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Sulphur Nutrition of the Corn Plant: II. Sulphur Amide –N and Glucose Interrelationships

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Abstract

Corn (*Zea mays* L. var Dekalb 805) seedlings were grown in culture solutions containing two levels of sulphur - 156.2 uM S (S) and 6.0 uM S (-S). High levels of amide - N (0.5 - 1.2% dry wt. basis) accumulated in the (-S) plants. The accumulating amide was asparagine. Only trace amounts of amide-N (.07%) were found in the (+S) plants. In contrast to the amide trend, the glucose level in (+S) plants was very high (3.98%) compared to the trace quantities which were found in the (-S) plants. Thus, there is an inverse relationship between glucose and amides. Exogenous supply of glucose to sulphur deficient (-S) plants resulted in an increase (88%) in tissue glucose level and this increase was accompanied by a decrease (43%) of amide-N levels in the (-S) plants. Also amelioration of S stress resulted in an increase in tissue glucose level and a decrease in amide-N level. Artificial imposition of S stress on sulphur adequate (+S) plants led to a drop (53.9%) in glucose level and a concomitant upsurge in amide-N, level, (170%). The above results although clear-out, do not constitute an unequivocal evidence for a cause - effect relationship between glucose and asparagine. However, they suggest very strongly that glucose may be functioning as an inhibitor of asparagine synthesis possibly through regulation of asparagine synthetase activity.

Introduction

Many workers using a number of different species have found evidence of a profound disturbance of nitrogen metabolism in plants grown under conditions of insufficient supply of sulphur. The accumulation of either or both of the amides, asparagine and glutamine, and of certain free amino acids, especially arginine, have been reported (Rendig and McComb, 1959; Coleman, 1957; Steward et al., 1959; Adams and Sheard, 1966; and Hanower and Bizozowska, 1964). An investigation by Rendig and McComb (1961) indicated that while asparagine accumulated in S-deficient alfalfa, there was almost a complete absence of glucose in these sulphur stressed plants. Preliminary studies with corn plants in our laboratory indicated similar effects.

The purpose of this study was to define the physiological role of S with particular reference to its interrelationships with amides and glucose in plant tissues.

Materials and Methods

Corn seedlings (*Zea mays L.* var Dekalb 805) were grown as previously described (Rendig et al. 1976). The chemical composition of the culture solution has been given in a previous report (Oputa et al 1990). In one experiment, the seedlings were grown for ten days in culture solutions supplied with adequate sulphur (156.2 uM S) or in solutions in which no sulphur was added. The shoots were analysed for amide-N, protein-N, total-S, SO_4-S, and organic-S. The reducing sugar, glucose, and total reducing sugars were also determined.

In another experiment, two sulphur levels were employed: 156.2 uM S designated (+S) and 15.62 uM S designated (-S). Corn seedlings were grown in these solutions for 10 days and then some (+S) plants were transferred into the sulphur deficient solution. At the same time, some (-S) plants were transferred into sulphur adequate solution, some (+S) and (-S) plants were left in their respective culture solutions to serve as controls. Before the transfers were made, shoot samples of (+S) and (-S) plants were collected for the determination of initial levels of amide-N, glucose and S fractions. The experiment was terminated 6 days after the transfer were made. The shoot samples of all the treatments: (+S), (-S) (+S- - - S) and (-S-- +S) were analysed for amide-N, glucose, total-S, SO_4-S and organic-S. This procedure made possible the monitoring of changes in these chemical fractions in response to sulphur supply.

The last set of experiments were designed to test if glucose introduced into sulphur deficient plants would affect the concentration of amide-N in the tissue. The corn seedlings were grown in (-S) culture solution (15.62 uM S.) Until they were just showing sulphur deficiency symptoms (faint chlorosis). The plants were then transferred into sulphur deficient culture solution with or without glucose. The glucose concentrations employed were .02%, 1.0%, 3.0% and 4.0% (W/V). Plants not supplied with glucose served as controls. Nutrient solutions were renewed every 12 hours to minimise the build up of bacterial population. The duration of glucose feeding was 3 days. In order to check whether glucose could exert its effect through alteration of osmotic pressure of solutions, some (-S) plants were fed with 3% (W/V) carbowax while others, the control, were not.

Chemical Analysis

Amide plus ammonia nitrogen was determined by an adaptation of the method of Pucher et al (1935). Asparagine was identified by paper chromatography as described in an earlier report (Rendig et al, 1976). SO_4-S was determined by the method of Johnson and Nishita (1952) and total-S by the same method after ashing by the procedure of Steinberg et al. (1962). Organic-S was determined by difference. For the determination of reducing sugars the procedure outlined in the Association of Official Agricultural Chemists (AOAC) (1960) for the extraction, clarification with ion exchange resigns, and Somogyi micro copper method were followed. Glucose was determined also by the AOAC (1960) procedure involving enzymatic oxidation of glucose to gluconic acid by glucose preparation ("Dee-O").

Results

Amide-N and Glucose

Figure 1 indicates that glucose was almost absent in the shoots of (-S) plants in which amide-N level was high (.73% dry wt.) and that (+S) plants containing high amounts of glucose (3.98% dry wt.) had very low levels of amide-N (.07% dry wt.).

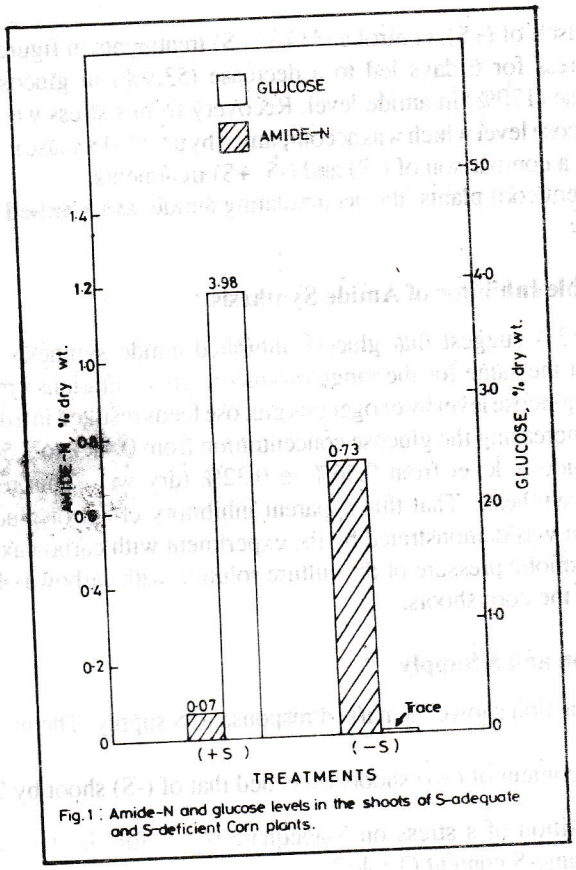

Fig. 1 : Amide-N and glucose levels in the shoots of S-adequate and S-deficient Corn plants.

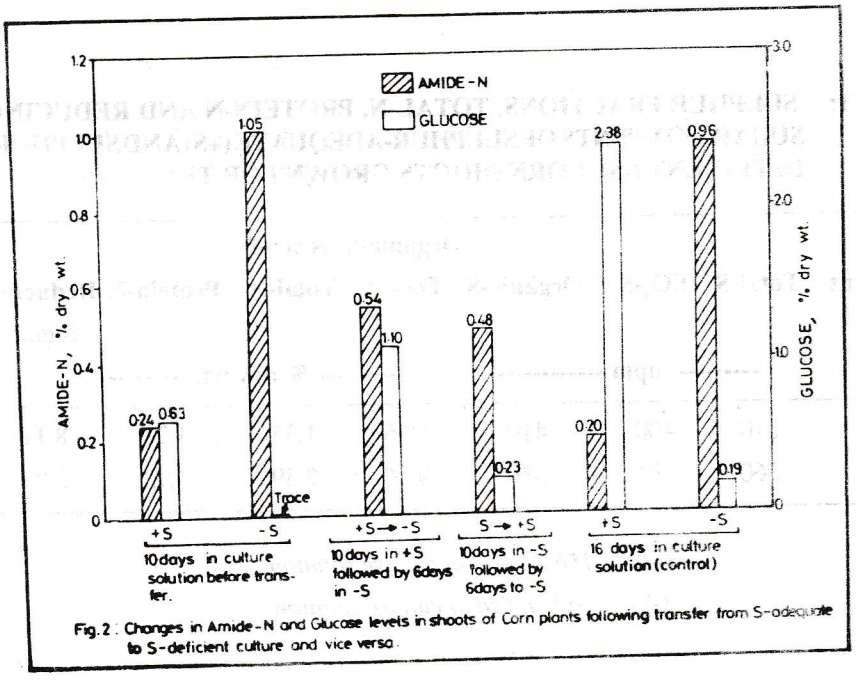

Fig. 2 : Changes in Amide-N and Glucose levels in shoots of Corn plants following transfer from S-adequate to S-deficient culture and vice versa.

A comparison of (+S), control and (+S -- S) treatments in figure 2 shows that the imposition of S stress for 6 days led to a decrease (53.9%) in glucose content with a concomitant increase (170%) in amide level. Recovery from s stress was characterised by 21% increase in glucose level which was accompanied by a 50% decrease in amide level. This fact is borne out by a comparison of (-S) and (-S +S) treatments.

In s-deficient corn plants, the accumulating amide, as identified by chromatography, was asparagine.

Glucose as a Possible Inhibitor of Amide Synthesis

Figures 3 and 4 suggest that glucose inhibited amide synthesis. The amount of inhibition was about the same for the range of glucose concentrations employed (Fig. 3). Increasing the tissue glucose level by exogenous glucose feeds resulted in a decrease in amide level. For example increasing the glucose concentration from 0.41% to 3.54% (dry wt.) led to a decrease in amide-N level from 0.56% to 0.32% (dry wt.). This represents a 43% inhibition of amide synthesis. That this apparent inhibitory effect of glucose was not an osmotic phenomenon was demonstrated by the experiment with carbowax. Table 4 shows that increasing the osmotic pressure of the culture solution with carbowax had no effect on the amide content of the corn shoots.

The Protein Fraction and S Supply

The protein fraction showed a marked response to S supply. The pieces of evidence are as follows:

(1) The protein-N content of (+S) shoots exceeded that of (-S) shoot by 23%

(2) Artificial imposition of s stress on S-adequate plants for six days led to a 59.1% decrease in organic-S content (Table 2).

TABLE 1: SULPHUR FRACTIONS, TOTAL-N, PROTEIN-N AND REDUCING SUGAR CONTENTS OF SULPHUR-ADEQUATE (+S) AND SULPHUR-*DEFECIENT (-S) CORNSHOOTS GROWN FOR TEN DAYS*

Treatment	Total-S	SO_4-S	Organic-S	Organic-S as % of Total-S	Organic-S as % of Total-N	Organic-S as % of Protein-N	Reducing Sugar
	ppm			% dry wt.			
+S	810	400	410	50.6	1.35	1.21	8.11
-S	560	30	530	94.6	2.39	0.93	2.91

(+S) = 156.2 uM S in culture solution

(-S) = No S added to culture solution

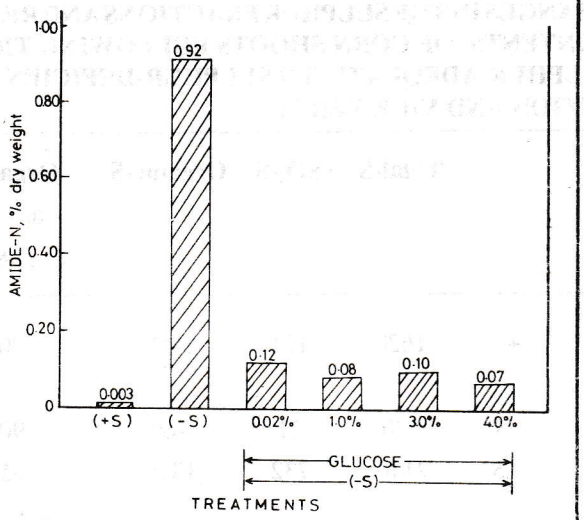

Fig.3 : Amide-N levels in S-deficient Corn plants and S-deficient Corn plants fed with four levels of glucose.

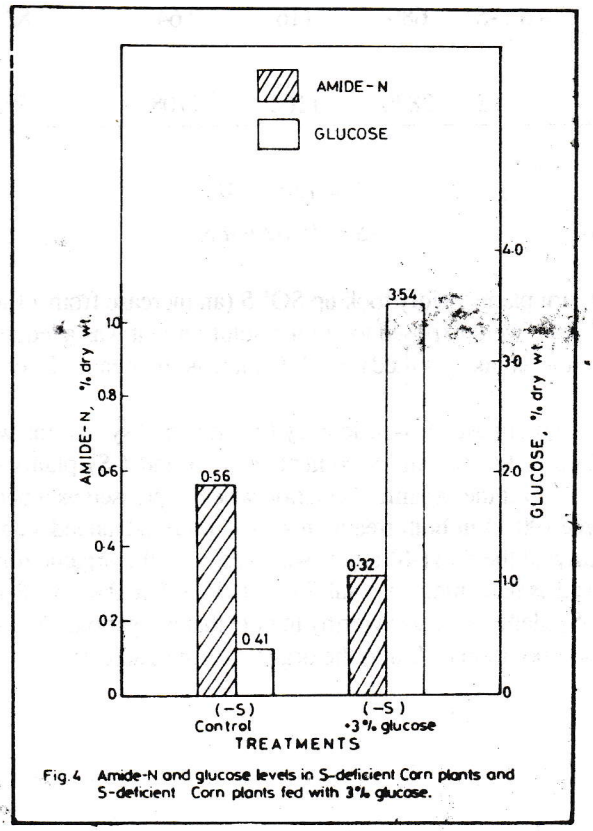

Fig.4 Amide-N and glucose levels in S-deficient Corn plants and S-deficient Corn plants fed with 3% glucose.

TABLE 2: CHANGES IN THE SULPHUR FRACTIONS AND REDUCING SUGAR CONTENTS OF CORN SHOOTS FOLLOWING TRANSFER FROM SULPHUR-ADEQUATE TO SULPHUR-DEFICIENT CULTURE SOLUTION AND VICE VERSA

Treatment		Total-S	SO_4-S	Organic-S	Organic-S as % of Total-s	Reducing Sugar % dry wt.
10 days in culture solution	+S	1620	161	1459	90.1	2.16
	-S	520	51	469	90.2	1.63
16 days in culture solution	+S	2110	732	1378	55.3	6.51
(CONTROL)-S	527	11	516	97.9	2.32	
6 days after transfer	+S -S	680	116	564	82.9	5.10
	-S +S	2870	1162	1708	59.5	2.67

+S = 156.2 uM S

-S = 15.62 uM S

(3) s-deficient corn plants avidly took up SO^4-S (an increase from 11 to 1162 ppm S in 6 days) when they were exposed to culture solution that was adequately supplied with sulphur; and this uptake resulted in a 231% increase in organic-S content of these plants (Table 2).

At the incipient phase of S-deficiency (when visual symptoms were hardly detectable) the difference in the organic-S content of (+S) and (-S) plants was already quite staggering (Table 2). But the organic-S fraction when expressed as a percentage of total-S was about the same (90%) in both treatments. At a more advanced stage of S-deficiency, however, practically all the S in (-S) plants was present in the organic form. As a result, the organic-S expressed as percentage of total-S rose to 97.9% in the (-S) plants as compared to only 55.3% for (+S) plants. It is also worthy to note that exogenous glucose supply resulted in an increased incorporation of S into the organic form (Table 3).

TABLE 3: REDUCING SUGARS, SULPHUR AND NITROGEN FRACTIONS IN SHOOTS OF SULPHUR-DEFICIENT CORN PLANTS AND SULPHUR-DEFICIENT CORN PLANTS FED WITH GLUCOSE FOR THREE DAYS.

Treatment	Reducing Sugar	Protein-N	total-N	Total-S	SO_4-S	Organic-S
	% dry wt.			ppm		
-S	1.41	1.72	3.56	913	204	709
-S + 3% glucose	9.23	1.22	1.42	929	93	836

-S = 15.62 uM S.

Discussion

The low glucose level in (-S) plants could be attributed to reduced photosynthetic activity in S-deficient plants (Chen, 1967). Ergle and Eaton (1951), however, gave a different interpretation: They explained that such low sugar levels in S-deficient plants was a result of depletion of carbon skeletons for the synthesis of organic nitrogen compounds and polysaccharides. Result in our investigation do not support this view. If glucose served as a precursor for asparagine, the exogenous supply of glucose to (-S) plants at a time they were actively synthesising asparagine would be expected to result in elevated levels of asparagine. On the contrary, glucose when introduced into the (-S) plants markedly decreased asparagine level.

The low protein content observed in (-S) plants in this study is a major biochemical syndrome of S deficiency and this fact has been reported by other workers (Chen, 1967; Thompson et al, 1970; Jordon and Ramsley, 1958). This finding is of special significance because around it revolves two theories that have been advanced to explain the phenomenon of asparagine accumulation in plant tissues. One theory (Schulze, 1919) attributes the low protein content to some blockage of protein synthesis. As a result of this blockage, surplus ammonia (either directly taken up by the plant or arising from nitrate reduction) which is not assimilated, is detoxified to asparagine. The second theory postulates that the low protein content is due to protein breakdown (Borodin, 1878; Chibnall, 1924; Butkwitsch, 1908; and Steward and Street, 1946). According to this theory, asparagine is derived primarily from protein degradation. Arguments in support of this latter view are rather weak since they are based mainly on studies involving detached leaves (Wood et al., 1943; wood et al., 1944.) and the protogenists of this theory have not eliminated the possible complications of sensecence physiology. In an intact plant system, the picture may be quite different. The proteins are relatively more stable (Richmond and Lang, 1957) because of a steady supply of stabilising factors like kinetin (6 - furfurylaminopurine). The bulk of the evidence seems to favour the first hypothesis - a denovo synthesis of asparagine. For example, Priannishnikow Shulow (1910) found that exogenous supply of ammonia to pea and barley seedlings resulted in asparagine formation. Also Schulze (1919) found that the amount of asparagine that accumulated in lupine seedlings was by far in excess of the amount contained in the reserve

of the seeds. In the present study, we found that although the organic-S content of (+S) plants was greater than that of (-S) plants the $\frac{\text{organic-S}}{\text{total-S}}$ ratio in both treatments was the same. This latter finding certainly does not suggest the occurrence of protein breakdown in the (-S) plants. If protein breakdown had occurred in (-S) plants. We would have observed a low $\frac{\text{organic-S}}{\text{total-S}}$ ratio in this tissue. In fact, the total-S subsequent observation (at a later stage of sulphur deficiency) that practically all the S in (-S) plants was in the organic form suggests, if anything, that at that state of s-stress, whatever SO_4-S the plant could scrounge was preferentially channelled to the synthesis of proteins. In the above statements we are assuming that the organic-S content represents the protein content. This assumption would hold good for the Gramineae although certainly not for the Brasscae which contain large amounts of organic sulphur in compounds other than proteins (Stewart and Porter, 1969). If indeed there was no protein breakdown, the logical conclusion would be that the accumulated asparagine was not derived from proteins. Rather, it was synthesised de novo.

It is this de novo formation of asparagine which is probably under the indirect control of S. Sulphur effect is considered indirect in the sense that its supply influences the glucose level and the glucose or glucose derivative there could act as a metabolic regulator of asparagine synthesis. A report by Oaks (1967) has established that glucose is a potent inhibitor of asparagine synthetase. The inverse relationship between the amide (asparagine) and glucose levels (Fig. 1) is in accord with Oaks' findings. Also, the fact that exogenous supply of glucose prevented the accumulation of asparagine (Fig. 3 and 4) adds further credence to Oaks results. A report by Yang (1958) also fits our observation.

Thus, a biochemical scheme that could link up sulphur with protein, glucose and asparagine may be stated as follows: Adequate S supply leads to a high glucose level inhibits asparagine synthesis. Under conditions of S deficiency, however, protein synthesis is impaired or blocked. The reduced nitrogen (NH_4^+) not utilised for protein synthesis accumulates and poses a toxicity hazard. Consequently, an ammonium detoxification mechanism is entrained to cope with this excess ammonia. Asparagine is probably the product of this detoxification process (Prianishnikow, 1924) and its synthesis proceeds unhindered because the concentration of the metabolic inhibitor, glucose, is very low in S-deficient tissues.

The above scheme may be operative in the corn plant but we cannot attest to its universality because there is the possibility that other biosynthetic pathways for asparagine synthesis exist in higher plants. For example, a B-cyanoalanine synthetase pathway involving cyanide assimilation has been observed in cyanophoric and some non cyanophoric plants (Blumenthal-GoldSchmidt et al, 1963). It is therefore necessary that a comparative study of the interrelationships of sulphur, asparagine and glucose in these two groups of plants (cyanophoric and non cyanophoric) be carried out.

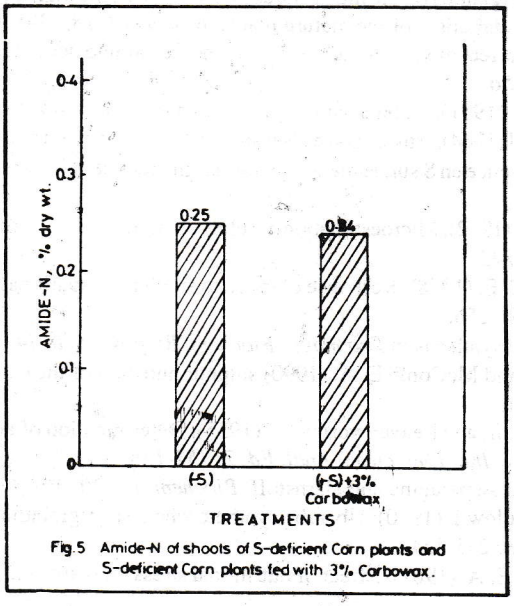

Fig.5 Amide-N of shoots of S-deficient Corn plants and S-deficient Corn plants fed with 3% Carbowax.

TABLE 4: AMIDE-N CONTENT OF SHOOTS OF (-S), CORN PLANTS AND (-S) CORN PLANTS FED WITH 3% CARBOWAX

Treatment	Amide-N (% dry wt.)
(-S)	0.25
(-S) + 3% Carbowax	0.24

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