Journal of Stress Physiology & Biochemistry, Vol. 7 No. 3 2011, pp. 165-184 ISSN 1997-0838

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Significance of Sulphur nutrition against metal induced oxidative stress in plants

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Received June 28, 2011

Sulphur (S) is available to plants as sulphate (SO_4^{2-}) in the soil to the roots and as gaseous SO_2 in the atmosphere to the shoots. Of all the macronutrient, S is perhaps the nutrient which has attracted the most attention in soil science and plant nutrition due to its potential defensive characteristics to pests, good nutritive potentiality to crops and its relative immobility in the soil-plant system. This review highlights the mechanisms of uptake and translocation of S from soil and its transport in xylem and phloem tissues of plants. Moreover, it also throws new insights, have led us to revisit the hypothesis of S chelation in reference to oxidative stress induced by heavy metals sequestration.

Key words: Xylem transport, phloem transport, oxidative stress, translocation, antioxidant, chelation.

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Of the total 17 naturally occurring essential elements, 7 are considered as macro-elements (Mengel and Kirkby, 1987), and all are of great biological importance. Among them nitrogen (N), potassium (K), calcium (Ca) are non-toxic to an extent at low to moderate concentration while Phosphorus (P) and S seems to be more or less toxic to plants at higher concentration (Niess, 1999). In fact, yield of most crop plants increase linearly with the amount of fertilizers that they absorb (Loomis and Conner, 1992) (Figure 1) (Table 1). Plants display a higher degree of physiological and developmental plasticity in response to their changing nutritional conditions (Forde and Lorenzo, 2001). Mineral elements those acquired primarily in the form of inorganic ions, continuously cycle through all organisms and their environments (Epstein, 1972) and enter the biosphere predominantly through the root system of plants, so in a sense, plants act as the Miners of the earth crust

(Epistein, 1994). Crop plants, however, typically use less than half of the fertilizer applied (Loomis and Conner, 1992) and the rest may leach out to water bodies and now becoming a major cause of water pollution (Hill, 1997) (Table 2).

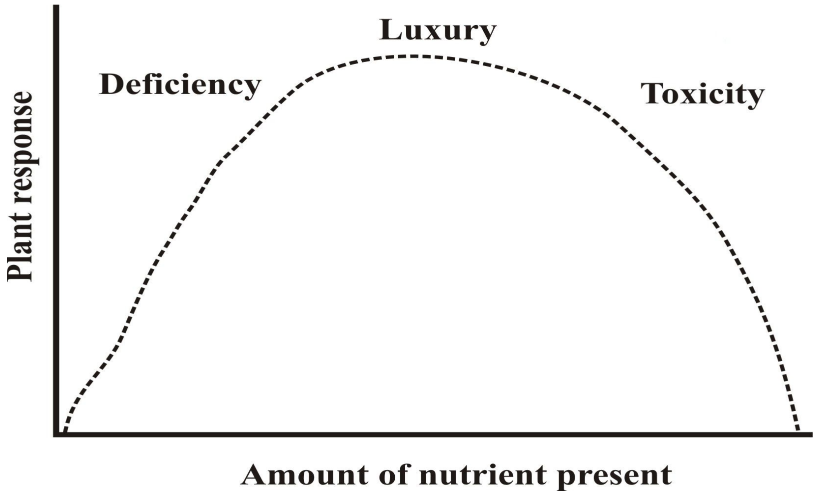

Figure 1. Response of plant to amount of one nutrient.

Table 1. Macro-elements classified on the basis of their mobility with in a plant and their tendency to re-translocate during deficiencies.

Mobile	Im-mobile
N	Ca
K	S
Mg	Si
P	

Note: Elements are listed in the order of their abundance in the plant.

Table 2. Adequate tissue levels of macro-nutrients that may be required by plants (Epstein, 1972, 1994).

Element Name	At. Wt.	Concentration in dry matter	%	Relative no of atoms with respect to molybdenum.
N	14.01	1,000	1.5	1,000,000
K	39.10	250	1.0	250,000
Ca	40.08	125	0.5	125,000
Mg	24.32	80	0.2	80,000
P	30.58	60	0.2	60,000
S	32.07	30	0.1	30,000
Si	28.09	30	0.1	30,000

S is the 9th and least abundant essential macronutrient in plants. The dry matter of S in plants is only about 1/15 of N and it is necessary to be assimilated in to organic metabolites as well as play critical roles in the catalytic or electrochemical function of the biomolecules in cells (Saito, 2004). It is found in amino acids (Cys and Met), oligopeptides (glutathione (GSH) and PCs), vitamins and cofactors (Biotin, thiamine Co A and SAM) and a variety of secondary products (GSL in Cruciferae and Allyl Cys sulfoxides in Liliaceae) (leustek, 2002). Cys is a component in organic compounds including GSH that have been implicated in the adaptation of plants to various types of stresses affects the kinetics of cysteine synthase (CS) formation leading to destabilization of the complex. CS, formed by the pyridoxal 5'-phosphate-dependent enzyme O-acetylserine sulfhydrylase (OASS) and serine acetyltransferase (SAT), the branch point of the S, C, and N assimilation pathways (Salsi et al., 2010). The regulation of CS activity through the transient formation of multiprotein assemblies plays an important role in the control of biosynthetic pathways. Cys-rich PR proteins, such as non-specific lipid transfer proteins (nsLTPs) and metallocarboxypeptidase inhibitors are candidates for the sequestration of metals (Harada et al., 2010). OAS-TL catalyses the final step of Cys biosynthesis and its isoforms are localised in the cytoplasm, the plastids and mitochondria but the contribution of individual O-acetyl serine (thiol) lyase (OASTL) isoforms to plant S metabolism has not yet been fully clarified. OAS-A1 (gene encoding the cytosolic OAS-TL) is involved in maintaining S and thiol levels and is required for resistance against cadmium (cd) stress (Shirzadian-Khorramabad et al., 2010). The antioxidant function of 2-Cys peroxiredoxin (Prx) involves the oxidation of its conserved peroxidatic Cys to sulphenic acid that is recycled by a reductor agent. During the oxidative stress, the peroxidatic Cys can be overoxidized to sulphinic acid inactivating the Prx.

Moreover, S nutrition significantly increased the Cys, GSH and alliin contents of leaves and bulbs in onion, at all harvesting dates and at maturity (Mohamed and Aly, 2008). The thiol (-SH) group of Cys in proteins takes a job of maintaining protein structures by forming disulphide bonds between two Cys residues via oxidation. The thiol of Cys and GSH is often involved in the redox cycle by two thiol↔disulphide conversions. This interchange is versatile for redox control and mitigation against oxidative stress in nearly all aerobic organisms including plants (Leustek and Saito, 1999). In addition, GSH contents increased in response to salt stress in leaves and stress-induced decreases in transpiration and net CO_2 assimilation rates at light saturation as well as enhanced rates of photorespiration cause accumulation of Gly and Ser in leaves immediate precursors of GSH (Herschbach et al., 2010). The nucleophilicity of the thiol group and in particular, GSH, play an important role in detoxification of xenobiotics by direct conjugation with sulfhydryl (-SH) group mediated by glutathione S-transferase (GST). Since, GSH function in xenobiotic and heavy metal detoxification, plant development and plant-pathogen interactions. As well as its emerging functions for plant GRXs and GSH concern the regulation of protein activity via glutathionylation and the capacity of some GRXs to bind Fe-S centres and for some of them to transfer Fe-S clusters into apoproteins (Rouhier et al., 2008). The level of GSH was lower and the redox state was significantly more oxidized, led to an up regulation of the cytosolic isoforms of GSH reductase and monodehydroascorbate reductase (MDHA), as well as alternative antioxidants like flavonoids and anthocyanins were increased in the S-deprived plants (Lunde et al., 2008). PCs are involved in detoxification of heavy metals by serving as chelating ligands through thiol groups. Therefore, it is well cleared that proper S nutrition is one of the good strategies to alleviate the damaging effects of Cd on plants, and to avoid its entry into the food chain, developing tolerance to Cd toxicity and thus, low Cd uptake and accumulation in different parts of crop plants (Sarwar et al., 2010).

S cycle in nature

S cycle is a good example to illustrate linkage between the air, water and soil and also provides excellent examples of the interaction and regulation that exists between different minerals cycles and demonstrates the complex biological and chemical regulation. Plants require a continuous supply of S and its compounds in order to synthesize some amino acids and proteins. Inorganic SO_4 is a major source of biologically significant S. Most of the biologically incorporated S is mineralized by bacteria and fungi in ordinary decomposition by species of Aspergillus and Neurospora. Some organic S enters the atmosphere as SO_2 threw incomplete combustion of fossil fuels, especially coal. Inorganic S as SO_4^{2-} may participate out, but since it is relatively soluble, it serves as a source of elemental S in many ecosystems. SO_4 is also reduced under anaerobic conditions to elemental S, or to sulphide, including H_2S by such heterotrophic bacteria as species of Beggiatoa, oxidises H_2S to elemental S and species of Thiobacillus, oxidise it to SO_4^{2-} as shown in the following reactions.

$$H_2S \xrightarrow{\text{Beggiatoa}} S \xrightarrow{\text{Thiobacillus}} SO_4$$

$$H_2S \xleftarrow{\text{Anaerobic } SO_4 \text{ reduction}} {}_4$$

Moreover, SO_4^{2-} is the principal biologically available form that is reduced by autotrophs and incorporated in to proteins, S being an essential constituent of certain amino acids. Not as much S is required by the ecosystem, nor it is often limiting to the growth of plants and animals. Nevertheless, the S is a key one in the general pattern of production and decomposition. Figure 2 provides estimates of amount of S in the reservoir pools (Lithosphere, Atmosphere and Oceans), and the annual fluxes in and out of these pools including inputs and outputs directly related to human activities. Once in the atmosphere, this gaseous phase is converted to other forms, principally SO_2, SO_4^{2-} and S aerosols. S aerosols, unlike CO_2, reflect sun light back in to the sky, thereby contributing to global cooling and to acid rain.

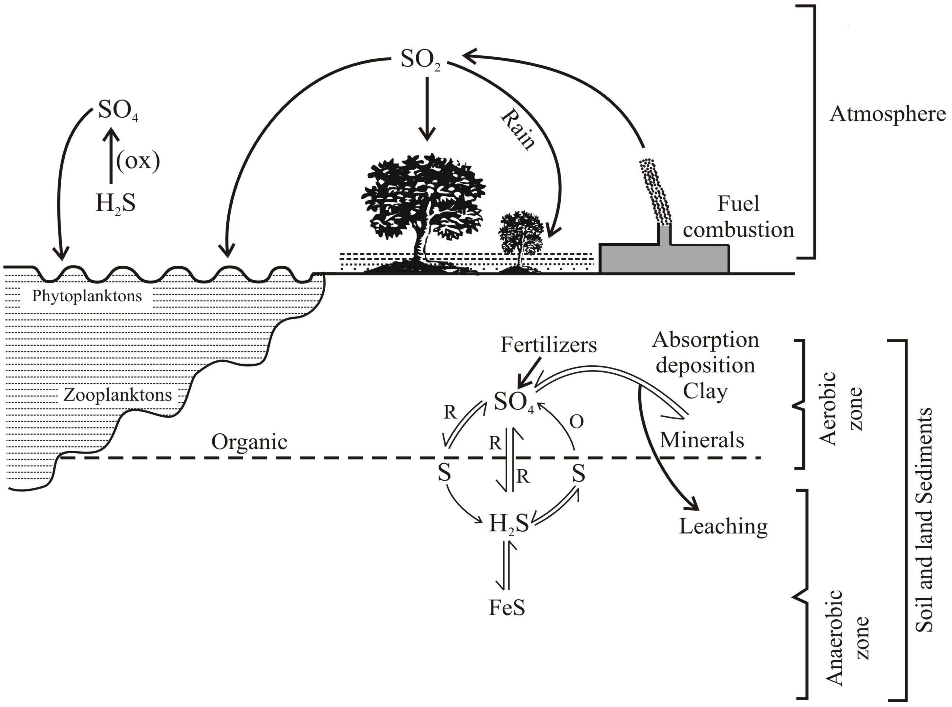

Figure 2. S cycle, linking air, water and soil (modified Odum, 1966).

Moreover, S cycle is increasingly being affected by industrial air pollution. The gaseous oxides of S are toxic to varying degrees. Normally they are only transitory steps in their respective cycles in most environments. They are present in very low concentrations and adversely affect important biotic components and processes of ecosystems. They constitute about 1/3 of industrial air pollutants discharged into the air. Coal burning emissions and automobile exhaust are major sources of SO_2 and SO_4 production. SO_2 is damaging to photosynthesis. The destruction of vegetation around copper smelters is largely caused by SO_2. Oxide of S along with water vapour produce acid rain (SO_2 + NO_2), a truly alarming development, has great impact on soft water lakes and already acidic soil that lack pH buffer. The increase in acidity in lakes has rendered them in capable of supporting their fauna and flora.

Mechanism of uptake and translocation

S plays a pivotal role in the cellular metabolism of many organisms. In plants, the uptake and assimilation of SO_4^{2-} is strongly regulated at the transcriptional level. Activity of SO_4^{2-} uptake system in higher plants is related to external SO_4^{2-} conditions. In higher plants, S metabolism is initiated by the uptake of SO_4^{2-} by roots from the environment. Plants assimilate inorganic SO_4^{2-} in to Cys. Thus plants serve as nutritional S sources for animals (Giovanelli et al., 1980). Uptake of SO_4^{2-} by plants is considered to be the key entry step of the S-cycle in the nature. Low experimental S supply is up regulating the expression of high affinity SO_4^{2-} transporter SULTRI 1 (HAST), present in the plasma-membrane of root hairs, epidermis and cortex cells (Maruyama-Nakashita et al., 2004). HAST also up regulates by Cd and Zn exposure, resulting higher SO_4^{2-} concentration in root (Nocito et al., 2006; Sun et al., 2007). The expression of HAST seems to be undetectable in plants growing in S-sufficient conditions (Herbette et al., 2006). Second SO_4^{2-} transporter, SULFTR 2 is abundantly expressed at higher SO_4^{2-} levels than HAST. The effect of Cd along with other heavy metals on the expression of SULFTR1, 2 has not been analysed till now, but it is well cleared that it have low affinity to SO_4^{2-} and involved in transport at their expression sites (Hawkesford and De Kok, 2006).

In addition, the study of SULFTR1, 2 activities will be very welcome to judge primary step of S-metabolism in plant growing in heavy metal containing richly areas. A decrease of this transporter activity may keep sufficient S in the roots for Cd detoxification as proved by increasing levels of sulphide, GSH and non-protein thiols (Sun et al., 2007). SULTR 3 localised in shoots, help to short out the diminished activity of SULFTR 1, 2 because SULTR 3 is not sensitive to Cd. The expression SULTR 4, 1 are interfering with short distance efflux of SO_4^{2-} from the vacuole into the cytoplasm, is not affected by exposure of Cu (Sehiavon et al., 2007). Therefore the influence of heavy metal stimulated expression of most of these S-suppliers certainly ensures an optimum S requirement for heavy metal detoxification. If this stimulation find in case of other heavy metals, has still to be investigated.

Moreover, the SO_4^{2-} is transported into plant root cells by SO_4^{2-} transporters and then mostly reduced to S^{2-}. The S^{2-} is then bonded to OAS through the activity of Cys synthase (OASTL) to form Cys. OASTL, a key enzyme of the plant S assimilatory pathway, catalyses the formation of Cys from S^{2-} and OAS. Transgenic hybrid poplar (*Populus sieboldi x P. grandidentata 'Y63'*) plants expressing cys1 found to accumulated up to several-fold higher Cys and GSH levels and were significantly more resistant in terms of foliar damage to the pollutants than control (Nakamura et al., 2009). Studies of Shivbagaki and Grossaman (2010) show that a root plasma membrane SO_4^{2-} transporter of Arabidopsis, SULTR1; 2, physically interacts with OASTL. The interaction was initially demonstrated using a yeast two-hybrid system and corroborated by both *in vivo* and *in vitro* binding assays. The domain of SULTR1; 2 shown to be important for association with OASTL is called the STAS domain. This domain is at the C terminus of the transporter and extends from the plasma membrane into the cytoplasm. The functional relevance of the OASTL-STAS interaction in yeast mutant cells devoid of endogenous SO_4^{2-} uptake activity but co-expressing SULTR1; 2 and OASTL. The analysis of SO_4^{2-} transport in these cells suggests that the binding of OASTL to the STAS domain in this heterologous system negatively impacts transporter activity. Therefore they suggests a regulatory model in which interactions between SULTR1; 2 and OASTL coordinate internalization of SO_4^{2-} with the energetic/metabolic state of plant root cells.

SO_4^{2-} transporters are differentially affected at metal exposure. S containing amino acids (Cys and Met) and stress response-related compounds (such as GSH), are derived from reduction of root-absorbed SO_4^{2-}. SO_4^{2-} distribution in cell compartments necessitates specific transport systems. The low-affinity SO_4^{2-} transporters SULTR4; 1 and SULTR4; 2 have been localized to the vacuolar membrane, where they may facilitate SO_4^{2-} efflux from the vacuole. The study of Zuber et al. (2010) revealed a role for SULTR4; 1 in determining SO_4^{2-} content of mature Arabidopsis seeds. Moreover, the adaptive response of sultr4; 1 mutant seeds as revealed by proteomics suggests a function of SULTR4; 1 in redox homeostasis, a mechanism that has to be tightly controlled during development of orthodox seeds. *Lycopersicon esculantum* SO_4^{2-} transporter gene (LeST 1.1) encodes a high-affinity SO_4^{2-} a transporter (HAST) located in root epidermis. Also studies of Abdin et al. (2010) clearly indicate that constitutive expression of LeST 1.1 gene in *Brassica juncea* had led to enhanced capacity of S uptake and assimilation even in S-insufficient conditions.

Selenate (Se) and SO_4^{2-} share the initial assimilation route, Se fertilization could interfere with S metabolism and plant growth. Se application does not affect plant growth, contents of Cys, GSH, total GSL, glucoraphanin (major aliphatic GSL) or the expression of BoMYB28 (encoding a functionally confirmed master regulator for aliphatic GSL biosynthesis). Conversely, due to the changed expression of SO_4^{2-} transporters (BoSULTR1; 1, 1; 2, 2; 1, and 2; 2), SO_4^{2-} and total S contents increased in the shoot of young plants while decreasing in the root (Hsu et al., 2010). Shinmachi et al. (2010) studied the interactions between S nutritional status and SO_4^{2-} transporter expression in field-grown wheat (*Triticum aestivum*) using Broadbalk +S and -S treatments and concluded that S fertilizer has a marked impact on Mo and Se distribution and accumulation, which is at least partially a result of altered gene expression of the SO_4^{2-} transporter family. Moreover, Se is thought to compete with SO_4^{2-} in the S assimilation pathway, and SO_4^{2-} therefore may have a protective effect against the toxic effects of high doses of Se in algae. The high concentration of SO_4^{2-} was able to counter Se-induced production of PCs and GSH. In addition, Simmons and Emery (2010) studied the interaction of Se and SO_4^{2-} with respect to the induction of PCs short metal detoxification peptides made from the S-rich molecule GSH and finally suggested the possible mechanisms for above generalization (i) A negative feedback system in the S assimilation pathway that affects PCs production when SO_4^{2-} is abundant (ii) Competition for uptake at the ion transport level between Se and SO_4^{2-}.

Moreover, regulatory factors are the demand of reduced S in organic or non-organic form and the level of OAS, the C precursor for Cys biosynthesis.

In plants, Cys is synthesized by action of the Cys-synthase complex (CSC) containing serine acetyltransferase (SAT) and OASTL, both enzymes are located in plastids, mitochondria and the cytosol. Subcellular metabolite analysis by non-aqueous fractionation revealed distinct changes in subcellular metabolite distribution upon short-term S starvation. In addition, Metabolite and transcript analyses of SERAT1.1 and SERAT2.1 mutants (Krueger et al., 2010) grown under S starvation conditions indicate that both isoenzymes do not contribute directly to the transcriptional regulation of genes involved in uptake SO_4^{2-} and assimilation (Krueger et al., 2010). Furthermore, recent findings of Takahashi (2010) provided evidence that the regulatory pathways are highly organized to balance the uptake, storage, and assimilation of SO_4^{2-} in plants. In addition to the physiological and biochemical functions diversified among the isoforms of SO_4^{2-} transporters, regulatory elements in transcriptional and posttranscriptional mechanisms were suggested to play significant roles in coordinating the assimilatory functions to adapt with varying S nutritional status that fluctuates in the environment.

In addition, after entry in to the roots cells, SO_4^{2-} is delivered to various parts of tissues through the vascular system. Today increasing SO_4^{2-} utilization efficiency (SUE) is an important issue for crop improvement. Little is known about the genetic determinants of SO_4^{2-} utilization efficiency. The function of SUE3 and SUE4 in low S tolerance confirmed either by multiple mutant alleles or by recapitulation analysis. Results of Wu et al. (2010) demonstrate that this recapitulation analysis is a genetic screen or a reasonable approach to isolate Arabidopsis mutants with improved low S tolerance and potentially with enhanced SO_4^{2-} utilization efficiency. The process of long-distance translocation of the SO_4^{2-} may require five different types of transporters in higher plants, responsible for cell to cell movement of SO_4^{2-} across the plasma membrane and also involved in translocation of SO_4^{2-} from roots to shoots (Hawkesford, 2003; Hawkesford and De kok, 2006; Clarkson et al., 1993). Because the SO_4^{2-} transporter protein is involved in this initial step, it may play a central role in the regulation of the entire S metabolism pathway by controlling the import of the available SO_4^{2-}. Loading of SO_4^{2-} in to vascular tissues in roots and unloading of SO_4^{2-} in to the leaf cells are assumed to be the essential steps in this process. With respect to the regulation of the entire SO_4^{2-} assimilation pathway, fluctuation of the extracellular SO_4^{2-} concentration may act as a signal for the modulation of the gene expression from the earlier physiological experiments on membrane vesicles and cell cultures (Smith, 1975), it is demonstrated that plants can adapt to low SO_4^{2-} availability by modulating the SO_4^{2-} transport activity.

(i) Xylem transport

The chemical composition of xylem sap is very different from phloem sap, xylem sap having a pH ranging from 5.0-6.0, a more oxidizing redox potential and a much lower concentration of organic compounds such as sugar, peptides and proteins (Glavac et al., 1989). During the growing season, concentrations of all S compounds like GSH and SO_4^{2-} in the xylem found highest and decreased later (Kostner et al., 1996). GSH is the main reduced S compounds (Thiol) in the xylem sap (Rennenberg and lamoureux, 1990) and concentration of inorganic SO_4^{2-} in xylem sap found up to 50 times higher than those of GSH, ranging from 60 to 230 μmol^{-1} in case of *picea abies* (Kostner et al., 1996). Thiol concentration in the xylem sap can originate from SO_4^{2-} reduction in the roots (Brunold, 1990), from internal cycling as a result of phloem-xylem exchange. On average, SO_4^{2-} concentrations in xylem sap were 30 percent higher at the declining site compared with the normal. Compounds like

GSH and Cys found significant high concentration during the night and low concentration during the day. Xylem sap concentrations of SO_4^{2-} and Cys twice as high and GSH concentrations were tenfold higher in surface roots than in branches. At both sites, transport of organic S was low (up to 30% of total S) compared to transport of SO_4^{2-} (Rennenberg and Polle, 1994).

(ii) Phloem transport

The composition of phloem translocate, moving down the stem toward the root was determined from the phloem exudates. 20-40% of the S moved in the form of organic S compounds. However the bulk of S transported as inorganic SO_4^{2-}. The most important organic S compounds translocated in phloem are GSH, carrying about 70% of level present in the organic fraction. In addition, Met and Cys were involved in phloem S transport and accounted for roughly 10%. Primarily, the reduced forms of both GSH and Cys are present in the sieve tubes. Previously published results have shown that tobacco plants export organic S compounds from mature leaves and translocate them towards sink regions i.e. shoot apex, immature leaves and the roots system. This transport occurred at least partially in the phloem. Separation and identification of the organic S translocated revealed that GSH is the major component of the translocate in phloem (Biddulph et al., 1958). Uptake of SO_4^{2-} by roots has been intensively studied (Cram, 1990) whereas the processes that control S nutrition and distribution at the whole-plant level have received little attention (Rennenberg, 1995). However, it is evident that SO_4^{2-} is not only transported in the xylem sap, but may also be exchanged between xylem and phloem resulting in the circulation of SO_4^{2-} in the vascular system of the stem (Rennenberg and Polle, 1994; Schneider et al., 1994b).

In addition, there are several previous studies reports that reduced S compounds are present in the xylem sap of deciduous trees in addition to SO_4^{2-}. For examples, Sauter (1981) reported that the S-containing amino acids Met is transported in the xylem sap of Salix. The thiol Cys and GSH have been detected in the xylem sap of beech (Schupp et al., 1991) and poplar (Suchneider et al., 1994a). Transport studies with Gymnosperms suggest that reduced S in form of GSH is transported in the xylem from mature needles to the going parts of the stem especially the new growing needles (Schneider et al., 1994b). However, it is not known to what extent GSH transport contributes to the S nutrition in plants. SO_4^{2-} is mainly translocated in upward (acropetal) direction and the capability of higher plants to move S in a downward (Basipetal) direction is relatively poor. For redistribution of stored S, the main transported form GSH undergoes by directional phloem-xylem exchange (Rennenberg and Herschbach, 1995). Although xylem transport of S in reduced form is important for the actual demands of all tree organs, SO_4^{2-} dominated the S transport in the xylem. S in the root and petioles found to be translocated towards the younger leaves while S of older leaves however, did not contribute to S supply of younger tissues (Singh et al., 1994). This shows that translocation against the transpiration stream did not occur. Randal (1988) opined that the mobility of S in the phloem is relatively lesser than N, leading to chlorosis of younger leaves and later shows necrotic symptoms and die (Singh et al., 1994).

Sulphur induced protective mechanism against heavy metal sequestration

Plants have evolved a variety of complex mechanisms to short out the problem of heavy metal sequestration. Of which, chelation is a common phenomenon induced by S fertilization. In previous studies Berry (1986) suggested that accumulation and detoxification as the main strategy that serve to control the uptake and accumulation of heavy metals. Different plant species, commercial varieties, cultivars and ecotypes vary widely in their tolerance to excess concentrations of heavy metals (Al-Hellal, 1995) and metal complexes of S-N chelating agents (Nag and Joardar, 1976). One recurrent general mechanism for excess heavy metal detoxification in plants and other organisms is chelation by a ligand and in some cases, the subsequent compartmentization of the ligand-metal complexes (Singh et al., 1981). Two major groups of complexes have been isolated from various higher plants are as follows:

(i) Meta-thioneine (MTs)

(ii) The Phytochelatins (PCs)

MTs are low molecular Cys-rich, metal binding proteins. They are present in many, but not in all plants. Plants MTs contain two structural metal-binding, Cys-rich domains in the amino and the carboxyl-terminal region (Cobbett, 2003a) which can bind Cd, Cu and Zn. Particularly, in Angiosperms they occur in four types MT1, MT2, MT3 and MT4 with different expressions in plant tissues during plant development and with obviously different metabolic functions (Roosens et al., 2005a; b; Zimeri et al., 2005; Domenech et al., 2006; Hassinen et al., 2007; Peroza and Freisinger, 2007). The role in Cd detoxification is supported by MT1 knock-down plants of *Arabidopsis thaliana* which become Cd hypotolerant despite a diminished Cd uptake (Zimeri et al., 2005). Independent of external metal exposure, expression of MT1 is enhanced during leaf senescence of all investigated based metal-tolerant plants (Kohler et al., 2004; Heise et al., 2007).

Moreover, the expression of MT2 genes depends on plant age (Guo et al., 2003; Kohler et al., 2004), plant tissues (Chang et al., 2004) and heavy metal exposure. Expression of MT2 seems to be restricted to leaves and slightly stimulated at exposure to Cd, Cu or Zn (Guo et al., 2003; Kohler et al., 2004; Zhang et al., 2005; Hassinen et al., 2007). MT2b is highly expressed in roots and leaves and the mRNA level is obviously species and ecotype specific. Treatments of *Brassica juncea* with Cu and Zn reduce the expression of MT2 (Schafer et al., 1997). The expression of MT3 genes is elevated with leaf ageing and at exposure to Cu, but not to Cd in leaves. The MT3 expression is not changed in roots of basal Cu-tolerant plants and ecotypes (Guo et al., 2003; Kohler et al., 2004; Roosens et al., 2004). MT4 is highly expressed in seeds *of Arabidopsis thaliana* (Guo et al., 2003). Therefore, MT4 may play a role in metal homeostasis during seed development and seed germination. Presently, some experimental data are available to evaluate the impact of the additional S need by MTs for metal detoxification on the total S pool.

Moreover, Clemens (2006a) favours a role of PCS in metal homeostasis. Another function of PCs may be degradation, however, is only possible if PCS is activated by Cd exposure (Beck et al., 2003; Blum et al., 2007; Grzom et al., 2006) and therefore, not possible when plants do not grow on metal-enriched soils. GSH depletion following Cd exposure has been observed in cultured cells and in roots (Klapheck et al., 1995). In maize, decreased GSH was accompanied by increase in γ-EC contents. Inhibition of PC accumulation by buthionine sulphoximine, demonstrated the importance of γ-EC activity in PC synthesis (Grill et al., 1987). Elevated γ-ECS activity was shown to correlate with Cd resistance in cultured tomato cells (Chen and Goldsborough, 1994) while inhibition of γ-ECS markedly enhanced the negative effects of Cd on growth in birch (Gussarsson et al., 1996). Although no extensive study has get been undertaken, the study of heavy metal tolerance will probably be one of the most important applications of plants over expressing γ-ECS. The up regulation of GSH during the biosynthesis to provide substrates for PCs. The total protein and GSH content of barley and maize roots declined with an increase in heavy metal concentration; however, this decrease was more in the roots than in the shoots (Shanthala et al., 2006). Thus GSH is somehow involved in the biosynthesis of PCs (Robinson et al., 1993) which in turn confers tolerance to the cells (Jackson et al., 1987).

Based on their solubility under physiological conditions, heavy metals may be available to living cells and have significance for the plant and animal communities with in various ecosystems (Weast, 1984). Among the heavy metals As, Hg, Ag, Sb, Cd, Pb and Al have no known functions as nutrients and seem to be more or less toxic to plants and microorganisms (Sogut et al., 2005; Beak et al., 2006). There are two types of causal relationships existing between the high concentration of heavy metals on soil and the expression of toxicity symptoms. On the one hand, heavy metals compete with essential mineral nutrients for uptake thereby disturbing the mineral nutrition of plants (Clarkson and Luttge, 1989) and on the other hand, after uptake by the plant, it accumulates in plant tissues and cell compartments and hampers the general metabolism of the plant (Turner, 1997).

The SO_4^{2-} concentration in non-metal-enriched environments is mostly in the range from 0.16 to 7 mM SO_4^{2-} in soil solution and surface near water (Stuyfzand, 1993; Haneklaus et al., 2003). The SO_4^{2-} concentration of soil solution varies from 13 mM SO_4 in the Cu mine of the Parys Mountains (Walton and Johnson, 1992) and up to 110 mM SO_4^{2-} in the Avoca Cu/Zn mine in Ireland (Gray, 1996). The SO_4^{2-} concentration in standard nutrient solution is moderate. When standard nutrient solution is diluted, the SO_4^{2-}concentration decreases to levels of S deficiency with impact on gene expression and enzyme activities (Nocito et al., 2006; Sun et al., 2007). Because of concomitant changes in metal sensitivity, it is difficult to compare plant reactions at extreme low SO_4^{2-} supply (Cazale and Clemans, 2001; Van Belleghem et al., 2007) with those of plants grown at 18-fold higher SO_4^{2-} concentration (Howden et al., 1995a; 1995b; Dominguez-Solis et al., 2004). In contrast to SO_4^{2-}, the exposure to heavy metals, especially Cd, surpasses environmentally relevant concentration in many physiological experiments. Investigation with high Cd levels can only show how a heavily disturbed metabolism of a nearly dead plant reacting to an extreme, environmentally never occurring plants. Cd and Pb exposure causes high mutation rates in *Arabidopsis thaliana* (Kovalchuk et al., 2005) with floral anomalies (bonding), poor seed production and malformed embryos. Other nutrients have also an impact on the uptake of heavy metals especially that of Cd and Pb. Up regulation of S related enzymes at Cd exposure such as PC synthase occurs also at insufficient N supply (Sarry et al., 2006).

In addition, the role of S along with other macronutrients like N, P, S and Ca and micronutrients like Zn, Fe, Mn and Si has play a role in decreasing Cd uptake and accumulation in crop plants (Sarwar et al., 2010). Excessive S supply may result in loss of rice yield, but it could effectively reduce Cd accumulation in brown rice exposed to Cd contaminated soils (Fan et al., 2010). Studies of Castillo-Michel et al. (2009) suggest that the concentration of S increased in roots of Cd treated plants but remained at normal concentration in shoots. Several S-containing metabolites like GCL and GSH have attractive targeted issues in recent years for engineering the tolerance against oxidative stress in crop plants (Foyer, 1997). Of which, GSH have major multiple roles in plant defences against both biotic and abiotic stresses by reducing the ascorbate in ascorbate-glutathione cycle (Nakano and Asada, 1980). In this pathway, GSH acts as a recycled intermediate in the reduction of H_2O_2 using electrons derived, ultimately, from H_2O (Foyer, 1997).

GSH accumulation is found to compensate for decrease in the capacity of other antioxidants, for example, in catalase-deficient mutants and in plants where catalase activity has been reduced by antisense technology (Chamnonngpol et al., 1996; Willekens et al., 1997). Furthermore, when GSH is depleted, increases sensitivity to oxidative stress has frequently been found (Kunert et al., 1990; Kushnir et al., 1995; Grant et al., 1996). In instances where GSH depletion has not increased sensitivity to oxidative stress (Greenberg and Demple, 1986; May et al., 1996), it is probable that other antioxidant molecules are increased to compensate for decreases in GSH. Since, GSH is a major water soluble antioxidant accounts only for 2% of the total organic S content in plants ranging from 120 to 380 m mol S kg/dry matter(Hawkesford and De-Kok, 2006; Sun et al., 2007) and directly reduces most AOS (Active oxygen species). GSH peroxidases are induced in plants in response to stress (Eshdat et al., 1997). These enzymes are involved in detoxification of lipid peroxidases rather than H_2O_2. Efficient recycling of GSH is ensured by glutathione reductase (GR) activity. The components of this cycle exist in both chloroplast and cytosol (Foyer, 1993) and evidence for their presence in mitochondria, glyoxysomes and peroxisomes has been recently reported (Jimenez et al., 1997). Therefore, it is crucial to establish the significance of GSH as a reductant in process of heavy metal tolerance against oxidative stress (Figure 3).

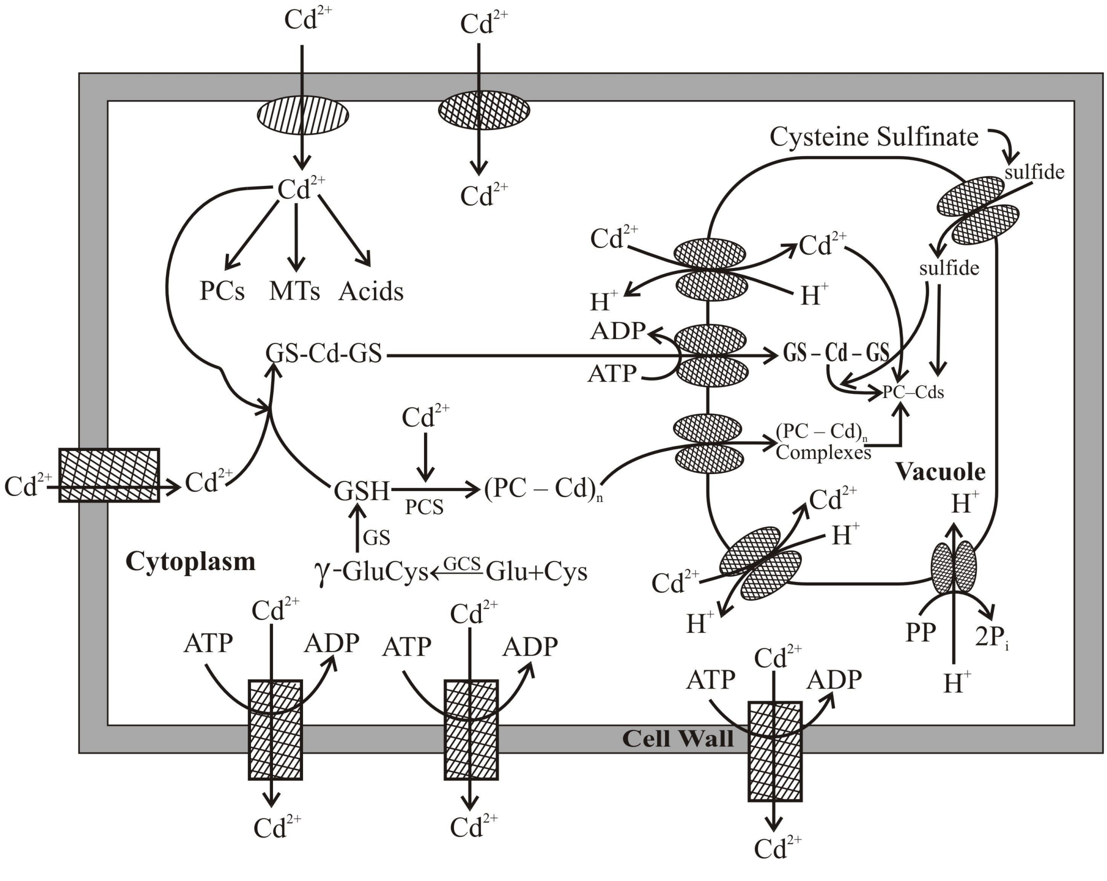

Figure 3. A detailed account of potential cellular mechanisms for metal detoxification and tolerance in plants. This figure is a composite view of various roles of S nutrition played in different plants. Here GS and PC stands for GSH synthase and phtochelatin synthase.

Conclusion and future guidelines

Although our knowledge about the role of S fertilization in plant metabolism and defense have been described in recent years for the tri-peptide thiol GSH (GSH) and other S assimilatory metabolites like GSL, particularly specific to family, Brassicaceae. In spite of this, the biosynthesis of such S assimilatory metabolites and their defense responses to oxidative and heavy metal stress conditions in higher plants as well as in the soil-plant system has still many gaps in our knowledge or only partly understood. Certainly more research work is needed regarding the mechanism of S-chelation towards heavy metal detoxification. Authors suggesting that researchers must elaborate the knowledge about the biochemistry of S-homeostasis factors, physical interactions of transporters and chelaters. The application of trans-gene technology to over-expressing of two enzymes (GS and –ECS) of S-assimilatory metabolic pathway, especially involved in enhancing the GSH content up to 10 fold greater than untransformed plants open up a promising vista of prospectives for industrial and environmental applications. Tissue culture (in vitro techniques) researches are very relevant to explain the display of S-chelation and also a priority approach to explain enzymological aspects as well as nutrients-heavy metal homeostasis.

ACKNOWLEDGEMENT

The authors are highly thankful for the facilities obtained at AMU Aligarh. Financial support from the Department of Science and Technology, New Delhi in the form of project (SR/FT/LS-087/2007) is gratefully acknowledged.

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