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Nitrate or ammonium nutrition in french bean

S. CHAILLOU, J.-F. MOROT-GAUDRY, C. LESAINT, L. SALSAC and E. JOLIVET
Laboratoire du Metabolisme et de la Nutrition des Plantes, I.N.R.A., route de st-Cyr, F-78000 Versailles, France

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Summary Bean Plants were grown in a greenhouse in sand irrigated with nutrient solutions containing either 2 m*M* NO_3^- or 2 m*M* NH_4^+. After 45 days fresh weight of NH_4^+ plants was half that of NO_3^- plants. Cation concentration in NH_4^+ plants was 30% less than in NO_3^- plants. Amino acids (SER, ASN, GLN) accummulated 3 to 10 times more in NH_4^+ plants. The concentration of organic acids (malic, malonic, citric) was 10 to 30 times higher in NO_3^- plants. The ATP-costings for the synthesis of amino acids and organic acids in NH_4^+ plants was half that of NO_3^- ones: therefore it could not account for the reduction of growth in the ammonium-fed plants.

Introduction

Ammonium nitrogen nutrition is known to depress plant growth as compared to nitrate nitrogen nutrition: the dry weight of tomato, mustard, buckwheat and Chenopodium grown in NH_4^+ nutrient solutions was 30 to 50% of that measured in NO_3^- solutions[2,6,7]. The opposite result could be expected, since NH_4 is a reduced N form, so that ammonium-fed plants save the energy required for the reduction of NO_3^-, i.e. 15 ATP per mole. NH_4^+ or urea fed plants, as compared to NO_3^--fed ones, are characterized by a smaller accumulation of cations[2,4,6,7], by a greater concentration of amino acids[3,10] and by a decrease in the pool of organic acids[2,6,7]. One hypothesis to explain the lower growth in NH_4^+-fed plants is that they spend much more energy (ATP) for the synthesis and accumulation of amino acids than do NO_3^--fed plants for originates.

To test this hypothesis, bean plants were grown in nutrient solutions containing either nitrate or ammonium or no nitrogen. After 1.5 month of culture fresh and dry weight, and the contents of cations, organic acids and amino acids of the plants were determined.

Materials and methods

Bean plants (*Phaseolus vulgaris* L. cv. Rugally) were grown in a greenhouse at Versailles during April and May 1984 on silica sand irrigated daily (100 ml every 15 min) with the appropriate nutrient solution. The nitrate solution contained: 1 m*M* $Ca(NO_3)_2$, 1.30 m*M* KH_2PO_4, 0.45 m*M* K_2SO_4, 0.75 m*M* $CaCl_2$, 0.50 m*M* $MgSO_4$, 0.15 m*M* $CaSO_4$, 0.20 m*M* NaCl and micronutrients. The ammonium solution contained: 1 m*M* $(NH_4)_2SO_4$, 1 m*M* KH_2PO_4, 0.30 m*M* K_2HPO_4, 0.05 m*M* K_2SO_4, 0.75 m*M* $CaCl_2$, 0.28 m*M* $MgSO_4$, 0.28 m*M* $CaSO_4$, 0.20 m*M* NaCl and micronutrients. The solution without nitrogen contained: 1 m*M* KH_2PO_4, 0.30 m*M* K_2HPO_4, 0.45 m*M* K_2SO_4, 0.75 m*M* $CaCl_2$, 0.40 m*M* $MgSO_4$, 0.75 m*M* $CaSO_4$, 0.20 m*M* NaCl and micronutrients. Fe (0.60 mg l^{-1}) was supplied with EDTA (ethylene-diamine tetraacetic acid) in each solution. The pH of the nutrient solutions were 5.5, 6.3 and 6.3, respectively, before use After use by the plants (drainage solution), the pH of NH_4^+ solution did not fall below 5.0; it did not rise over 6.0 in NO_3^- solution, and it was not modified in N-deprived solution. Plants grown in the N-deprived solution were inoculated with *Rhizobium phaseoli.* The inoculation took place twice: one time at the beginning of the culture, another time after one week. The nodules became effective after 3 weeks of culture. For nitrate- or ammonium-fed plants, one lot was inoculated, another lot was not. After 45 days plants were removed, weighed and dried or lyophilized. After tissue extraction and preliminary fractionation, the content of cations was determined by flame emission and atomic absorption spectrophotometry. The organic acids were separated by liquid-liquid chromatography. The amino acids were separated on a cationic resin (Hamilton HPAN 90 Li). They were eluted with a lithium citrate buffer. All the plants of each lot were treated together for biochemical analysis.

Results and discussion

Final fresh weight of NH_4^+-fed plants was reduced by 50% as compared to NO_3^- ones and plants grown without mineral N had 80% less fresh matter than NO_3^--fed plants (Table 1). Total cation content was 30% less in NH_4^+ plants than in NO_3^- ones. Plants grown without N had an intermediate content between NO_3^- and NH_4^+ ones (Table 2). The greatest differences were found in the roots for K^+ and Ca^{2+} (data not shown). The concentration of amino acids was 30% less in leaves of NO_3^--fed plants, 60% in stems, 75% in roots, as compared to NH_4^+ plants (Table 3). The lowest amino acid concentration was observed in plants without N in the nutrient solution. The difference between NO_3^--and NH_4^+-fed plants were especially marked for SER in leaves (4 to 5 times higher in NH_4^+ plants), and ASN and GLN in stems and roots (3 to 10 times higher in NH_4^+ plants) (data not shown). Organic acid content was 5 to 8 times greater in leaves and stems of NO_3^--fed plants as compared to NH_4^+ ones (Table 3). Differences were less marked in roots. Plants grown without N had intermediate concentrations. Malonate (the characteristic acid of french bean), malate and citrate were the most affected organic anions (10 to 30 times higher in NO_3^- plants) (data not shown). It may be noted that the inoculation with *Rhizobium* had very little effect on the mineral and organic acid content of NO_3^--or NH_4^+-fed plants. The nodules formed on the nitrate or ammonium nutrient solutions were very small and ineffective, as measured by the acetylene reduction test.

Table 1. Fresh weight of 45 days old bean plants grown on nutrient solutions containing either nitrate or ammonium or no nitrogen. Each value is the mean of 9 or 18 replicates ± confidence interval at $p = 0.05$

	Fresh weight (g plant^{-1})				
	NO_3^-	NO_3^- inoculated	NH_4^+	NH_4^+ inoculated	No N inoculated
Leaves + stems	63.2 ± 5.8	55.3 ± 7.2	29.4 ± 4.4	26.9 ± 3.1	9.9 ± 0.8
Roots + nodules	9.9 ± 0.9	16.5 ± 2.1	5.8 ± 0.9	8.5 ± 1.0	3.8 ± 0.3

Table 2. Total cation content in 45 days old bean plants grown on nutrient solutions containing either nitrate or ammonium or no nitrogen

	Cation content (mg g^{-1} DW)				
	NO_3^-	NO_3^- inoculated	NH_4^+	NH_4^+ inoculated	No N inoculated
Leaves + stems	66.5	63.4	42.5	41.3	49.0
Roots	54.1	58.5	33.7	40.3	56.6

Table 3. Total concentration of amino acids and organic anions in 45 days old bean plants grown on nutrient solutions containing either nitrate or ammonium or no nitrogen; inoc = inoculated

	Total amino acids concentration (μmol g^{-1} DW)					Total organic anions concentration (μeq g^{-1} DW)				
	NO_3^-	NO_3^- inoc	NO_4^+	NO_4^+ inoc	No N inoc	NO_3^-	NO_3^- inoc	NO_4^+	NO_4^+ inoc	No N inoc
Leaves	66	69	99	90	66	1896	1622	241	201	548
Stems	128	109	302	276	69	1200	1071	213	224	616
Roots	41	61	168	221	33	354	396	146	165	547

The synthesis of organic and amino acids by the plants requires a certain amount of energy, which has been called "prices of metabolits" by Atkinson[1], and expressed in ATP equivalents for each compound. We used this Atkinson's table to calculate the total ATP equivalents spent by the plants for organic and amino acids synthesis. It appears that the whole plant grown on the nitrate medium have spent 40 mmoles ATP per g DW, against 20 for the ammonium-fed plants and 22 for the plants grown without nitrogen in the nutrient solution. Therefore the hypothesis initially proposed cannot account for the reduced growth in NH_4^+-fed plants. Another hypothesis was stated[9]: nitrate and organic anions accumulating in the vacuole together with cations constitute the main osmoticum for turgor pressure in plants. In purely ammonium nutrition, contents of organic acids and cations are low, so that the osmotic adjustment may only be realized by amino acids. Those compounds could therefore be immobilized and no longer available for synthesis of macromolecules, causing a pertubation in the normal growth of the plant. The present results stress the importance of the relations between C and N[5]. Our next purpose is to investigate, after Marques *et al.*[8], the effect of different N sources on the assimilation and distribution of C in leguminous plants.

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