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Biological sulfur acquisition: an update

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ABSTRACT

Sulfur is an essential macronutrient for plants. As a constituents of sulfure containing amino acids, methionine and cysteine are also important for animal and human nutrition. That is why understanding of how inorganic sulfure is taken up by plants and built into organic molecules in the process of sulfur assimilation is important. The process of sulfur uptake and assimilation is an integral part of sulfur metabolism. The major physiological problem connected with sulfur metabolism is a sulfur deficiency stress which can be diagnosed in two states. The short -term response is characterized by the development of enhance lateral roots exploring the space in search for lacking nutrients. The long- term response is characterized by decrease in internal lipids, and chlorophyll content and increase in photorespiration. These factor provide-cause effect connections to decrease photosynthesis leading to limitation in energy assimilation, which is turn induces in decline of plant metabolisms.

Introduction

Sulfur is an essential element for growth and physiological functioning of plants, however, its content strongly varies between species from 0.1 to 6% of the dry weight(0.3 to 2 m mol g^{-1}) reported by De Kok *et al*. (2002a). Sulfur in the form of sulfate (SO_4^{2-}) is an essential, yet chemically inert, nutrient required by plants for the synthesis of a large array of metabolites (Schachtman and Shin, 2007; Yi *et al*., 2010; Takahashi *et al*., 2011; Ravilious and Jez, 2012). Sulfur is the least abundant macronutrient in plants with highest functional versatility. Sulfur metabolism has been characterized in details at the biochemical and molecular levels (Saito, 1999; Hell *et al*., 2002; Hesse and Hoefgen, 2003) and has been shown to be intimately interactive with many parts of the plant metabolism and physiology (Fig. 1). It functions in plants in its oxidation states from $SO4^{-2}$ as major cationic constituents, as $SO3^{-2}$ in the form of sulfated compounds and sulfolipids as elemental S^o in pathogen defense, and as S^{-2} in all organic cell compounds containing reduced sulfur in Cysteine and Methionine and is the fundamental chemical and physical state of these organosulfur compounds that account for their biochemical functions (Giles *et al*., 2003). The presence of sulfur in structural and functional compounds is a very common feature of biological system (Giordano and Hell, 2001). In contrast, CO_2 and NO_3^- are also assimilated as inorganic oxidized ions but rarely function in oxidation states other than in their fully reduced state. Sulfur is typically involved in redox processes (Falkowski and Raven, 1997; Saito, 2000; 2004; Pfannschmidt, 2003). The nucleophilic character of sulfide makes it highly reactive and an ideal mediator of redox reactions including electron transport (Fe-S cluster), enzyme catalysis (*e.g*. proteases), activation of reactive groups (Co enzyme A) and disulfide bridges with structural and regulatory roles. Sulfur also participates along with the Fe^{+2}/Fe^{+3} redox pair in the integral redox systems of chloroplast and mitochondria constituting the indispensable energy generating processes of life. In fact, it has been speculated that sulfur and iron served as the first electron transport systems for proto life of the early earth. With increasing oxygen concentration brought about by the development of photosynthesis, the sulfide/disulfide redox system was retained as a detoxification mechanism (*e.g*. glutathione) for reactive oxygen radicals and as a signaling mechanism (*e.g*. control of Calvin cycle enzymes by thioredoxin) needs to coordinate photosynthetic light and dark reactions, thereby preventing the destructive production of excess reactive oxygen radicals. In addition reduced sulfur compounds, such as hydrogen sulfide, serve as electron donor for chemotrophic or phototrophic growth in a large and diverse group of bacteria and archae, including purple and green sulfur bacteria (Truper and Fischer, 1982).

Plants, bacteria and fungi can assimilate inorganic sulfur as sulfate (oxidation state +6) for reduction to sulfide leading to the synthesis of sulfur containing amino acids (Marzluf, 1996; Thomas and Surdinkerjan, 1997; Leustek *et al*., 2000). But sulfur assimilation in plants plays a key role in the sulfur cycle in nature. Sulfur from both pedospheric *i.e*. sulfate ion in soil and atmospheric *i.e*. SO_2 and H_2S gas, is also fixed into Cys. by the sulfur assimilation pathway in plants (Saito, 2000). In contrast animals and humans lack the capability to reduce sulfate. As a consequence human and most animals rely on their diet for provision of reduced sulfur in terms of Cys. and Met. (Tabe and Higgins, 1998; Zhao *et al*., 2000). Thus plants are the most important source of essential sulfur amino acids for human and animals, which constitute general economic interest of sulfur amino acid biosynthesis in higher plants (Zhao *et al*., 1999). In addition to the atmospheric and pedospheric sulfate is usually well accessible in aquatic ecosystems also (Giordano *et al*., 2005). The ocean represents huge reservoirs of sulfur as dissolved sulfate, with typical concentrations around 29 mM (Strauss, 1997; Pilson, 1998; Norici *et al*., 2005). Phytoplankton plays a pivotal role in sulfur (S) biogeochemistry, mostly as a consequence of dimethyl sufide (DMS) emission, a potent effecter on global climate released by micro algal cells

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(Giordono *et al.*, 2005). This trace gas, in fact, directly affects global climate and biogeochemistry by being the main natural source of reduced S to the global boundary layer. The oxidation of DMS yields sulfate that participates to cloud condensation nuclei and represents one of the ways of entry of bioaccessible S in the biogeochemical cycle (Giordano *et al.*, 2005).

Sulfur is mainly taken up by the plant as inorganic sulfate from the soil after incorporation from the soil through root specific transporters; sulfate is distributed into plant cells through a family of transporters needed to cross all the cell barriers (Buchner *et al.*, 2004). Minor part of sulfate is used for sulfation of proteins in the cytosol, whereas most part of sulfate is activated and reduced into a three step mechanism to sulfide exclusively in plastids before its incorporation in to a serine derivative leading to the first organic sulfur amino acid, cysteine (Droux, 2004; Wirtz and Droux, 2005). Although root plastids contain all of the enzymes of the sulfate assimilatory pathway, it is evident that the sulfate assimilated in the shoot chloroplasts is the primary source of reduced sulfur in the plants.

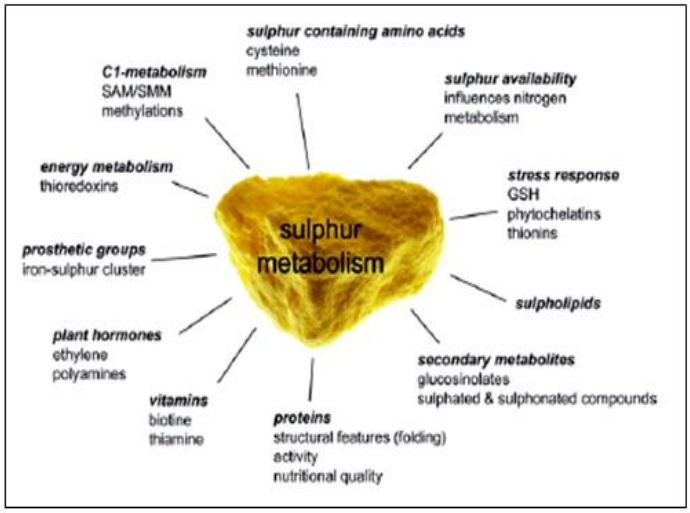

Fig. 1. Schematic representation of the involvement of sulfur in the biosynthesis of plant metabolites or physiological functions. As deduced from current biochemical, molecular and physiological knowledge sulfur metabolism is intimately involved in plant metabolic and physiological processes. Sulfur-containing metabolites are either directly involved as constituents or indirectly, for example, as cofactors, or prosthetic groups, methyl group donor, hormone precursor, or generally as integral part of proteins and enzymes (Buchner *et al.*, 2004b).

Multiple points of control with in the sulfate assimilatory pathway have been described with much emphasis on the cellular control of flux through the pathway (Vauclare *et al.*, 2002; Hawkesford and De Kok, 2006). Recent researches have begun to determine the molecular components of the signal transduction pathway responsible for the transcriptional regulation controlling the expression of sulfate transporters (Maruyama-Nakashita *et al.*, 2005). Recently a combination of transcriptome and metabolome analysis has significantly increased our knowledge of the cellular processes affected by sulfur starvation, which is becoming a serious problem in agriculture (Hirai *et al.*, 2003; 2004; 2005; Maruyama-Nakashita *et al.*, 2003; Nikiforova *et al.*, 2003; 2005). The sequestration of sulfur by plants has become an increasingly important concern for the agricultural industry due to decreasing trends of S-emissions from industrial sources and the consequent limitation of inputs from deposition. The recognition of the importance of sulfate for plant growth and vigor and hence crop yield as well as the nutritional importance of sulfur for human and animal diets has led to an increased emphasis on research on the process of sulfate uptake, transport and assimilation.

Biological SO_2 acquisition

Fundamental importance to plant sulfur assimilation in the effective delivery of sulfate to plastid which is a major site of assimilatory reductive pathway. In addition, the requirement for cytosolic ion homeostasis leads to a flux of surplus sulfate into the vacuoles, which serves as a nutritional reservoir (Buchner *et al.*, 2004 b). It is assumed that sulfate concentrations in the cytoplasm and in the chloroplast are quite similar (Kaiser *et al.*, 1989). The cytosolic and plastidic sulfate homeostasis is important to avoid toxification. The transfer of sulfate across the tonoplast has been investigated in details only in yeast and barley mesophyll vacuoles (Hirata *et al.*, 2002) (Fig. 2). Intracellular sulfate is further metabolized into a large variety of primary and secondary metabolites. The reduction of sulfate to sulfide occurs in three steps. Prior to its reduction sulfate is activated by adenylation to adenosine 5' phosphosulfate (APS) in a reaction catalyzed by ATP sulfurylase (ATPS: EC 2.7.7.4). APS is reduced to sulfite by ATP reductase (APR: EC 1.8.4.9); the electrons are derived from glutathione. Sulfite is further reduced by a ferredoxin dependent sulfite reductase (SiR: EC 1.8.7.1) to sulfite which is incorporated by O-acetylserine (thiol) lyase (OASTL; EC 2.5.1.47) into the amino acid skeleton of O-acetylserine (OAS) to form cysteine. OAS is synthesized by acetylation of serine with acetyl co-enzyme A catalyzed by serine acetyl transferase (SAT: EC 2.3.1.30) (Leustek *et al.*, 2000; Kopriva and Koprivova, 2003).

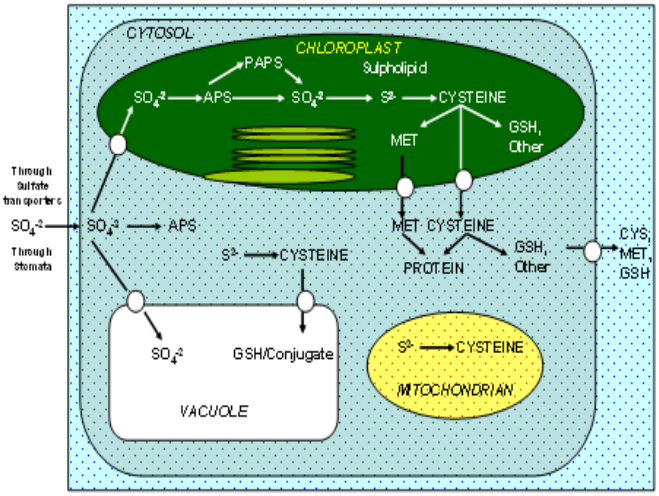

Fig. 2. Sub-cellular compartmentation of major reaction and compounds of sulfur metabolism in a typical plant cell (Hell *et al.*, 2002).

Plant shoot also form a sink for atmospheric sulfur gases, their foliar uptake is generally directly dependent on the degree of the opening of stomates. SO_2 is highly soluble in the apoplastic water of the mesophyll, where it dissociates under formation of bisulfite (HSO_3^-) and sulfite (SO_3^{-2}). Sulfite may directly enter the sulfur reduction pathway and be reduced to sulfide, incorporate into cysteine, and subsequently into other sulfur compounds. Sulfite may also be oxidized to sulfate, extra and intracellularly by peroxidases or nonenzymatically catalyzed by metal ions or super oxide radicals and subsequently reduced and assimilated again. Excessive sulfate is transferred to the vacuole. The foliar uptake of H_2S appears to be directly dependent on the rate of H_2S metabolism into cysteine and

subsequently into other sulfur compounds (De Kok *et al*., 2002 a,b). There is strong evidence that O-acetylserine(thiol)lyase is directly responsible for the active fixation of atmospheric H_2S by plants. plants are able to transfer from sulfate to foliar absorbed SO_2 or H_2S as sulfur source(De Kok *et al*., 2000 a,b; Yang *et al*., 2003) and levels of 0.06ul appear to be sufficient to cover the sulfur requerments of plants(Yang *et al*., 2003; Buchnuer *et al*., 2004) (Fig. 4). There is an interaction between atmospheric and pedospheric sulfur utilization. Forinstance, H_2S exposure resulted in adepresed sulfate uptake iin Brassica oleracea (Westerman *et al*., 2001; De Kok *et al*., 2000b). However, H_2S solely affected the expression of the different sulfate transporters in the shoot, but not in the roots (Buchner *et al*., 2004).

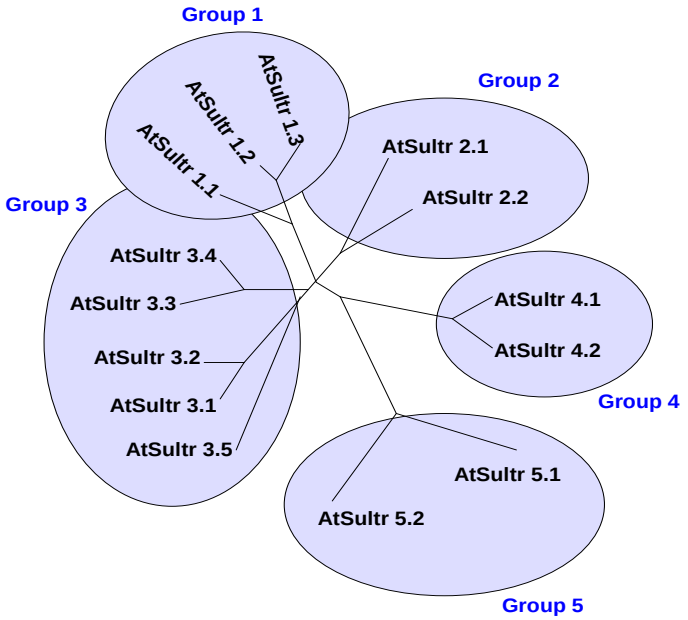

Fig. 3: A phylogenetic tree of the *Arabidopsis* sulphate transporter gene family (Hawkesford, 2003).

Molecular mechanism associated with biological sulfur acquisition

The major source of sulfur for plants is inorganic sulfate, which is taken up by the roots with high affinity and the maximal sulfate uptake rate is generally already reached at pedospheric sulfate levels of 0.1 mM and lower (Hawkesford, 2000; Hawkesford *et al*., 2003 a,b). Although plants are able to used reduced sulfur compounds from the atmosphere, such as sulfur dioxide (SO_2) or hydrogen sulfide (H_2S) (Leustek *et al*., 2000). Sulfate is actively taken up across the plasma membrane of the root cells through a proton/sulfate (presumably $3H^+/SO_4^{-2}$) co-transport (Clarkson *et al*., 1993). After entry into the plant, sulfate is the major form of transport as well as stored sulfur. The delivery of sulfate into the plastid for assimilation, sulfate storage within the vacuoles, and the long distance transport between organs in order to fulfill the source/sink demand during plant growth requires specific sulfate transporter proteins (Buchner *et al*., 2004). Transport of sulfate into the cell is considered to be a major regulated step (Vauclare *et al*., 2002). There are several distinct transport steps that are independently, but probably coordinately regulated and which serve to maintain constant cytoplasmic sulfate levels and prevent excess accumulation. To facilitate the complex movement of sulfate around the plant, the sulfate transporters themselves are encoded by a gene family consisting of 14 members in *Arabidopsis* (Hawkesford, 2003) (Fig. 3). The transcription of the genes encoding the transporters involved in initial uptake at the soil root interface, cell to cell transfer and vascular transportation as well as the vacuolar efflux transporter is controlled by plant sulfur status Buchner *et al*. (2004).

The coordinated expression of this gene family facilitates optimum management of plant sulfate under varying condition of supply and demand. According to their cellular and sub-cellular expression, and possible functioning the sulfate transporter family comprises of 14 genes which are classified in up to 5 different groups (Hawkesford *et al*., 2003 a,b; Buchner *et al*., 2004). Some groups are expressed exclusively in the roots or shoots or expressed both in the roots and shoots. Group 1 are high affinity sulfate transporters predominantly but not exclusively expressed in roots. (Smith *et al*., 1997) which are involved in the uptake of sulfate by roots; many of these groups are transcriptionally regulated in response to sulfur availability. A unique specific localization of one isoform in this group. *At Sultr* 1;3, to the sieve elements companion cells in the phloem is indicative of a specialized role in the redistribution of sulfur from source to sink tissues (Yoshimoto *et al*., 2003). Initial uptake of sulfate from the soil solution is mediated by high affinity sulfate transporter, *At Sultr* 1:1 (Takahashi *et al*., 2000). Expression studies indicated that group 2 are vascular transporters and therefore, of potential significance in considering tissue distribution of sulfate and are low affinity sulfate transporters. Transporters of sulfate from roots to leaves is mediated by the two low-affinity sulfate transporters, *At Sultr* 2;1 and *At Sultr* 2;2 (Takahashi *et al*., 2000). In *Brassica*, only isoform of *At Sultr* 2:1 is expressed substantially in roots, stem and leaves, while *At Sultr* 2;2 is also expressed in roots. In *Arabidopsis* both isoforms are expressed in roots and leaves. In *Brassica*, (Buchner *et al*., 2004; Buchner *at al*., 2004b), the expression of *At Sultr* 2;1 only occurs during sulfur starvation in the roots, however, in the leaves expression occurs also under sulfur-replete conditions, but is increased upon sulfur starvation. *At Sultr*2;2 expressions in the roots increased by sulfur starvation (Takahashi *et al*., 2000; Hawkesford and De Kok, 2006). In *Arabidopsis* (Takahashi *et al*., 2000), *At Sultr* 2;1 is noticeably induced by sulfur starvation in the roots, as found in *Brassica*. Group 3 is the so called 'leaf group'. This is a rather larger group with five examples in *Arabidopsis*. Genes encoding sulfate transporters *At Sultr* 3;1, *At Sultr* 3;2 and *At Sultr* 3;3 appear to be exclusively expressed in leaves and their expression does not appear to be significantly modulate by the sulfur status of the plants (Takahashi *et al*., 2000). One of the isoform *At Sultr* 3;5 functions as a heterodimer with *At Sultr* 2;1 (Kataoka *et al*., 2004 a). This was supported from the observation in yeast where expression of *At Sultr* 3;5, itself failed to catalyze sulfate transport, but contributes to uptake when co expressed with *At Sultr* 2;1. A homologue of *At Sultr* 3;5 has been described for *Lotus japonicus* which is localized on the symbiosome membrane in N_2 fixing nodule (Krusell *et al*., 2005). This transporter is essential for sulfur delivery to the bacteroid and for an efficient N2 fixation. By contrast to the plasma membrane location of the sulfate transporters of group 1-3, group 4 and 5 putative sulfate transporters have been localized to the Tonoplast membrane. The group 4 transporters have been implicated in efflux of sulfate from the vacuole and are up-regulated by the sulfur stress, thus providing the unloading of sulfate from the vacuole (Kataoka *et al*., 2004b). The role of group 5 transporters has yet to be established.

Toxicity of sulfate: Sulfitolysis

The impact of sulfurous air pollutants on plant functioning is paradoxial, since they may both act as toxin and nutrient (De Kok et al., 2002a,b). SO_2 is known for many years to damage plants. As nucleophilic agent, sulfite is able to attack diverse substrate, where it opens S-S bridges and thereby causes inactivation of these compounds i.e. sulfitolysis. Several enzymes are inhibited when incubated with sufite or when plants are exposed to high concentration of SO_2. Sulfitolysis can occur also under physiological conditions which further underpines the phytoxicity of SO_2 (Hansch and Mendle, 2005). Plant sulfite oxidase (SO) a peroxisomal enzyme possibly serve as 'safety valve' to detoxify excess amounts of sulfite and protect the cell from sulfitolysis. Indeed there is an example supporting this assumption it has been shown that peroxisomal catalase is inhibited when leavas were treated with sulfite (Veljovic-Jovanovic *et al*., 1998). The half maximal inhibition was below of 500µM sulfite. Here, plant SO could playa role for protecting this important enzyme from sulfite damage.

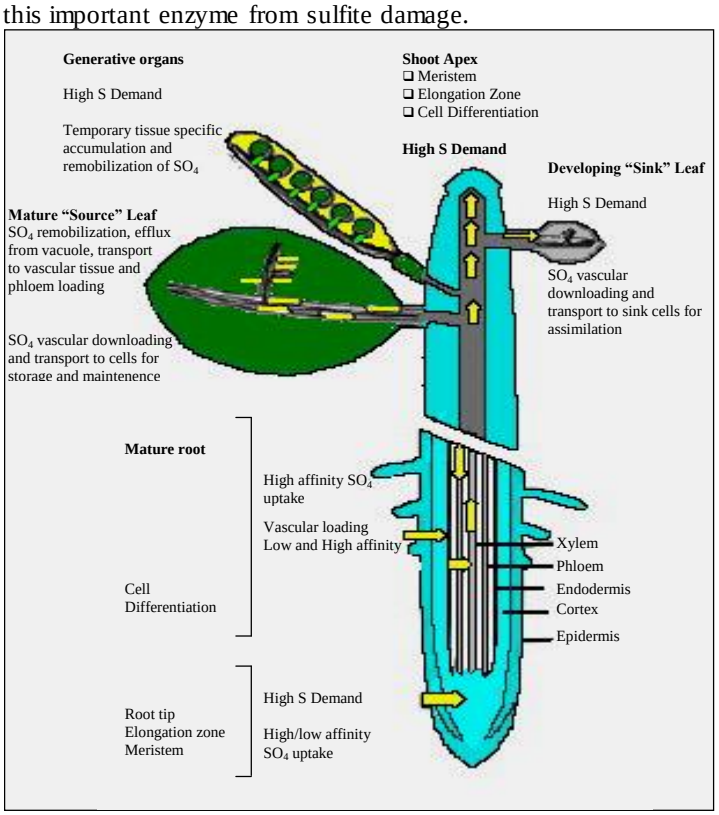

Fig. 4: Model of sulphate uptake and movement on the whole plant level. Illustration of the proposed uptake, distribution, import, remobilization, and export of sulphate based on the function and tissue as well as developmental specific demand of sulphate. Arrows indicate uptake and potential movement of Sulphate (Buchner *et al*., 2004b).

Transcriptome, metabolome vs. Sulfur nutritional stress

The major physiological problem connected with sulfur metabolism is sulfur deficiency, the extent to which this condition affects plant metabolism was investigated by expression profiling (Hirai *et al*., 2003; Maruyama-Nakashita *et al*., 2003) and combined transcriptome and metabolome analysis (Hirai *et al*., 2005; Nikiforova *et al*., 2005; Hoefgen and Nikiforova, 2008).

Long term sulfur deficiency response

In the process of plant adaptation to low sulfur the internal lipid content is strongly decreased. Decreased amounts of sulfur containing molecule, S-adenoyl-methionine (SAM), is followed by decreased chlorophyll content, for which the biosynthesis of SAM is required and increased photorespiration. These factors provide cause-effect connection to decreased photosynthesis leading to limitation in energy assimilation, which in turn conduces to general decline of metabolism. Insufficient sulfur supply reach to its disbalance with nitrogen being further enforced by the alterations isn tetrahydrofolate, a central cofactor in C1 metabolism that link photorespiration (Ser/Gly metabolism), sulfur assimilation. (met biosynthesis) and dumping of disbalance nitrogen (through enforced purine metabolism, influenced also by decreased SAM). Mutual influences between these processes shown in Fig. 5 (A).

Short term sulfur deficiency response

When sulfate in the growth medium is depleted, internal sulfur quickly drops down, followed by fast decrease in cysteine (the first organic molecule into which inorganic is incorporated) and its derivative glutathione (Hirai *et al*., 2003). Red traffic light on the biochemical pathway to cysteine leads to accumulation of its direct biosynthetic precursor O-acetyl L-serine(OAS) as well as the immediate OAS precursor serine, and to the subsequent re-channeling of the metabolic flow of Glycine and tryptophan (Hirai *et al*., 2003, Nikiforova *et al*., 2005).

Next set of response event activation of glucosinolate catabolism. Glucosinolate are sulfur rich compound stored in vacuole. In short term sulfur depletion following biochemical changes takesplace.

- Excess tryptophan (Nikiforova *et al*., 2003)
- Down regulate glucosinolate biosynthesis (Hirai *et al*., 2004) and activated glucosinolate catabolism
- Strong overexpression of nitilases (Kutz *et al*., 2002)

The general positive alternation in auxin flux. Exess auxin provides the causal connection with enhanced lateral root formation. Development of enhanced lateral root can be considered as the end point physiological reaction for the primary state limited sulfur Fig 5 (B). The genes induced by sulfur deficiency including those coding for sulfate transporters and APR, other genes of sulfate assimilation were not significantly and/or consistently affected. In *Arabidopsis* , OAS induced mRNA accumulation of all genes of sulfate assimilation and dramatically increased flux through sulfate assimilation (Koprivova 2006). OAS has been identified as a limiting factor for cysteine synthesis (Rennenberg, 1983) and was shown to induce APR activity and rate of thiol synthesis in *Lemna minor* (Neuenschwander *et al*., 1991). OAS also strongly affects the cysteine synthase complex; even a less than two fold increase in OAS concentration results in dissociation of the complex and inactivation of the SAT (Berkowitz *et al*., 2002). Because OAS accumulation during sulfur deficiency and because of its effect on cysteine synthase and expression of sulfate assimilation genes, OAS was proposed to act a mediator of plant sulfur status (Hell *et al*., 2002).

This conclusion was further strengthened by a transcriptome analysis suggesting the role of OAS as general regulator of gene expression (Hirai *et al*., 2003). Transcript level of other genes also regulated by sulfur deficiency, such as SIR, APS Kinase or group 3 sulfate transporters, by contrast, were not correlated with OAS showing that OAS can not be the sole sensor of sulfur deficiency (Hirai *et al*., 2005). Metabolome analysis revealed that approximately 6000 analyzed metabolites, 11.5% were significantly affected by 13d of sulfur starvation (Nikiforova *et al*., 2005). Glutathione (GSH) is one of the compounds that

directly regulate sulfate assimilation. Reduced form of sulfur, such as cysteine, H_2S or GSH, trigger a strong decrease in sulfate uptake and assimilation (Lappartient *et al*., 1999; Westerman *et al*., 2001). In Arabidopsis thaliana root cultures APR activity and transcript levels were decreased by feeding either cysteine or GSH (Vauclare *et al*., 2002).

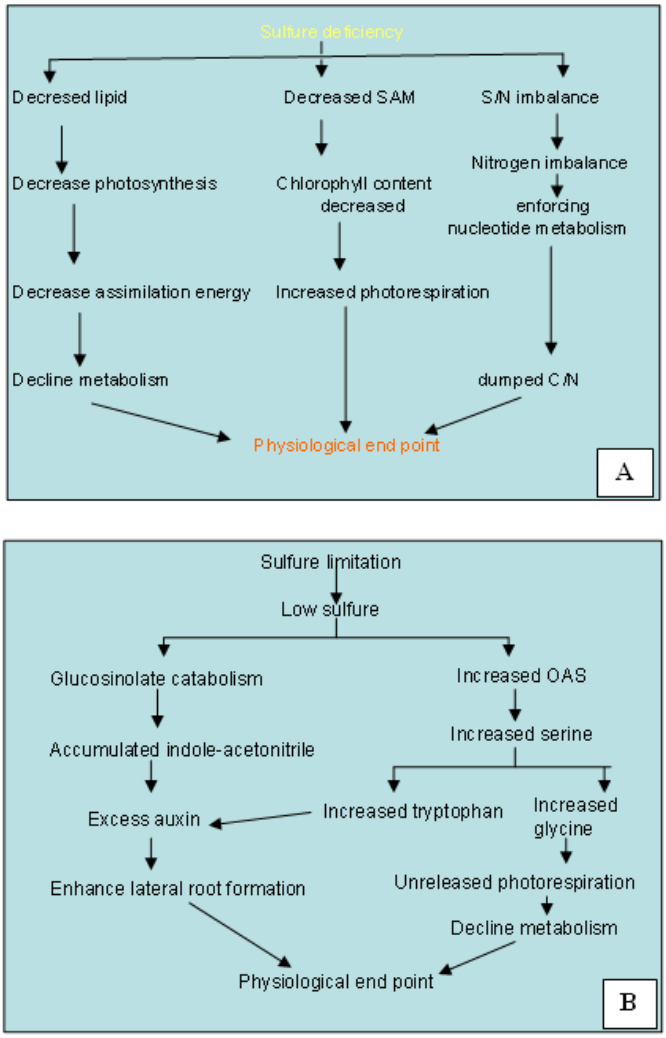

Fig. 5. Long term (A) and Short term (B) response of sulfur deficiency

Signalling pathways involving methyl jasmonate and auxin seen to be involved in the sulfur stress response. Primary and secondary metabolic pathways in volving amino acids, carbohydrates and glucosinolates are modulated in response to sulfur deficiency stress. Nontargeted metabilome analysis indicates that the level of glucosinolates from the array of approximately 3000 putative metabolites decrease rapidly upon sulfur deficiency concomitant (Hirai *et al*., 2004). Glucosinolates contain two or three sulfur atoms per molecule, and thus are regarded as storage and possibly mobilizing forms of assimilated sulfur in response to acute sulfur deficiency (Saito, 2004). GSH functions as thiol buffer and in protection of plants against oxidative and environmental stress and it depress / scavenge the formation of reactive oxygen species *e.g*. super oxide, H_2O_2 and lipid hydroperoxides (Tausz *et al*., 2003). GSH is precursor of Phytochelatins which play a role in heavy metal homeostasis and detoxification by buffering of the cytoplasmic concentration of essential heavy metals. GSH is also involved in the detoxification of the xenobiotics compounds without direct nutritional value or significance in metabolism, which at too high levels may negatively affect plant functioning (Gullner *et al*., 2001).

Regulation of sufur assimilation through plant hormone

Some phytohormones are also involved to control gene expression of sulfur assimilation, recent developments indicate that this group of compounds is very important for regulation of sulfur nutrition (Ohkama *et al*., 2002; Maruyama-Nakashita *et al*., 2004, 2005). Cytokinin signaling appears to be involved in gene expression related to sulfur metabolism. Cytokinins have been shown to down regulate the expression of high affinity transporter genes in *Arabidopsis* roots (Maruyama-Nakashita *et al*., 2004). A NIT 3 nitrilase involved in synthesis of indole 3 acetic acid (IAA) leading to change in root morphology, belongs to genes strongly induced by sulfur deficiency (Kutz *et al*., 2002). The cis-acting element conferring sulfur starvation response recently identified in *Arabidopsis Sultr* 1:2 promotor contains an auxin response factor (ARF) binding sequence (Maruyama-Nakashita *et al*., 2005). Jasmonic acid (JA) did not affect the expression of the sulfur responsive promotor element (Ohkama *et al*., 2002), but is nevertheless involved in regulation of sulfate assimilation. Treatment of Arabidopsis with methyl jasmonate resulted in a fast but transient increase in mRNA levels of many genes involved in sulfate assimilation and GSH synthesis, but without affecting sulfur metabolite levels (Jost *et al*., 2005). The mRNA for sulfate transporters was not affected, confirming that JA does not participate in the regulation by sulfur nutrition although genes of jasmonate biosynthesis are among those induced by sulfur starvation (Hirai *et al*., 2003; Maruyama-Nakashita *et al*., 2003; Nikiforova *et al*., 2003). The induction of sulfate assimilation by JA is not surprising as JA is known to participate in the transduction of stress response (Reymond and Farmer, 1998) and sulfur compounds often play an important role in plant stress defense (Foyer and Rennenberg, 2003). The interaction of sulfur assimilation an GSH synthesis with stress defense is further corroborated by the finding that the level of GSH increased in plants treated with abscisic acid (ABA) (Jiang and Zhang, 2001) and salicylic acid (SA) (Fodor *et al*., 1997). ABA plays an important role in adaptive responses to environmental stresses (Chandler and Robertson, 1994) and leads to increased production of reactive oxygen species (Guan *et al*., 2000). SA plays a central role in plant defense against pathogens. SA accumulates upon pathogen attack, induces expression of pathogenesis- related genes and is a necessary component of systemic acquired resistance (Kunkel and Brooks, 2002). The physiological significance of so called secondary sulfur compounds, *viz*., glucosinolates in *Brassica* (Glawisching *et al*., 2003) and r-glutamyl peptides and allins [S-alk (en) yl cysteine sulfoxides] in *Allium* (Coolong and Randle, 2003 a) is still ambiguous though they are considered to function as sink compounds in situations of sulfur excess. Upon tissue disruption glucosinolates are enzymatically degraded by myrosinase and may yield a variety of biologically active products such as isothiocynates, thiocynates, nitriles and oxazolidine-2-thiones (Graser *et al*., 2001; Peterson *et al*., 2002; Wittstock and Halkeir, 2002). The glucosinolates-myrosinase system is assumed to play a role in plant -herbivore and plant-pathogen interactions. Furthermore, glucosinolates are responsible for the flavor properties of Brassicaceae and recently have received attention in view of their potential anticarcinogenic properties (Graser *et al*., 2001; Reichelf *et al*., 2002).

Effect of sulfure on sugarcane

Sulfur is fourth major nutrient beyond N, P and K nutrient. The sugarcane responses to sulfure application are increasingly reported from different part of India (Tandon, 1991). Sulfure is indespensible element for carbohydrate metabolism and crop production (Singh *et al*., 2007). Application of sulfure significantly increased the shoots and number of sugarcane upto 80 kg/ha and significantly effect on brix% of cane juice. Sucrose percent in cane juice recorded significantly upto 80 kg/ha. Maximum sucrose (15.68%) was recorded with sulfure application, which was (6.95%) higher than without sulfure (Singh *et al*., 2007**).**

Conclusion

Life in the presence of very high/ low concentrations of a nutrient may require metabolic adjustments aimed at keeping a balanced cell composition so that (near) optimal activity of key pathway is maintained. In many industrialized regions, anthropogenic sulfur emissions have been restricted in recent years as a result of environmental legislation resulting in an incidence of sulfur deficiency in crops world wide. Growing plants have a constitutive demand for sulfur to synthesize protein, sulpholipid and other essential S containing molecules for growth. Furthermore, the different tissues and organs differ in their demands for sulfur which in turn may depend upon developmental stage and function. The uptake and subsequent distribution of sulfate is regulated in response to demand and environmental factors. In the perspective of whole plant sulfur metabolism, the requirement is the provision of adequate sulfur to optimize vegetative plant growth and hence reproductive potential and ultimately to provide sulfur for seed tissues to maximize fecundity. In green crops, the delivery of adequate sulfur to seed tissues is needed for maximal production and for quality aspects in terms of maximizing sulfur amino acid content. At the whole plant perspective, the uptake of sulfate its distribution assimilation will be coordinated and balanced with the actual sulfur demand for growth, sulfur uptake is optimized for growth, and when supply is in excess, uptake mechanisms are down regulated. Sulfate is taken up in excess is loaded in to vacuoles from where it can be subsequently re-mobilized which varies between plant species and depends upon leaf maturity. Re-mobilization of reserves and redistribution around the plants are employed to maximize the usefulness of limited resources. In cereals during grain filling, sulfur along with nitrogen is remobilized from vegetative tissue to the grain as vegetative tissues senesce. Redistribution is important if the overall supply is limiting or if the supply is intermittent. In some cases, the rate of remobilization may cause young developing leaves to be sulfur deficient (Clarkson *et al*., 1983); however, a controlled rate of redistribution may be necessary to optimize availability in the whole plant context.

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