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Nitrogen and Sulphur Interaction in Higher Plants

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Nitrogen and sulphur are assimilated primarily into protein and they occur in a molar ratio of about 20:1 in most plants. A mechanism to coordinate and balance the flow of these two essential components to meet the needs of net protein synthesis seems warranted. Evidence exists for coregulation of sulphate and nitrate transport in higher plants. The involvement of carrier proteins was suggested but the nature of these proteins has not been elucidated. A clear understanding on the localization of the enzymes of nitrogen and sulphur metabolism in plant cells is established. Studies on the regulation of these enzymes led to the conclusion that metabolic links exist between the two pathways. O-acetylserine, a product of nitrogen metabolism is considered to be a limiting factor for sulphate assimilation. Investigations on the volatile emission of nitrogen and sulphur from the leaves into the atmosphere were carried out in higher plants. Need arises to quantify such emissions at field scale under different environmental conditions. Studies on the interaction of N and S in terms of H_2S- and NH_3-release can yield useful information especially in crop plants. The ratio of total N to total S in the plant indicates the relative requirement of these nutrients. The central role of N and S in protein synthesis makes it necessary to visualize a coregulation between N- and S-metabolism.

Key Words: Nitrogen, Sulphur, Nitrogen and sulphur interaction, Nitrate uptake, NO_3^- assimilation, SO_4^{2-} assimilation, Gaseous emissions

Introduction

Nitrogen(N) and sulphur(S), the two major plant nutrients serve as constituents of proteins and several other important organic compounds. These N- and S-compounds are involved in the functioning of primary and secondary metabolism in higher plants. A close interaction between these elements in terms of uptake, reduction and assimilation has been reported in the literature. Both these nutrients, predominantly in the form of NH_3 and H_2S, are known to be released into the atmosphere through the foliage. Such emissions are very likely due to imbalance in N and S nutrition, particularly in crop plants. In this communication, possible regulatory connections between nitrogen and sulphur at various metabolic levels are discussed.

Uptake and Reduction

The bulk of nitrogen and sulphur taken up by the plant roots is in the form of NO_3^- and SO_4^{2-}, respectively. In general, N and S concentration in plants is in the ratio of 20 to 1 (Cram 1990). The N/S ratios in the whole plant arise predominantly by the ratio in which these are taken up by the root. It can be assumed that the mechanism of uptake of NO_3^- and SO_4^{2-} should, therefore, match with the mechanism of their reduction in these proportions. The finding of a coupling between NO_3^- and SO_4^{2-} reduction (Reuveny et al. 1980) becomes extremely important in that the linkage can be extended to root uptake of these anions. NO_3^- and SO_4^{2-} reduction predominantly occurs in the leaves and, therefore, N to S uptake in the

required proportion, must take place at the root. Clarkson et al. (1989) have demonstrated that at the whole plant level the apparent matching of supply to demand is accompanied by an apparent linkage of SO_4^{2-} to NO_3^- uptake in barley plants. When the sulphate transport system is derepressed by withholding sulphate for 1-5 days, there is a concomitant decrease in both the influx and net uptake of nitrate and ammonium eventhough growth is not affected during this period. Sulphate deprivation depresses total nitrogen intake in advance of any major effect on growth. Similarly, in nitrate deprived plants, an inhibition of sulphate influx was observed. This suggests that there may be a mutual regulation of N and S transport in plants. In the nutrient medium, uptake of NO_3^- by plant roots takes place concurrently with that of SO_4^{2-} and other anions. This is compensated for by OH^- efflux from the roots (Dijkshoorn 1962). It was suggested that the OH^- excreted came from the reduction of NO_3^- and SO_4^{2-}.

$$NO_3^- + 8H^+ + 8e^- \rightarrow NH_3 + 2H_2O + OH^-$$

$$SO_4^{2-} + 8H^+ + 8e^- \rightarrow S^{2-} + 2H_2O + 2OH^-$$

Nitrate reduction provides a primary source for OH^- compared to sulphate reduction because much more nitrogen is taken up than sulphate.

The biophysical and biochemical nature of uptake of these nutrients at cellular level is poorly understood. However, similarities were found in the movement of NO_3^- and SO_4^- ions into the cells against the prevailing membrane potential. Cells accumulate nitrate in concentration much higher than that found in the external medium thereby showing the operation of an active energy dependent uptake mechanism for nitrate. Clarkson et al. (1992) proposed proton-sulphate cotransport at the plasmamembrane and suggested the involvement of transporters. Symport of sulphate with two protons and nitrate with one would allow the intracelluar concentration to reach that of the extracellular medium, but active transport by such a mechanism would only be possible when *p*H inside is significantly higher than outside. Such circumstances are not expected to be common, so consideration should be given to the possibility that sulphate is taken up with three protons and nitrate with two, so that the membrane potential would act as a driving force. The role for carrier proteins in the transport of NO_3^- and SO_4^{2-} is hypothesized and studies initiated at the molecular level would reveal the nature of these proteins. The available information so far does not suggest any competition existing between NO_3^- and SO_4^{2-} uptake. It is, therefore, viewed that the carrier proteins involved may be different for each of these anions.

Nitrogen and sulphur are also taken up in small amounts in gaseous form through the leaves from the atmosphere. Plants exposed to atmospheric pollutants such as SO_2, H_2S, NO_2 and NO may use these gases as a source of S and N. Experiments with radioactive SO_2 and H_2S showed that S from both gases could be incorporated into organic sulphur compounds, cysteine and glutathione (De Kok et al. 1985, Rennenberg 1984). Similarly, entry of NO_x through the stomata and its participation in the metabolism was demonstrated (Dueck et al. 1986, Freer-Smith 1985, Shimizu et al. 1984). Simultaneous exposure of plants to a mixture of SO_2 and NO_2, frequently occurs in polluted environments. However, foliar uptake of N and S compounds may not have a regulatory control, as the entry is via the stomates. Toxic effects of both SO_2 and NO_2 alone or in combination, on several physiological processes was demonstrated (Darrall 1989). Interactions between the pollutants SO_2 and NO_2 and water stress have been found in *Betula pendula* and *B. pubescens*. Following exposure for 40 d to 20 ppb SO_2 + 20 ppb NO_2, the ability of excised leaves to conserve

water under conditions of limited availability was considerably reduced (Wright et al. 1986). The ability of tissues to conserve water was lower, possibly as a result of an increased permeability of the cuticle to water.

The reduction reactions of sulphate (Schmidt & Schwenn 1971) and nitrate (Abrol et al. 1983) are largely dependent on the photosynthetic reductant supply in higher plants. The eight electron transfers involved in the reduction of NO_3^- and SO_4^{2-} to ammonia and sulphide, respectively, illustrate the possible mechanistic connections between N- and S-assimilation. Reduced ferridoxin with its Fe atoms bound to sul phur acts as electron transmitter from the 'Z compound to $NADP^+$. In the chloroplast, it also mediates reduction of NO_2^- and SO_4^{2-}. Thus both nitrite and sulphate compete with $NADP^+$ for reduction. In addition to the generation of reductant, photosynthesis supplies simultaneously organic carbon skeleton required for assimilation of the resulting ammonia and sulphide. The synthesis of cysteine as a result of the incorporation of sulphide moiety into O-acetylserine, thus appears to be the meeting point between N- and S-metabolism. O-acetylserine was considered a rate limiting factor of cysteine biosynthesis in cucurbit cells (Rennenberg 1983). When nitrogen deficient tobacco cell cultures were supplied with ammonium plus nitrate, the amount of O-acetylserine in the cells increased in a manner similar to the increase in extractable cysteine synthase (Smith 1980). Therefore, O-acetylserine might possibly be the product of nitrogen metabolism, positively regulating cysteine synthase under conditions of net nitrate assimilation.

Intracellular localization of the enzymes of S- and N-metabolism is fairly established in higher plants. While the enzymes of N-metabolism are distributed in the cytoplasm, chloroplast and possibly mitochondria, the entire sequence of SO_4^{2-} reduction reactions take place in the chloroplast. In the leaves of C_4 plants, an intercellular compartmentation of the enzymes of N- and S-assimilation exists. A scheme (figure 1) on the division of labour between the mesophyll cells and the bundle sheath cells of maize leaves concerning assimilation of nitrate and sulphur compounds was proposed by Brunold

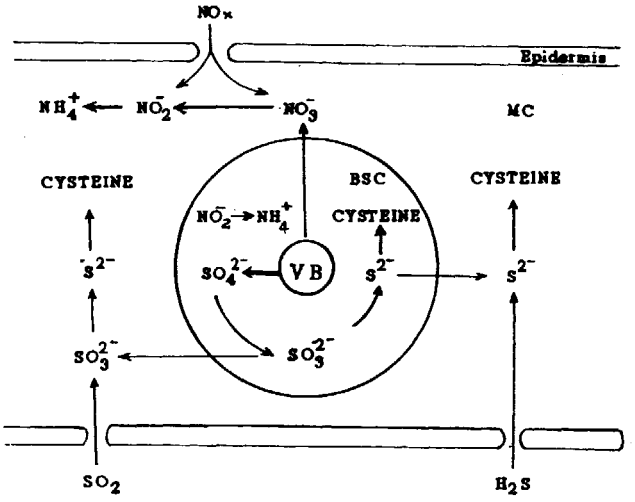

Figure 1 Intercellular localization of assimilatory sulphate and nitrate reduction in maize leaves [*BSC*, bundle sheath cells; *VB*, vascular bundle; *MC*, mesophyll cells]

(1990). It is still not clear as to what extent the epidermal cells contribute to nitrate reduction and assimilation of S compounds.

Regulatory Coupling of Nitrate and Sulphate Assimilation

Assimilatory pathways of nitrate and sulphate are functionally convergent leading to the synthesis of proteins and other S and N containing compounds. Several studies have established regulatory interactions between assimilatory sulphate and nitrate reduction (Barney & Bush 1985, Deboer & Duke 1982, Haller et al. 1986, Schmutz & Brunold 1985, Zink 1984). Based on results with cultured cells of tobacco a scheme (figure 2) has been proposed (Reuveny et al. 1980) in which each pathway is regulated down by its own internal signal when the other pathway is not limiting. This type of regulation is combined with a regulation by positive signals originating in the other pathway, thus establishing a coordination of both pathways. Since they converge in the synthesis of proteins, this coordinate regulation can be envisaged as a mechanism aimed at the production of appropriate amounts of non-sulphur amino acids and sulphur amino acids. The effects of varying sulphur and nitrogen nutrition are especially clearly evident with cell cultures. In sulphate-sufficient cell cultures of tobacco, ATP sulfurylase activity increased at a rate corresponding to the initial NO_3^- concentration. The enzyme from *Ipomea* cell cultures increased with NH_4^+ and NO_3^- as nitrogen sources, as compared to cultures with NO_3^-, whereas lack of a nitrogen source decreased the enzyme activity to 80% of the controls within 24 hr (Zink 1984). The effects of nitrogen and sulphur sources on nitrate reductase (NR), ATP-sulfurylase and APS sulfotransferase have also been studied in cell cultures of *Rosa* species (Haller et al. 1986). Without a sulphur source, APS sulfotransferase activity increased by 200% and nitrate reductase activity decreased to 30% as compared to controls. Omission of both sulphur and nitrogen sources did not affect ATP sulfurylase, whereas nitrate reductase and APS sulfotransferase are more efficiently regulated by these nutrients in *Rosa*. Thiol compounds such as dithiothreitol, dithioerythreitol and mercaptoethanol have been shown to depress the initial activity of the partially purified wheat leaf nitrate reductase (Aryan et al. 1984). It was suggested that the thiols mediate the re-

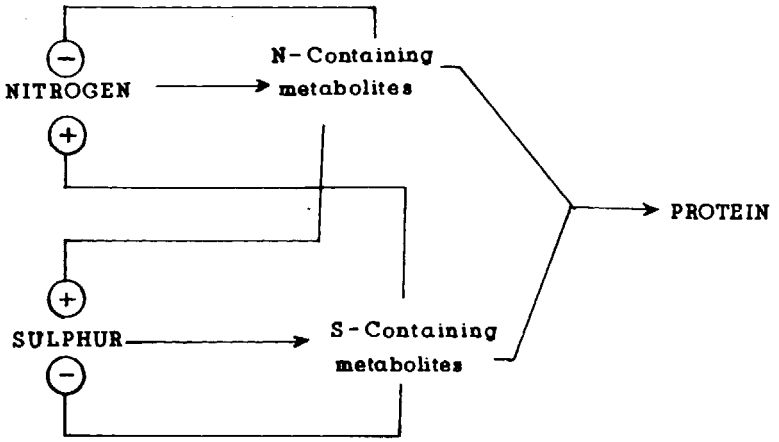

Figure 2 Diagram of the regulatory coupling between the nitrogen and sulphur assimilation pathways [− and + represent the negative and positive control mechanisms]

duction of NAD^+ to NADH which in turn inactivated NR. However, *in vivo* studies in our laboratory revealed 25-85% enhancement in NR activity in the leaves of wheat and *Brassica* treated with the above mentioned thiols at low concentrations and cysteine and glutathione at a higher concentration (Lakkineni & Rene, unpublished data). The exogenous supply of thiols and NAD to the leaf discs resulted in a further increase in the activity of nitrate reductase. It is possible that the thiols activate nitrate reductase by the supply of NADH under *in vivo* situation which assures the presence of nitrate.

Several lines of evidence indicate that O-acetylserine a product of nitrate assimilation, is a limiting factor for sulphate assimilation. Consistent with this idea, exogenous O-acetylserine has a regulatory effect on assimilatory sulphate reduction in light and darkness (Brunold 1992). The derepression of sulphateadenyl transferase in tobacco cells requires the presence of a nitrogen-containing compound (Reuveny & Filner 1977), as yet unidentified. It is an attractive speculation that O-acetylserine also plays this role in plants, thereby regulating cysteine synthesis not only by its requirement as a substrate for cysteine synthase, but also by its requirement for derepression of sulphateadenyl transferase (Giovanelli 1990). Such a regulatory scheme would provide a mechanism for coupling nitrogen assimilation into O-acetylserine, with sulphur assimilation into cysteine. This regulatory coupling can be described as metabolic interlock which seems likely to be encountered throughout the plant kingdom.

N- and S-Emissions

Emission of nitrogen and sulphur by plants into the atmosphere was observed separately by various investigators. Both the nutrients are emitted predominantly in the gaseous form mainly through stomata of leaves. Synthesis of these compounds and the circumstances leading to their emission with a possibility of a co-regulation between N- and S-metabolism, are discussed.

Numerous laboratory experiments have shown that green cells of higher plants can release volatile sulphur compounds such as hydrogen sulphide (Karuna Chand et al. 1990, Rennenberg 1989), methyl mercaptan (Schmidt et al. 1985) sulphur dioxide, dimethyl sulphide and carboxyl sulphide (Rennenberg et al. 1990) into the atmosphere. Many naturally occurring sulphur compounds were analysed as potential substrates for the emission of volatile sulphur by plant cells. It was observed that volatile sulphur is released by plant cells in response to sulphur dioxide, sulphate and L- and D-cysteine (Rennenberg 1984). The compound emitted in response to these substrates was found to be predominantly hydrogen sulphide (H_2S). The H_2S emitted in response to the external source of L-cysteine appears to be produced directly from L-cysteine by a pyridoxal phosphate-dependent, L-cysteine specific cysteine desulphydrase enzyme (Rennenberg 1983). Besides the generation of pyruvate and SH^- this reaction also gives rise to ammonium, a product of N-metabolism.

$$\text{Cysteine} \xrightarrow{\text{cysteine desulphydrase}} \text{Pyruvate} + SH^- + NH_4^+$$

Hydrogen sulphide can also arise from cysteine in a cyanide dependent reaction catalyzed by β-cyanoalanine synthase enzyme (Miller & Conn 1980).

$$\text{Cysteine} + HCN \xrightarrow{\beta\text{-cyanoalanine Synthase}} \beta\text{-cyanoalanine} + H_2S$$

However, its activity is found in several non-cyanogenic plants as well. Activities of both cysteine desulfhydrase and β-cyanoalanine synthase, were detected during most part of the growth period in *Brassica* species (Lakkineni & Abrol 1993), indicating a consistent release of H_2S in these species.

Both the enzymes known to have different catalytic functions in the metabolism, however, yield a common product, hydrogen sulphide. Apparently, the pathways leading to H_2S generation and its subsequent emission are part of an intracellular sulphur cycle (Rennenberg 1984) which may operate to maintain cysteine concentration at a safer level in plant cells.

Volatilization losses of nitrogenous compounds through plants are well documented in the literature. These compounds include ammonia, some amines, oxides of nitrogen, hydrogen cyanide etc. Ammonia is considered a major nitrogenous compound lost through the leaves into the atmosphere. The possible mechanisms leading to the N losses through volatilization are dealt in detail in Chapter by Maheswari et al.

O-acetylserine, a product of N-metabolism was suggested to have a regulatory control on the H_2S emitted in response to sulphate (Rennenberg 1983). Availability of O-acetylserine to sulphate fed cucurbit cells declined the rate of H_2S emission by incorporating the sulphide released from the carrier into O-acetyleserine to form cysteine. O-acetylserine enhanced cysteine synthesis at the cost of H_2S emission in response to sulphate. This indicates that the availability of O-acetylserine is the rate limiting factor of cysteine synthesis in cells supplied with excess sulphate. Alterations in light energy resulted in transients in H_2S emission which coupled with changes in the rate of the biosynthesis of O-acetylserine (Rennenberg 1983). Such adjustments seem to be suitable for the regulation of cysteine synthesis and may be a tool to coordinate sulphur and nitrogen metabolism in a constantly changing environment. N and S interaction in relation to H_2S emission in some crop species was investigated in our laboratory (Lakkineni et al. 1990). The enhancement in the emission of H_2S with an external supply of sulphate and cysteine was inhibited when nitrate was provided concomitantly with either sulphate or cysteine in both mustard and groundnut (table 1). Similarly, external supply of cysteine to the leaves of *B. juncea* considerably induced the activity of the enzyme cysteine desulphydrase (Lakkineni et al. 1990). Provision of NO_3^- in the incubation medium brought down the activity of the enzyme nearly by two fold. Such a phenomenon suggests a strong interaction between S- and N-metabolism in the above mentioned crop species. It was proposed that nitrogen interferes with the system responsible for H_2S emission at the level of substrate availability and diverts the sulphur that is being emitted, towards protein synthesis (Lakkineni et al. 1990). Further studies designed to investigate the protein labelling by ^{35}S- cysteine as influenced by nitrogen supply are needed to substantiate the above hypothesis. There is no information available at the present with regard to whether sulphur metabolism has a role in monitoring N-volatilization. It is possible that N-emissions (NH_3 losses) could have a regulatory control by S nutrition. Since regulatory interactions have been shown to occur at the level of uptake and reduction of both SO_4^{2-} and NO_3^-, it would be an attractive proposition to ascribe a coregulation between N- and S-metabolism in terms of N- and S-emissions. As discussed,

Table 1 *Emission of H_2S from the leaf extracts of mustard and groundnut as affected by sulphate, cysteine and nitrate*

Treatment	H_2S esmission n mole g^{-1} fwh^{-1}	
	B. carinata	Groundnut
Control (H_2O)	0.224	0.149
Sulphate (5 mM)	0.300	0.243
Cysteine (5 mM)	0.296	0.342
Sulphate + NO_3^- (5 mM)	0.243	0.171
Cysteine + NO_3^- (5 mM)	0.211	0.206

Each value is a mean of two replicates

H_2S is generated out of the reaction catalyzed by L-cysteine desulphydrase. However, the fate of NH_4 that comes out of the same reaction has not been investigated. It is possible that it could either be emitted as NH_3 or assimilated by the primary N-metabolic pathway. In the cells of higher plants cysteine desulphydrase appears to be an inducible enzyme (Renneberg 1983). Cysteine can, therefore, be viewed to have a regulatory role not alone on H_2S emission but also on the release of NH_3. Elaborate experiments are needed to suggest conclusively whether a coregulation occurs in the emission of ammonia and hydrogen sulphide.

Emission of volatile sulphur (Filner et al. 1984) and nitrogen (Stutte & Weiland 1978) was also observed under field conditions. Such emissions from the foliage of crop plants into the atmosphere may result in loss of the important plant nutrients. Based on the studies on H_2S emission from the leaf discs, we calculated that a wheat crop with optimum leaf area index (LAI) of 5 would lose about 2.07kg sulphur per hectare over a growing season. Non-availability of nitrogen in required amounts may possibly lead to inefficient utilization of sulphur and vice-versa. The proportions of N and S in plants thus seem to be important in view of the interdependency between the two metabolic pathways. The possibility of N and S interaction in relation to the emission of H_2S and NH_3 and the associated changes in the metabolism can be checked by varying N and S nutrition levels. There is a need to quantify nitrogen and sulphur losses in elaborate field scale experiments.

N and S Nutrition in Crop Plants

In field crops, it is sometimes difficult to distinguish between N- and S-deficiency. In S deficient plants the sulphate-S levels are very low; whereas amide – N and nitrate-N are increased (Marschner 1986). This contrasts with N deficiency where soluble N is depressed and the sulphate level is normal. Inhibition of protein synthesis during sulphur deficiency leads to chlorosis just as it does during nitrogen deficiency. Furthermore, the distribution of sulphur in S-deficient plants is also affected by the nitrogen supply (Robson & Pitman 1983). It appears that the extent of sulphur retranslocation from older leaves depends on the rate of nitrogen deficiency-induced leaf senescence. In legumes, during early stages of S-deficiency, nitrogen deficiency in the root nodule is much more depressed than photosynthesis (Deboer & Duke 1982). The lower S content of proteins influences nutritional quality considerably. Lack of S-amino acids was suggested as the main factor limiting the biological value of proteins (Chopra & Kanwar 1966). Methionine, an essential amino acid in human nutrition, is often a limiting factor in diets in which seeds are a major source of protein. Furthermore, a decrease in the cysteine content of cereal grains reduces the baking quality of flour, since disulphide bridging during dough preparation is responsible for the polymerization of the glutelin fraction (Marschner 1986).

The ratios of total N to total S have long been used as a diagnostic tool for S deficiency (Dijkshoorn & Van Wijk 1967). The N:S ratios were also used as a measure to indicate the relative requirements of these nutrients in crop plants. In the seeds of rapeseed-mustard, groundnut and wheat N:S was shown to be 4.5, 28.3 and 12.9, respecttively (Lakkineni & Abrol 1992). The additional S requirement in rapeseed-mustard can be attributed to the presence of various glucosinolates (Booth & Walker 1991, Schung 1990) and a number of neutral volatile S- and N-components (Hashimoto & Kameoka 1985). The extent to which nitrogen goes into the making of glucosinolates has not been critically evaluated in Brassicas. Studies in this direction would give an estimate of N diverted for the incorporation into glucosinolates. Tripathi and

Hazra (1988) reported a significant NXS fertilizer interaction for the concentration of N and S, and forage yield in Chinese cabbage. The relative amounts of sulphur and nitrogen can also have an important influence on grain yield (Booth et al. 1991). Work by Aulakh et al. (1980) with mustard, and Janzen and Bettany (1984) with rapeseed, showed that there was a significant interaction between nitrogen and sulphur applications and that the maximum yield was only obtained when sulphur and nitrogen applications were balanced. Similarly N and S relationship was established in many studies (Dev & Saggar 1974, Ishwari et al. 1987, Lakkineni 1988, Masih & Sharma 1989) in terms of dry matter and yield in several crop species. There appears to be a very narrow range in the N:S ratio that ensures optimum yield and quality of the crop. This relationship has important implications for nutrition especially in those regions where plant sources supply the bulk of required proteins.

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