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Copper toxicity in plants: a review and a case study on tea

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Abstract

Copper in trace amounts is essential for various metabolic processes in the plant such as photosynthesis, carbohydrate distribution, and protein metabolism but at high concentration it causes physiological stress through generation of free radicals that induce the production of reactive oxygen species (ROS) via Haber-Weiss and Fenton reactions. Copper-induced generation of hydrogen peroxide, hydroxyl radicals, or other reactive oxygen species has been directly correlated with the damage to protein and lipids that may lead to reduced growth and even death. Tea (*Camellia sinensis* L. (O.) Kuntze) is an economically important plantation crop in India with round the year productivity. Copper based fungicides are cheap and effective in controlling fungal diseases and are used consistently throughout the year to combat different fungal diseases that pose a major threat to tea production. Excess Cu^{2+} has been found to alter several physiochemical parameters in the tea plants. A more detailed study on mechanisms of Cu^{2+} toxicity at the gene level is warranted.

Key words: Copper, stress, tea, reactive oxygen species, antioxidative enzymes.

Introduction

The role of copper in plants depends greatly on its concentration. Copper in trace amounts is an essential micronutrient for algae and higher plants for its role as a cofactor for metabolic processes like photosynthesis, respiration, carbohydrate distribution, nitrogen fixation, protein metabolism, ethylene perception, oxidative stress reduction, cell expansion and cell-wall lignification. At higher concentrations, copper can induce several negative effects including generation of reactive oxygen species, exchange of essential metal ions from the active sites and visible symptoms such as chlorosis, necrosis and growth inhibition (Marschner 1995; Prasad, 2004; Rehman et al. 2019). A well coordinated procedure of uptake, buffering, translocation and storage processes is necessary to uphold essential concentrations of the metal in various tissues and compartments within the narrow physiological limits (Clemens et al. 2002). Copper is transported into the plant cell by COPT family of transporters on the plasma membrane which has been described as a group of highly hydrophobic proteins; all its members contain 3 trans-membrane domains and specific Cu^{2+} binding site rich in methionine and histidine residues at the amino terminus (Kampfenkel et al. 1995; Sancenon et al. 2003; Andres-Colas et al. 2006). Copper homeostasis is maintained inside the cell by copper chaperones which sequester copper to a non-reactive form and also interact with other transport proteins for delivering copper to its necessary destinations (Himelblau and Amasino 2000; Company and Gonzalez-Bosch 2003; Chu et al. 2005). Two P-type ATPases, PAA1 and PAA2, are required for efficient copper delivery across the plastid envelope and the thylakoid membrane, respectively, in *Arabidopsis*

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(Shikanai et al. 2003; Abdel-Ghany et al. 2005). Inside the root, Cu^{2+} is said to be strongly accumulated in the cortex and the concentration decreases sharply from the outer to the inner cell layers (Adruini et al. 1996; Ducic and Polle 2005). Copper is poorly translocated by xylem and thus uptake by shoots is very low (Liao et al. 2000).

The aim of this review is to summarize the toxic effects of Cu^{2+} and focus on the recent developments on the various underlying metabolic changes that bring about such toxic effects. We also focus on tea, which is the most popular drink in the world after water. Tea (*Camella sinensis* L. O. Kuntze) is a perennial evergreen plantation crop with productivity round the year. The harvest includes tender shoots that are plucked normally at one to three weeks interval. This induces further vegetative growth and ensures continuous supply of green flushes (Burgess and Carr 1997; Karmakar and Banerjee 2005). Fungal pathogens such as *Exobasidium vexans* are capable of infecting the pluckable tender leaves thereby warranting a regular spraying of copper fungicides in heavy doses especially during the six month long monsoon period (May-October) when fungal infections assume massive proportions. This causes a buildup of Cu^{2+} in the soil over the years and the concentration of Cu^{2+} can easily overcome the threshold limit for toxicity.

Copper in plants

One of the major sites of copper accumulation in plants is the chloroplast. This metal is directly involved as a component of plastocyanin (PC) in the photosynthetic electron transport chain. PC is one of the most abundant proteins of thylakoid lumen (Kieselbach et al. 1998) and is essential for electron transfer between the cytochrome b6f complex and photosystem 1 (Weigel et al. 2003). The metal has a distinct regulatory role in electron transport between the photosystems as the constituent of PC (Maksymiec 1997). In the chloroplast stroma, Cu/Zn superoxide dismutase (SOD) requires Cu^{2+}, along with Zn, as cofactors to catalyze the dismutation of superoxide radicals (O_2^-) threby forming H_2O_2 and O_2^-. In *Arabidopsis thaliana*, out of seven identified SOD genes, the most active CSD1 and CSD2 genes both encode a Cu/Zn SOD with CSD1 activity in the cytosol and CSD2 activity in the stroma (Kliebenstein et al. 1998). Polyphenol oxidase is another Cu^{2+} protein found in the thylakoids of some plants, such as spinach (Kieselbach et al. 1998), but not in other species such as *A. thaliana* (Schubert et al. 2002). The enzyme has been proposed to be involved in the photoreduction of O_2^- by PS1 (Vaughn et al. 1988). Cu^{2+} mediates the activity of several other enzymes such as ascorbate oxidase which catalyses the reduction of O_2^- to water. The enzyme contains 8 Cu^{2+} ions which participate in the transfer of electrons in presence of ascorbate, the reducing substrate (Maksymiec 1997). Other important Cu containing proteins within plant cells include the mitochondrial cytochrome-C oxidase enzyme, the ethylene receptors in the endomembrane system and various apoplastic oxidases (Cohu and Pilon 2007). Copper is also necessary for amine oxidase function where it catalyses oxidative deamination of polyamines with the simultaneous formation of aldehyde, ammonia and H_2O_2 (Maksymiec 1997).

Copper as a toxic element

Inspite of the indispensability of copper in plant metabolism, excess copper has strong toxic effects. Copper can be limiting to plant productivity in crops when below 5 μg g^{-1} dry weight (DW),

whereas toxicity is reported above 30 µg g^{-1}DW (Marschner 1995). The most common feature of copper toxicity is the decrease in mass of roots. Copper toxicity can be damaging to plant roots, with symptoms ranging from disruption of the root cuticle and reduced root hair proliferation, to severe deformation of root structure (Sheldon and Menzies 2005; Lequex et al. 2010, Rehman et al. 2019). Cu^{2+} is toxic to plant cell which lead to plant retardation and leaf chlorosis (Rhoads et al. 1989; Yadav 2010). High Cu^{2+} concentrations predisposes photosystem II to photoinhibition (Patsikka et al. 2002), causes reduction in chlorophyll content arising from partial destruction of grana and modification of the protein-lipid composition of thylakoid membranes (Lidon ane Henriques 1991; Maksymiec 1997). Copper toxicity can also results in significant alteration in the concentration of minerals such as Fe, Mg, Ca, Zn, K and Na in both root and shoot (Lidon and Henriques 1993; Lequex et al. 2010).

Copper is relatively abundant in the earth's crust and better soluble, therefore more mobile than other heavy metals in the surface environment (Flemming and Trevors 1989). Copper concentration in non-polluted soils range from 10 to 80 ppm Cu^{2+} but soils located near mining areas or metal-processing industries may be contaminated by very large amounts of Cu^{2+} (Hagemeyer 2004). The bioavailability is determined by the form taken by the metal (ionic, complex or precipitated) which depends on environmental factors and therefore, varies widely, giving rise to possible conditions of toxicity (Flemming and Trevors 1989 Greger 2004). The level of bioavailable copper is increased by human activities which either increases the abundance or causes changes in soil chemistry thus affecting the solubility (Rhoads et al. 1989; Flemming and Trevors 1989). In the soil, copper remains immobilized onto the organic materials such as fulvic and humic acids and to clay and mineral surfaces. The bioavailibility in soil is strongly dependent on factors such as pH, cation exchange capacity (CEC), clay content, water hardness and organic matter content (Flemming and Trevors 1989; Greger 2004; Rooney et al. 2006, Rehman et al. 2019). Low pH increases the metal availability since the hydrogen ion has a higher affinity for negative charges on the colloids, thus competing with the metal ions of these sites, therefore releasing metals (Greger 2004). Rhoads et al. (1989) found that growth of tomato plants was reduced at soil pH below 6.5 with soil-copper levels above 150 mg. Thus soil properties have a significant impact in the expression of toxicity of copper in plants.

Agricultural soil in many parts of the world is contaminated by heavy metals (Brun et al.2001; Ballabio et al. 2018). The use of Bordeaux mixture for almost one century against vine downy mildew has caused severe copper contamination of soil in many wine-producing regions (Van-Zwieten et al. 2004). Copper contamination also caused serious problems in cereals such as rice (Lidon and Henriquesa 1993), wheat (Lanaras et al. 1993) and barley (Vassilev et al. 2003). Graham et al. (1986) found that excess fungicidal copper reduced seedling growth in citrus and also inhibited colonization of the roots by mycorrhizal fungus. In citrus orchards, stunted tress were produced with less mycorrhizal colonization under higher Cu concentrations and low pH (<5) conditions of the soil. In India, the major tea cultivation area comprises the eastern sub-Himalayan region where the soil is mainly acidic in nature (pH 4.2-5.8) (Singh and Singh 2006). While this is good for tea cultivation (Sarkar 1994), but it increases the possibility of Cu^{2+} ions

accumulated in the tea garden soils to become more available for absorption by plants which may lead to toxicity.

Copper in tea gardens

An example of an industry in India which depends primarily on copper fungicides is the tea industry. India is second only to China in tea production and the largest consumer of tea in the world. Currently, India produces 23% of total world production. It is the second largest industry in terms of employment and generally drives the economies of the regions where the tea gardens are concentrated, for example Assam and sub-Himalayan West Bengal (Selvakumar and Jeyaselvam 2012). Tea plants are cultivated extensively as large plantations where it is often allowed to grow under variant soil and climatic condition thereby making them prone to attacks by fungal pathogens. Major diseases include blister blight, brown blight, grey blight and black rot in leaves, and branch canker, thorny blight and pink disease in stems. To control the diseases, copper-based fungicides are used excessively in tea gardens of North East India including Assam and sub-Himalayan West Bengal (Barua 1988). The fungicides that are used most commonly include basic copper sulphate, Bordeaux mixture (a combination of hydrated lime and copper sulphate), Bicoxy (a new formulation of copper oxychloride 50% WP) and various customized formulations of copper sulphate and copper oxychloride (Worthing 1983; Singh 2005). A survey covering several tea gardens of the Darjeeling and adjoining Jalpaiguri district of sub-Himalayan West Bengal conducted by the authors has revealed that copper-fungicides are extensively used in the tea gardens of the Dooars and Terai region and also in the hilly regions of West Bengal. Copper based fungicides are used in large scale because they have multisite activity with a low risk of pathogens developing resistance (Van-Zwieten et al. 2004) and are relatively less phytotoxic than Ni based fungicides. In fact, copper based fungicides are highly recommended in literature and are often regarded as the most efficacious and economic fungicide for controlling the foliar diseases of tea (Singh 2005).

Mechanisms of Cu^{2+} toxicity

Copper is a redox active metal with an electrochemical potential of -260V. The redox nature of Cu^{2+} ions makes it very useful as a cofactor in electron transfer reactions (Ducic and Polle 2005). However, the reversible oxidation–reduction property of Cu^{2+} could also result in oxidative stress if Cu^{2+} would be present as a free ion. Heavy metals in general have been recognised as a major toxicant in plant cells due to their capability of generating reactive oxygen species (ROS) such as hydroxyl radical ($OH^{\bullet}$) superoxide (O_2^-) and hydrogen peroxide (H_2O_2), which can damage the biomolecules such as membrane lipids, proteins and nucleic acids. During the reduction of oxygen to water, ROS may be produced by a chain of reactions which initially needs energy input but subsequently occur spontaneously. O_2^- is a short-lived and moderately reactive ROS which reduces quinines and transition metal complexes of Fe^{3+} and Cu^{2+} thereby affecting the metal containing transporters and enzymes. O_2^- can additionally combine with protons in aqueous medium and form hydroperoxyl radicals ($HO_2^{\bullet}$) which can induce lipid auto-oxidation in membranes (Shaw et al. 2004). H_2O_2 is relatively long-lived and moderately reactive which oxidises the thiol groups of some enzymes (e.g. enzymes of the Calvin cycle and Cu-Zn SOD) and inactivates them (Vranova et al. 2002). However, the

most reactive of all the ROS is the hydroxyl radical ($OH^{\bullet}$) which can potentially react with all types of biomolecules and in excess can cause cell death because cells do not have any enzymatic antioxidant system to quench it. The radical is formed from H_2O_2 by the Haber Weiss and Fenton reactions and Cu^{2+} being a redox active metal catalyzes the formation of this most harmful active radical (Arora et al. 2002; Vranova et al. 2002) as summarized below:

$$H_2O_2 + O_2^{-} \xrightarrow{Cu+,\ Cu2+} OH^{-} + OH^{\bullet} + O_2^{-}$$

Haber Weiss reaction

One of the richest sources of ROS in plants is the chloroplast. These can be formed due to the highly energetic electron transfer reactions triggered by chlorophyll excitation along with an excess supply of oxygen. Singlet oxygen ($^1O_2$) can formed during de-excitation of chlorophyll which causes major oxidative damage to biomolecules. High light intensity can cause over reduction of PS1 and generation of excessive NADPH which cannot be utilized by the CO_2 fixation process thereby reducing the $NADP^+$ pools. O_2^-which is abundant in the chloroplast can take up electrons from PS1 in such a situation, which leads to production of ROS through the Mehler reaction (Sharma et al. 2012). Under conditions of low CO_2 fixation such as cold temperature or low CO_2 availability, excess reduction of PS1and increase in ROS levels can occur even at moderate light intensities. As H_2O_2 or O_2^- are only moderately reactive, therefore, the main responsible factor for the intense biological damage is the metal ion which catalyzes the formation of the highly toxic hydroxyl free radical ($OH^{\bullet}$) from H_2O_2 (Maksymiec 1997). Thus ROS may be generated in the plant due to several abiotic as well as biotic causes but true damage is caused by the additional metal toxicity.

The hydroxyl radical ($OH^{\bullet}$) can either add onto the biological molecules or eliminate hydrogen from them by forming water. The hydroxylated biomolecules can in turn hydroxylate other molecules thereby initiating a chain of reaction or change to stable oxidised products. The activated hydroxylated molecules can also dismute themselves by forming intermolecular cross links (Shaw et al. 2004). Oxidised Cu^{2+} ions can be actively involved in electron transfer during formation of stable oxidized products. In reactions where the $OH^{\bullet}$radical eliminates H from biomolecules, it leaves an unpaired electron in the organic molecule thereby forming a reactive organic radical which can then react with oxygen to form peroxy radical ($ROO^{\bullet}$). The peroxy radical is again a reactive species and can eliminate hydrogen from other biomolecules and change them into organic radical products thereby creating a chain of reactions. The peroxidation reaction is evident in lipid peroxidation reactions that take place in cell membranes to form lipid peroxides (ROOH) (Shaw et al. 2004; Arora et al. 2002). However, in presence of reduced Cu^{2+} ions which can participate in Fenton reaction (shown below), the highly reactive alkoxy radical (RO^{-}) is formed from the ROOH which is as damaging as the hydroxyl radical thus opening up another cascade of immensely damaging oxidative reactions.

$$ROOH + Cu^{+} \longrightarrow OH^{-} + Cu^{2+} + RO^{-}$$

A study on the toxicity mechanisms suggest that the generation of reactive oxygen species is a natural phenomenon but is increased to alarming proportions due to presence of stress factors. Presence of Cu^{2+} ions above the threshold limit is

immensely stressful to plants due to its redox nature as it can catalyze and enhance the formation of all types of ROS by participating actively in several types of oxidative reactions.

Plant response to Copper toxicity

Plants have developed a wide range of protective mechanisms for mitigating copper toxicity. Primary defence mechanisms prevent metal to enter into the cell via exclusion, or binding of metal to cell wall and other ligands, organic acids, amino acids, glutathione (GSH) or phytochelatins (PCs) to render them harmless (Antosiewicz and Wierzbicka 1999; Rehman et al. 2019). Antioxidative mechanisms that control the level of ROS and shield the system before the sensitive parts of the cellular machinery gets damaged are mediated by molecules which have been broadly divided into two types, the high molecular weight enzymatic catalysts and the low molecular weight antioxidants (Pinto et al. 2003). The enzymes involved in scavenging ROS include SOD, catalase (CAT), peroxidases (POD) and glutathione peroxidase and those involved in detoxifying lipid peroxidation products include glutathione-S-transferases (GST), phospholipid-hydroperoxide glutathione peroxidase and ascorbate peroxidase (APX). Table 1 enlists the different enzymes which have been studied in relation to copper toxicity. The low molecular weight compounds that act as cellular antioxidants are ascorbate, glutathione, phenolics, flavonoids, carotenoids and tocopherols. Besides these, a whole array of enzymes is needed for the regeneration of active forms of the antioxidants such as monohydroascorbate reductase and glutathione reductase (Blokhina et al. 2003; Pinto et al. 2003).

Binding of copper and its sequestration

Plant adapt to heavy metal stress by acquiring several strategies, the most prominent being the synthesis of phytochelatins and metallothioneins which contribute to metal detoxification by chelation of the metal ions. Phytochelatins are simple thiol rich metal binding peptides containing glutamate, cystein and glycine in ratios of 2:2:1 to 11:11:1 (Grill et al. 1985; Prasad 2004). These peptides are synthesized non-translationally from glutathione in the presence of heavy metals by the enzyme phytochelatin synthase (Grill et al. 1989). Apart from being a precursor to phytochelatins, glutathione is also an important antioxidant molecule, which plays a predominant role in protection against free radicals (Alscher 1989). Copper induced metallothioneins are low molecularincrease in phytochelatin synthesis results in oxidative stress through the depletion of the antioxidant glutathione. De Vos et al. (1992) showed that copper tolerance in the plant species*Silene cucubalus* does not depend on the production of phytochelatins but is related to the ability of this plant to prevent glutathione depletion resulting from copper-induced phytochelatin production weight proteins with high cystein content, which bind metal ions to form metal thiolates and metal thiolate clusters. Class III metallothioneins are found in plants and is reported to be induced by the presence of a variety of metals including Cd, Cu, Zn, Pb, Hg and Ag (Hamer 1986; Prasad 2004). However, phytochelatins rather than metallothioneins are mainly responsible for detoxification of toxic heavy metals (Yadav 2010). Moreover, metal binding ability is higher in phytochelatins than in metallothioneins on a per-cysteine basis (Mehra andMulchandani 1995).

Table 1 Enzymes/Metabolites whose levels have been studied after copper exposure

Enzyme/Metabolite	Plant	Location	Reference
Peroxidase	*Zinnia elegans* and *Cosmos sulfureus*	shoots and roots	Tsay et al. 1995
	Zea mays L.	leaves and roots	Mocquot 1996
	Helianthus annuus	leaves and roots	Garcia et al. 1999
	Oryza sativa	leaves	Fang and Kao, 2000
	Capsicum annum	seedlings	Diaz et al. 2001
	Phaseolus vulgaris	leaves and roots	Cuypers et al. 2002
	Allium sativum	leaves and roots	Meng et al. 2007
	Erica andevalensis	leaves, roots	Oliva et al. 2010
	Zea mays	roots	Zhao et al 2010
	Vigna mungo	seedlings	Solanki et al. 2011
	Beta vulgaris L.	leaves	Morales et al. 2012
	Camellia sinensis	leaves	Saha et al. 2012
Catalase	*Avena sativa*	leaves	Luna et al. 1994
	Lycopersicon esculentum	leaves, stem and roots	Mazhoudi et al. 1997
	Oryza sativa	seedlings	Chen et al. 2000
	Camellia sinensis	root	Ghanati et al. 2005
	Prunuscerasifera	seedlings	Lombardi and Sebastiani, 2005
	Zea mays	roots and shoots	Pourakbar et al. 2007
		leaves and roots	Moravcová et al, 2018
	Vigna mungo	seedlings	Solanki et al. 2011
	Atriplex halimus	leaves	Brahim and Muhamed, 2011
	Cucumi sativus	roots	Iseri et al. 2011
	Lens culinaris	shoots	Hossain et al. 2020
Superoxide dismutase	*Nicotiana tabacumGlycine max*	leaves	Pitcher et al. 1991
		root	Chongpraditnun et al. 1992
	Nicotiana tabacum and *Pisum sativum*	leaves	Sen Gupta et al. 1993
	Holcus lanatus	root	Hartley-Whitaker et al. 2001
		roots	Wang et al. 2004
	Brassica juncea	root	Ghanati et al. 2005
	Camellia sinensis	root and shoot	Lombardi and Sebastiani, 2005
	Prunuscerasifera	root, stem and	Peng et al. 2006
	Elsholtzia splendens	leaves	Ke et al. 2007
		roots and leaves	Meng et al. 2007

	Daucus carota	leaves and roots	Zhang et al. 2008
	Allium sativum	root	Gao et al. 2008
	Elsholtzia haichowensis	root, stem and leaves	Nie et al. 2012
	Jatropha curcas	leaves	Moravcová et al, 2018
	Zea mays	leaves and roots	Azooz et al. 2012
	Triticum aestivum cv. Hasaawi	seedlings	
Ascorbate peroxidase	*Avena sativa*	leaves	Luna et al. 1994
	Lycopersicon esculentum	leaves, stem and roots	Mazhoudi et al. 1997
	Phaseolus vulgaris	leaves and roots	Weckx and Clijsters, 1996
	Oryza sativa	root	Chen et al. 2000
	Camellia sinensis	root	Ghanati et al. 2005
	Morus rubra	leaves	Tewari et al. 2006
	Oryza sativa	root and shoot	Thounaojam et al. 2012
	Camellia sinensis	root and shoot	Hajiboland and Bastani, 2012
	Camellia sinensis	leaves	Saha et al. 2012
	Lens culinaris	shoots	Hossain et al. 2020
γ-glutamylcysteinyl synthetase	*Camellia sinensis*	leaves	Yadav and Mohanpuria, 2009
	Triticum aestivum	leaves	Shan et al. 2012
Glutathione reductase	*Silene cucubalus*	root	De Vos et al. 1992
	Panax ginseng	roots	Ali et al. 2006
	Morus rubra	leaves	Tewari et al. 2006
	Zea mays	roots and leaves	Pourakbar et al. 2007
	Oryza sativa	root and shoot	Thounaojam et al. 2012
	Triticum aestivum	leaves	Shan et al. 2012
	Zea mays	roots	Wang et al. 2011
	Zea mays	leaves	Nie et al. 2012
	Lens culinaris	shoots	Hossain et al. 2020
Dehydroascorbate reductase	*Cucumis sativus*	roots and leaves	Arora et al. 2002
	Panax ginseng	roots	Ali et al. 2006
	Triticum aestivum	leaves	Shan et al. 2012
	Lens culinaris	shoots	Hossain et al. 2020
Phenylalanine ammonia lyase	*Phyllanthus tenellus*	leaves	Santiago et al. 2000 Basak et al. 2001
	Camellia sinensis	leaves	Chakraborty et al. 2002

	Camellia sinensis	leaves	Kovacik and
	Matricaria recutita	root and leaves	Backor, 2007
	Glycine max	roots	Chmielowska et al. 2008
	Jatropha curcas	root, stem and leaves	Gao et al. 2008
Polyphenol oxidase	*Camellia sinensis*	leaves	Basak et al. 2001
	Jatropha curcas	root, stem and leaves	Gao et al. 2008

In addition, phytochelatins possess the ability to scavenge ROS and thereby aid in mitigating oxidative stress (Tsuji et al. 2002).

Accumulation of amino acids like proline has been observed in response to several biotic and abiotic stresses in plants. Content of free proline has been found to be related to Cu^{2+} tolerance in plants (Backor et al. 2003; Chen et al. 2004). Excess Cu^{2+} has been found to result in inadequate proline (Thomas et al. 1998) and lead to the malfunctioning of copper exclusion machinery (Chen et al. 2004). Copper complexes with amino acids such as proline, histidine or nicotinamine play important role in xylem sap transport (Liao et al. 2000).

Antioxidant response

Plants possess well developed defence system against ROS which restricts its formation and maneuver its removal. Inside the plant cell, superoxide dismutases (SOD) provide the first line of defence against ROS. The enzyme is located in different cell compartments including mitochondria, chloroplast, glyoxisomes, peroxisomes, microsomes, apoplast and cytosol (Alscher et al. 2002) and catalyzes the disproportionation of O_2^- to H_2O_2 and molecular oxygen (Scandalios 1993). SOD enzymes are classified based on the metal cofactors: the Cu-Zn SOD, the Mn-SOD and Fe-SOD (Bowler et al. 1994). Although each type of SOD predominates in specific cell compartments, their occurrences are not restricted, and all types can be detected in most of the cellular locations (Arora et al. 2002). An increased level of SOD has been correlated to enhanced oxidative stress protection in plants (Sen Gupta et al. 1993). Increase in SOD activity has been reported against copper induced stress in tolerant plants such as *Prunuscerasifera* (Lombardi and Sebastiani 2005); *Elsholtzia haichowensis* (Zhang et al. 2008); *Elsholtzia splendens* (Peng et al. 2006); *Jatropha curcas* (Gao et al. 2008); *Holcus lanatus* (Hartley-Whitaker et al. 2001); *Daucus carota* (Ke et al. 2007); *Ceratophyllum demersum* (Rama Devi and Prasad 1998); *Brassica juncea* (Wang et al. 2004); *Hydrilla verticillata* (Srivastava et al. 2006); *Zea mays* (Nie et al. 2012), *Triticum aestivum* cv. Hasaawi (Azooz et al. 2012), *Allium sativum* (Meng et al. 2007) etc. However, Weckx and Clijsters (1996) observed that SOD was not involved in the defence mechanism against copper induced oxidative stress in primary leaves of *Phaseolus vulgaris*. Contradictory results have also been recorded regarding the response of catalase (CAT) against copper stress. Both CAT and peroxidase (POD) are involved in the removal of H_2O_2 that accumulates due to dismutation of O_2^- by SOD. Catalase activity did not increase in Cu^{2+} stressed roots of rice seedlings (Chen et al. 2000) or in black gram (Vigna mungo) seedlings (Solanki et al. 2011) and decreased in *Lens culinaris* seedlings (Hossain et al. 2020). On the other hand,

CAT activity was reported to increase in *A. halimus* leaves (Brahim and Muhamed 2011) *Prunuscerasifera* (Lombardi and Sebastiani 2005), *C. sativus* roots (Iseri et al. 2011) and in maize roots, shoots and leaves (Pourakbar et al. 2007, Moravcová et al. 2018) in response to excess Cu^{2+} concentrations. The mobilization of POD in response to Cu^{2+}induced oxidative stress in plants is well accepted (Fang and Kao 2000; Diaz et al. 2001; Cuypers et al. 2002; Meng et al. 2007; Solanki et al. 2011). Apart from POD and CAT, the enzymes and metabolites of the ascorbate-glutathione cycle are also involved in the removal of H_2O_2. The majority of these enzymes [ascorbate peroxidase (APX), glutathione reductase (GR), and dehydroascorbate reductase (DHAR)] have been found in chloroplasts, cytosol, mitochondria, and peroxisomes (Dat et al. 2000). Glutathione and ascorbate accumulate in these cellular compartments and their redox state is maintained through glutathione reductase (GR), monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR).

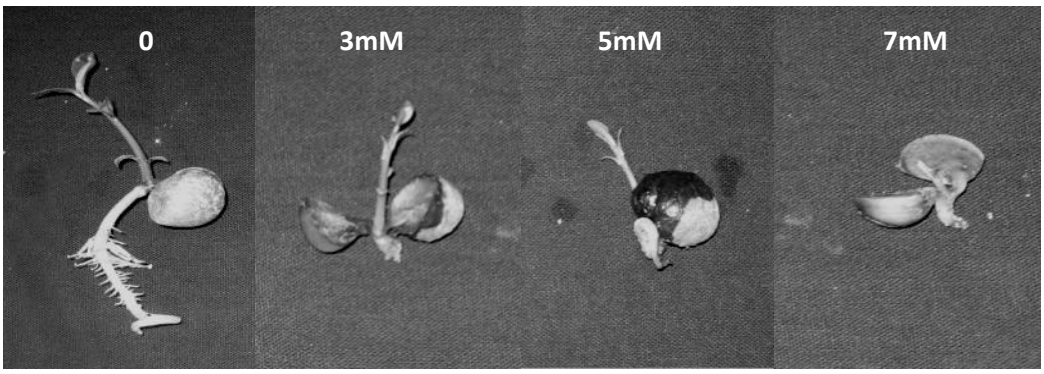

Fig. 1 Effect of excess copper on germination of tea seeds: reduction in root and shoot lengths of germinated tea seeds of TS 462 variety on exposure to different concentrations of $CuSO_4$ (indicated in the figure) photographed after 27 days of treatment

All these enzymes along with ascorbate and glutathione have a pivotal role in defence against ROS induced oxidative damage (Arora et al. 2002; Yruela 2005; Sharma and Dietz 2008; Shan et al. 2012). De Vos et al. (1992) observed that glutathione depletion is the major cause of Cu^{2+} induced oxidative damage in Cu^{2+} sensitive *SiIene cucubalus* plants. It has been shown that tolerance to a copper-enriched environment, and the accompanying oxidative stress in *Enteromorpha compressa* occurs through the accumulation of copper, activation of ascorbate peroxidase, synthesis of ascorbate (accumulated as dehydroascorbate) and consumption of glutathione and water-soluble phenolic compounds (Ratkevicius et al. 2003).

Stress in tea

A literature survey revealed that several studies have been conducted on different types of abiotic stresses in tea. Plants of different cultivars of tea have been grouped into the tolerance classes: susceptible and resistant, in response to drought stress (Chakraborty et al. 2002; Damayanti et al. 2010), cold stress (Upadhyay 2012) and heavy metal stress (Yadav and Mohanpuria 2009). Several parameters have been identified such as rates of photosynthesis and transpiration, relative water content, stomatal

conductance and leaf total soluble sugar content (Damayanti et al. 2010), root and shoot extension (Burgess and Carr 1997), levels of proline and antioxidative enzymes (Chakraborty et al. 2002; Upadhyay and Panda 2004; Upadhyay et al. 2008), morphological characters (Waheed et al. 2012) etc. in order to screen tea cultivars for drought tolerance. Additionally, studies on alterations in bioconstituents that determined quality of tea in the tea clones under soil moisture revealed a decrease in PAL activity in both tolerant and susceptible clones which correlated with a lower flavonol content and quality deterioration (Jeyaramaja et al. 2003).

Tea plants exposed to excess heavy metals have shown several alterations in physiological and biochemical parameters. Increased level of lipid peroxidation and a reduction in photosynthetic rate, transpiration rate, chlorophyll and protein content and biomass production were found in plants exposed to excess Cd (Mohanpuria et al. 2007; Shi et al. 2008). Oxidative stress was evident as the transcript levels of glutathione biosynthetic genes showed up-regulation while glutathione-S-transferase (GST), the enzyme which help in sequestration of high levels of metal ions to vacuole, did not show any change on Cd exposure (Mohanpuria et al. 2007). Hajiboland and Bastani (2012) observed that CO_2 assimilation and dry matter production decreased while antioxidant enzyme activity and proline content increased significantly in tea plants under Boron deficiency and water stress. Mukhopadhyay et al. (2013) observed that both deficiency and excess in zinc caused a considerable decrease in shoot and root fresh and dry masses. Zinc stress decreased net photosynthetic rate, transpiration rate, stomatal conductance, and content of chlorophylls *a* and *b* and increased the content of superoxide anion, malondialdehyde, hydrogen peroxide, and phenols. Although the activities of ascorbate peroxidase, catalase, superoxide dismutase, and peroxidase as well as expression of respective genes were up-regulated, the authors concluded that the overall antioxidant system did not afford sufficient protection against oxidative damage (Mukhopadhyay et al. 2013). Treatment of tea plants with excess heavy metals such as mercury (II) and nickel (II) decreased the chlorophyll content of the leaves, along with a significant reduction in Hill activity (Basak et al. 2001). The activities of antioxidative enzymes viz. Superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) was increased by Aluminium in the roots of cultured tea cells and also in intact plants (Ghanati et al. 2005). Aluminum (Al) inhibited tea pollen tube growth but the effect was found to be alleviated by fluorine (Konishi and Miyamoto 1983) which is accumulated by tea plants normally in high excess (Ruan et al. 2004). Tea plants tolerated fluorine at concentrations < 0.32 mM (Li et al. 2011). Fresh and dry mass, chlorophyll content and net photosynthetic rate decreased while proline, malondialdehyde and hydrogen peroxide contents increased with increasing fluorine concentrations. Activity of antioxidant enzymes also showed significant alterations thereby suggesting that antioxidant defence system of leaves did not sufficiently scavenge excessive reactive oxygen species generated due to excess fluorine (Li et al. 2011).

Cu^{2+} stress in tea

Although copper-based fungicides are being used in tea gardens for several decades (Sarmah 1960), we know little about the role of excess Cu^{2+} on tea plants and at what concentrations it may be considered as a pervasive threat (Saha et al. 2012). Only a few studies have focused

on Cu^{2+} toxicity in tea (Basak et al. 2001; Yadav and Mohanpuria 2009; Saha et al. 2012; Dey et al. 2014, 2015) and these have revealed that number physiochemical parameters are altered on exposure to excess copper. For example, the chlorophyll and protein contents were found to decrease in Cu^{2+} treated plants (Basak et al. 2001; Yadav and Mohanpuria 2009; Saha et al. 2012). Germination of tea seeds were also affected in presence of excess copper. Substantial reduction in the length and biomass of root and shoot (Fig.1) was observed (Mandal et al, 2013). Excess Cu^{2+} caused an increase in lipid peroxidation, phenolics and antioxidative enzyme levels such as POD, SOD and APX in multiple cultivars of tea (Saha et al. 2012; Dey et al. 2015). A significant difference among cultivars was noted where the more sensitive cultivar seemed to lose its antioxidative capacity at Cu^{2+} concentrations higher than 400 µM while the more tolerant cultivar was able to withstand a maximum of 600 µM of Cu^{2+} ions. Two new isozymes were also found to be induced in the leaves of tea exposed to high concentration of Cu^{2+} (Saha et al. 2012). Yadav and Mohanpuria (2009) observed that expression of the enzymes γ-glutamylcysteinyl synthetase, glutathione synthetase and phytochelatin synthase was elevated more in the tolerant tea cultivar than the susceptible one when exposed to excess Copper and Aluminium.

Conclusion

Heavy metal stress is one of the major problems that limit agricultural productivity of plants. Plants show relative differences in their heavy metal tolerance capacity among the species and also among cultivars of the same species. Copper stress in general induces ROS and generates oxidative stress. It has been found that in addition to accumulated metal ions, high levels of ROS adversely affected the plants. Such ROS related damages have been observed in tea cultivars also. Although of the negative impact of excess Cu^{2+} in tea plants have been documented, the level of Cu^{2+} accumulation caused due to long term application of Cu^{2+}-based fungicides in tea gardens and its bioavailability under tea garden conditions are yet to be studied. Additionally, more detailed studies on mechanisms of Cu^{2+} toxicity in the tea plant, especially at the gene level are necessary. Identification of genetic determiners of tolerance may make the resistant cultivars a potential source for genetic manipulation of other important elite cultivars.

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