+) exposure impacts on auxin, nitric oxide (NO) metabolism and morphology of *Arabidopsis thaliana* L. Plant Growth Regul. 68, 151–159.

Krupa, Z., 1988. Acyl lipids in the supramolecular chlorophyll–protein complexes of photosynthesis-isolation artifacts or integral components regulating their structure and functions? Acta Soc. Bot. Pol. 57, 401–418.

Ku, H.M., Tan, C.W., Su, Y.S., Chiu, C., Chen, C.T., Jan, F.J., 2012. The effect of water deficit and excess copper on proline metabolism in *Nicotiana benthamiana*. Biol. Plant 56, 337–343.

Lee, C.W., Jackson, M.B., Duysen, M.E., Freeman, T.P., Self, J.R., 1996. Induced micronutrient toxicity in 'Towndown' Kentucky bluegrass. Crop Sci. 36, 705–712.

Lee, S., Kang, B.S., 2005. Phytochelatin is not a primary factor in determining copper tolerance. J. Plant Biol. 48, 32–38.

Lequeux, H., Hermans, C., Lutts, S., Verbruggen, N., 2010. Response to copper excess in *Arabidopsis thaliana*: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiol. Biochem. 48, 673–682.

Lidon, F.C., Henriques, F.S., 1991. Limiting step on photosynthesis of rice plants treated with varying copper levels. J. Plant Physiol. 138, 115–118.

Lidon, F.C., Henriques, F.S., 1993. Changes in the thylakoid membrane polypeptide patterns triggered by excess Cu in rice. Photosynthetica 28, 109–117.

Liu, J.J., Wei, Z., Li, J.H., 2014. Effects of copper on leaf membrane structure and root activity of maize seedling. Bot. Stud. 55, 47.

Liu, Q., Zheng, L., He, F., Zhao, F.J., Shen, Z., Zheng, L., 2015. Transcriptional and physiological analyses identify a regulatory role for hydrogen peroxide in the lignin biosynthesis of copper-stressed rice roots. Plant Soil 387, 323–336.

Lv, Y., Deng, X., Quan, L., Xia, Y., Shen, Z., 2013. Metallothioneins BcMT1 and BcMT2 from *Brassica campestris* enhance tolerance to cadmium and copper and decrease production of reactive oxygen species in *Arabidopsis thaliana*. Plant Soil 367, 507–519.

Maksymiec, W., 2007. Signaling responses in plants to heavy metal stress. Acta Physiol. Plant 29, 177–187.

Maksymiec, W., Krupa, Z., 2006. The effects of short-term exposition to Cd, excess Cu ions and jasmonate on oxidative stress appearing in *Arabidopsis thaliana*. Environ. Exp. Bot. 57, 187–194.

Maksymiec, W., 1997. Effect of copper on cellular processes in higher plants. Photosynthetica 34, 132–342.

Maksymiec, W., Bednara, J., Baszynski, R., 1995. Responses of runner bean plants to excess copper as a function of plant growth stages: effects on morphology and structure of primary leaves and their chloroplast ultrastructure. Photosynthetica 31, 427–435.

Maksymiec, W., Russa, R., Urbanik-Sypniewska, T., Baszynski, T., 1994. Effect of excess Cu on the photosynthetic apparatus of runner bean leaves treated at two different growth stages. Physiol. Plant 91, 715–721.

Malec, P., Maleva, M., Prasad, M.N.V., Strzałka, K., 2009. Copper toxicity in leaves of *Elodea canadensis* Michx. Bull. Environ. Contam. Toxicol. 82, 627–632.

Mani, S., Van de Cotte, B., Van Montagu, M., Verbruggen, N., 2002. Altered levels of proline dehydrogenase cause hypersensitivity to proline and its analogs in *Arabidopsis*. Plant Physiol. 128, 73–83.

Marschner, H., 2011. Marschner's Mineral Nutrition of Higher Plants. Academic Press, London, p. 672.

Mateos-Naranjo, E., Gallé, A., Florez-Sarasa, I., Perdomo, J.A., Galmés, J., Ribas-Carbó, M., Flexas, J., 2015. Assessment of the role of silicon in the Cu-tolerance of the C_4 grass *Spartina densiflora*. J. Plant Physiol. 178, 74–83.

Mattle, D., Zhang, L., Sitsel, O., Pedersen, L.T., Moncelli, M.R., Tadini-Buoninsegni, F., Gourdon, P., Rees, D.C., Nissen, P., Meloni, G., 2015. A sulfur-based transport pathway in Cu^{+}-ATPases. EMBO Rep. 16, 728–740. http://dx.doi.org/10.15252/embr.201439927.

Mertens, J., Vangronsveld, J., Van Der Straeten, D., Poucke, M., 1999. Effects of copper and zinc on the ethylene production of *Arabidopsis thaliana*. In: Kanellis, A.K., Chang, C., Klee, H., Bleecker, A.B., Pech, J.C., Grierson, D. (Eds.), Biol Biotechnol Plant Horm Ethyl II SE - 60. Kluwer Academic Publishers, Dordrecht, pp. 333–338.

Miotto, A., Ceretta, C.A., Brunetto, G., Nicoloso, F.T., Girotto, E., Farias, J.G., Tiecher, T.L., De Conti, L., Trentin, G., 2014. Copper uptake, accumulation and physiological changes in adult grapevines in response to excess copper in soil. Plant Soil 374, 593–610.

Mira, H., Martínez-Garcia, F., Penñarrubia, L., 2001. Evidence for the plant-specific intercellular transport of the *Arabidopsis* copper chaperone CCH. Plant J. 25, 521–528.

Mohanty, N., Vass, I., Demeter, S., 1989. Copper toxicity affects Photosystem II electron transport at the secondary quinone acceptor (Q_B). Plant Physiol. 90, 175–179.

Monteoliva, M.I., Rizzi, Y.S., Cecchini, N.M., Hajirezaei, M.R., Alvarez, M.E., 2014. Context of action of proline dehydrogenase (ProDH) in the hypersensitive response of *Arabidopsis*. BMC Plant Biol. 14, 21.

Morita, S., Kaminaka, H., Masumura, T., Yanaka, K., 1999. Induction of rice cytosolic ascorbate peroxidase mRNA by oxidative stress signaling. Plant Cell Physiol. 40, 417–422.

Mostofa, M.G., Seraj, Z.I., Fujita, M., 2014. Exogenous sodium nitroprusside and glutathione alleviate copper toxicity by reducing copper uptake and oxidative damage in rice (*Oryza sativa* L.) seedlings. Protoplasma 251, 1373–1386.

Munzuroglu, O., Zengin, F.K., Yahyagil, Z., 2008. The abscisic acid levels of wheat (*Triticum aestivum* L. cv. cakmak 79) seeds that were germinated under heavy metal (Hg^{++}, Cd^{++}, Cu^{++}) stress. Gazi Univ. J. Sci. 2, 1–7.

Murata, N., Higashi, S.I., Fujimura, Y., 1990. Glycerolipids in various preparations of photosystem II from spinach chloroplasts. Biochim. Biophys. Acta 1019, 261–268.

Nair, P.M.G., Chung, I.M., 2015. Study on the correlation between copper oxide nanoparticles induced growth suppression and enhanced lignification in Indian mustard (*Brassica juncea* L.). Ecotox. Environ. Safe 113, 302–313.

Orzechowska, A., Bock, R., Piramowicz, M.O., Strzałka, K., Burda, K., 2008. Cu^{2+} binding sites in PSII. In: Allen, J.F., Gantt, E., Golbeck, J., Osmond, B. (Eds.), Photosynthesis: Energy from the Sun. Springer, Netherland, pp. 657–660.

Palmgren, M.G., Axelsen, K.B., 1998. Evolution of P-type ATPases. Biochim. Biophys. Acta 1365, 37–45.

Panda, S., Panda, S., 2009. Effect of mercury ion on the stability of the lipid-protein complex of isolated chloroplasts. Indian J. Biochem. Biophys. 46, 405–408.

Panou-Filotheou, H., Bosabalidis, A.M., 2004. Root structural aspects associated with copper toxicity in oregano (*Oreganum vulgare* sudsp. *hirtum*). Plant Sci. 166, 1497–1504.

Panou-Filotheou, H., Bosabalidis, A.M., Karataglis, S., 2001. Effects of copper toxicity on leaves of oregano (*Origanum vulgare* subsp. *hirtum*). Ann. Bot. 88, 207–214.

Parry, M.A.J., Andralojc, J., Scales, J.C., Salvucci, M.E., Cormo-Silva, A.E., Alonso, H., Whitney, S.M., 2012. Rubisco activity and regulation as targets for crop. J. Exp. Bot. 63, 695–709.

Peñarrubia, L., Andrés-Colás, N., Moreno, J., Puig, S., 2010. Regulation of copper transport in *Arabidopsis thaliana*: a biochemical oscillator? J. Biol. Inorg. Chem. 15, 29–36.

Peng, H., Kroneck, P.M.H., Küpper, H., 2013. Toxicity and deficiency of copper in *Elsholtzia splendens* affect photosynthesis biophysics, pigments and metal accumulation. Environ. Sci. Technol. 47, 6120–6128.

Perea-García, A., Garcia-Molina, A., Andrés-Colás, N., Vera-Sirera, F., Pérez-Amador, M.A., Puig, S., Peñarrubia, L., 2013. *Arabidopsis* copper transport protein COPT2 participates in the cross talk between iron deficiency responses and low-phosphate signaling. Plant Physiol. 162, 180–194.

Pilon, M., 2011. Moving copper in plants. New Phytol. 192, 305–307.

Posmyk, M.M., Kuran, H., Marciniak, K., Janas, K.M., 2008. Presowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations. J. Pineal Res. 45, 24–31.

Puig, S., 2014. Function and regulation of the plant COPT family of high-affinity copper transport proteins. Adv. Bot. 2014. http://dx.doi.org/10.1155/2014/476917.

Puig, S., Andres-Colas, N., Garcia-Molina, A., Peñarrubia, L., 2007. Copper and iron homeostasis in *Arabidopsis*: responses to metal deficiencies, interactions and biotechnological applications. Plant Cell Environ. 30, 271–290.

Quartacci, M.F., Pinzino, C., Sgherri, C.L.M., Dalla Vecchia, F., Navari-Izzo, F., 2000. Growth in excess copper induces changes in the lipid composition and fluidity of PSII-enriched membranes in wheat. Physiol. Plant 108, 87–93.

Raven, J.A., 2013. Rubisco: still the most abundant protein of Earth? New Phytol. 198, 1–3.

Rejeb, K.B., Abdelly, C., Savouré, A., 2014. How reactive oxygen species and proline face stress together. Plant Physiol. Biochem. 80, 278–284.

Ritter, A., Dittami, S.M., Goulitquer, S., Correa, J.A., Boyen, C., Potin, P., Tonon, T., 2014. Transcriptomic and metabolomic analysis of copper stress acclimation in *Ectocarpus siliculosus* highlights signaling and tolerance mechanisms in brown algae. BMC Plant Biol. 14, 116.

Rouillon, R., Piletsky, S.A., Breton, F., Piletska, E.V., Carpentier, R., 2006. Photosystem II biosensors for heavy metals monitoring. In: Teresa, M., Giardi, P.E.V. (Eds.), Biotechnological Applications of Photosynthetic Proteins: Biochips, Biosensors and Biodevices. Springer, USA, pp. 166–174.

Rout, J.R., Sahoo, S.L., 2013. Antioxidant enzyme gene expression in response to copper stress in *Withania somnifera* L. Plant Growth Regul. 71, 95–99.

Rozentsvet, O.A., Nesterov, V.N., Sinyutina, N.F., 2012. The effect of copper ions on the lipid composition of subcellular membranesin *Hydrilla verticillata*. Chemosphere 89, 108–113.

Sagasti, S., Bernal, M., Sancho, D., Castillo, M.B., Picorel, R., 2014. Regulation of the chloroplastic copper chaperone (CCS) and cuprozinc superoxide dismutase (CSD2) by alternative splicing and copper excess in *Glycine max*. Funct. Plant Biol. 41, 144–155.

Sancenon, V., Puig, S., Mateu-Andres, I., Dorcey, E., Thiele, D.J., Penarrubia, L., 2004. The *Arabidopsis* copper transporter COPT1 functions in root elongation and pollen development. J. Biol. Chem. 279, 15348–15355.

Sanchez-Pardo, B., Fernandez-Pascual, M., Zornoza, P., 2014. Copper microlocalisation and changes in leaf morphology, chloroplast ultrastructure and antioxidative response in white lupin and soybean grown in copper excess. J. Plant Res. 127, 119–129.

Schafer, C., Simper, H., Hofmann, B., 1992. Glucose feeding results in coordinated changes of chlorophyll content, ribulose-1,5-biphosphate carboxylase-oxygenase activity and photosynthetic potential in photoautrophic suspension cultured cells of *Chenopodium rubrum*. Plant Cell Environ. 15, 343–350.

Schellingen, K., Van Der Straeten, D., Vandenbussche, F., Prinsen, E., Remans, T., Vangronsveld, J., Cuypers, A., 2014. Cadmium-induced ethylene production and responses in *Arabidopsis thaliana* rely on *ACS2* and *ACS6* gene expression. BMC Plant Biol. 14, 214.

Sharma, P., Bhardwaj, R., 2007. Effects of 24-epibrassinolide on growth and metal uptake in *Brassica juncea* L. under copper metal stress. Acta Physiol. Plant 29, 259–263.

Shin, L.J., Lo, J.C., Yeh, K.C., 2012. Copper chaperone antioxidant protein1 is essential for copper homeostasis. Plant Physiol. 159, 1099–1110.

Sibbald, P.R., Green, B.R., 1987. Copper in photosystem II: association with LHC II. Photosynth. Res. 14, 201–209.

Siborova, M., 1988. Cd^{2+} ions affect the quaternary structure of ribulose-1,5-bisphosphate carboxylase from barley leaves. Biochem. Physiol. Pflanzen 183, 371–378.

Simova-Stoilova, L., Demirevska-Kepova, K., Stoyanova, Z., Smilova, E., 2002. Effect of Cu and Mn toxicity on leaf protein pattern and rubisco quantity of young barley plants. Compt. Rend. Acad. Bulg Sci. 55, 79–84.

Singh, R., Gautam, N., Mishra, A., Gupta, R., 2011. Heavy metals and living systems: an overview. Indian J. Pharmacol. 43, 246–253.

Smeets, K., Opdenakker, K., Remans, T., Forzani, C., Hirt, H., Vangronsveld, J., Cuypers, A., 2013. The role of the kinase OXI1 in cadmium- and copper-induced molecular responses in *Arabidopsis thaliana*. Plant Cell Environ. 36, 1228–1238.

Son, J.A., Narayanankutty, D.P., Roh, K.S., 2014. Influence of exogenous application of glutathione on rubisco and rubisco activase in heavy metal-stressed tobacco plant grown in vitro. Saudi J. Biol. Sci. 21, 89–97.

Souza, V.L., Almeida, A.A.F., Souza, J.S., Mangabeira, P.A.O., Jesus, R.M., Pirovani, C.P., Ahnert, D., Baligar, V.C., Loguercio, L.L., 2014. Altered physiology, cell structure, and gene expression of *Theobroma cacao* seedlings subjected to Cu toxicity. Environ. Sci. Pollut. Res. 21, 1217–1230.

Sun, X.H., Yu, G., Li, J.T., Jia, P., Zhang, J.C., Jia, C.G., Zhang, Y.H., Pan, H.Y., 2014. A heavy metal-associated protein (AcHMA1) from the Halophyte, *Atriplex canescens* (Pursh) Nutt. confers tolerance to iron and other abiotic stresses when expressed in *Saccharomyces cerevisiae*. Int. J. Mol. Sci. 15, 14891–14906.

Szabados, L., Savoure, A., 2010. Proline: a multifunctional amino acid. Trends Plant Sci. 15, 89–97.

Tan, D.X., Hardeland, R., Manchester, L.C., Korkmaz, A., Ma, S., Rosales-Corral, S., Reiter, R.J., 2012. Functional roles of melatonin in plants, and perspectives innutritional and agricultural science. J. Exp. Bot. 63, 577–597.

Tan, D.X., Manchester, L.C., Helton, P., Reiter, R.J., 2007. Phytoremediative capacity of plants enriched with melatonin. Plant Signal Behav. 2, 514–516.

Tapken, W., Ravet, K., Pilon, M., 2012. Plastocyanin controls the stabilization of the thylakoid Cu-transporting P-type ATPase PAA2/HMA8 in response to low copper in *Arabidopsis*. J. Biol. Chem. 287, 18544–18550.

Taylor, G.J., Foy, C.D., 1985. Differential uptake and toxicity of ionic and chelated copper in Triticum aestivum. Can. J. Bot. 63, 1271–1275.

Tewari, R.K., Hahn, E.J., Paek, K.Y., 2008. Modulation of copper toxicity-induced oxidative damage by nitric oxide supply in the adventitious roots of *Panax ginseng*. Plant Cell Rep. 27, 171–181.

Thounaojam, T.C., Panda, P., Mazumdar, P., Kumar, D., Sharma, G.D., Sahoo, L., Sanjib, P., 2012. Excess copper induced oxidative stress and response of antioxidants in rice. Plant Physiol. Biochem. 53, 33–39.

Tie, S.G., Tang, Z.J., Zhao, Y.M., Li, W., 2012. Oxidative damage and antioxidant response caused by excess copper in leaves of maize. Afr. J. Biotechnol. 11, 4378–4384.

Tripathi, B.N., Singh, V., Ezaki, B., Sharma, V., Gaur, J.P., 2013. Mechanism of Cu- and Cd-induced proline hyperaccumulation in *Triticum aestivum* (Wheat). J. Plant Growth Regul. 32, 799–808.

Turchi, A., Tamantini, I., Camussi, A.M., Racchi, M.L., 2012. Expression of a *metallothionein A1* gene of *Pisum* sativum in white poplar enhances tolerance and accumulation of zinc and copper. Plant Sci. 183 (2012), 50–56.

Upadhyay, R., Panda, S.K., 2010. Zinc reduces copper toxicity induced oxidative stress by promoting antioxidant defense in freshly grown aquatic duckweed Spirodela polyrhiza L. J. Hazard. Mater 175, 1081–1084.

van Assche, F., Clijsters, H., 1990. Effects of metals on enzyme activity in plants. Plant Cell Environ. 13, 195–206.

Vázquez, M.N., Guerrero, Y.R., González, L.M., de la Noval, W.T., 2013. Brassinosteroids and plant responses to heavy metal stress. An overview. Open J. Metal 3, 34–41.

Verma, A., Bhatia, S., 2014. Analysis of some physicochemical parameters and trace metal concentration present in the soil around the area of Paricchа thermal power station in Jhansi, India. Int. J. Inno Res. Sci. Eng. Technol. 3, 10482–10488.

Vijayarengan, P., Jose, M.D., 2014. Changes in growth, pigments and phytoremediating capability of four plant species under copper stress. Int. J. Environ. Biol. 4, 119–126.

Wang, L., Yang, X., Ren, Z., Hu, X., Wang, X., 2014. Alleviation of photosynthetic inhibition in copper-stressed tomatoes through rebalance of ion content by exogenous nitric oxide. Turkish J. Bot. 38 http://dx.doi.org/10.3906/bot-1312-17.

Wang, L., Yang, L., Yang, F., Li, X., Song, Y., Wang, X., Hu, X., 2010. Involvements of H_2O_2 and metallothionein in NO-mediated tomato tolerance to copper toxicity. J. Plant Physiol. 167, 1298–1306.

Wang, Y.Z., Nie, L.H., Tie, S., Xie, D., Zhu, W., Qi, J., Yue, R., 2011. Effects of excess copper on the oxidative stress in roots of maize seedlings. Afr. J. Agric. Res. 6, 4998–5004.

Wen, J.F., Gong, M., Liu, Y., Hu, J.L., Deng, M.H., 2013. Effect of hydrogen peroxide on growth and activity of some enzymes involved in proline metabolism of sweet corn seedlings under copper stress. Sci. Hort. 164, 366–371.

Whitacre, D.M., 2011. Reviews of Environmental Contamination and Toxicology, vol. 213. Springer, USA, p. 180.
Xia, Y., Qi, Y., Yuan, Y., Wang, G., Cui, J., Chen, Y., Zhang, H., Shen, Z., 2012. Overexpression of *Elsholtzia haichowensis* metallothionein 1 (EhMT1) in tobacco plants enhances copper tolerance and accumulation in root cytoplasm and decreases hydrogen peroxide production. J. Hazard Mater. 234, 65–71.
Xu, Q., Qiu, H., Chu, W., Fu, Y., Cai, S., Min, H., Sha, S., 2013. Copper ultrastructural localization, subcellular distribution, and phytotoxicity in *Hydrilla verticillata* (L.f.). Royle. Environ. Sci. Pollut. Res. 20, 8672–8679.
Yamasaki, H., Hayashi, M., Fukazawa, M., Kobayashi, Y., Shikanai, T., 2009. SQUAMOSA promoter binding protein-like7 is a central regulator for copper homeostasis in *Arabidopsis*. Plant Cell 21, 347–361.
Yau, P.Y., Loh, C.F., Azmil, I.A.R., 1991. Copper toxicity of clove (*Syzygium aromaticum* (L.) Merr. and Perry) seedlings. MARDI Res. J. 19, 49–53.
Yang, J., Chen, Z., Wu, S., Cui, Y., Zhang, L., Dong, H., Yang, C., Li, C., 2015. Overexpression of the *Tamarix hispida ThMT3* gene increases copper tolerance and adventitious root induction in *Salix matsudana* Koidz. Plant Cell Tiss. Organ Cult. 121, 469–479.
Yeh, C.M., Chien, P.S., Huang, H.J., 2007. Distinct signalling pathways for induction of MAP kinase activities by cadmium and copper in rice roots. J. Exp. Bot. 58, 659–671.
Yruela, I., 2009. Copper in plants: acquisition, transport and interactions. Funct. Plant Biol. 36, 409–430.
Yruela, I., 2005. Copper in plants. Braz. J. Plant Physiol. 17, 145–156.
Yruela, I., Pueyo, J.J., Alonso, P.J., Picorel, R., 1996. Photoinhibition of Photosystem II from higher plants–effect of copper inhibition. J. Biol. Chem. 271, 27408–27415.
Yu, C.C., Hung, K.T., Kao, C.H., 2005. Nitric oxide reduces Cu toxicity and Cu-induced NH^{4+} accumulation in rice leaves. J. Plant Physiol. 162, 1319–1330.
Yuan, H.M., Liu, W.C., Jin, Y., Lu, Y.T., 2013. Role of ROS and auxin in plant response to metal-mediated stress. Plant Signal Behav. 8, e24671.
Yuan, M., Li, X., Xiao, J., Wang, S., 2011. Molecular and functional analyses of COPT/Ctr-type copper transporter-like gene family in rice. BMC Plant Biol. 11, 69.
Zengin, F.K., Kirbag, S., 2007. Effects of copper on chlorophyll, proline, protein and abscisic acid level of sunflower (*Helianthus annuus* L.) seedlings. J. Environ. Biol. 28, 561–566.
Zhang, Y., Han, X., Chen, X., Jin, H., Cui, X., 2009. Exogenous nitric oxide on antioxidative system and ATPase activities from tomato seedlings under copper stress. Scientia Hortic. 123, 217–223.
Zhao, S., Liu, Q., Qi, Y., Duo, L., 2010. Responses of root growth and protective enzymes to copper stress in Turfgrass. Acta Biol. Cracov Ser. Bot. 52, 7–11.
Zlobin, I.E., Kholodova, V.P., Rakhmankulova, Z.F., Kuznetsov, V.V., 2014. *Brassica napus* responses to short-term excessive copper treatment with decrease of photosynthetic pigments, differential expression of heavy metal homeostasis genes including activation of gene NRAMP4 involved in photosystem II stabilization. Photosynth. Res. 125, 141–150. http://dx.doi.org/10.1007/s11120-014-0054-0.