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Effect of ammonium nutrition on uptake and metabolism of nitrate in wheat

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Summary

One-month old wheat plants grown in NO_3 medium were exposed for 2 days to media with 4 meq NO_3 and 0 to 4 meq NH_4 per litre. In mixed (NO_3 + NH_4) nutrition, NH_4 uptake exceeded NO_3 uptake even at the lowest NH_4 level. Ammonium metabolism also predominated over nitrate assimilation and caused a higher free amino acid content, a lower carboxylate content, and a lower nitrate reductase activity (NRA) in the tops than nitrate alone.

In vitro experiments with desalted cell-free nitrate reductase extracts of wheat tops showed that NH_4 ions inhibited NRA, but that a selection of amino acids did not depress NRA.

The drop in NRA in wheat tops caused by NH_4 nutrition could not be related to the low level of tissue NH_4, nor to any other single factor. It was suggested that changes in the redox situation in the cells are involved.

Introduction

A still inadequately explained phenomenon in plant nutrition is the severe suppression of NO_3 uptake by concomitant NH_4 uptake in, for example, perennial ryegrass (Lycklama, 1963), wheat (Minotti et al., 1969) and *Chlorella* (Syrett & Morris, 1963). Conversely, the effect of NO_3 uptake on NH_4 uptake in small.

Weissman (1951) and Lyklama (1963) found that NH_4 nutrition affected nitrate reduction rather than nitrate accumulation in the plant. The latter author and Jackson et al. (1973) suggested the existence of an assimilation-linked rate of nitrate uptake.

The present communication deals with the effect of NH_4 uptake on uptake and metabolism of nitrate in wheat plants. The additional study of the inhibition of the nitrate reductase activity (NRA) by NH_4 nutrition was considered of interest, because those factors associated with NH_4 nutrition which actually repress NRA in plants are still unknown.

Materials and method

Spring wheat seeds (*Triticum aestivum* cv. Orca) were germinated in quartz sand moistened with demineralized water. The seedlings were transferred to a well-aerated

Table 1. Composition of the nutrient solutions (meq/litre).

Medium	NH_4	Na	K	Ca	Mg	NO_3	H_2PO_4	SO_4	Cl
NO_3	–	–	6.0	2.5	4.0	6.0	1.0	2.5	3.0
NO_3+NH_4	0-4.0	1.0	3.0	2.0	1.0	4.0	1.0	1.0	1.0-5.0

Trace elements: 0.5 mg B, 0.5 mg Mn, 0.4 mg Fe, 0.05 mg Zn, 0.02 mg Cu and 0.01 mg Mo/litre. N serve (2-chloro-6-trichloromethyl pyridine) 0.01 mg/litre was added to the (NO_3 + NH_4) media.

nutrient solution (Table 1, NO_3 medium), renewed at regular time intervals. After 1 month, the plants were distributed over 5 well-aerated solutions with constant nitrate but different ammonium concentrations of 0, 1, 2, 3 and 4 meq NH_4/litre (Table 1, NO_3 + NH_4 media). The plants grew for 2 days in these media which were renewed 4 times. The plants were grown in the spring of 1972 in a heated greenhouse.

For in vitro determinations, nitrate reductase was extracted from tops of plants remaining on the NO_3 medium.

Analytical methods

Nitrate reductase (E.C.1.6.6.1.) activity was determined according to Sanderson & Cocking (1964) with a variety of modifications. A sample of tops was excised, immediately taken to a cold room (1 °C) and clipped into small pieces. 2.00 g were homogenized together with 25 ml of a cold extraction medium, consisting of 200 ml 0.5 *M* sucrose + 50 ml 0.1 *M* tris. HCl buffer pH 7.5 + 25 ml 0.01 *M* cysteine + 2.5 ml 0.003 *M* EDTA, having a final pH of 7.5 to 7.6. A Bühler homogenizer with an ice-water cooled container was used for 30 seconds at 40 000 rev/min. The homogenates were centrifuged (15 minutes, 0 °C, 40 000 *g*) and the supernatant filtered through glass-wool. Of this crude enzyme extract 1 ml was pipetted into a centrifuge tube and mixed with 5 ml incubation medium (20 ml 0.1 *M* KNO_3 + 60 ml reduced NAD solution of 0.5 mg/ml + 120 ml 0.1 *M* tris. HCl buffer pH 7.5). Incubation proceeded at 30 °C for 30 minutes. Nitrate reduction was stopped by placing the tubes in ice-water and adding 1 ml 1 % (w/v) sodium lauryl sulphate, 2 ml ice-cold 96 % (v/v) ethanol and 1 ml 2 *M* barium acetate. After each addition the tubes were shaken vigorously. The mixture was centrifuged (20 000 *g*, 10 minutes) and nitrite was determined by mixing the supernatant with 2 ml 1 % (w/v) sulphanylamide in 2.5 *M* HCl and then with 2 ml 0.02 % (w/v) N-1-naphtylethylene diamine.HCl.

After 30 minutes, the optical density was measured with a Uvichem H 1620 spectrophotometer at 540 nm. Standard series of 0-0.3 m*M* KNO_2 were treated in the same way. The coefficient of variation of the measurements was ± 5 %.

Gel filtration chromatography of the crude enzyme extracts was used to desalt extracts for in vitro experiments. Through a column (ø 1.5 cm) of 6.0 g Sephadex G-25 (medium) 4 ml of crude extract were passed, eluted with extraction medium, and eluate ml 13-18 were collected. This 6-ml fraction contained 98 % of the NRA. The nitrate concentration was 0.05 m*M* (0.5 % of the concentration in the crude extract).

Desalted and crude extracts were tested for NRA in the same way.

For methods of determination of carboxylates, free nitrogenous compounds, water-soluble carbohydrates, nitrogen and inorganic constituents, reference is made to Breteler (1973).

Experimental

1. Wheat plants grown for 1 month in NO_3 medium were exposed to the (NO_3 + NH_4) test media for 2 days. The pH of the nutrient solution was measured at regular intervals. After exposure the tops were harvested and analysed for NRA, free ammonium ions, amides and amino acids, water-soluble carbohydrates, total N, NO_3, carboxylates, K, Na, Mg, Ca, SO_4, Cl and H_2PO_4.

In vitro tests with desalted enzyme extracts of wheat tops:

2. The effect of the addition of NH_4 ions (0-100 meq/litre as NH_4Cl) to the incubation medium on NRA was tested.
3. The effect of NH_4 on NRA was compared with the effect of other cations by substitution of the nitrates of Na, Mg, Ca and NH_4 for KNO_3 in the incubation medium.
4. The effect of some major amino acids of wheat, glutamine, asparagine, glutamic acid, aspartic acid and γ-amino butyric acid (Mengel & Helal, 1970) on NRA was tested by adding these compounds in a concentration of 10m*M* to the incubation medium.

Results

Experiment 1. Changes in pH of the nutrient solution during growth on (NO_3 + NH_4) media are given in Table 2. With NH_4 the pH dropped so that cation absorption must have exceeded anion absorption even where NH_4 constituted only 20 % of all the N in the medium. This indicates preferential NH_4 uptake or inhibited NO_3 uptake.

NRA in the tops after 2 days of growth in (NO_3 + NH_4) media (Fig. 1) decreased with NH_4 concentration and NH_4 uptake by ultimately 77 %.

A similar decrease in NRA by NH_4 was found by Smith & Thompson (1971a) for excised barley roots and Joy (1969) for *Lemna minor*.

Fig. 2 shows the carboxylate content of the tops. Their sum decreased with increasing NH_4 concentration mainly by a decrease in citrate, succinate and malate. The drop indicates that in (NO_3 + NH_4) nutrition assimilation of ammonium predominated over the assimilation of nitrate since substitution of ammonium for nitrate is known to reduce the accumulation of carboxylates.

Table 2. Change in nutrient solution pH caused by wheat plants after 16 hours growth in (NO_3 + NH_4) media. The initial pH is the average of 5 fresh media (range 5.17 – 5.46).

Medium	pH
Initial	5.31
$4NO_3$ + ONH_4	7.18
$4NO_3$ + $1NH_4$	3.72
$4NO_3$ + $2NH_4$	3.38
$4NO_3$ + $3NH_4$	3.07
$4NO_3$ + $4NH_4$	3.17

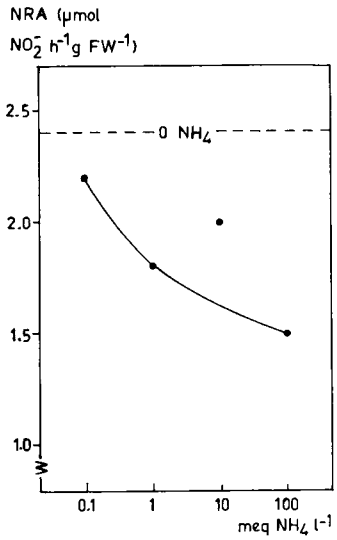

Fig. 1. The effect of two days of mixed (NO_3 + NH_4) nutrition on the NRA of the tops. Experiment 1.

Fig. 3 shows that ammonium increased the concentration of free amino acids and amides in the dry matter and as % of total N. NH_4 accumulated in the tops of NH_4-fed plants at a highest value of 26 meq/kg DM, whilst with nitrate alone the plants contained 18 meq/kg DM.

Fig. 4 shows that increased NH_4 uptake decreased the total inorganic cation content (C) more than the total inorganic anion content (A). The resulting drop in (C-A) followed closely the decrease in carboxylate content (Fig. 2). Changes in C and A were mainly

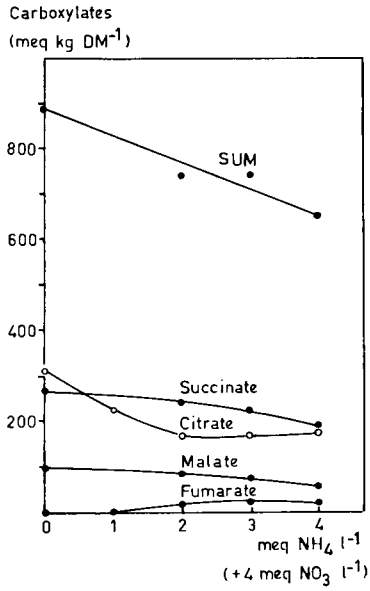

Fig. 2. The effect of two days of mixed (NO_3 + NH_4) nutrition on the carboxylate content of the tops. Experiment 1.

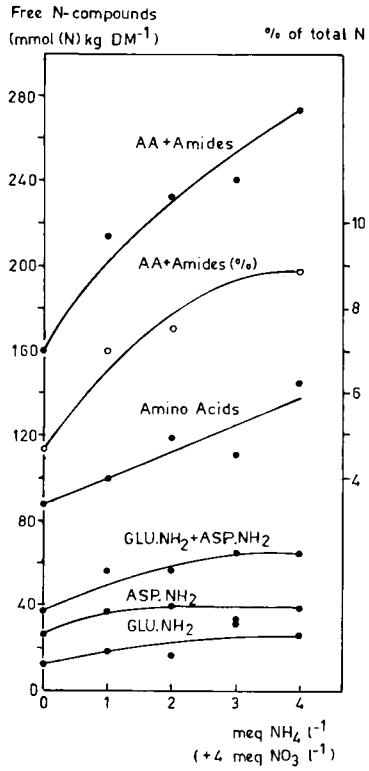

Fig. 3. The effect of two days of mixed (NO_3 + NH_4) nutrition on the contents of some free nitrogenous compounds in the tops. Amides and amino acids in mmol/kg DM, sum of amides and amino acids in mmol N/ kg DM or as % of total N (right hand ordinate). Experiment 1.

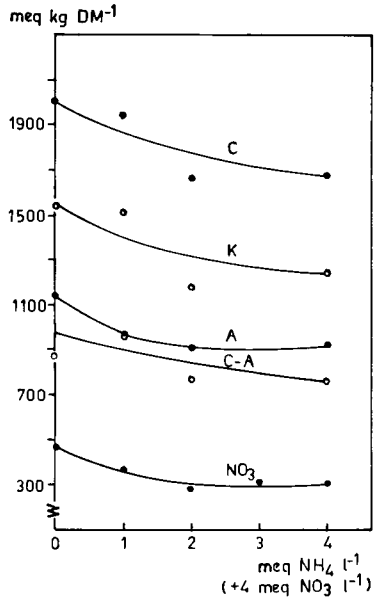

Fig. 4. The effect of two days of mixed (NO_3 + NH_4) nutrition on the contents of inorganic cations (C = Na + K + Mg +Ca + NH_4), inorganic anions (A = Cl + H_2PO_4 + NO_3 + SO_4), (C-A), K and NO_3 in the tops. Experiment 1.

due to changes in K and NO_3 content. Potassium was lower, probably as a consequence of competitive inhibition. Decreased nitrate assimilation caused no increase in nitrate accumulation in the tops, because uptake of NO_3 was also inhibited. The water-soluble sugar content of the tops increased slightly from 6.4 to 7.4 % of DM with increase in NH_4 concentration. This agrees with results of Kirkby (1968) on white mustard. Breteler (1973) found a lower sugar content in sugar-beet plants supplied with ammonium in place of nitrate, but in his experiments pH of the medium was kept accurately constant. Perhaps, the effect of acidity of the nutrient medium on plant growth is involved.

Experiment 2. Ammonium concentrations up to 100 meq/litre in the incubation medium decreased NRA by ultimately 38 % in vitro (Fig. 5). As NRA in Experiment 1 (Fig. 1) dropped by 77 % in vivo, whilst the incubated plant extracts contained not more than 0.5 meq NH_4/litre, the decrease in nitrate metabolism in the intact wheat tops could not be due to NRA inhibition by NH_4 ions in the tissue.

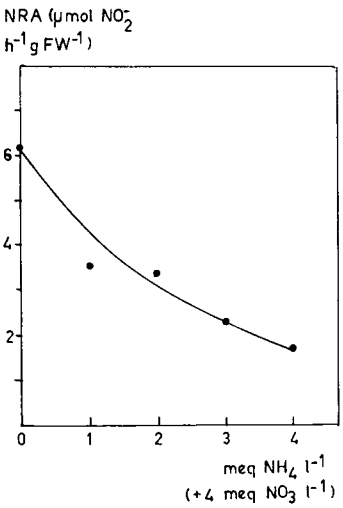

Fig. 5. The effect of the NH_4 concentration in the incubation medium on NRA of a desalted extract of wheat tops. The dashed line represents NRA without NH_4. Experiment 2.

Experiment 3. Highest in vitro NRA's were obtained with the nitrates of K and Na (Table 3). The divalent cations Ca and Mg gave lower NRA's, and NH_4NO_3 was intermediate in its effect. Compared with the non-desalted crude extract (no GFC) NRA was stimulated only by K and Na. In the presence of NH_4, the NRA was still considerable. Nitsos & Evans (1966) found the highest induction rate of NR in *Neurospora crassa* with K present in the nutrient solution.

Experiment 4. The presence of some amides and amino acids in the incubation medium (in vitro tests) increased rather than decreased the NRA. Stimulation ranged between 7 % (γ-amino butyric acid) and 66 % (asparagine). Apparently, the increased content of free amino acids in the plants grown in NH_4 nutrition (Fig. 3), bore no relation to

Table 3. Effect of several cations on in vitro NRA of a desalted extract of wheat tops. Cations present as nitrates (10 meq/litre incubation medium). NRA in μmol NO_2-h^{-1}gFW^{-1}. Bottom line represented NRA of a non-desalted extract.

Nitrate of	NRA
Ca	1.5
Mg	1.9
Na	2.6
K	3.0
NH_4	2.1
No GFC	2.4

their decreased NRA. Morton (1956) found that amino acids in a casein hydrolysate did not reduce NRA in mould fungi. Smith & Thompson (1971a, b) found that ammonium and a variety of aminoacids did not affect the in vitro NRA from barley roots and *Chlorella,* whereas these compounds reduced NR induction in vivo in *Chlorella,* indicating that NRA *per se* was not involved.

Discussion

Data of Experiment 1 prove that NH_4 uptake prevailed after 2 days in (NO_3 + NH_4) nutrition. Keeping pace with the ammonium concentration in the nutrient solution, NH_4 was also assimilated at a higher rate than NO_3.

Concerning plant composition, NH_4 assimilation increased free amino acids and amides and decreased carboxylates (C-A), alkali cations (especially K) and NO_3 in the tops. The decrease in K and NO_3 in the tissue seems too small to account for the decrease in NRA.

In sugar-beet plants, the contribution of the youngest leaves to the total NRA of the aerial parts was considerable and high NRA in those leaves coincided with low nitrate contents (van Egmond & Breteler, 1972). Thus, the fall in NO_3 uptake and NO_3 content in the tops may affect total NRA more than can be accounted for by the decrease in average NO_3 content. Data of Experiments 2, 3 and 4 indicate that the activity *per se* of the investigated enzyme was suppressed by NH_4 ions, and not by free amino compounds. However, free NH_4 accumulation was low and also occurred in the tops of NO_3 plants, although at a still lower level than in plants with additional NH_4 in the medium.

Since half-life time of NR is about 4 hours (Beevers & Hageman, 1969), its synthesis plays an important role in the in vivo activity. Investigations into the effect of NH_4 nutrition on NR induction produced variable results. In radish and corn (Beevers et al., 1965; Ingle et al., 1966; Schrader & Hageman, 1967), perennial ryegrass (Bowerman & Goodman, 1971) and wheat (Minotti et al., 1969), NR induction was not depressed by NH_4 absorption. In a review Filner et al. (1969) concluded that in contrast with

higher plants, NR induction in fungi and algae is inhibited by NH_4 in the nutrient medium. However, the data of Joy (1969) on *Lemna*, Smith & Thompson (1971a) on barley roots and Candela et al (1957) on cauliflower give evidence of decreased NR induction on media containing NH_4 in higher plants as well.

The relatively small depressing effect of NH_4 ions on enzyme inactivation and the stimulatory effect of amino acids readily synthesized from ammonium are indications of hampered enzyme synthesis during the 2 days of Experiment 1, unless factors associated with other constituents, changed by NH_4 nutrition, are involved. One of these could be the change in reducing power in the cells. Kessler (1964) stated that the nitrate reductase activity is linked to all processes that generate reduced pyridine nucleotides. In cotton leaf discs under anaerobiosis, the ratio oxidized: reduced NAD increased from 0.15 to 0.43 as a consequence of NH_4 nutrition (Radin, 1973). Mould fungi contained 20 % less H donors in NH_4 medium than in NO_3 medium (Morton, 1956). The interaction between NH_4 nutrition, oxygen and NO_3 reduction in *Aerobacter aerogenes* (van 't Riet et al., 1968) and in *Escherichia coli* (Showe & DeMoss, 1968) suggested the existence of a redox-sensitive repressor which mediates NR regulation. Smith & Thompson (1971a) also suggest that the supply of reducing power acts as a limiting factor in the NH_4 – NRA interaction. All cited data suggest that the influence of nitrogen source on the redox situation is responsible for the regulation of enzymatic nitrate reduction.

Our conclusion is that up till now there is no conclusive evidence that one single factor is responsible for the NH_4-induced repression of nitrate reduction in plants.

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