Nitrogen Assimilation and Protein Synthesis in Wheat Seedlings As Affected by Mineral Nutrition. I. Macronutrients[1]

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Abstract. **Deficiencies of each macronutrient (N, P, K, Ca, Mg, S, and Fe) decreased the specific activity of nitrate reductase from *Triticum aestivum* L. seedlings. Nitrate content was decreased by N, P, K, Ca, and Mg deficiencies and unaffected by S and Fe deficiencies. Glutamic acid dehydrogenase activity was decreased by N, P, and S deficiencies, unchanged by K deficiency, and increased by Ca, Mg, and Fe deficiencies. Glutamine synthetase activity closely paralleled nitrate reductase activity and was decreased by deficiencies of N, P, K, Ca, Mg, and S. Glutamic-oxaloacetic transaminase was not sensitive to macronutrient deficiencies. High ^{14}C-leucine incorporation into tissue sections of N-, P-, K-, Ca-, and S-deficient seedlings did not appear indicative of protein synthesis rates in intact seedlings. Nutritional deficiencies apparently depleted endogenous amino acid pools and caused less inhibition of exogenous ^{14}C-leucine incorporation into protein.**

The influence of mineral nutrition of higher plants on enzymes involved in nitrogen assimilation has received limited treatment. Nitrate reductase, which has been studied most extensively, is substrate inducible (1, 6, 7, 27) and requires the presence of nitrate for maximum activity. Phosphorus appears to be essential for activity of nitrate reductase (25) and possibly for all flavoprotein enzymes involved in reduction of nitrate to ammonia (19). Calcium deficiency has been shown to repress nitrate reductase activity indirectly (23), and the close association between nitrate assimilation and chloroplasts (24) suggests magnesium deficiency might affect activity of the enzymes involved. Similarly, the presence of sulfhydryl groups (3, 6) suggests sulfur is essential for nitrate reductase synthesis. Nitrate reductase from *Neurospora* (21) and *Pseudomonas* (5) has received more attention and is affected by deficiencies of most macronutrients and iron.

Enzymes functioning in nitrogen incorporation into organic forms do not appear as sensitive as nitrate reductase to nutrient deficiencies. Brief nitrogen deficiency which decreased nitrate reductase activity had no effect on glutamic acid dehydrogenase activity (12). Magnesium is required for glutamine synthetase activity (4), and phosphorus-containing cofactors function with most enzymes involved in nitrogen assimilation. Nitrate reductase and glutamic acid dehydrogenase require reduced pyridine nucleotides, glutamine synthetase requires ATP, and transaminases require pyridoxal phosphate.

A number of nutrients have been implicated directly or indirectly in protein synthesis. Phosphorus, potassium, and magnesium are required for cell-free amino acid incorporation into protein (28) and presumably for protein synthesis by intact plant tissue. Nutrients involved in amino acid synthesis and structure can likewise be considered essential for protein synthesis.

Numerous studies emphasize the importance of mineral nutrition on nitrogen assimilation by plants. The effects of nutrient deficiencies on nitrogen assimilation have not been systematically determined, however, and information on several nutrients and many enzymes is lacking. The present studies compared nitrogen assimilation and protein synthesis in wheat seedlings under deficiencies of 7 macronutrients.

Materials and Methods

'Pawnee' winter wheat (*Triticum aestivum* L.) was germinated in moist vermiculite and transplanted to nutrient solutions after 5 days. Twelve seedlings were suspended through the covers of 2-liter styrene containers holding partial nutrient solutions similar to those described by Hoagland and Arnon (10). The compositions of the solutions are shown for each macronutrient in table I. The deficient nutrient in each instance (N, P, K, Ca, Mg, S, or Fe) was varied individually at 5 concentrations, 0, 5, 10, 50, and 100 %, relative to the levels supplied to controls. All macronutrients other than the variable were supplied at the 100 % level; control treatments contained all nutrients at the maximum level. The

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Table I. *Concentration of Macronutrients in Nutrient Solutions*

Each nutrient was varied individually at the 5 levels shown while the other nutrients were held constant at the 100 % level.

Nutrient variable	Nutrient level (% of control) 0	5	10	50	100
	mM	mM	mM	mM	mM
N	0	1.25	2.5	12.5	25.0
P	0	0.05	0.1	0.5	1.0
K	0	0.3	0.6	3.0	6.0
Ca	0	0.25	0.5	2.5	5.0
Mg	0	0.1	0.2	1.0	2.0
S	0	0.1	0.2	1.0	2.0
Fe	0	0.043	0.065	0.086	0.215

solutions were prepared with reagent quality salts, and the variable nutrients were generally supplied in the Na or Cl form. Iron (43 μmoles) was supplied every 2 days as a 0.6 % (w/v) $FeSO_4 \cdot 7H_2O$ − 0.4 % (w/v) tartaric acid solution. Micronutrients were supplied at levels suggested by Johnson *et al.* (13). The nutrient solutions were adjusted periodically to pH 5.0 with HCl and were aerated continuously.

Iron was considered a macronutrient because the severity of growth reduction caused by iron deficiency was similar to that caused by deficiencies of the other nutrients. The iron added initially to the nutrient solutions did not remain available, and for the experiment involving iron deficiency, the nutrient was supplied periodically at concentrations found to cause approximately 0, 5, 10, 50, and 100 % of the plants' leaf area to have no chlorotic deficiency symptoms.

The 5 treatments were replicated 4 times for each nutrient and randomized in environmental chambers maintained at 20° day and 10° night temperatures. Lighting was provided from sixteen 160-watt fluorescent lamps and six 300-watt incandescent lamps for a 16-hr light period and an 8-hr dark period. Relative humidity in the chambers was approximately 40 %.

Plant leaf blades were sampled for analysis 21 days after transplanting. One-g samples of blade tissue were homogenized in 20 ml of chilled medium containing 33 mM tris (pH 7.2), 3.3 mM cysteine, and 0.1 mM Na_2EDTA. The homogenate was filtered and centrifuged at 20,000*g* for 15 min at 2°. The supernatant was filtered through glass wool and held at 2° for assay of nitrate reductase, glutamic acid dehydrogenase, glutamine synthetase, and glutamic-oxaloacetic transaminase. All enzyme assays were incubated 15 min at 30°. Activities were based on protein content of the enzyme extracts measured by the Folin phenol method modified by Miller (20).

Nitrate reductase was assayed by the method of Hageman and Flesher (7). The assay mixture contained 1 ml of 0.2 M potassium phosphate (pH 7.2), 0.2 ml of 0.1 M KNO_3, 0.5 ml of 1.4 mM NADH, and 0.3 ml of enzyme extract. Glutamine synthetase activity was determined by the method of Elliot (4) in an assay mixture containing 1 ml of 0.8 M tris (pH 7.2), 1 ml of 0.5 M glutamate, 0.2 ml of 1 M $MgSO_4$, 0.2 ml of 1 M NH_2OH, 1.0 ml of 25 mM ATP, and 1 ml of enzyme extract. Glutamic acid dehydrogenase was assayed by a modification of the method of Lowry, Roberts, and Lewis (16). Preliminary trials showed over 95 % of the activity was recovered in the supernatant although the enzyme has been reported in mitochondria (24). The reaction mixture contained 1 ml of 0.2 M potassium phosphate (pH 7.2), 0.5 ml of 0.1 M $(NH_4)_2SO_4$, 0.5 ml of 1.4 mM NADH, and 0.5 ml of enzyme extract. Reactions were stopped by adding 1 ml of 1 N HCl. The NAD formed was determined by adding 0.1-ml aliquots of reaction mixture to 10 ml of 5 N NaOH and measuring the fluorescence after 30 min. Glutamic-oxaloacetic transaminase was assayed by the method of Karmen, Wroblewski, and LaDue (14). One ml of enzyme extract was incubated with 1 ml of reaction mixture containing 0.02 M aspartate and 0.02 M α-ketoglutarate in 0.2 M potassium phosphate (pH 7.2). The reaction was stopped with 1 ml of color reagent (1 mg 2,4-dinitrophenylhydrazine in 1 ml of 1.35 N HCl). After 20 min, 10 ml of 0.4 N NaOH was added followed by 10 ml of distilled water. The absorbance was read at 504 mμ against reagent blanks containing extraction medium substituted for enzyme extract; color due to the enzyme itself was negligible. Standard curves were prepared from oxaloacetic acid.

Incorporation of ^{14}C-L-leucine by leaf blade sections was conducted by the method of Key (15) and measured by the method of Mans and Novelli (17). One g of fresh tissue sections was preincubated in unbuffered 1 % (w/v) sucrose for 2 hr at 2°. The sections were removed, blotted dry, and transferred to 5 ml of incubation medium containing 5 mM potassium phosphate (pH 6.0), 1 % sucrose (w/v), 80 μg streptomycin, and 0.25 μc uniformly labeled ^{14}C-L-leucine (248 μc per μmole). The tissue sections were vacuum-infiltrated and incubated in the medium for 2 hr at 30° with room lighting and constant shaking. The incubation medium was decanted and protein was extracted by homogenizing the samples in chilled 0.1 M tris (pH 7.5) containing 0.2 mg of ^{12}C-L-leucine per ml. The homogenate was filtered through glass wool and the protein was precipitated with an equal volume of 10 % (w/v) trichloroacetic acid. The protein pellet formed after centrifuging at 5000*g* for 10 min was dissolved in 1 ml of 3 N NaOH. Duplicate 0.1-ml aliquots were spotted on Whatman No. 3 filter paper disks (2.3 cm diameter) and were prepared and counted by the technique of Mans and Novelli (17) using a Packard liquid scintillation system. Results were expressed as incorporation per mg protein determined by the Folin phenol method modified by Miller (20).

Nitrate content was determined by the method of

Woolley, Hicks, and Hageman (29) after drying fresh leaf blade samples at 70°.

Results

Each of the macronutrient deficiencies decreased the specific activity of nitrate reductase (fig 1). Nitrate and calcium deficiencies decreased nitrate reductase activity more than deficiencies of the other nutrients. Relatively high levels of nitrate and phosphorus were required to maintain maximum nitrate reductase activity, while only low levels of the other nutrients were necessary for near-maximum activity. The nitrate content of the leaf blades was decreased by all the macronutrient deficiencies except sulfur (table II). Nitrogen deficiency had the greatest effect, but lack of calcium, phosphorus, and potassium also decreased the nitrate concentrations. The effect of iron was inconsistent.

Activity of glutamic acid dehydrogenase, in comparison with nitrate reductase, was increased by deficiencies of several macronutrients (fig 2). Deficiencies of calcium, magnesium, and iron increased activity of the enzyme to approximately similar extents. Nitrogen, phosphorus, and sulfur deficiencies, on the other hand, decreased glutamic acid dehydrogenase activity. Potassium nutrition had no effect on the enzyme. The response of glutamine synthetase to macronutrient nutrition was similar to that of nitrate reductase (fig 3). Deficiencies of each of the macronutrients, except iron, decreased the specific activity of the enzyme. Nitrogen and phosphorus deficiencies, respectively, had the greatest effect on glutamine synthetase. Glutamic-oxaloacetic transaminase was least sensitive to nutrient deficiencies of the enzymes studied (fig 4). The enzyme responded to calcium, but that response was inconsistent.

Table II. *Nitrate Content of Leaf Blades From Wheat Seedlings*

Seedlings were grown 3 weeks under 5 levels of the 7 macronutrients varied individually as shown in table I. Mean nitrate content of controls was 454 μmoles per g dry wt.

Nutrient variable	Nutrient level (% of control) 0	5	10	50	100 (control)
	Nitrate content (% of control)				
N	3.2 a[1]	1.0 a	0.8 a	65.4 b	100 c
P	29.7 a	28.4 a	42.6 a	78.4 b	100 c
K	67.4 b	25.4 a	32.3 a	72.7 a	100 c
Ca	14.5 a	81.0 b	81.3 b	91.9 b	100 b
Mg	66.1 a	100.0 b	87.5 b	87.5 b	100 b
S	108.0	91.1	94.1	81.7	100
Fe	106.7 c	83.9 a	93.4 ab	93.6 ab	100 bc

[1] Treatment means of individual nutrients followed by different letters differ significantly at the 5 % level.

Extremely high levels of ^{14}C-leucine were incorporated into leaf blades of wheat seedlings grown under several macronutrient deficiencies (fig 5). Nitrogen and sulfur deficiencies most markedly increased incorporation of the label, but phosphorus, potassium, and calcium deficiencies also did so to significant extents. Only magnesium and iron deficiencies had no effect on ^{14}C-leucine incorporation. The effect of nutrient deficiencies appeared due to the response of endogenous amino acid pools to plant nutrition (26) and the subsequent effect of those pools on ^{14}C-leucine incorporation (2, 11, 18). The necrotic and partially desiccated condition of seedlings grown without several macronutrients increased their fresh-weight protein content (fig 6). The intermediate nitrogen levels and the lowest sulfur levels were the only treatments that decreased protein contents.

Discussion

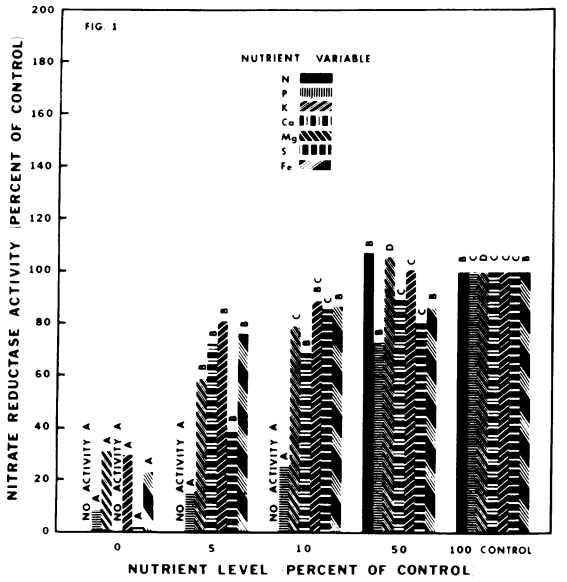
The labile nature of nitrate reductase from wheat seedlings was indicated by the detrimental effect of macronutrient deficiencies on specific activity of the enzyme. Nitrate reductase activity and nitrate content were concurrently low under nitrogen, phosphorus, calcium, and magnesium deficiencies. The substrate-inducible nature of nitrate reductase (1, 7, 27) likely accounted for decreased enzyme activity when low nitrate levels were supplied. Similarly, low concentrations of nitrate in wheat seedlings deficient in other nutrients may have limited enzyme induction. However, phosphorus has been shown necessary for both nitrate reductase activity (25) and nitrate uptake (9) and may influence the enzyme directly or indirectly. The lack of nitrate in calcium-deficient seedlings indicated that reductase activity may have been limited by low substrate levels. Alternatively, a previous study indicated nitrite in calcium-deficient wheat seedlings (23) repressed nitrate reductase. Similar accumulation of nitrite was observed in the present study.

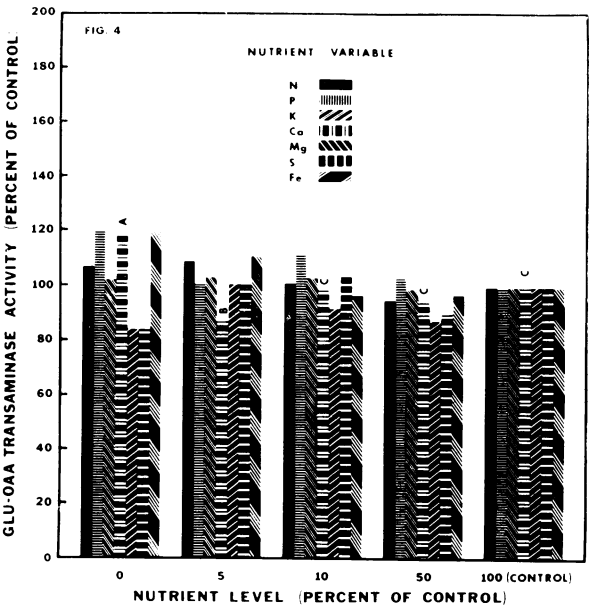
Sulfur and iron deficiencies caused slight nitrate accumulation and the effect of those deficiencies on nitrate reductase was from factors other than limited substrate. Nitrate reductase activity requires ability of the tissue to synthesize protein (12). The involvement of sulfur in amino acid structure and the sulfhydryl requirement for activity of nitrate reductase (3) implied that sulfur directly influenced enzyme synthesis.

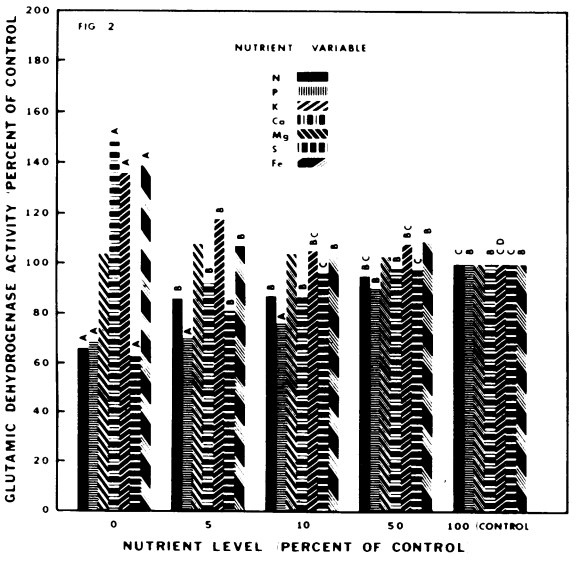
The similar effects of nitrogen, phosphorus, and sulfur deficiencies on nitrate reductase and glutamic acid dehydrogenase activities implied a direct relationship between the 2 enzymes, as has been suggested for *Neurospora* (22). However, increased glutamic acid dehydrogenase activity in seedlings deficient in calcium, magnesium, and iron showed that the enzyme was not strictly dependent on nitrate reductase activity. Increased glutamic acid dehydrogenase activity may have been due to *in vivo*

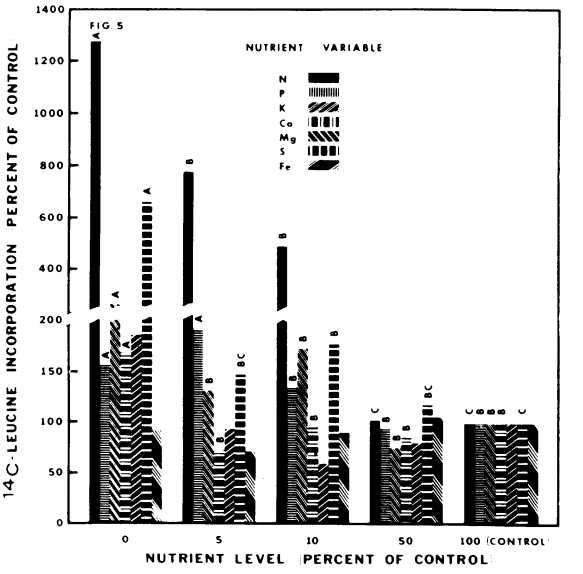
oxidative deamination instead of reductive amination. Glutamine synthetase more closely paralleled nitrate reductase, as indicated by significant positive correlations (not shown) between activities of the 2 enzymes under each treatment. Nitrate reductase appears to be rate-limiting to nitrogen assimilation (8); thus, decreased glutamine synthetase activity in nutrient-deficient seedlings may have been due indirectly to decreased nitrate reductase activity. However, the phosphorus, magnesium, and sulfur requirements of glutamine synthetase (4) suggest that the enzyme was affected directly by certain nutrient deficiencies.

The observed ^{14}C-leucine incorporation rates by tissue sections of nutrient-deficient seedlings did not appear indicative of protein synthesis rates in intact seedlings. The effects of nutritional deficiencies on amino acid pools, and the subsequent effect of those pools on ^{14}C-leucine incorporation, may explain the present results. Many nutrient deficiencies deplete endogenous amino acid pools by restricting nitrate uptake and assimilation more than protein synthesis (26). Holeman and Key (11) demonstrated that depleting amino acid pools increased incorporation of ^{14}C-leucine. Furthermore, prior incubation of plant tissue with ^{12}C-leucine (11) or addition of ^{12}C-leucine to an active system (18) inhibited incorporation of ^{14}C-leucine. Bamji and Jagendorf (2) likewise demonstrated that a mixture of ^{12}C-amino acids inhibited ^{14}C-leucine incorporation in chloro-









plasts, the most active site of protein synthesis in wheat seedlings. Thus, it appeared that fewer endogenous amino acids were present in nutrient-deficient seedlings, as compared with normal seedlings, and less inhibition of ^{14}C-leucine incorporation occurred. Low nitrate reductase activity in nutrient-deficient seedlings having high ^{14}C-leucine incorporation rates further suggested that lower levels of amino acids were present to inhibit ^{14}C-leucine incorporation in deficient seedlings than in normal seedlings.

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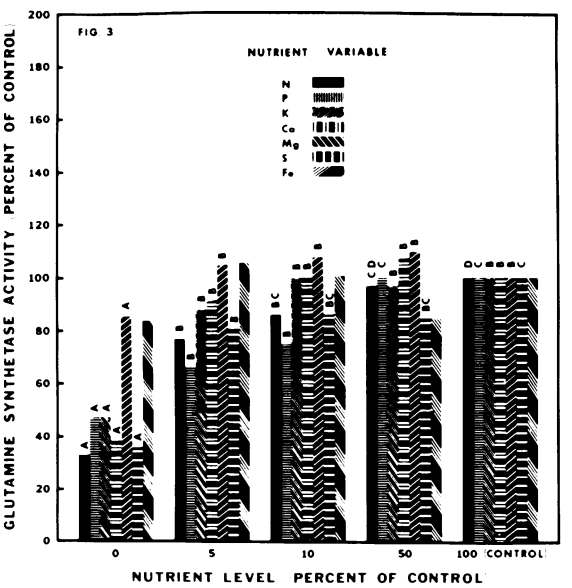

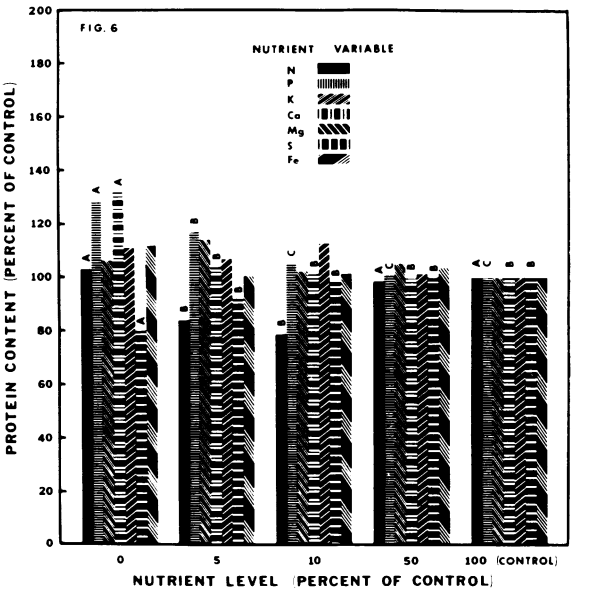

Fig. 1 to 6. Activities of nitrate reductase (fig 1), glutamic acid dehydrogenase (fig 2), glutamine synthetase (fig 3), glutamic-oxaloacetic transaminase (fig 4), and protein synthesis (fig 5), and content of soluble protein (fig 6) as percentages of controls. Mean activities of controls were 0.11, 0.75, 3.51, and 4.83 μmoles product (mg protein hr)$^{-1}$, respectively, for nitrate reductase, glutamic acid dehydrogenase, glutamine synthetase, and glutamic-oxaloacetic transaminase; 2.88 $\mu\mu$moles ^{14}C-leucine (mg protein hr)$^{-1}$ for protein synthesis; and 30.8 mg protein per g fresh wt for protein content. Treatment means of individual nutrients followed by different letters differ significantly at the 5 % level.

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