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Sulphur Nutrition of the Corn Plant: I. Effect on the Nitrate — Nitrogen Fraction

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Abstract

Corn plants (Dekalb 805) were grown in nutrient solutions supplied with adequate (156.2 uM S) or inadequate (0, 6.0 or 15.62 uM s) sulphur.The shoots and roots were analysed for nitrate–nitrogen (NO –N) content as well as for sulphate sulphur (SO_4–S), total sulphur and organic sulphur. Also the effect of varying the nitrogen supply (from 3.57 to 7.14 uM N) was studied. The activity of nitrate reductase in the leaves of sulphur deficient (–S) and sulphur adequate (+S) plants was determined.

In (–S) corn leaves, the NO_3–N content was relatively low (.42% dry wt.) and the nitrate reductase activity was also low (0.48 u moles NO_2 (g fr wt.)$^{-1}$ hr^{-1}). In contrast, (+S) corn leaves accumulated more NO_3–N (.64% dry wt.) and had a corespondingly higher nitrate reductase activity (5.64 u moles NO_2–(g fr wt.)$^{-1}$ hr^{-1}). The soluble protein (expressed in mg. per g. fresh weight) in (+S) corn leaves was threefold the amount in sulphur deficient corn leaves.

Introduction

The NO -N fraction is of special interest to animal producers because high NO_3-N levels in forage or herbage lead to toxicity problems. Reports on the effect of sulphur (S) on the NO_3-N fraction are conflicting. Some investigators (Eaton, 1941; Anderson and Spencer, 1950, and Thomas, 1958) have reported that the level of both ammonia and NO -N increased in sulphur deficient plants. Other (Wooding et al, 1970) reported that there was no accumulation of ammonia or NO -N in sulphur deficient plants. However, a report cited by Tisdale and Nelson (1970) indicates that the danger of high NO_3-N levels in plants could be reduced with adequate sulphur fertilization.

The purpose of the present study was to explore how sulphur nutrition influences NO -N levels in the corn plant - a crop that could be used for forage.

Materials and Methods

Corn seedlings (Zea mays L. var. Dekalb 805) were grown as previously described (Rendig et al, 1976) and transplanted into experimental culture solutions.

Composition of Culture solutions and Preparation of Plant Samples:

Two levels of N (3.57 and 7.15 uM) and two levels of S (6.0 and 156.2 uM) were employed to give four treatment combinations designated High N and High S (HH), High N and Low S (HL), Low N and High S (LH) and Low N and Low S (LL). Each treatment was replicated four times. The concentrations of the other macronutrients were 1.0 mM P, 1.0 nM Ca, 0.5 mM Mg, 2.5 mM K and 2.5 mM Cl. The concentration of micronutrients were 25.0 uM B, 2.5 uM Mn, 2.0 uM Zn, 0.5 uM Cu, and 0.5 uM Mo. Iron was supplied in the chelate form, fe-EDTA, at a concentration of 0.8mM. The pH of the nutrient media genrally varied from 4.0 to 4.5.

Culture solutions were renewed every other day. Height measurements were regularly taken. Plants were harvested at predetermined times; the roots were thoroughly rinsed in large volumes of distilled water before drying. Plant samples were dried in forced - draft oven at a temperature of 65°F for 24 hours.

Chemical Analysis:

Nitrate nitrogen (NO_3-N) was determined by an adaptation of the phenoldisulphonic acid method (Kitchen, 1938) sulphate sulphur (SO_4-S) was determined by the method of Johnson and Nishita 1952, and total sulphur (total-S) by the same method after ashing by the procedure of Steinbergs et al 1962. Organic sulphur (Organic-S) was determined by difference. Amide plus ammonia nitrogen (Amide-N) was determined by an adaptation of the method of Pucher et al (1935). Total nitrogen (Total-N) was determined by the improved micro-kjeldahl method of A. O. A. C. (1960) involving predigestion with salicylic acid.

Determination of Nitrate Reductase Activity:

(a) Leaf Samples:

In the first experiment (Figure 3) the 12th leaf of 35 day-old corn reciving either 156.2 or 15.62 uM S were used for assay of nitrate reductase activity. In the Second experiment, (Figure 4) 48 day-old plants were used and three S levels 0,6.0 and 156.2 uM were employed. All the leaves (5 leaves) of the corn shoot were harvested and representative samples were used for enzyme assay. The remaining leaf samples were saved for other chemical analysis. Leaf harvests were always done at noon when nitrate reductase activity is believed to be highest (Hageman et al 1961). Enzyme assays were in duplicates.

(b) Preparation of Cell free extracts:

Leaves were homogenized with mortat and pestle in 3 volumes of 1% polyvinylpyrrolidone (PVP) and 3 volumes of 0.2 M phosphate (PH 7.4) containing 10^{-3}M EDTA and centrifuged 15 minutes at 30,000g. The supernatant was used as the source of enzyme and for soluble protein and nitrate determinations. All procedures after harvest were carried out at 0-3°C.

(c) Enzyme Assay:

The reaction mixture (0.2 ml, pH 7.4) contained the following n u moles: phosphate 37.5, EDTA 40. KNO_3 15, and riboflavin 5 - phosphate 1.2. The reaction was initiated by adding 0.3 ml sodium dithionite solution (2.5 mg ml^{-1}).

After 15 minutes incubation at 29°C the reaction was stopped by shaking the tubes in a virbration mixer for 20 seconds. The nitrate was determined by adding 1.5 ml colour reagent containing 0.5% (W/V) Sulfanilamide in 1.5 N HCl and 0.01 percent (W/V) N - (1-naphthyl) ethylenediamine dihydrochloride, (Hageman and flesher 1960). After 15 minutes, absorbancy was read at 540 nm with a spectronic 20.

(d) Soluble Protein and Nitrate Analyses:

Soluble protein was precipitated with trichlorocetic acid (final concentration of trichloroacetic acid, 5%) and determined by the method of Biuret (layne, 1957). The standard was bovine serum albumin, fraction V. Nitrate was determined with a nitrate ion activity electrode (Model 92-07, Orion Research. Cambridge, Massachusetts).

Results

The growth curves of corn plants under three S levels (0.6.0 and 156.2 uM S) are shown in Figure 1. Growth which was stunted at the O level (S_o) was markedly enhanced at the 156.2 uM S level (+S), whereas at 6.0 uM S level (S_1), growth was intermediate. Apart from the stunting effects, the leaves of S deficient plants were chlorotic

Table 1 shows the differences in the chemical composition of S-adequate and S-deficient corn plants: The NO_3-N content of (+S) plants was .64% (dry weight) as compared to .42% (dry weight) in (S_o) plants. Figure 2 also indicates that the levels of NO_3-N in S-adequate tissues (shoots and roots) exceeded those in S-deficient tissues. Direct tests for NO_3-N on cell free extracts by means of a specific nitrate ion activity electrode further confirmed that corn leaves which were adequately supplied with sulphur accumulated more NO_3-N than those under acute sulphur stress (Table 2).

The effect of different N and S combinations were evaluated because NO_3-N level in tissues are said to be related to N:S ratios (Tisdale and Nelson, 1970). Figure 2 reveals that different N and S combinations influence NO_3-N distribution or partitioning between shoots and roots. For clarity, the NO_3-N redistribution patterns are categorised below under four nutritional regimes:

(i) High Nitrogen Regime (7.14mMN) i.e. HH and HL.
In HH (i.e plants receiving high N and high S supply), the NO_3-N levels in shoots and roots were about the same. In contrast, HL plants (i.e. plants receiving high N and low S supply) showed a remarkable differential in NO_3-N partitioning between shoots and roots. Thus while the roots contained only .41% NO_3-N the shoots contained as much as 1.0%.

(ii) Low Nitrogen Regime (3.57mM N) i.e LH and LL.
under this nutritional regime there was not much difference between the NO_3-N levels in shoots and roots. However, there was a tendency for roots to retain more NO_3-N than shoots.

(iii) High sulphur Regime (156.2 uM S) i.e HH and LH.
Under high sulphur supply, lowering the N supply from 7.15 to 3.57 mM appeared to increase the NO_3-N levels in both shoots and roots. (Compare HH and LH). Aiso, lowering the N supply resulted in higher level of NO -N in roots (1.42%) than in the shoots (1.17%).

(iv) Low sulphur Regime (6.0 uM S) i.e. HL and LL.

Under low S regime, increasing the N supply in the bathing solution appeared to enhance nitrate accumulation more in the shoots than in the roots.

Results in Table 1 show that the level of amide-N was much higher in S-deficient than in S-adequate plants. For example, the level of amide-N in (S_0) plants was about fifteeen times that in (+S) plants. The total-N level was also higher in S-deficient plants than in s-adequate plants. The levels of total-S and organic-S were higher in plants receiving adequate S supply than in those under s stress.

The activity of nitrate reductase in the leaves of (+S) and (-S) plants was determined because this enzyme complex is responsible for the reduction of NO_3-N to NH_4-N and so would be expected to directly affect the NO_3-N level. Figures 3 and 4 indicate that the activity of nitrate reductase was higher in S-adequate plants than in S-deficient plants. In Figure 3, the enzyme activity in (+S) leaves was almost sixfold that in (-S) leaves. In Figure 4, the activity of the enzyme in (+S) leaves was almost twelvefold that in (S_o) and only slightly

TABLE 1: THE CONCENTRATIONS OF N AND S FRACTIONS IN THE LEAVES OF 48 DAY-OLD CORN PLANTS SUPPLIED WITH THREE DIFFERENT LEVELS OF S.

Treatment	Total-N	No_3-N	Amide-N	Total-S	SO_4-S	Organic-S
	.....	(% dry weight)		.....	.....ppm	
(+S)	3.96	0.64	0.13	2963	773	2190
($-S_1$)	4.24	0.56	0.23	1884	218	1666
($-S_0$)	5.94	0.42	1.90	1341	31	1310

(+S) = 156.2 uM S in culture solution
($=S_1$) = 6.0 uM S in culture solution
($-S_0$) = No S in culture solution.

TABLE 2: NITRATE REDUCTASE ACTIVITY, NITRATE NITROGEN AND SOLUBLE PROTEIN CONCENTRATIONS IN THE LEAVES OF 48 DAY-OLD CORN PLANTS SUPPLIED WITH THREE DIFFERENT LEVELS OF S**

Treatment*	Nitrate Reductase Activity uM NO_2-(g fr wt)$^{-1}$ hr^{-1}	Nitrate-N ug (g fr wt)$^{-1}$	Soluble Protein mg (g fr wt)$^{-1}$
(+S)	5.64	911.4	10.2
($-S_1$)	4.68	849.4	6.3
($-S_0$)	0.48	337.3	3.3

* (+S) = 156.2 uM S in culture solution
($-S_1$) = 6.0 uM S in culture solution
($-S_0$) = no S in culture solution
** Determined by means of a nitrate ion activity electrode.

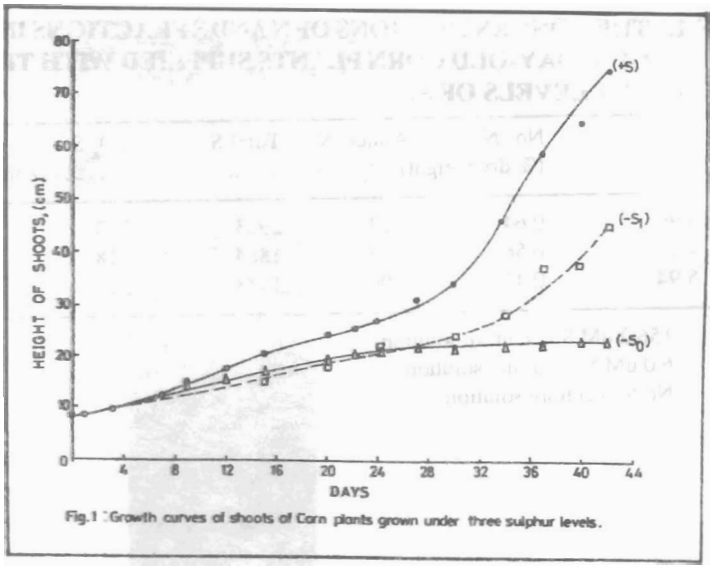

Fig.1 : Growth curves of shoots of Corn plants grown under three sulphur levels.

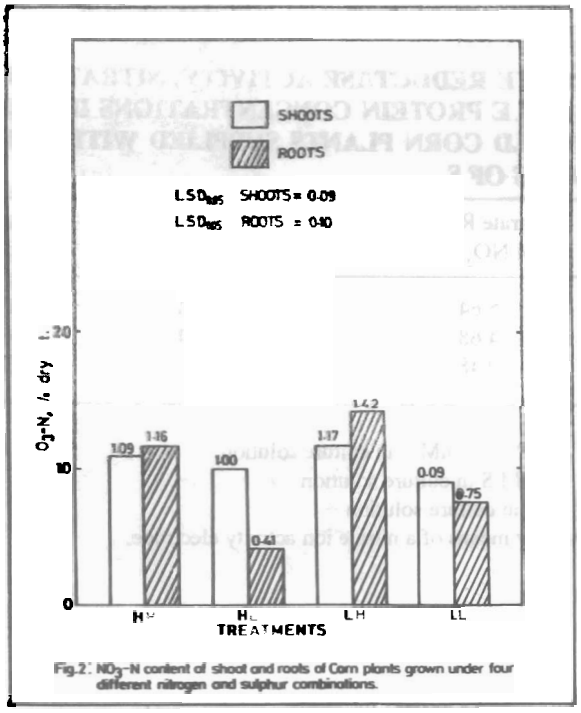

Fig.2: NO_3-N content of shoot and roots of Corn plants grown under four different nitrogen and sulphur combinations.

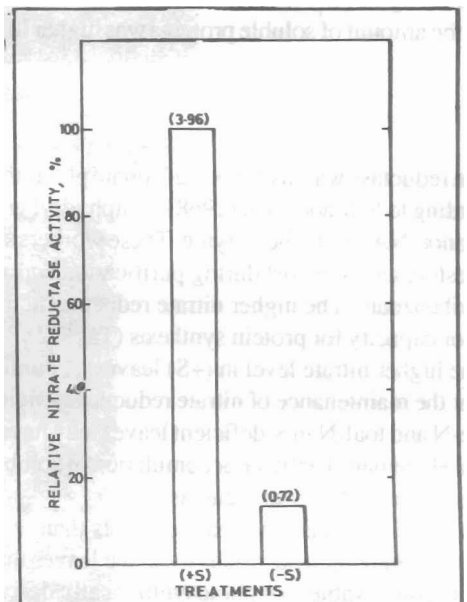

Fig.3 : Relative nitrate reductase activity in the 12th leaf of S-adequate and S-deficient Corn plants.

Figures in bracket represent the actual activities of reductase expressed in μM NO_2^- (g fr wt)$^{-1}$ hr^{-1}

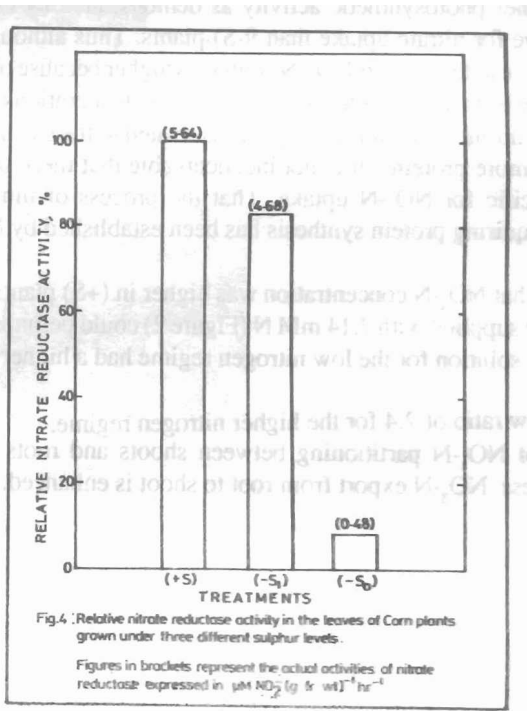

Fig.4 : Relative nitrate reductase activity in the leaves of Corn plants grown under three different sulphur levels.

Figures in brackets represent the actual activities of nitrate reductase expressed in μM NO_2^- (g fr wt)$^{-1}$ hr^{-1}

higher than the enzyme activity in (S_1) leaves.

Table 2 shows that the amount of soluble proteins was higher in S-adequate leaves than in S-deficient leaves.

Discussion

That the activity of nitrate reductase was higher in s-adequate plants than in S-deficient plants is not unexpected. According to Schrader et al (1968) a sulphydryl group is implicated in the binding of the electron donor, NADH, to the enzyme. These workers showed that the addition of sulphydryl reagents restores activity lost during purification and alleviates the inhibitory effects of P-chlor-mecuribenzoate. The higher nitrate reductase activity in (+S) plants may be related to their superior capacity for protein synthesis (Table 2) It may also be accounted for, at least in part, by the higher nitrate level in (+S) leaves: A continuous supply of nitrate is said to be important for the maintenance of nitrate reductase activity (Jackson et al, 1973). The high levels of amide-N and toal-N in S-deficient leaves may have a deleterious effect for according to Beevers and Hageman (1969) the accumulation of soluble nitrogenous products specifically repress the synthesis of nitrate reductase.

The concentration of NO_3-N was lower in the shoots than in the roots of (+S) plants probably because of higher nitrate reductase activity in the leaves than in the roots (Beevers and Hageman, 1969). It is conceivable that photosynthetically derived reductant for nitrate reductase would be more accessible to leaves than to roots (Beevers and Hageman, 1969).

Since nitrate reductase activity was higher in (+S) than in (-S) plants, one would have expected a lower NO_3-N level in the (+S) plants. On the contrary, results in this study indicate that NO_3-N level was higher in (+S) than in (-S) plants. This apparent paradox could perhaps be xplained by differences in the rate of nitrate uptake. Corn plants with adequate s supply because of their higher photosynthetic activity as demonstrated by chen (1967), possess higher energy reserve for nitrate uptake than 9-S)-plants. Thus although (+S) plants have higher nitrate reductase activity, their NO_3-N content is higher because of their greater ability to take up more nitrate from the nutrient solution. Energy considerations aside, the possibility exists that the higher nitrate content in (+S) plants is related to the superior capacity of these plants to synthesise more proteins. It is not inconceivable that these proteins may include carrier proteins specific for NO_3-N uptake. That the process of nitrate uptake is under metabolic control requiring protein synthesis has been established by Neyra and Hageman (1975).

The anomaly that NO_3-N concentration was higher in (+S) plants receiving 3.57 mM N than in (+S) plants supplied with 7.14 mM N (Figure 2) could perhaps be explained by the fact that the nutrient solution for the low nitrogen regime had a higher $\frac{NO_3\text{–N}}{NH_4\text{–N}}$ ratio of 11.5 as compared to a low ratio of 2.4 for the higher nitrogen regime.

The pattern of NO_3-N partitioning between shoots and roots suggests that under conditions aof S stress, NO_3-N export from root to shoot is enhanced.

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