RESPONSE OF CROP PLANTS UNDER SULPHUR STRESS TOLERANCE: A HOLISTIC APPROACH

Mohd Mazid¹*, Taqi Ahmed Khan², Firoz Mohammad¹

¹Plant Physiology Division, Department of Botany, Faculty of Life Sciences, AMU, Aligarh, India. 202002.
²Department of Biochemistry, Faculty of Life Sciences, AMU, Aligarh, India. 202002.

E-mail: mazidmohd699@gmail.com
Telephone: +91-0571-2702016
Fax num: +91-0571-2706002

Received April 23, 2011

Sulphur (S) is a part of every living cell and is a constituent of two of the 20 amino acids which form proteins. 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. The benefits from S fertilisation of crops can be traced to its role in protein development, to improvement of nitrogen use, etc. However, the availability of S needed for profitable crop production continues to decline. This review highlights the prominent effects of S in plants (i.e. growth retardation, alternations of photosynthesis, stomatal movement, enzymatic activities, protein synthesis, interferences with various stress conditions, membrane functioning etc.) but also includes the mechanism of uptake and translocation. Moreover, it also throws new insights, have led us to revisit the hypothesis of S chelation.

Key words: antioxidant/ chelation/oxidative stress/Sulphur deficiency/Xylem transport.
Sulphur stress tolerance...

ORIGINAl ARTICLE

RESPONSE OF CROP PLANTS UNDER SULPHUR STRESS TOLERANCE:

A HOLISTIC APPROACH

Mohd Mazid1*, Taqi Ahmed Khan2, Firoz Mohammad1

1Plant Physiology Division, Department of Botany, Faculty of Life Sciences, AMU, Aligarh, India. 202002.
2Department of Biochemistry, Faculty of Life Sciences, AMU, Aligarh, India. 202002.

E-mail: mazidmohd699@gmail.com
Telephone: +91-0571-2702016
Fax num: +91-0571-2706002

Received April 23, 2011

Sulphur (S) is a part of every living cell and is a constituent of two of the 20 amino acids which form proteins. Of all the macro nutrient, 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. The benefits from S fertilisation of crops can be traced to its role in protein development, to improvement of nitrogen use, etc. However, the availability of S needed for profitable crop production continues to decline. This review highlights the prominent effects of S in plants (i.e. growth retardation, alternations of photosynthesis, stomatal movement, enzymatic activities, protein synthesis, interferences with various stress conditions, membrane functioning etc.) but also includes the mechanism of uptake and translocation. Moreover, it also throws new insights, have led us to revisit the hypothesis of S chelation.

Key words: antioxidant/ chelation/oxidative stress/Sulphur deficiency/Xylem transport.

Macro-elements are defined as nutrient having a concentration of 1to10 mg per gram of dry matter in plant tissues (Arnon and stout, 1939). 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 (Goldbol and Huttermann, 1985; Niess, 1999). Some macro-elements are significant environmental pollutants (Hill, 1997) and their availability in soils depends on natural processes,
especially lithogenic and pedogenic activities, but also on anthropogenic factors such as mining, combustion of fossil fuels, urban waste disposal, soil run off, metal working industries, boating activities, phosphate fertilizer application, sewage treatment plant effluents, and municipal solid waste (MSW) disposal sites (U S Congress Office of Technology Assessment, 1990).

In fact, yield of most crop plants increase linearly with the amount of fertilizers that they absorb (Loomis and Conner, 1992). Plants display a higher degree of physiological and developmental plasticity in response to their changing nutritional conditions. 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 (Epstein, 1994). To meet increased demand for food, world consumption of the primary fertilizers especially in terms of macro-nutrients rose steadily from the 112 million metric tons in 1980 to 143 million metric tons in 1990 (Laurient, 1995). 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). Soil pH is an essential critical factor to availability of all these nutrients (Fig. 1).

![Figure 1](Image)

**Figure 1** Influence of soil pH on the availability of nutrient elements in-organic soil. The width of the shaded areas indicates the degree of nutrient availability to the plant root. All these nutrients are available in pH range of 5.5 to 6.5. (modified Lucas and Davis, 1961).
Classification of plant macro-nutrients according to biochemical function

**Group-1** (Nutrients that form the organic compounds of plants)

**Nitrogen** - Constituents of amino acids, amides, proteins, nucleic acids, nucleotides, co-enzymes and hexo-amines etc.

**Sulphur** - Components of cysteine (Cys), cystine, methionine (Met) and proteins. Constituents of lipoic acids, co-enzyme-A, thiamine pyrophosphate, glutathione (GSH), biotin, adenosine-5-phospho sulphate and 3-phosphoadenosine.

**Group-2** (Nutrients that are important in energy storage or structural integrity)

**Phosphorus** - Components of sugar phosphates, nucleic acids, nucleotides, co-enzymes, phospholipids, phytic acid etc. has a key role in reaction in which ATP is involved.

**Silicon** - Deposited as amorphous silica in cell walls. Contributes to cell wall mechanical properties including rigidity and elasticity.

**Group-3** (Nutrients that remain in ionic form)

**Potassium** - Required as a co-factor for more than 40 enzymes of biological reactions. Principal cation in establishing turgor and maintaining cell electro-neutrality.

**Magnesium** - Required by many enzymes involved in phosphate transfer. Constituents of the chlorophyll molecule.

**Calcium** - Constituents of the middle lamella of cell wall. Required as a co-factor by the enzymes involved in the hydrolysis of ATP as a second messenger in metabolic regulation.

S represents 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 (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 Allium) (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 and affects the kinetics of CS formation leading to destabilization of the complex. Cysteine synthase (CS), formed by the pyridoxal 5'-phosphate-dependent enzyme O-acetylserine sulphydrylase (OASS) and serine acetyltransferase (SAT), the branch point of the S, C, and N assimilation pathways (Salsi et al., 2010). 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). O-acetyl serine (thiol) lyase (OASTL) catalyses the final step of Cys biosynthesis and its isoforms are localised in the cytoplasm, the plastids and mitochondria but the contribution of individual OAS-TL 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 oxidative stress, the peroxidatic Cys can be overoxidized to sulphinic acid inactivating the Prx. An enzyme recently discovered, named sulfiredoxin (Srx), reduces the sulphinic 2-Cys Prx...
Mohd Mazid et al

The activity of sulfiredoxin, determined by a new method, is dependent on the concentration of the sulphinic form of Prx and the conserved Srx is capable of regenerating the functionality of both pea and Arabidopsis Prx-SO$_2$H (Iglesias-Baena et al., 2010). 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). Results of Holler et al., (2010) demonstrates a link between the activation of Cys and GSH metabolism and the induction of SIR/SED during a compatible plant-virus interaction in tobacco plants, indicating a general mechanism behind SIR/SED. 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). High S application in combination with low N fertilization increased the allin concentration in garlic significantly during main growth until the beginning of ripening (Bloem 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 sulphydryl (-SH) group mediated by glutathione S-transferase. In addition, GSH and glutaredoxins (GRXs) are implicated in the response to oxidative stress through the regeneration of enzymes involved in peroxide and methionine sulfoxide reduction. As well as emerging functions for plant GRXs and GSH concern the regulation of protein activity via glutathionylation and the capacity of some GRXs to bind iron S centres and for some of them to transfer FeS clusters into apoproteins (Rouhier et al., 2008).

Moreover, 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 plant parts of crop plants (Sarwar et al., 2010). S-containing secondary products often have a characteristic smell and are regarded not only as defensive compounds against herbivores and pathogenic organisms but also signaling molecules for fundamental cellular functions (Matsubayashi et al., 2002).

**S as a nutrient in crop management**

S is an essential macronutrient, taken up as SO$_4^{2-}$ from soil, reduced and incorporated into bioorganic compounds in plant cells. The pathway of SO$_4^{2-}$ assimilation is highly regulated in a demand-driven manner in seed plants (Hermsen et al., 2010). Plants as autotrophic organisms have a set of transporters and enzymes that mediate uptake and assimilation of inorganic SO$_4^{2-}$ and subsequent metabolic conversion to organic S compounds. Studies in higher plants indicate the individual component of SO$_4^{2-}$ transport system and enzymes for SO$_4^{2-}$ assimilation are consisted of multiple isoforms.
Among these isoforms, several essential components are shown to have specific biochemical properties and localize in specific cellular and subcellular compartments.

S is a vital ingredient in nutrient management plans designed for high-yield, top profit cropping systems and an adequate amount is necessary for optimum plant use of N and K especially. These three nutrients are essential for protein synthesis, photosynthesis and other plant growth developmental processes. A shortage of any one of the three will restrict the effectiveness of the others. The functions of S and its influence on plant growth show why S is a full season secondary nutrient. It promotes plant growth from the seedlings stage until the crop reaches physiological maturity. SO$_4^{2-}$-S is very mobile in soil solution but considered non-mobile within the plant. Elemental S must be converted to the SO$_4^{2-}$ in the soil before taken up by the plant and its size determines the amount of S expected to be available in the season of application. A severe shortage of S often develops visible deficiency symptoms on new growth like pale green colouration. S is a mobile nutrient in the soil and its reservoirs are smaller greatly and vary for different textured soils. S assimilation in plants plays a key role in the S-cycle in nature. The inorganic S in the environment, SO$_4^{2-}$ in soil and SO$_2$ in the air, is fixed in to Cys by S assimilation pathway in plants (Saito, 2000). Sulfite oxidase (SO) catalyses the physiologically vital oxidation of S$^2-$ to SO$_4^{2-}$, the terminal reaction in degradation of S containing amino acids, Cys and Met. SO from vertebrate sources is among the best studied molybdenum enzymes. Existence of this enzyme in plants has been established recently by identification of a cDNA from Arabidopsis thaliana encoding a functional SO (Ahmad and Sarfraz, 2010). S uptake varies greatly among major agricultural crops. Crops having high N needs also have a high S need due to their mutual functions in plant growth such as protein formation. Forage grasses and certain vegetables crops require S for both high yield and quality.

A plethora of agronomic studies showed the importance of a balanced nutrient program including S on N-uptake efficiency. To avoid an imbalance many suggest the use of 20 to 30 pounds per acre of fertilizer S. Modern agriculture requires adequate fertilisation of S to achieve maximum crop yield and performances (Crawford et al., 2000). S ranks thirteen in terms of abundance in earth crust. It is one of the 13 mineral nutrients which are essential for the growth and development of all plants. S is increasingly being recognised as the fourth major plant nutrient after N, P and K. S-induced resistance, also known as S-enhanced defense (SIR/SED). Sufficient SO$_4^{2-}$ supply resulted in a suppressed and delayed symptom development and diminished virus accumulation over a period of time after inoculation as compared with -S conditions. SO$_4^{2-}$ withdrawal from the soil was accelerated at the beginning of the infection, whereas it declined in the long term, leading to an accumulation of S in the soil of plants grown with SO$_4^{2-}$.

But currently, S deficiency has been reported in previously S sufficient areas. One survey found that 240 districts of India are under S deficiency and problem is spreading rapidly (Singh, 1998), and now areas of S deficiency are becoming widespread throughout the world (Scherer, 2001). Therefore in recent years, S deficiency has become an increasing problem for agriculture resulting in decreased crop quality parameters and yield (McGrath et al., 1996; Zhao et al., 1999; Hawkesford, 2000). The main reasons for this include: (1) The environmental
control of SO$_2$ emission in industrial areas and ensures declines in S-deposition (McGrath et al., 1996). (2) The increasing use of P-fertilizers with low S-content. (3) The increase in yields obtained as a result of other technological improvements. (4) The decreasing use of S-containing pesticides (Blair, 2002). Subsequently this widespread S deficiency and yield responses have been reported in a number of arable crops, e.g. oil seed rape (McGrath et al., 1995; 1996), Barley (Withers et al., 1995), wheat (McGrath et al., 1996; Zhao et al., 1999) and grassland (Syers et al., 1987).

S deficiency has been shown to influence the quality of many crops including the GSL levels in oilseed rape (Schnug et al., 1989; Zhao et al., 1993), baking quality of wheat flour (Zhao et al., 1999; Randall et al., 1986; McGrath et al., 1995), nutritional value of legume storage proteins (Spencer et al., 1990; Gayler et al., 1985) and herbage quality of grassland (Murphy et al., 1989). Reliable early diagnostic plant indicators are such as total S concentration, SO$_4^{2-}$-S concentration, N/S ratio, ratio of SO$_4^{2-}$-S to total S and GSH concentration has been used in many crops to predict S deficiency (Zhao et al., 1996; Spencer et al., 1980). S deficiency increases the concentrations of non-protein and in particular amide N (Zhao et al., 1996; Josefsson, 1970; Karmoker et al., 1991). The accumulation of N-containing compounds (NCCs) in response to minerals stress has also been reported (Rabe, 1990). Today, our main target is to understanding how specific crops utilize S for improved growth and how fertilizers provide crop S needs will help to improve crop yield and profitability and we should also targeted to practical approaches for reducing S losses and improving the overall synchrony between supply and demand.

**Basic properties of S**

S is the element of group VI A (oxygen family) in the periodic table and its atomic number is 16.2. It shows chemical similarity especially in non-metallic characteristics with the other element of this group (P-block elements) specifically with oxygen (O), Selenium (Se) and Tellurium (Te). S (II) is commonly associated with P and Zinc (Zn) in natural geologic settings. S (II) is relatively more unstable in positive valency of two and occurs in most of natural aquatic systems in this state. This ability of S (II) to form oxides like SO$_2$ (Angular shape) and SO$_3$ (Planar triangular) and chlorides like SCl$_2$, SCl$_3$ with O$_2$ and Cl$_2$ respectively, indicate similarity with most of the macro-elements (Table 1). Electronic configuration of S represent that after taking two electrons, it can acquire stable electronic configuration of nearest noble gas (Argon-18). The atoms of S forms single bonds only and therefore it is more complex and are octa-atomic molecule, represented by S$_8$ with puckered-8-atom ring structure as shown in Figure 2.

**(i) Catenation**

It shows the property of catenation i.e. a tendency to form chains and rings of identical atoms. The Chains of S atoms are present in poly-Sulphuric acid, HO$_3$SS$_n$SO$_3$H, and different allotropic forms of the element containing S$_8$ rings of different sizes. S has several allotropic forms which may be classified into three types as follows:

(a) Homocyclic species having 6-20 S atoms. e.g., S$_6$.

(b) Different chain polymers known as catenation-S$_n$-Sulphur.

(c) Unstable small molecules S$_n$ (n= 2 to 5) existing in different concentrations in liquid S at higher temperatures and in S vapour.
Sulphur stress tolerance ...

Figure 2 Puckered 8-atom ring structure of S molecule (S₈).

Table 1. Some important oxides and halides of S (II)

<table>
<thead>
<tr>
<th>Oxides</th>
<th>Mono-oxide</th>
<th>Di-oxide</th>
<th>Tri-oxide</th>
<th>Other oxides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SO</td>
<td>SO₂</td>
<td>SO₃</td>
<td>S₂O₇</td>
</tr>
<tr>
<td>Halides</td>
<td>Fluorides</td>
<td>Chlorides</td>
<td>Bromides</td>
<td>Iodides</td>
</tr>
<tr>
<td></td>
<td>SF₂, SF₄,</td>
<td>SCl₂,</td>
<td>S₂Br₂</td>
<td>(not exist)</td>
</tr>
</tbody>
</table>

Table 2. Different allotropic forms of S and their irrespective physical and chemical characteristic.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular form</td>
<td>S₈</td>
<td>S₈</td>
<td>Polymeric</td>
<td></td>
</tr>
<tr>
<td>Colour</td>
<td>Pale yellow</td>
<td>Amber</td>
<td>Dark amber</td>
<td></td>
</tr>
<tr>
<td>Nature</td>
<td>Crystalline</td>
<td>Crystalline</td>
<td>Amorphous</td>
<td></td>
</tr>
<tr>
<td>Crystal shape</td>
<td>Octahedral</td>
<td>Needle shaped</td>
<td>No definite shape (Zigzag)</td>
<td>It is prepared by passing H₂S through a solution of an oxidising agent or water or by treating sodium thiosulphate with dil HCL.</td>
</tr>
<tr>
<td>Melting point</td>
<td>1128 °C</td>
<td>119 °C</td>
<td>No sharp M.P</td>
<td></td>
</tr>
</tbody>
</table>

(ii) Allotrophy

S exist in four allotropic forms, differ generally in their physical properties, such as colour and hardness and molecular structure, but similar in most chemical properties (Table 2).

Deposition and mineralogy

An approximation of present day sea water can be made by dissolving 2.2 g of MgSO₄ and 1.4 g of CaSO₄ in a litre of pure water. Since there are roughly 2.65 g of SO₄²⁻ per litre, the seas probably contain most of the crusts S as SO₄²⁻ (Jones, 1949). In volcanic areas, the gases H₂S and SO₂ are often evolved in quantity. They may react according to 2H₂S + SO₂ → 3S + 2H₂O and deposit elemental S, sometimes in well formed crystals. The H₂S may be oxidized by atmospheric oxygen with the same effect. The bacterium *Rhodospirillum rubrum* obtains energy by oxidizing H₂S to elemental S. Volcanic deposits of elementary S exist but they are not a major source of S because of difficult access (Pauling, 1989). Most of the sources of elemental S are biogenic. Deposits of gypsum and anhydrite are processed by anaerobic bacteria, especially where hydrocarbons are available as food for the bacteria, which reduce the SO₄ to S with the emission of CO₂ and H₂S. The H₂S may escape or especially, if
trapped, may be oxidized by oxygen containing surface water that has percolated in to the area to elemental S, or by biological action (Hurlburt and Dana, 1952).

In this way, bedded S is created, associated with lime stone and gypsum or anhydrite. The S-bearing rocks are of Miocene age. Everything was now perfect for the \( \text{SO}_4^{2-} \) reducing bacteria to make elemental S and create brecciated (broken) limestone layers above the anhydrite. The S filled fractures and voids in this limestone, so that about 25% of the rock was S (Bates, 1969). Studies with isotopic tracers have confirmed the biological origin of salt-dome S. Drilling for oil revealed the abundance of the S in some (not all) salt domes. If a salt dome reached the surface, the S was removed by erosion. The S in sub-surface salt domes was at depths of 1000 ft. One dome can produce 30,000,000 tons of S (Kay and Colbert, 1965).

Besides elemental S, S minerals are chiefly metal sulphides and \( \text{SO}_4^{2-} \), produced by hot, active gases and fluids containing the \( \text{SO}_4^{2-} \). Pyrite (FeS\(_2\)) and Galena (PbS) are two familiar sulphide minerals. Regular sulphides, FeS and Fe\(_2\)S\(_3\) are not found in nature in pure form. \( \text{SO}_2 \) produced from roasting pyrite often contains arsenic as an impurity.

Two modes of origin of the S in solfatars have been suggested by geologists and chemists.

(i) Oxidation of \( \text{H}_2\text{S} \), probably according to the equation
\[
2\text{H}_2\text{S} + \text{O}_2 = 2\text{H}_2\text{O} + 2\text{S}
\]

(ii) The mutual reaction of \( \text{H}_2\text{S} \) and \( \text{SO}_2 \), accordingly to equation
\[
2\text{H}_2\text{S} + \text{SO}_2 = 3\text{S} + 2\text{H}_2\text{O}
\]

The \( \text{H}_2\text{S} \) and \( \text{SO}_2 \) emanating from the conduit, from numberless bubbles in the lake and where they are in contact with the water the S is deposited. Thus larger after layer of S may accumulate in a lake and bedded deposits be formed.

**Natural resources, crop requirement and present scenario of S availability**

Crop plants, however, typically use less than half of the fertilizer applied (Lauriente, 1995). The remaining minerals may leach in to ground water become fixed in the soil or contribute to air pollution. S can be applied as broadcast or banded fertilizers material or applied through irrigation systems. Most S containing minerals are metal sulphides and the best known is perhaps pyrite. The most common \( \text{SO}_4^{2-} \) containing mineral is gypsum.

The fact that crop deficiencies of S have been reported with increasing frequency over the past several years. Today, the greater attention has been focused on the importance of this element in plant nutrition. In many regions of the world S deficiency has been recognized as a limiting factor for crop production. Although not readily plant available, organic S compounds may potentially contribute to the S supply of plants by mineralization. While \( \text{SO}_4^{2-} \) esters play an important role in the short term release of \( \text{SO}_4^{2-} \), carbon bonded S seems to be responsible for long term mineralization. To predict S delivery of soils different soil tests may be suggested which take the most important soil characteristics in to considerations. Besides soil test, tissue analysis assists in identifying S deficiencies. However, in general they are used very seldom for making fertilizers recommendations. To achieve very high yields and to minimize S leaching, rates of fertilizer S should be recommended on the basis of available soil S and crop requirement (Scherer, 2001) (Table 3).

**Chelation in S**

In order to cope with toxicity of heavy metals or to maintain its concentration with in a suitable
physiological range, plants have evolved a variety of complex mechanisms such as chelation, a common phenomenon in S. 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). Formation of these complexes is termed as chelation and these complexes are known as chelaters. Two major groups of complexes have been isolated from different higher plants.

(i) 8-14 K Da complexes, similar to those of meta 40-thioneine (MTs)

(ii) 1.5-4 K Da complexes, ThePCs= (γ-Glu-Cys)n, Gly (Steffens, 1990)

These complexes contain a number of amino acids with glutamic acid, Cys and glycine as the major constituents.

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. 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 (Guo et al., 2003; Roosens et al., 2005a, b; Zimeri et al., 2005; Domenech et al., 2006; Hassinen et al., 2007; Peroza and Freisinger, 2007). Only a few MTs, are chemically characterized (Peroza and Freisinger, 2007). At present, a correlation cannot be established. MT1a and MT1b are expressed at higher levels in roots at exposure to Cd, Cu and Zn and in senescent leaves, but only at low levels in young leaves of almost all plants species investigated (Zhou and Goldsborough, 1995; Ma et al., 2003; Kohler et al., 2004). 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 (Foley et al., 1997; Kohler et al., 2004; Heise et al., 2007).

The expression of MT2 genes depends on plant age (Garcia-Hernandez et al., 1998; 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). In contrast, exposure to Ag, Cd, Cu, Ni and Zn enhances the concentration of MT2 mRNA in several ecotypes of Arabidopsis thaliana but does not restore root growth to the Cu-free control (Murphy and Taiz, 1995; Guo et al., 2003; Zhigang et al., 2006). 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;
Mohd Mazid et al

Roosens et al., 2004). MT4 is highly expressed in seeds of Arabidopsis thaliana (Guo et al., 2003). The MT4 protein will not play a role in metal decontamination because seeds of plants growing on metal enriched soils are highly protected from a surplus of heavy metals (Ernst, 1974; Mesjasz-Przybylowicz et al., 1999). 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.

PCs are synthesized enzymatically by PC synthase (PCS) in higher plant (Gekeler et al., 1989; Kneer and Zenk, 1992). PCS is present in all so far investigated higher plants and some fungi (Clemens, 2006a). PCS activity is measured in crude or partially purified extract of plants grown either with or without a metal treatment by addition of extreme levels of 100 to 500 µM metals to extraction mixture (Grill et al., 1989; Yan et al., 2000; Heiss et al., 2003; Peterson and Oliver, 2000; Picault et al., 2006). This enzyme removes a γ-Glu-Cys residue from one molecule of GSH (γ-Glu-Cys-GYy) and couples to another GSH, a precursor of PCs (Grill et al., 1989). It was purified to homogeneity in cell cultures of Silene cucufalus, Beta vulgaris and Equisetium giganteum (Robinson et al., 1993). PCs could reduce cytoplasmic toxicity by complexing intracellular metals. The PC metal complexes would be expected to be less toxic to cellular metabolism then free metal ions. Cd, a well known inducer of PCs is thought to be chelated in complexes in the cytosol and transported to the vacuole (Rauser, 1995). PCs contain between 2 to 11 repeated γ-EC units usually followed by a C-terminal amino acid which may be glycine, alanine, serine or glutamine (Rauser, 1995; Zenk, 1996).

A direct evidence of PCs in protecting plant enzymes was reported in suspensions cell cultures of Rauvolfia serpentine that were treated with Cd (Meuwly and Rauser, 1992). 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 sulfoximine, 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 accumulation of GSH observed under certain conditions will require increased synthesis of γ-ECS. The impact of heavy metals on PCs introduction has been extensively reviewed (Hirata et al., 2005; Clemens 2006a; Mendoza-cozotl and Morenosanchez, 2006). Therefore, we showed focus on those aspects which are not sufficiently considered still.

Co-production of PCs and MTs upon exposure to trace metals was reported in yeast (Candida glabrata) (Mehra, 1988). In addition to PCs, other
intracellular ligands such as GSH may play a role in complexing heavy metals like Cd. 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). SO$_4^{2-}$ salts have also been reported to afford protection to nutrient toxicity by enhancing SO$_4^{2-}$ uptake, leading to increased synthesis of GSH-a tripeptide exists either in a reduced form with a free thiol or in an oxidised form with a disulfide between two identical molecules (Rochier et al., 2008).

Effect of S on oxidative stress induced by heavy metal accumulation

High concentration of heavy metals in the soil is toxic to most plant (Baker, 1986; Ernst, 1980; Macnair, 1993; Wool House, 1983). 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 (Goldbol and Huttermann, 1985; Niess 1999; Sogut et al., 2005; Beak et al., 2006). Of the known metals Cd Ni Zn and Cu are toxic to plants at elevated levels, whereas Pb has generally observed to cause phyto-toxicity (Foy et al., 1978). 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; Thurman and Collins, 1983; Tayler, 1988).

Heavy metal accumulation in plants has multiple direct and indirect effects on the plant growth and alters many physiological functions (WoolHause, 1983) by forming complexes with O, N and S ligands (Van Asche and Clijsters, 1990). They interfere with mineral uptake (Yang et al., 1998; Zhang et al., 2002; Kim et al., 2003; Shukla et al., 2003; Drazic et al., 2004; Adhikari, 2006) water relations (Kastori et al., 1992) and Seed germination (Iqbal and Siddiqui, 1992; Al-Hellal, 1995). Moreover, they cause metabolic disturbance by altering essential biochemical reactions (Krupa, 1988; Hermens et al., 2004; Epstein and Bloom, 2005). The demand of plant species for S as an essential major nutrient is very species specific, with enhanced need of plant species belonging to the family Brassicaceae (Ernst, 2000).

High SO$_4^{2-}$ levels alone demand already specific adaptations (Ernst, 1997) in combination with high heavy metal concentrations; the selection pressure is very tough. In arid regions of the world, SO$_4^{2-}$ in irrigation water ranges from 3 to 16 mM SO$_4$ (Eriksen et al., 1998). 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-Solís et al., 2004). In contrast to SO\(_4^{2-}\), the exposure to heavy metals, especially Cd, surpasses environmentally relevant concentration in many physiological experiments. As soon as the threshold level of a metal is surpassed, the impact on plants performance increases in a linear or sigmoid manner. The degree of toxicity is the result of exposure time and exposure concentration, that is, the role of dose-response relationship in toxicology.

As a consequence, the metal level of causing 50% loss of plant performance of basal Cd-tolerant plants decline from 50 µM Cd in short term experiment (Sun et al., 2007) to less than 5 µM Cd in long term experiments (Van Belleheim et al., 2007). 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 PCS synthase occurs also at insufficient N supply (Sarry et al., 2006). An analysis of more than a single metal under investigation will help to detect interactions with other parts of the mineral metabolism (Zhu et al., 1999a; Larson et al., 2002). Rhizosphere bacteria can facilitate the mobilization of heavy metals in the root zone and stimulate root growth (Whiting et al., 2001; Belimov et al., 2005). Fungi and bacteria are another factors affecting metal uptake and translocation with exception of non-mycorrhizal plants (Brassicaceae & caryophyllaceae), AMF (arbuscular mycorrhizal fungi) cause quiet different effects in higher plants growing in metal enriched substrates, either diminishing or enhancing uptake of metals or being neutral (Gohre and Parz-Kowski, 2006; Hildebrandt et al., 2006). Facilitation of S supply by AMF and EMF (endo-mycorrhizal fungi) to their host (Hepper, 1986; Mansouri-Bauly et al., 2006) may have impacts on metal toxicity, but it is not yet investigated.

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. The concentration of S and the production of LMWT were found to increase significantly upon exposure to Cd, confirm a strong linkage between S uptake and the production of LMWT upon exposure to Cd. Results of Zhang et al., (2010) suggest that H\(_2\)S could increase antioxidant capability in wheat seeds leading to the alleviation of Al\(^{3+}\) stress. 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 (Foyer and Halliwell, 1976; Groden and Beck, 1979; Nakano and Asada, 1980). In this pathway, GSH acts as a recycled intermediate in the reduction of H$_2$O$_2$ using electrons derived, ultimately, from H$_2$O (Foyer, 1997).

Stimulation of GSH biosynthesis is frequently observed in stress conditions. Similarly, 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 (Smith et al., 1984; Smith, 1985; Chamnonngpol et al., 1996; Willekens et al., 1997). Furthermore, when GSH is depleted, increases sensitivity to oxidative stress has frequently been found (Hibberd et al., 1978; 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. Confocal laser scanning microscopy (CLSM) showed that GSH levels in tip cells of both long and short trichomes were higher than those in other types of leaf cells, indicating the presence of an active S-dependent protective system in trichomes (Harada et al., 2010).

GSH is a major water soluble antioxidant accounts only for 2% of the total organic S content in plants ranging from 120 to 380 mmol S kg/dry matter (Mc Mahon and Anderson, 1998; Ernst, 2000; Hawkesford and De-Kok, 2006; Sun et al., 2007) and directly reduces most AOS (Active oxygen species). It reacts rather slowly with H$_2$O$_2$ and GSH-dependent reduction of H$_2$O$_2$ is not a major route of H$_2$O$_2$ reduction in plants. GSH peroxidases are induced in plants in response to stress (Eshdat et al., 1997). These enzymes are involved in detoxification of lipid peroxides rather than H$_2$O$_2$. In plants; the major substrate for reductive detoxification of H$_2$O$_2$ is ascorbate, which must therefore be continuously regenerated from its oxidized forms. Efficient recycling of GSH is ensured by 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 tolerance against oxidative stress.

**Effect on various parameters**

**(i) Photosynthesis**

Under the condition of effect of S limitation the pulse crops showed reduced growth and photosynthetic rates (Giordano et al., 2000). S$^{2-}$ a major form of SO$_4^{2-}$ in aqueous phase of apoplast, may reduce photosynthesis rate and thereby crop yield through inducing ROS, involved in S$^{2-}$-induced stress, and the S$^{2-}$-induced enhancements in levels of ROS (Li et al., 2007). A decrease in the yield of some crops due to decreased atmospheric S input has been reported for northern Europe (Dammgen et al., 1998). S is one of the limiting plant nutrients threatening the sustainability of crop production (Subba Rao and Ganeshamurthy, 1994). General responses to S limitation are reduced growth and photosynthesis (Davies and Grossman, 1998) and strong reduction in photosynthesis under S-limited growth correlates with a substantial decline of rubisco and chlorophyll a/b binding protein (Jamal, 2006). According to Sexton et al., (1997) S deficiency at first influences the protein synthesis and later on photosynthesis and work of Wheeler (1971) and Lawn and Brun (1974) also confirms this
findings in case of soya bean. High S deposition may reduce photosynthetic capacity in Sphagnum, but the negative effects may be relaxed under high N availability (Granath et al., 2009) (Table 4).

Table 3. Plant removal of S

<table>
<thead>
<tr>
<th>Crops</th>
<th>Yield</th>
<th>S kg/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat (grain)</td>
<td>1 tonne/hectare</td>
<td>2</td>
</tr>
<tr>
<td>Sunflower (grain)</td>
<td>1 tonne/hectare</td>
<td>5.2</td>
</tr>
<tr>
<td>Conola (grain)</td>
<td>1 tonne/hectare</td>
<td>10</td>
</tr>
<tr>
<td>Potato (tuber)</td>
<td>1 tonne/hectare</td>
<td>5</td>
</tr>
<tr>
<td>Banana (fruit)</td>
<td>1 tonne/hectare</td>
<td>0.4</td>
</tr>
<tr>
<td>Grape (fruit)</td>
<td>1 tonne/hectare</td>
<td>1.5</td>
</tr>
<tr>
<td>Cotton (lint and seed)</td>
<td>1 bale/hectare</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Table 4. S uptake by major crops

<table>
<thead>
<tr>
<th>Crops</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cron (180 bu/a)</td>
<td>28</td>
</tr>
<tr>
<td>Soya bean (60 bu/a)</td>
<td>25</td>
</tr>
<tr>
<td>Bermudagrass (8t/a)</td>
<td>44</td>
</tr>
<tr>
<td>Wheat (55 bu/a)</td>
<td>13</td>
</tr>
<tr>
<td>Rice (75t/a)</td>
<td>12</td>
</tr>
<tr>
<td>Cotton (1500t/a)</td>
<td>40</td>
</tr>
<tr>
<td>Alfalfa (8t/a)</td>
<td>40</td>
</tr>
</tbody>
</table>

(ii) Growth, yield and quality

S-deficient plants generate a lower yield and have a reduced nutritional value. The process of S acquisition and assimilation play an integral role in plant metabolism, and response to S deficiency involves a large number of plant constituents (Table 4). The photosynthetic apparatus was severely affected under S deficiency. The Chl content was reduced because of a general reduction of PSII and PSI and the associated light-harvesting antenna. The Rubisco content was also significantly reduced in the S-deprived plants. The imbalances between PSII and PSI, and between photosynthesis and C fixation led to a general over-reduction of the photosynthetic electron carriers. Chromatographic analysis showed that the level of monosaccharides was lower and starch content higher in the S-deprived plants. While no changes in metabolite levels were found in the TCA or Calvin cycle (Lunde et al., 2009).

S deficiency in legume crops affect yield, quality and nutritional value of seeds (Sexton et al., 1998), because Met is usually the most limiting essential amino acid in pulse seeds (Friedman, 1996). The yield response to optimum S application, however, differs any crop species, being lower in Medicago sativa and Pisum sativum as compared to Trifolium pratense and Vicia fava, suggesting that legumes differ in their S-requirement (Scherer and Lang, 1996). Adverse effect of S deficiency on inorganic nutrition and biochemical processes might lead to the observed decrease in growth and finally may result in a decrease yield of chickpea (Badruddin and Karmoker, 2001). Since, the yield and quality of legume seeds are limited by the amount of S partitioned to the seeds. The amino acid S-
methylMet (SMM), a Met derivative and a long-distance transport form of reduced S and whether SMM phloem loading and source-sink translocation are important for the metabolism and growth of pea plants. The changes in SMM phloem loading affected plant growth and seed number, leading to an overall increase in seed S, N, and protein content. The phloem loading and source-sink partitioning of SMM are important for plant S and N metabolism and transport as well as seed set (Tan et al., 2010).

Under S limiting conditions, in pot experiments with different legumes, a lower N accumulation and a yield reduction was found (Scherer and Lange, 1996). S also affect leguminous plant species growth through its effect upon N\textsubscript{2} fixation by rhizobium microorganisms because of the relatively high S content of nitrogenase (Jannsen and Vitosh, 1974; Mortenson and Thornley, 1979; Lange, 1998) and ferredoxin (Yoch, 1979). S is an essential macronutrient and at an optimum concentration accelerates the plant growth (Thomas et al., 2000). S deficiency has been shown to influence the quality of many crops including glucocinolates levels in oil seed rape, baking quality of wheat flour, nutritional value of legume storage proteins and herbage quality of grassland (Thomas et al., 2000). Lawn and Brun (1974) indicate that decline in nodule activity coincided with the time when pod growth rate first exceeded total crop growth rate. S has profound effect on creating assimilation area absorbing PAR and as a consequence on yield of crops (Scherer, 2001; Lambers et al., 1990; Blake-Kalff, 1998).

Plants grown on S-deficient soils have suppressed development of reproductive organs that, in rapeseed, can even lead to pod abortion. Reproductive growth and the proportion of the reproductive tissues in total dry matter are significantly increased by the application of S during pod development. The presence of S maximizes the seed and oil yield of other plants (Ahmad et al., 2007). Positive effect of S on seed yield and its quality is visible and significant when S content in soil is low (Ahmad et al., 2007; Malhi et al., 2007). Analogically, S fertilisation does not influence the yield when S content in soil is quite high (Nowosielski, 1961). S supply only increase the S-concentration of the plants without enhancing the yield. Growth of Broccoli (Brassica oleracea var.) did not respond to the S supply, but GSL concentrations showed a sharp response to the whole range of S applications and high S assimilation into aliphatic GSL were consistently observed in the florets than other parts (Omirou et al., 2010). In addition, high menthol content in Japanese mint grown in nutrient formulas on supplementation with a combination of S fertilisation and amino acid mixture yields the highest quantity of volatile oil (Vimolmangkang et al., 2010).

(iii) Nodulation and N-fixation

Biological N\textsubscript{2}-fixation, nodulation and yield of Peanut crops are reduced with S-deficiency. Varin et al., (2010) examined whether the effect of SO\textsubscript{4}\textsuperscript{2-} addition on N fixation resulted from a stimulation of host plant growth, a specific effect of S on nodulation, or a specific effect of S on nodule metabolism. The application of SO\textsubscript{4}\textsuperscript{2-} increased whole plant dry mass, root length, and nodule biomass, expressed on a root-length basis. N uptake proved less sensitive than N\textsubscript{2} fixation to the effects of S-deficiency, and decreased as a consequence of the lower root length observed in S-deficient plants. N\textsubscript{2} fixation was drastically reduced in S-deficient plants as a consequence of a low nodule development, but also due to low nitrogenase and leghaemoglobin production. This effect is likely to
be due to down-regulation by an N-feedback mechanism, as, under severe S-deficiency, the high concentration of whole plant N and the accumulation of N-rich amino acids indicated that the assimilation of N exceeded the amount required for plant growth. As compared to subterranean clover supplied with S nodule was markedly decreased in S-deficient clover. This is attributed to the decline in the requirement for N with reduced S supply.

However, the observed increase in the number of nodules by S-fertilization of legumes was not the result of increased nodulation per unit length of roots, but rather due to enhanced root growth (Scherer and Lang, 1996). S nutrition was found to improve nodulation activity in soya bean (Andrew and Robins, 1969; De Moy et al., 1973; Lluch et al., 1982) and in combination with FYM promoted the nodule production and dry weight in soya bean and wheat to an extent in terms of total number of nodules and number of active nodules (Ganeshamurthy and Reddy, 2000). Although the dry weight content of the nodules at higher levels of applied S showed a tendency to increase also (Vance et al., 1998; Ganeshamurthy and Reddy, 2000). Pulse crop obtain N mainly from symbiotic N-fixation which may be affected by S deprivation (Scherer et al., 2006) and it is quite sensitive to supply of photosynthetic assimilates which indicate that decline in nodule activity under S deprivation is associated with development of pods in soya bean (Wheeler, 1971). Nitrogenase and ferredoxin which play vital roles in N₂-fixation are rich in S and contain Fe-S clusters. With S-deficiency, amino acids and other N forms accumulate due to the impaired protein synthesis. This could be due to the feedback repression of N-fixation (Janssen and Vitosh, 1974). Meanwhile Lang (1998) suggested that S affect leguminous species through its influence on N-fixation by Rhizobium species. If symbiotic N₂-fixation has a greater requirement for a nutrient than the growth of host plants, a negative interaction between the addition of that nutrient and inorganic N on plant growth is expected.

(iv) Chlorophyll and Protein

The chlorophyll content of the leaf is influenced by optimum dose of S nutrition and it significantly increased in case of soybean leaves (Gameshamurthy and Reddy, 2000) but at higher rates of applied S did not further increase the chlorophyll content of leaves and significantly reduced in case of rapeseed. Chlorosis is a common symptom of S deficiency in plant species (Stewart and Porter, 1969). Circumstantial evidences indicate that S-deficiency greatly diminishes Carbon-fixation of Medicago sativa, which is assumed to be caused by the reduction in the synthesis of key carbon metabolic enzymes as a result of reductions in the pods of the free S-containing amino acids.

S is a constituent of Met (21%), the first amino acid incorporated during protein synthesis and it also linked to proper functioning of NR (Ahmad et al., 1999), the enzyme regulating the flow of NO₃-N in to the amino acid and subsequently in to protein. S deficiency markedly reduced the mole percent of Cys and Met in the total leaf protein fraction of Lucerne (Medicago sativa L.), while storage proteins in legume seeds like vicilin, which contain no Cys and Met increased. While arginine content in shoot tissues of sugar beet increases in S deficiency (Thomas et al., 2000). Application of S also increased soluble protein and chlorophyll content in the cell of groundnut (Jamal et al., 2006) and improves the status of S-containing amino acids in the seed protein of chickpea because seeds have some capacity to increase their rate of S assimilation.
and S amino acids biosynthesis in response to an added demand (Chiaiese et al., 2005). Evaluation of amino acid profile under S-starvation conditions showed two- to fourfold enhancement in the contents of arginine, asparagine and OAS, whereas the contents of Cys and Met were reduced heavily. Exogenous supply of metabolites (arginine, asparagine, Cys, glutamine, OAS, and Met) also affected the uptake and assimilation of NO$_3^-$, with a maximum for OAS. Therefore this tight interconnection of S-nutrition with NO$_3^-$ assimilation and that OAS plays a major role in this regulation and must be helpful in developing a nutrient-management technology for optimization of crop productivity in future (Kaur et al., 2010).

(v) NR activity and ATP sulfurylase

Scherer and Lang (1996) investigated the effect of S nutrition on the activity of key enzymes of the C and N metabolism of *vicia faba* and *Pisum sativum*. S deficiency results in a reduction of NR activity and an accumulation of amino acids in a variety of plants (Reuveny et al., 1980; Migge et al., 2000; Prosser et al., 2001). The application of S to groundnut may result in increased NR and ATP-Sulfurylase activities (Jamal et al., 2006; Ahmad et al., 1999). ATP-sulfurylase, is the first enzyme of the S assimilation pathway and NR play a key regulatory role in the NO$_3^-$ assimilation, is to be exploited that the activities of these enzymes are related to root, growth and yield (Reuveny et al., 1980; Barney and Bush, 1985; Clarkson et al., 1989; Ahmad et al., 1999). Chlorophyll content, ATP sulfurylase activity and protein content were also higher in transgenic plants than untransformed plants under sulfur-insufficient conditions (Abdin et al., 2010).

Deficiency of S in plants results in a reduction of NR activity and an accumulation of amino acids or soluble protein in chickpea seedlings of non-nodulating genotypes than nodulation genotypes and this results hints to a genetic variability of NR activity (Migge et al., 2000; Prosser et al., 2001). Earlier, it was reported that NR was genetically controlled (Badruddin and Karmoker, 2001). However the reduction of NR activity and mRNA levels seen to be a relatively late process in plant adaptation to S limiting conditions (Prosser et al., 2001).

(vi) Nitrate content

N-metabolism is strongly affected by the S-status of the plant. S deficiency decreases the concentration of N in the shoot of legumes (Andrew, 1977; Zhao et al., 1999). NO$_3^-$ dramatically increased in S limited cells, probably as a consequence of the lowered activity of NR. For higher plants, it has been suggested that the down regulation of NR is mediated by products of N-accumulation like Gln, Asn and Arg (Migge et al., 2000). It has been reported that the NR is extremely susceptible to ammonium rather than to its metabolic products (Franco et al., 1985). The increased accumulation of NO$_3^-$ may be correlated with a reduced synthesis of soluble protein due to the reduced availability of SO$_4^{2-}$.

Impact on food and pharmaceuticals and global stress responses

S assimilation in plants plays a key role in the S cycle in nature. S deficiency in agricultural areas in the world has been recently observed because emission of S air pollutants in acid rain has been diminished from industrialized area. S deficiency severely affects plant growth and their agricultural productivity leading to diverse changes in development and metabolisms. Molecular mechanisms regulating gene expression under low S conditions remain largely unknown. Fertilization of
S is required in these areas; otherwise, low crop quality and productivity are seen. In seed proteins, the level of S containing amino acids, Cys and Met, are low from the nutritional point of view for animals. Enhancing Met levels via genetic engineering has been shown to increase the nutritive value of seed crops for animals (Molvig et al., 1997). Animals, however, do not have the assimilatory mechanisms for inorganic S; they require Met as an essential amino acid for their source of S nutrient. Arabidopsis plants were exposed to S depletion alone, changes of photosynthetic parameters and metabolite abundances were quantified. Photosynthetic electron transport rates (ETRs) of plants exposed to S depletion and high light decreased strongly at day 2 of the acclimation period. However, at metabolic level, the stress combination had a profound effect on central metabolic pathways such as the tricarboxylic acid (TCA) cycle, glycolysis (EMP), pentose phosphate cycle (PPP) and large parts of amino acid metabolism. Under these conditions, central metabolites, such as sugars and their phosphates, increased, while S-containing compounds were decreased (Wulff-Zottele et al., 2010) (Fig. 3).

Figure 3 A diagram for sequential events occurring in plants at conditions of enough S supply.

Recently increased attention has been paid to the neutral and pharmaceutical value of S containing plant products. Epidemiologic and experimental studies have suggested that S-containing phytochemicals from cruciferous plants such as isothiocyanates (ICN) may prevent cancer (Talalay and Fahey, 2001). Nutritional stress of S most notably modulates \( \text{SO}_4^{2-} \)-S assimilation. Upon S deficiency, S is remobilized less efficiently than N, resulting in chlorosis of young leaves by S deficiency, while the first appearance of N deficiency is in old leaves (Hawkesford, 2000). Studies of Astolfi et al., (2010) support the idea that the extent to which the plant is able to cope with Fe starvation is strongly associated with its S nutritional status and the barley plants fully recover their capability to cope with Fe shortage after the supply of S is restored to S-deficient plants.
AtSLIM1, a member of the EIN3-like (EIL) family was reported to be a central transcriptional regulator of the plant S response. Studies of Wawrzynska et al., (2010) suggest that a promoter region of UP9C (a tobacco gene) strongly induced by S limitation. A 20-nt sequence (UPE-box), also present in the promoters of several Arabidopsis genes. The UPE-box, consisting of two parallel tebs sequences (TEIL binding site), proved to be necessary to bind the transcription factors belonging to the EIL family. The interactions of NtEIL2, and its homologue from Arabidopsis, AtSLIM1, with DNA were affected by mutations within the UPE-box. Interestingly, activation by NtEIL2, but not by AtSLIM1, was dependent on the S-deficiency of the plants.

Analyses of transcriptome (Hirai et al., 2003; 2004; Maruyama-Nakashita et al., 2003; Nikiforova et al., 2003; Wang et al., 2003) and metabolome (Hirai et al., 2004) in Arabidopsis indicate the presence of global multiple networks responding to nutritional deficient stress. Long-term S deficiency results in similar global changes in the transcriptome and metabolome, as doe’s N deficiency (Hirai et al., 2004). These are general responses common to S deficiencies. These general nutritional responses differ in leaves and roots. Signaling pathways involving methyl jasmonate and auxin seem to be involved in the S stress response. Primary and secondary metabolic pathways, involving amino acids, carbohydrates and GSL are modulated in response to S deficiency stress (Hirai et al., 2004). GSL are regarded as storage and possibly mobilizing forms of assimilated S in response to acute S deficiency. Since integrated -omics studies have just started; further investigation will provide more detailed information on holistic networks of S metabolism.

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. Additionally, the major forms of S-containing secondary metabolites in various staple plant foods (e.g. rice, corn wheat legume potato and tomato) need to more research necessary for their identification. Authors suggesting that researchers must elaborate the knowledge about the biochemistry of S-homeostasis factors, physical interactions of transporters and chelaters. A genetic approach as opposed to physiological/biochemical investigation may assist by increasing the capability of plant system for more GSH synthesis, may prove useful for eco-detoxification through the trapping of xenobiotics pollutants permitted by an increased capability of GSH mediated conjugation. Up regulation of GSH synthesis provide substrates for the PCs. Similarly, plants with enhanced capabilities for PCs synthesis could be applied to bio-remediation of poor soils through the overcoming the excess of heavy metals by increasing the rate and dose of S containing fertilizers, pesticides and insecticides.

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. Therefore, a lot of research work needs in all above crucial areas to elucidate molecular and genetic basis of mechanisms involved in S-chelation to overcome the heavy metal excess (stress) and their homeostasis with SO$_4^{2-}$ in soil-plant system.

ACKNOWLEDGMENTS

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.

REFERENCES


Badruddin, M., Karmoker, J.L. (2001). The effect of sulphur deficiency on ion accumulation with special references to $^{15}$N and $^{35}$S transport in chickpea. Ph.D. Thesis, Dhaka University,
Sulphur stress tolerance

Dhaka, Bangladesh.


Eriksen, J., Murphy, M.D., Schnug, E. (1998). The


Harada, E., Kim, J.A., Meyer, A.J., Hell, R.,


Biosynthetic regulation of phytochelatins, heavy metal-binding peptides. *Journal of Bioscience and Bioengineering, 100*: 593–599.


Sulphur stress tolerance...


Salsi, E., Campanini, B., Bettati, S., Raboni, S.,
Sulphur stress tolerance


U.S. Congress office of Technology Assessment, (1990). Beneath the bottom line: Agriculture approaches to reduce agrichemical


