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Root Respiration Associated with Nitrate Assimilation by Cowpea[1]

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ABSTRACT

Nitrate uptake by roots of cowpea (*Vigna unguiculata*) was measured using $^{15}NO_3^-$, and the energy cost to the root was estimated by respirometry. Roots of 8-day-old cowpea seedlings respired 0.6 to 0.8 milligram CO_2 per plant per hour for growth and maintenance. Adding 10 millimolar NO_3^- to the root medium increased respiration by 20 to 30% during the following 6 hours. This increase was not observed if the shoots were in the dark. Removal of NO_3^- from the root medium slowed the increase of root respiration. The ratios of additional respiration to the total nitrogen uptake and reduced nitrogen content in roots were 0.4 gram C per gram N and 2.3 grams C per gram N, respectively. The latter value is close to theoretical estimates of nitrate assimilation, and is similar to estimates of 1 to 4 grams C per gram N for the respiratory cost of symbiotic N_2 fixation.

Applying nitrate to legumes usually stimulates vegetative dry matter production and improves seed yield compared with plants dependent on symbiotic N_2 fixation throughout growth. Soybean plants grown in the field are estimated to obtain from 25 to 60% of their total N from symbiotic N_2 fixation (17). The remainder of the N in plants must be derived from soil, primarily as NO_3^-.

Nitrate assimilation, like symbiotic N_2 fixation, requires energy to provide the ATP and reducing power to reduce NO_3^- to NH_4^+, and to incorporate the NH_4^+ into organic compounds. The energy costs to legumes using nitrate have been estimated in several studies (1, 9, 12, 15). Almost all experiments, however, have been done by comparing energy costs between non-nodulated NO_3^- fed plants and nodulated N_2-fed plants. Pate *et al.* (12) estimated CO_2 loss per unit N assimilated in white lupin roots as 10.2 mg C/mg N by the nodulated root, and 8.1 mg C/mg N by the nonnodulated NO_3^--fed roots. Neves *et al.* (9) estimated C consumption by nodulated and nonnodulated cowpea roots as 8.0 and 4.5 mg C/mg N assimilated, respectively. Atkins *et al.* (2) reported that CO_2 loss of below-ground parts of cowpea is less in nonnodulated, NO_3^--fed plants than in nodulated, N_2-fed plants. Ryle *et al.* (15) also reported for soybean, cowpea, and white clover that plants fixing all their nitrogen respire 11 to 13% more of their carbon than equivalent plants lacking nodules and using NO_3^-. These values include energy costs for maintenance and growth during N_2 fixation or NO_3^- assimilation. There is little information on energy cost to the legumes of taking up, transporting, and reducing NO_3^-.

Recently Mahon (7) proposed an equation in order to consider the relationship between total root and nodule respiration and N_2 fixation in a symbiotic system. The equation could be modified for NO_3^- assimilation as follows:

$$R = Rm\ W + Rg\ dw/dt + Ra\ (NR\text{-ases})$$

where R is the total respiration of roots, W is the root dry weight, NR-ases is NO_3^- assimilating activity, and Rm, Rg, and Ra are the maintenance, growth, and assimilation coefficients, respectively. If NO_3^- assimilating activity can be varied in a short-term experiment, the change of weight or growth rate will not be significant. Then we can determine the relationship between respiration and NO_3^- assimilating activity. With this approach, we measured NO_3^- uptake and incorporation in cowpea (*Vigna unguiculata*) using $^{15}NO_3^-$, and estimated the energy cost to the root by respirometry.

MATERIALS AND METHODS

Plant Material. Cowpea seeds (*Vigna unguiculata* cv California blackeye) were germinated in well-washed vermiculite in a greenhouse. At 5 d after planting, seedlings were transferred to, four per pot, 18 cm-diameter pots containing 3 L of N-free culture solution (1.6 mM K_2SO_4, 2 mM $MgSO_4 \cdot 7H_2O$, 0.8 mM KH_2PO_4, 0.2 mM K_2HPO_4, 7.0 mM $CaCl_2 \cdot 2H_2O$, 0.022 mM Fe-EDTA, 0.116 mM H_3BO_3, 1 μM $NaMoO_4 \cdot 2H_2O$, 30 μM $MnSO_4 \cdot H_2O$, 3.8 μM $ZnSO_4 \cdot 5H_2O$, and 2.5 μM $CoCl_2 \cdot 6H_2O$) and grown hydroponically for 3 d in a lightroom. The day/night regime was 15 h/9 h, and light intensity at plant level was 500 μE m^{-2} s^{-1}. The culture solution was aerated at 1.5 L/min^{-1}. At 8 d after planting, the cotyledons were almost withered, but unifoliolate leaflets had not yet shown symptoms of N deficiency.

Respiration Experiments. The 8-d-old seedlings were transferred, three per respirometer, to respirometers in which the shoots were separated from the roots with a nontoxic sealant Terostat (Teroson GnGH, Heidelberg, FRG). The shoots were under a bank of metal halide lamps giving a PAR of 350 μE m^{-1} s^{-1}. The shoots were covered with a glass chamber (45 L) through which air (10 L min^{-1}) was passed. In some experiments the chamber was covered with black cloth to exclude light. The root chambers, 425 ml volume, contained 180 ml of culture solutions (pH 6.0). The composition of the culture solution was the same as described above. After 1 h, N as $NaNO_3$ or KNO_3 was added to the solution for some experiments. Air, 0.75 L min^{-1}, was passed through the root chamber to an IR gas analyzer (model 865, Beckman Instruments, Inc.) for ΔCO_2 measurement relative to the input air.

N Analyses. Plant parts were air dried at 60°C, ground, and 50 mg subsamples used for N determinations. Total N was estimated after Kjeldahl digestion using Devarda's alloy to reduce NO_3^--N (6). The dry leaves were extracted by vigorous shaking for 1 h with six volumes (ml/g dry weight) of extracting solution consisting of: 25 ml $Al_2(SO_4)_3$, 20 mM H_3BO_3, 15 mM Ag_2SO_4, 25 mM NH_2SO_3H (pH 3). Nitrate in the extracts was determined by a nitrate ion electrode (model 93-07, Orion Research Inc.,

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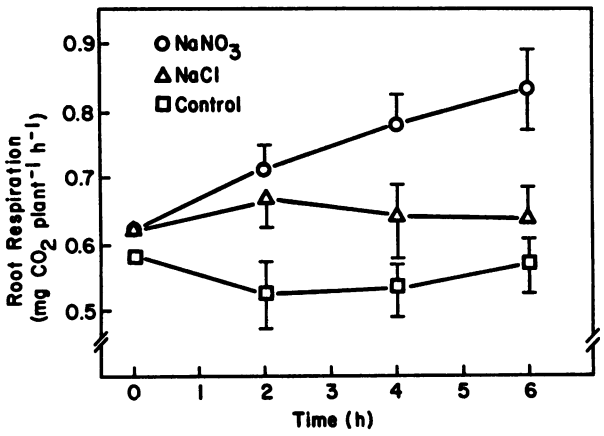

FIG. 1. Respiration of cowpea roots during 6 h after treatment with 10 mM $NaNO_3$ (O), 10 mM NaCl (Δ), and unamended nutrient (□). There were three replicates respirometers in each treatment. The average dry weights per root were 75, 81, and 66 mg, respectively.

Cambridge, MA). Nitrate in roots and stems was extracted with 0.2 M NH_4Cl solution adjusted to pH 8.5 with 0.1 M NH_4OH, as described in the Orion Analytical Methods Guide. Nitrate was reduced to nitrite using a Cu-Cd column, and the nitrite determined by reaction with sulfanilamide and N-1-naphthylethylenediamine dihydrochloride (18). Reduced-N was calculated by subtraction of NO_3^--N from total N.

^{15}N enrichment of total N was measured by a Micromass mass spectrometer (model 622, VG Isotopes Ltd. Winsford, Cheshire CW7 3BX).

Nitrate Reductase Activity. The plant parts were homogenized in a mortar and pestle with 0.25 M K-phosphate (pH 7.5) containing 1 mM L-cysteine and 1 mM EDTA at 4°C. The homogenate was centrifuged at 25,000*g* for 10 min and nitrate reductase activity in the supernatant was measured by the procedure of Hageman and Hucklesby (5). The assay mixture contained 100 μmol K-phosphate (pH 7.5), 10 μmol KNO_3, 0.4 μmol NADH, and enzyme extract in a final volume of 2 ml. The reaction was terminated at 15 min by additions of 0.2 ml of 0.5 M zinc acetate and 0.2 ml of phenazine methosulfate (46 mg/L) (10). After standing 30 min, the mixtures were centrifuged at 1,000*g* for 5 min. The nitrite concentration of the supernatant was determined with sulfanilamide and N-1-naphthylethylenediamine dihydrochloride.

RESULTS

CO_2 production by cowpea roots was measured for 6 h after adding 10 mM $NaNO_3$ (Fig. 1). There was an increase in respiration from roots receiving 10 mM $NaNO_3$ but not from those treated with 10 mM NaCl or fresh unamended culture solution. The requirement for NO_3^- to maintain increased root respiration is shown in Figure 2. At 6 h, the root medium was replaced with fresh medium containing 10 mM $NaNO_3$, or with fresh medium lacking nitrate. Root respiration was measured for another 6 h. Replacement with fresh medium containing NO_3^- maintained the respiration increase. On the contrary, after removal of NO_3^- the respiration rate hardly changed. The increase in root respiration was only observed if the shoots were in the light (Fig. 3).

Nitrate reductase activity was induced in the seedlings within 2 h after addition of 10 mM $NaNO_3$ (Fig. 4). The activity in roots was lower than in leaves and stems.

The change in respiration rate of three samples, consisting of three plants each, was monitored for 8 h after addition of 10 mM KNO_3 containing 97.9 atom % ^{15}N excess. These samples were used for analysis of total-N, NO_3-N, and ^{15}N. ^{15}N enrichment of total-N was highest in roots and was almost twice that in unifoliolate leaflets. ^{15}N enrichment in stems with petioles and trifolioliate leaves was quite low, and was a third of that in unifoliolate leaves (Table I). Forty-three percent of the total N taken up during 8 h remained in roots, while 51% and 6% were partitioned to the unifoliolates, and stem with petioles and bud, respectively. Sixty-three percent of the N taken up and remaining in the roots was NO_3^-. Approximately 95% of the N in unifoliolates was reduced-N. The proportions of NO_3^--N and reduced-N were equal in stem with petioles and trifoliolate bud.

There was no detectable lag in the increase in respiration. During 8 h, the additional respiration stimulated by NO_3^- was 164 μg C $plant^{-1}$ (Table I). The energy cost of NO_3^- assimilation in cowpea roots was calculated from the data. The values of ΔC loss from roots/Δ total-N in whole plant (g/g ± SE) and ΔC loss from roots/Δ reduced-N in roots (g/g ± SE) were 0.4 ± 0.03 and 2.60 ± 0.55, respectively.

DISCUSSION

At 8 d, cowpea seedlings have almost exhausted their cotyledonary reserves. At this age, roots respired 0.6 to 0.8 mg CO_2 $plant^{-1}$ h^{-1} for maintenance and growth and this rate was constant for several h. When NO_3^- was supplied to the roots, root respiration increased approximately 20 to 30% (Fig. 1). This increase in respiration required the continued presence of NO_3^- (Fig. 2).

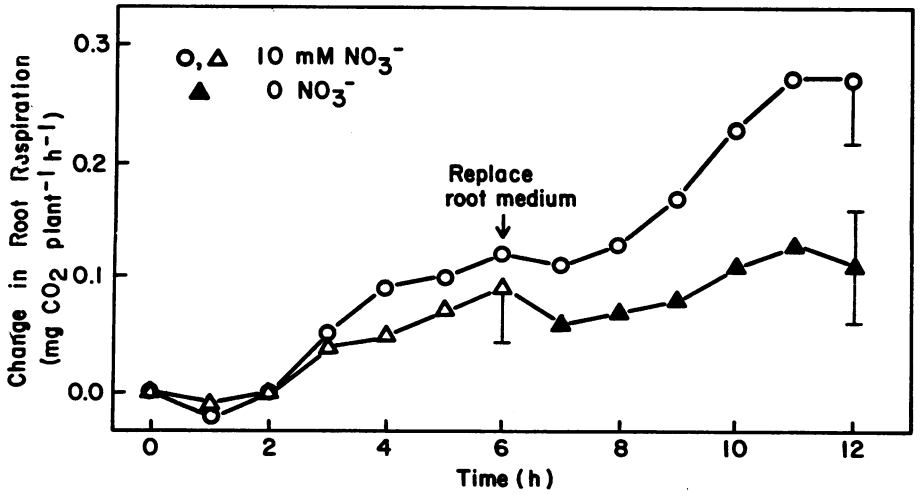

FIG. 2. Influence of presence or removal of nitrate on respiration (±SE) of cowpea roots. Both sets of plants (O, Δ) were incubated in 10 mM $NaNO_3$. At 6 h, root medium was replaced with fresh medium containing 10 mM $NaNO_3$ (O) or fresh medium lacking nitrate (▲). There were three replicate respirometers in each treatment. The root respirations at the beginning of the experiment were 0.74 and 0.64 mg CO_2 $plant^{-1}$ h^{-1}, respectively. The average dry weights per root were 73 and 70 mg, respectively.

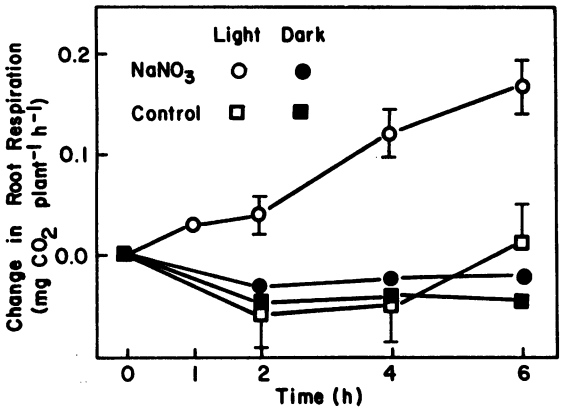

FIG. 3. Changes in root respiration (±SE) of cowpea seedlings with shoots in the light during 6 h after treatment in unamended nutrient (□) or with 10 mM $NaNO_3$ (○). (■, ●). The same treatments with shoots in the dark. The initial root respirations before treatment were from 0.63 to 0.66 mg CO_2 $plant^{-1}$ h^{-1}. There were three replicates in each treatment. The dry weights per root were from 66 to 70 mg.

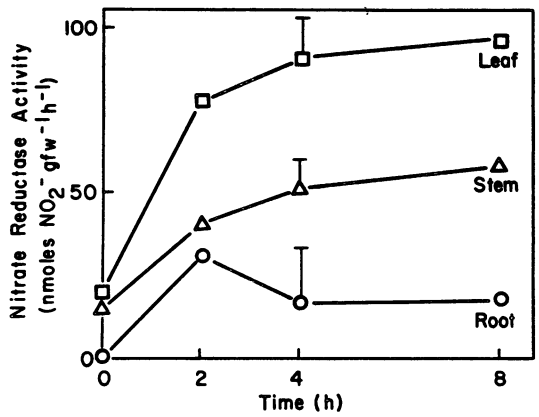

FIG. 4. Induction of nitrate reductase (±SE) in cowpea seedlings after addition of 10 mM $NaNO_3$. The activities of root (○), stems (△), and unifoliolate and first trifoliolate leaflets (□) were measured with the *in vitro* assay. The average dry weight of root, stem, and leaflets of companion seedlings was 69, 60, and 112 mg, respectively.

Table I. *Nitrogen Content, Atom Percent ^{15}N Excess, Increase in Plant N, and Increase in C Loss by Root Respiration during 8 h in the Presence of 10 mM KNO_3 Containing 97.9 atom % ^{15}N Excess*

The values are averages and SE of three replicates, each consisting of three plants. Reduced N was calculated as the difference between Δ total-N and Δ NO_3^--N.

Plant Part	Total N	^{15}N excess	Δ Total N	Δ NO_3-N	Δ Reduced-N	Δ C loss
	mg plant^{-1}	*atom %*		*μg plant^{-1}*		
Roots	2.026 ± 0.140	8.36 ± 0.08	173 ± 13	109 ± 3	64 ± 10	164 ± 9
Unifoliolate leaves	4.317 ± 0.207	4.46 ± 0.19	206 ± 12	10 ± 1	197 ± 13	
Stem with petioles + trifoliolate bud	1.560 ± 0.051	1.44 ± 0.20	23 ± 3	11 ± 1	12 ± 3	

The increase of root respiration required light on the shoots (Fig. 3), indicating that photosynthate supply from shoot to root may be necessary (11). A lag phase before the increase of root respiration was frequently observed in some lots of seedlings (*cf.* Fig. 2). Similar lags have been reported previously for NO_3^- absorption (10, 16) and nitrate reductase induction (10) in roots.

The energy cost of nitrate assimilation in cowpea roots was calculated as 2.60 ± 0.55 g C/g reduced-N based on ΔC loss from roots/Δ reduced N in roots. In our experiments, the respiration rate of roots of untreated plants was almost constant over 6 to 8 h. Therefore, the estimates presented here could indicate the total energy cost of nitrate uptake, transport, reduction, and incorporation occurring in the root. However, the value is slightly high compared to the theoretical value, 2.21 g C/g N assimilated, which is calculated assuming that NO_3^- is reduced and incorporated into asparagine (1, 12). In our calculations, transport of the reduced N which was transported from roots to shoots through xylem was not considered. However, it is likely that in the present study only a small proportion of the N taken up during the measurement period was transported to shoots as reduced N. Rufty *et al.* (14) reported that when soybean roots are fed ^{15}N-NO_3, considerable amounts of reduced ^{14}N from preexisting pools are transported into xylem, but almost all of the ^{15}N which is exported to the shoot in xylem was in the $^{15}NO_3^-$ form.

Crafts-Brander and Harper (4) also reported that a low percentage of newly taken up nitrate is in the form of reduced N *versus* NO_3^--N in xylem exudate: 5.2% at 1.5 h and 10.7% at 3 h after transferring plants into ^{15}N-NO_3^- solution (these values were calculated from their data). These values are probably high compared to those obtained in our study because they were obtained from plants which had an active nitrate reduction system in the roots when ^{15}N-NO_3^- was applied. The roots used here did not have nitrate reductase activity when the NO_3^- was supplied, and when the nitrate reductase acticity was induced in roots it was much lower in activity than that in leaves or stems (Fig. 4). This indicates that almost all of the N was transported from root to shoot as NO_3^-. We conclude that the estimated cost of 2.6 g C/g reduced-N for NO_3^- assimilation in roots is consistent with our knowledge of the process. The cost is of the same order of magnitude as estimates of 1 to 4 g C/g N for the respiratory cost of N_2 fixation (3, 8, 13).

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