Nitrate Reduction in Roots and Shoots of Barley (*Hordeum vulgare* L.) and Corn (*Zea mays* L.) Seedlings

I. ¹⁵N STUDY

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ABSTRACT

Nitrate reduction in roots and shoots of 7-day-old barley seedlings, and 9-day-old corn seedlings was investigated. The N-depleted seedlings were transferred for 24 h or 48 h of continuous light to a mixed nitrogen medium containing both nitrate and ammonium. Total nitrate reduction was determined by ¹⁵N incorporation from ¹⁵NO₃⁻, translocation of reduced ¹⁵N from the roots to the shoots was estimated with reduced ¹⁵N from ¹⁵NH₄⁺ assimilation as tracer, and the translocation from the shoots to the roots was measured on plants grown with a split root system. A model was proposed to calculate the nitrate reduction by roots from these data. For both species, the induction phase was characterized by a high contribution of the roots which accounted for 65% of the whole plant nitrate reduction in barley, and for 70% in corn. However, during the second period of the experiment, once this induction process was finished, roots only accounted for 20% of the whole plant nitrate reduction in barley seedlings, and for 27% in corn. This reversal in nitrate reduction localization was due to both increased shoot reduction and decreased root reduction. The pattern of N exchanges between the organs showed that the cycling of reduced N through the plant was important for both species. In particular, the downward transport of reduced N increased while nitrate assimilation in roots decreased. As a result, when induction was achieved, the N feeding of the roots appeared to be highly dependent on translocation from the leaves.

It is now well established that nitrate reduction can occur in both roots and shoots of higher plants (3). Nevertheless, the contribution of these organs to whole plant nitrate reduction remains generally uncertain. Only indirect methods have been involved.

The nitrate reduction in roots is believed to be significant when the major form of nitrogen translocated by xylem sap is the reduced form (23). However, alteration in exudation rate due to detopping and the unknown origin of this exudate reduced N led some authors to criticize this criterion as an indicator of nitrate reduction by the roots (4, 28).

Incorporation of ¹⁵N in the reduced N fraction is a direct determination of the ¹⁵NO₃⁻ reduction, but it does not take into account endogenous ¹⁴NO₃⁻ when it is present. Furthermore, the distribution within the plant of the resulting reduced ¹⁵N may not be related to the distribution of assimilation, because of the exchanges between organs (12). As a consequence, short time

labelings are generally performed to avoid significant movement of reduced ¹⁵N from and to the roots. By this method, numerous authors (1, 4, 30) have found that root contribution to whole plant nitrate reduction may be important during the early phase of nitrate utilization by seedlings. Unfortunately, there are no results to confirm this when induction is fully achieved.

Concerning barley and corn, contradictory conclusions are found in publications. In both species, NRA[1] is predominantly located in the shoots (13, 26). Nevertheless, the reduced N contents of the xylem saps are relatively high (23), and excised roots seem to be capable of significant reduction (8, 18).

The aim of this work was to investigate the root nitrate reduction in N-deprived seedlings by ¹⁵NO₃⁻ incorporation. An experimental procedure and a calculation model are proposed in order to estimate transports of reduced ¹⁵N between roots and shoots in intact seedlings and consequently to determine how much assimilation can be attributed to each organ. This labeling is long enough to enable us to characterize the use of nitrate once the induction process is established.

MATERIALS AND METHODS

Culture. Barley (*Hordeum vulgare* L. var Bérénice) seeds were germinated for 2 d on cheesecloth at the surface of distilled H₂O, and the seedlings were grown on a N-free basal nutrient solution containing 0.2 mM MgSO₄, 0.2 mM KH₂PO₄, 0.5 mM CaCl₂, 20 μM Fe citrate, 1 μM MnSO₄, 0.1 μM CuSO₄, 6 μM H₃BO₃, 0.2 μM (NH₄)₆Mo₇O₂₄ at pH 5.6. Photoperiod and temperature were 16 h with 40 W·m⁻² at 25°C and the 8-h-dark period was at 22°C. Seven-d-old seedlings were used for subsequent labeling. At this stage, the seedlings had five or six developed roots and one fully expanded leaf.

Corn (*Zea mays* L. var INRA 508) seedlings were obtained using the same procedure, except for a few modifications: the seedlings with the PS root, one SS root, and their first leaf were grown until the age of 9 d on a N-free solution containing 0.2 mM CaSO₄.

Labeling. The 7-d-old barley seedlings were divided into three

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[1] Abbreviations: NRA, nitrate reductase activity; NR, nitrate reductase; Ar, accumulation in roots of reduced ¹⁵N from ¹⁵NO₃⁻; As, accumulation in shoots of reduced ¹⁵N from ¹⁵NO₃⁻; Anl, accumulation of reduced ¹⁵N from ¹⁵NO₃⁻ in the side of the split root system grown on the nonlabeled nutrient solution; Tx, translocation to the shoots of root-reduced ¹⁵N from ¹⁵NO₃⁻ via xylem; Tp, translocation to the roots of shoot-reduced ¹⁵N from ¹⁵NO₃⁻ via phloem; Rr, ¹⁵NO₃⁻ reduction in roots; PS, primary seminal root; SS, secondary seminal root.

groups. Each group was transferred to a special container with separate compartments allowing culture of the plants with a split root system, and containing the basal nutrient solution plus 1.5 mM KNO_3, 0.5 mM NH_4Cl, and 50 mg/L chloramphenicol. The three groups of seedlings were characterized by different ^{15}N labelings (Fig. 1):

Experiment 1. The first group of seedlings was supplied on each side of the split root system with a nutrient solution containing $^{15}NO_3^-$ (^{15}N excess = 16.2%).

Experiment 2. The second group of seedlings was supplied on each side of the split root system with a nutrient solution containing $^{15}NH_4^+$ (^{15}N excess = 13.5%).

Experiment 3. The third group of seedlings was supplied with a nutrient solution containing $^{14}NO_3^-$ on one side of the split root system and $^{15}NO_3^-$ (^{15}N excess = 52.2%) on the other side.

The experiments were carried out in continuous light for 48 h and at a constant temperature (25°C). The pH of the nutrient solution was maintained at 5.6 with a pH-stat apparatus.

Labelings with corn seedlings were made according to the same procedure. In experiment 3, the split root system was constituted by the PS root for the ^{14}N-fed side, and by the SS root for the ^{15}N-fed side. The nutrient solution common to the three experiments contained 0.2 mM $CaSO_4$, 1.4 mM KNO_3, and 0.1 mM NH_4Cl. The duration of the experiments was 24 h. The ^{15}N excesses were 28.4, 24.1, and 85.5% for experiments 1, 2, and 3, respectively. The ammonium concentration in the media was monitored every hour, and maintained close to 0.1 mM by additions of 0.1 M NH_4Cl. No control of medium pH was made.

Harvest and Measurements. For each experiment the root and shoot contents of reduced ^{15}N were measured in 10 samples of four barley seedlings harvested 24 and 48 h after the transfer and in 10 samples of two corn seedlings harvested 12 and 24 h after the transfer. The roots were washed carefully with distilled H_2O and separated from the shoots. In experiment 3, the roots were separated into two samples corresponding to each side of the split root system. The fresh organs were weighed and dried at 70°C.

The dry material was submitted to three successive extractions with boiling 80% ethanol to separate soluble and insoluble nitrogen fractions. Each extract was then dried and treated with 2 ml H_2O, 1 ml H_2O_2 (30%), and 0.1 ml 36 N H_2SO_4 and heated

FIG. 1. Schematic representation of the labeling procedure used to determine the contribution of the roots and the shoots to the whole plant nitrate reduction. The three experiments were conducted at the same time, using the same lot of N-depleted seedlings. The composition of the nutrient solution was similar in the three experiments: 1.5 mM KNO_3 + 0.5 mM NH_4Cl (barley), and 1.4 mM KNO_3 + 0.1 mM NH_4Cl (corn).

for 48 h at 100°C to remove nitrate from the sample (22). The remaining soluble reduced nitrogen was added to the insoluble reduced nitrogen fraction. The total reduced nitrogen was then mineralized by Kjeldahl procedure. Ammonium was recovered after diffusion in Conway's dishes (7). Part of the solution obtained was used for colorimetric determination of ammonium using Nessler's reagent. The other part was prepared by Dumas' procedure for isotopic determination of ^{15}N: A sample equivalent to 3 μg of ammonium nitrogen was put in a Pyrex tube and dried at 70°C. A few pieces of CaO and CuO were then added in the tube which was subsequently vacuum sealed at 10^{-3} Torr. Ammonium nitrogen was finally converted into N_2 by heating the tube for 4 h at 570°C. The ^{15}N excess was determined by molecular emission with a SOPRA GS1 spectrometer (9, 15). Each sample was analyzed in duplicate.

Root and shoot nitrate contents were measured in five samples of four barley seedlings harvested 6, 12, 24, 36, and 48 h after transfer, and in five samples of two corn seedlings 24 h after the transfer. The nitrate was extracted by 0.1 N HCl and assayed colorimetrically after reduction in a cadmium column (26).

Ammonium concentration in the nutrient solution was measured with Nessler's reagent.

Calculation Model for Root Nitrate Reduction. After transfer to the nutrient solution containing $^{15}NO_3^-$ (experiment 1), the quantity of reduced ^{15}N accumulated in roots (Ar) is equal to the amount of $^{15}NO_3^-$ reduced by the roots (Rr), minus the part of this root-reduced ^{15}N translocated upward to the leaves via xylem (Tx) and plus the shoot-reduced ^{15}N translocated downward to the roots via phloem (Tp). This is expressed by the equation:

$$Ar = Rr + Tp - Tx \qquad (1)$$

Ar can be measured in experiment 1: it is the root content of reduced ^{15}N when the seedlings are harvested.

Tx can be approached with experiment 2, exactly similar to the first one, except that ^{15}N is in NH_4^+ form. Nearly all ammonium is estimated to be assimilated in roots (14, 16). Therefore, the reduced ^{15}N appearing in the shoots is derived from translocation by the xylem. This translocation to the shoots was evaluated by the ratio (γ) of the amount of reduced ^{15}N present in the shoot to the amount of reduced ^{15}N of the whole seedling. This ratio represents the proportion of reduced N assimilated from NH_4^+ in roots which was subsequently translocated to the shoots. In experiment 2, it is estimated with exogenous NH_4^+. We assume (assumption 1) that this ratio could be applied to endogenous NH_4^+ resulting from root nitrate reduction (Rr), from which, consequently, the part translocated to the shoots (Tx) would be:

$$Tx = \gamma \cdot Rr. \qquad (2)$$

Tp was estimated in experiment 3 where only one side of the split root system absorbed $^{15}NO_3^-$. In this experiment, the reduced ^{15}N accumulated in the roots growing in the nonlabeled nutrient solution (Anl) was the result of the phloem transport from the shoots, and consequently constitutes a direct measurement of this downward translocation. However, for two reasons, it represents only a part of the total allocation to the whole root system. First, the reduced ^{15}N translocation to the $^{15}NO_3^-$-fed roots is not determined. If we assume this allocation is proportional to the fresh weight of the roots (assumption 2), the total phloem transport of reduced ^{15}N is Anl/α, where:

$$\alpha = \frac{\text{fresh wt of } ^{14}NO_3^--\text{fed roots}}{\text{fresh wt of the whole root system}}.$$

Second, this reduced ^{15}N accumulation (Anl) takes into account only the reduction of the nitrate absorbed by the $^{15}NO_3^-$-fed roots. The amount of reduced N ($^{14}N + ^{15}N$) from nitrate
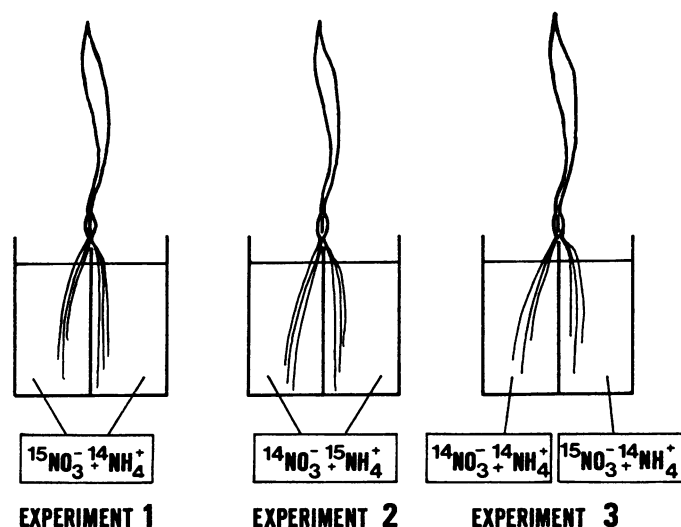
reduction in the shoot, and consequently its total allocation to the roots (Tp) can be inferred only if we correct Anl by the fraction of the root mass in contact with the isotope. Thus, assuming that nitrate uptake is proportional to the root fresh weight (assumption 3), it follows that:

$$Tp = Anl/\alpha\beta \qquad (3)$$

where

$$\beta = \frac{\text{fresh wt of } {}^{15}NO_3{}^--\text{fed roots}}{\text{fresh wt of the whole root system}}$$

As the three experiments are exactly similar, equations 2 and 3 may be used together in equation 1 to obtain:

$$Ar = Rr + Anl/\alpha\beta - \gamma \cdot Rr.$$

Then:

$$Rr = \frac{Ar - Anl/\alpha\beta}{1 - \gamma}. \qquad (4)$$

RESULTS

Reduced N and Nitrate Accumulation. The roots of barley and corn seedlings represented about 40% of the seedling mass, whereas the proportion of total reduced N accumulated in roots was 22% for barley and 25% for corn (Table I). Nitrate accumulation in barley seedlings followed a typical saturation kinetics with a higher rate in shoots than in roots (Fig. 2). In barley seedlings, the root and shoot nitrate contents were respectively 2.38 and 5.37 μmol·plant^{-1} 48 h after the transfer to the nutrient solution containing nitrate and 7.51 and 13.8 μmol·plant^{-1} in corn 24 h after the transfer (data not shown). Thus, the roots accounted for 31 (barley) and 35% (corn) of the whole plant nitrate accumulation.

Nitrate Assimilation. In barley and corn the reduced ^{15}N contents of the roots (Ar) and the shoots (As) indicated significant reduction of ^{15}NO$_3{}^-$ depending on the time following the transfer (Table II). In both species the accumulation of reduced ^{15}N was greater in the shoots. This was particularly true for barley where only 20 and 17% of the reduced ^{15}N was found in roots 24 and 48 h, respectively, after the transfer, as opposed to 43 and 32% for corn 12 and 24 h after the transfer. Total nitrate reduction by the corn seedlings increased with time following the transfer: 57% of the reduced ^{15}N was accumulated during the second

Table I. *Fresh Weight and Total Content of Reduced N in Barley and Corn Seedlings*

After growing on a N-free medium, 7-d-old barley seedlings and 9-d-old corn seedlings were transfered in light to a nutrient solution containing 1.5 mM K^{15}NO$_3$ + 0.5 mM ^{14}NH$_4$Cl (barley), or 1.4 mM K^{15}NO$_3$ + 0.1 mM ^{14}NH$_4$Cl (corn) (experiment 1). Plants were harvested 48 h (barley) or 24 h (corn) after the transfer. Values are means of 10 replicates, and SE are given in parentheses.

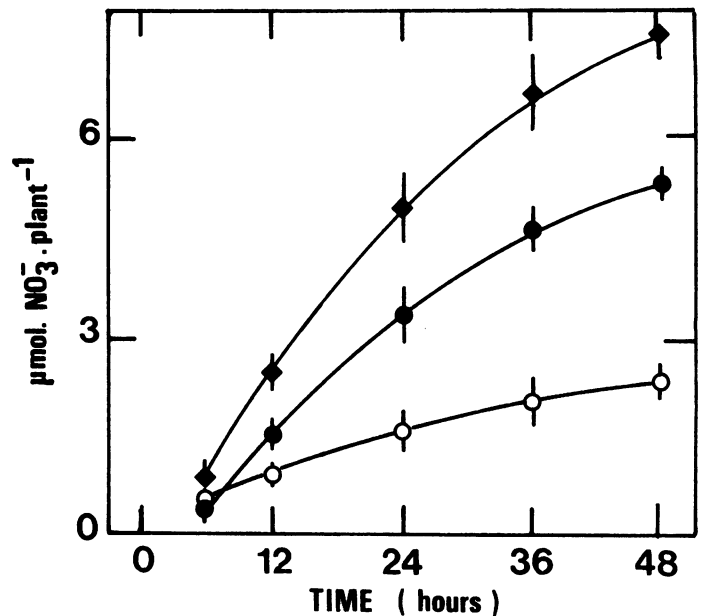| Plant Part	Fresh Weight		Total Reduced N	
	Barley	Corn	Barley	Corn
	g·plant^{-1}		*μmol·plant^{-1}*	
Roots	0.11	0.22	12.7	28.9
	(0.01)	(0.02)	(1.9)	(5.7)
Shoots	0.16	0.35	45.9	85.5
	(0.01)	(0.02)	(4.3)	(10.7)
Total plant	0.27	0.57	58.6	114.4
	(0.01)	(0.05)	(5.2)	(12.2)
	% of total			
Roots	40.7	38.6	21.7	25.3

FIG. 2. Time course of nitrate accumulation in the roots (O), the shoots (●), and whole plant (◆) of barley seedlings, after the transfer to 1.5 mM KNO$_3$ + 0.5 mM NH$_4$Cl. Vertical bars on symbols indicate SE of five replicates.

Table II. *Accumulation of Reduced ^{15}N in Barley and Corn Roots (Ar) and Shoots (As) after Exposure to ^{15}NO$_3{}^-$*

Plants were from the experiment described in Table I and harvested 24 h and 48 h (barley) or 12 and 24 h (corn) after the transfer to the labeled nutrient solution. Values are means of 10 replicates, and SE are given in parentheses.

Plant Part	Barley		Corn	
	24 h	48 h	12 h	24 h
	μmol·plant^{-1}			
Roots (Ar)	0.31	0.49	1.01	1.79
	(0.05)	(0.09)	(0.23)	(0.38)
Shoots (As)	1.23	2.33	1.33	3.72
	(0.20)	(0.27)	(0.27)	(1.16)
Total plant	1.54	2.82	2.34	5.51
	(0.25)	(0.34)	(0.48)	(1.55)
	% total			
Roots	20.1	17.4	43.2	32.5

period (12–24 h). Barley seedlings exhibited a slight decrease in their reduced ^{15}N accumulation rate between 24 and 48 h where only 46% of the total assimilation was achieved.

Ammonium Assimilation and Reduced ^{15}N Translocation to the Shoots. Barley and corn seedlings incorporated appreciable quantities of ^{15}N from ^{15}NH$_4{}^+$ (Table III). In spite of the low NH$_4{}^+$ concentration in the nutrient solution (0.1 mM for corn and 0.5 mM for barley), ammonium assimilation was equivalent to nitrate reduction in corn seedlings and much higher in barley seedlings. In this species, 57% of total ^{15}N incorporation from ^{15}NH$_4{}^+$ took place during the 2nd d after the transfer, showing no marked modification in function of time of the capacity of plants to use ammonium. At the opposite, in corn, 78% of the total reduced ^{15}N was already present in the seedlings 12 h after the transfer. This can be explained by the regular decrease with time of ammonium uptake (Fig. 3). The proportion of reduced ^{15}N from exogenous ammonium which was found in the shoots (γ) increased slightly in corn from 48 to 55%, respectively, 12 and 24 h after the transfer, and was quite steady for barley: 73

Table III. *Accumulation of Reduced ^{15}N in Barley and Corn Roots and Shoots after Exposure to $^{15}NH_4^+$*

After growing on a N-free medium, 7-d-old barley seedlings and 9-d-old corn seedlings were transferred in light to a nutrient solution containing 1.5 mM K^{14}NO$_3$ + 0.5 mM ^{15}NH$_4$Cl (barley) or 1.4 mM K^{14}NO$_3$ + 0.1 mM ^{15}NH$_4$Cl (corn) (experiment 2). Plants were harvested 24 and 48 h (barley) or 12 and 24 h (corn) after the transfer. Values are means of 10 replicates and SE are given in parentheses.

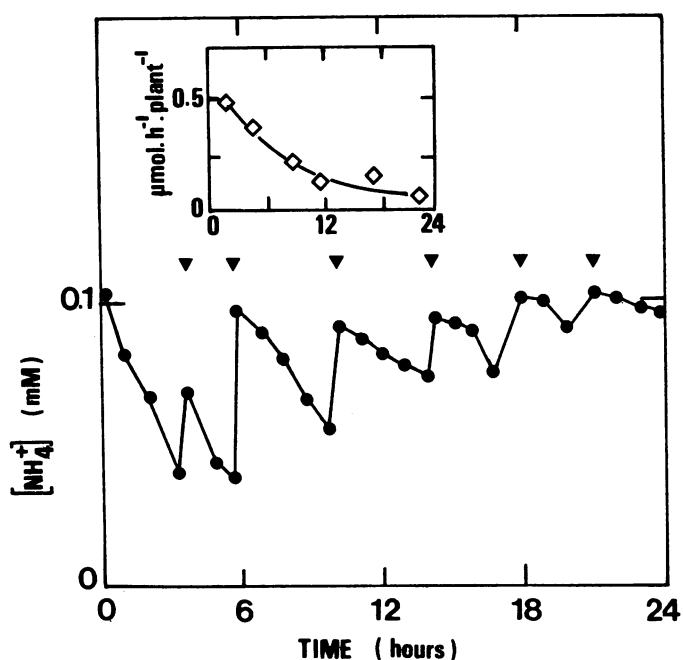| Plant Part	Barley		Corn	
	24 h	48 h	12 h	24 h
	μmol·plant^{-1}			
Roots	1.00	2.25	2.17	2.39
	(0.20)	(0.39)	(0.41)	(0.35)
Shoots	2.72	6.49	1.99	2.95
	(0.64)	(1.49)	(0.38)	(0.54)
Total plant	3.72	8.75	4.16	5.34
	(0.80)	(1.83)	(0.31)	(0.83)
	% of total			
Roots	26.9	25.7	52.2	44.8

FIG. 3. Time course of ammonium concentration in the nutrient solution (1.4 mM KNO$_3$ + 0.1 mM NH$_4$Cl) following transfer of the N-depleted corn seedlings to the solution. The arrows (▼) indicate the additions of ammonium to maintain the concentration close to 0.1 mM. Insert: Time course of uptake rate of ammonium.

and 74% at 24 and 48 h after the transfer, respectively (Table IV). As ammonium assimilation occurred in roots, this percentage can be taken as an estimate of net export to the shoots of the reduced N assimilated in roots (see "Materials and Methods"). It is then noteworthy that this export seemed to affect an important and apparently constant proportion of the nitrogen assimilated by the roots.

Reduced ^{15}N Translocation to the Roots. Reduced ^{15}N content of the ^{14}NO$_3^-$-fed roots (Anl) was low at each harvest (Table V), but it represents only a part of the reduced ^{15}N translocation to the roots (see "Materials and Methods"). Nevertheless, in both species, the downward translocation increased with time following the transfer.

Root Contribution to the Whole Plant Nitrate Reduction. In

Table IV. *Values of the Coefficients α, β, and γ*

See calculation model in "Material and Methods." SE are given in parentheses.

Coefficient	Barley		Corn	
	24 h	48 h	12 h	24 h
α	0.50	0.49	0.71	0.70
	(0.03)	(0.03)	(0.05)	(0.04)
β	0.50	0.51	0.28	0.30
	(0.04)	(0.03)	(0.02)	(0.03)
γ	0.73	0.74	0.48	0.55
	(0.03)	(0.04)	(0.05)	(0.02)

Table V. *Accumulation of Reduced ^{15}N (Anl) in $^{14}NO_3^-$-Fed Roots of Barley and Corn Seedlings Grown with a Split Root System*

After growing on a N-free medium, 7-d-old barley seedlings and 9-d-old corn seedlings were transferred in light to a nutrient solution containing 1.5 mM KNO$_3$ + 0.5 mM NH$_4$Cl (barley) or 1.4 mM KNO$_3$ + 0.5 mM NH$_4$Cl (corn). The root system was split into two parts, one supplied with ^{15}NO$_3^-$, the other one with ^{14}NO$_3^-$. Plants were harvested 24 and 48 h (barley) or 12 and 24 h (corn) after the transfer. Values are means of 10 replicates, and SE are given in parentheses.

Plant Part	Barley		Corn	
	24 h	48 h	12 h	24 h
	μmol·plant^{-1}			
Roots fed with ^{14}NO$_3^-$	0.009	0.040	0.030	0.140
(Anl)	(0.005)	(0.010)	(0.010)	(0.060)

Table VI. *Root Contribution to Nitrate Reduction by Barley Seedlings*

The data of root (Rr) and shoot (Rs) reduction, and xylem (Tx) and phloem (Tp) translocations of reduced ^{15}N are calculated as described in "Materials and Methods" from data of Tables II to V. The 24 to 48 h period data are calculated by the difference between 0 to 48 h and 0 to 24 h periods.

Period	Ar	As	Tx	Tp	Rr	Rs	Reduction in Roots
h		μmol·plant^{-1}					%
0–24	0.31	1.23	0.73	0.04	1.00	0.54	64.9
24–48	0.18	1.10	0.20	0.12	0.26	1.02	20.3
0–48	0.49	2.33	0.93	0.16	1.26	1.56	44.6

barley, the roots accounted for 65% of the whole plant nitrate reduction during the first 24 h after the transfer (Table VI). However, between 24 and 48 h, only 20% of the total nitrate reduction occurred in roots. This fall in root contribution was the consequence of a marked decrease in root nitrate assimilation (1.00 μmol·plant^{-1} between 0 and 24 h and 0.26 μmol·plant^{-1} during the 24 to 48 h period), and an increase in shoot nitrate assimilation (0.54 versus 1.02 μmol·plant^{-1} during the first and second period, respectively). The data calculated for corn (Table VII) led to the same conclusions, root contribution to the whole plant nitrate reduction was 70% during the first 12 h, and 27% during the subsequent 12 h. Increase in shoot nitrate reduction between the two periods was very marked (0.70 versus 2.30 μmol·plant^{-1}), while the decrease in root assimilation, although significant (1.64 versus 0.87 μmol·plant^{-1}), was not so drastic as for barley. During the whole experiment, the root contribution to the whole plant nitrate reduction was about 45% in both species (Tables VI and VII) and was markedly different from the proportion of the total reduced ^{15}N accumulated in roots at the end of the experiment (17 and 32% in barley and corn, respectively, Table II). This discrepancy can be explained by translocations of reduced N: During the first period, the xylem export

Table VII. *Root Contribution to Nitrate Reduction by Corn Seedlings*

The data of root (Rr) and shoot (Rs) reduction, and xylem (Tx) and phloem (Tp) translocations of reduced ^{15}N are calculated as described in "Materials and Methods" from data of Tables II to V. The 12 to 24 h period data are calculated by the difference between 0 to 24 h and 0 to 12 h periods.

Period	Ar	As	Tx	Tp	Rr	Rs	Reduction in Roots
h			$\mu mol \cdot plant^{-1}$				%
0–12	1.01	1.33	0.78	0.15	1.64	0.70	70.1
12–24	0.78	2.39	0.60	0.52	0.87	2.30	27.4
0–24	1.79	3.72	1.38	0.67	2.51	3.00	45.5

of reduced ^{15}N resulting from reduction in roots (Tx) was much higher than the quantity entering from the shoots (Tp, Tables VI and VII). Nevertheless, the change in translocation pattern between the two periods indicated that upward translocation to the shoots decreased while downward allocation to the roots increased. Thus, xylem and phloem transports of reduced ^{15}N appeared to be relatively balanced during the second period, especially for corn.

Fate of the Absorbed Nitrate. The fate of nitrate entering the roots of barley and corn seedlings is shown in Table VIII. Nitrate reduction accounted for 23% of the total nitrate absorbed by barley seedlings during the first 24 h of the experiment, and 27% for the total period of exposure to nitrate solution. This distribution of nitrate between reduction and accumulation was quite similar in corn seedlings as only 20% of the nitrate taken from the nutrient solution was assimilated. Consequently, in both species, the main fate of the absorbed nitrate was accumulation, predominantly in leaves.

DISCUSSION

Validity of the Model. The model proposed to calculate nitrate reduction by roots is based on three assumptions (see "Materials and Methods"). Assumption 1, *i.e.* the ability of exogenous ammonium to trace the upward translocation of reduced N from nitrate assimilation in roots, is first supported by the fact that all exogenous ammonium assimilation is believed to occur in roots (14, 16). As a consequence, ammonium is absent in xylem exudate of barley plants (14). Second, the assimilatory pathway in roots is the same for both endogenous (resulting from nitrate reduction) and exogenous (uptake from the solution) ammonium (6). Thus, if the two sources of ammonium are not distinguished

by assimilation, the proportion of amino acids translocated to the shoots should be similar for both origins. Furthermore, as this proportion appeared to be rather constant during the experiment (see γ values, Table IV), endogenous ammonium appearance and exogenous ammonium uptake do not necessarily have similar time courses.

The estimation of downward translocation is performed using the split root system technique, which implies that the two sides are functionally equivalent and that direct exchanges between these two sides are negligible. Thus, we assume (assumptions 2 and 3) that the reduced N downward translocation to both sides of the split root system and their nitrate uptake are proportional to their relative fresh weights. For barley, as the roots were not selected for planting in separate compartments, the two sides of the split root system were statistically identical. This supports the validity of assumptions 2 and 3. For corn, $^{15}NO_3^-$ was supplied to the SS root. The same experiment carried out supplying $^{15}NO_3^-$ to the PS root did not modify the estimation of this downward translocation (Table IX). This again confirms the two assumptions.

Although the procedure requires N-starved plants and the use of ammonium, it has three advantages for investigating N metabolism of the plant. First, assimilation is measured by ^{15}N incorporation which is a reference method and allows to distinguish exogenous and endogenous nitrogen. Second, reduced N translocations are investigated (experiments 2 and 3) using intact seedlings under the same conditions and simultaneously to the measurement of the whole plant nitrate reduction (experiment 1). Thus, limitations due to the use of excised organs are avoided. Third, this procedure can be applied to a wide range of species which allows the use of the split root system technique.

Nitrogen Assimilation in Roots. The overall experiment consisted of two periods with different physiological significance. The first period (0–12 h for corn and 0–24 h for barley) was an induction phase where nitrate reduction occurred mainly in the roots (Tables VI and VII). The second period (12–24 h for corn and 24–28 h for barley) may be more representative of a stable behavior of the seedlings, corresponding to the general case of continuous nitrate supply. During this period, roots accounted for only a minor part of the total assimilation (Tables VI and VII). The only difference between the two species was the more rapid evolution in corn seedlings, since assimilation in their shoots became predominant after 12 h. These results support both hypotheses that roots play a significant role in nitrate reduction during the initial nitrate utilization (1, 4, 8, 30), and that leaves are generally the main organ for reducing nitrate in

Table VIII. *Nitrate Accumulation, Reduction, Translocation, and Uptake in Barley and Corn Seedlings*

Plants were from the experiment described in Table I and were harvested 24 and 48 h (barley) or 12 and 24 h (corn) after the transfer.

Species and Period	NO_3^- Accumulation		NO_3^- Reduction		NO_3^- Translocation to Shoots	NO_3^- Uptake
	Roots (1)	Shoots (2)	Roots (3)	Shoots (4)	(2+4)	(1+2+3+4)
			$\mu mol \cdot plant^{-1}$			
Barley						
0–24 h	1.63[a]	3.39[a]	1.00[b]	0.54[b]	3.93	6.56
24–48 h	0.75[a]	1.98[a]	0.26[b]	1.02[b]	3.00	4.01
0–48 h	2.38[a]	5.37[a]	1.26[b]	1.56[b]	6.93	10.57
Corn						
0–12 h	—[c]	—	1.64[b]	0.70[b]	—	—
12–24 h	—	—	0.87[b]	2.30[b]	—	—
0–24 h	7.51	13.80	2.51[b]	3.00[b]	16.80	26.82

[a] Calculated from Figure 2.   [b] Calculated from Tables VI and VII.   [c] — = not determined.

Table IX. *Accumulation of Reduced* ^{15}N *(Anl) in SS or SP* $^{14}NO_3^-$*-Fed Roots of Corn Seedlings*

The plants were from an experiment similar to the one described in Table V. In one case, $^{15}NO_3^-$ was supplied to the SP root and in the other case to the SS root. The $^{14}NO_3^-$-fed roots were harvested 24 h after the transfer to the nutrient solution.

$^{14}NO_3^-$-Fed Root	Anl
	$\mu mol \cdot plant^{-1}$
SS root	0.052
SP root	0.058

barley (2, 5, 13, 14) and corn (26).

The decrease in nitrate reduction by roots with time (Tables VI and VII) could have several causes: (a) The NR level in roots could be depressed by the supply of amino acids from the shoots. This is supported by the increased downward translocation during the second period following transfer (Tables VI and VII), and by the effect of amino acids on NRA level in roots (20). (b) The lower nitrate uptake during the second period (Table VIII) could be responsible for a limitation of the nitrate supply to root NR, particularly because translocation, which seems to compete with reduction in roots (27), was less affected than uptake (Table VIII). This slowing of uptake could result from the increasing nitrate efflux associated with the higher nitrate content of the cells at the end of the experiment (18), or from the increase of reduced N compounds from the shoots (10). (c) Both nitrate and ammonium assimilation during the first period could have depressed the carbohydrate content of the roots and curtailed further nitrate uptake and reduction which appear to depend on it (11, 17).

The root contribution to the whole plant nitrate reduction (20–30%) during the second period (when shoot nitrate reduction was fully expressed) was higher than the estimated root contribution (10%) from *in vitro* NRA (13, 26). This indicates a relatively greater efficiency of the root nitrate reductase in assimilating nitrate.

In our experiments, ammonium is used to trace reduced N translocation from the roots to the shoots. This ion is reported to decrease nitrate reduction in roots (19). Thus, the root contribution to the whole plant nitrate reduction could even be higher if nitrate was the only source of nitrogen.

Nitrogen Translocation and Physiological Significance of Root Nitrate Reduction. For both species and for each period after the transfer, $^{15}NO_3^-$ reduction in the roots (Rr) was always greater than accumulation of reduced ^{15}N in these organs (Ar, Tables VI and VII). This supports the hypothesis of Radin (25) that the physiological significance of nitrate reduction occurring in the roots is the satisfaction of their reduced N demand, and that only the excess reduced N is exported to the shoots. However, in our experiment, a major proportion of amino acids synthesized by the roots was shown to be translocated to the shoots (see γ values, Table IV). Thus, the fate of this root reduced N did not seem to be the N feeding of the roots. Furthermore, the amounts (Tp) of reduced ^{15}N translocated from the shoots to the roots via phloem during the second period represented a large part of reduced ^{15}N accumulation in the roots (Ar, Tables VI and VII). This clearly shows that, in this case, assimilation in the shoots was an important source for N feeding of the roots. Together, these data are consistent with the occurrence, in barley and corn seedlings, of a large "cycling" of reduced N through the plant, already described for other species (21, 29). Particularly, our results confirm that the downward translocation of reduced N may be very great (24). Consequently, the nitrate reduction performed by roots and shoots is not obviously used for the self nutrition of each organ.

Then, it can be concluded that neither nitrate reduction in roots nor phloem translocation from shoots is the exclusive N source for the nutrition of the roots. Both inputs may coexist, with each one predominating under different circumstances.

It is however noteworthy that in roots of both species, $^{15}NO_3^-$ reduction (Rr) became close to the accumulation of reduced ^{15}N (Ar) during the second period after the transfer (Tables VI and VII). Thus, it may be proposed that barley and corn seedlings are capable of modulating the two inputs in the "cycling nitrogen pool" (*i.e.* reduction in root and shoot) in function of the relative magnitude of the two outputs (protein synthesis in root and shoot), leading in this case to a relative balance between upward and downward transports.

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