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Exogenous $^{15}NO_3^-$ Influx and Endogenous $^{14}NO_3^-$ Efflux by Two Maize (*Zea mays* L.) Inbreds during Nitrogen Deprivation[1]

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ABSTRACT

The influence of nitrogen stress on net nitrate uptake resulting from concomitant $^{15}NO_3^-$ influx and $^{14}NO_3^-$ efflux was examined in two 12-day-old inbred lines of maize. Plants grown on $^{14}NO_3^-$ were deprived of nitrogen for up to 72 hours prior to the 12th day and then exposed for 0.5 hour to 0.15 millimolar nitrate containing 98.7 atom % ^{15}N. The nitrate concentration of the roots declined from approximately 100 to 5 micromolar per gram fresh weight during deprivation, and $^{14}NO_3^-$ efflux was linearly related to root nitrate concentration. Influx of $^{15}NO_3^-$ was suppressed in nitrogen-replete plants and increased with nitrogen deprivation up to 24 hours, indicating a dissipation of factors suppressing influx. Longer periods of nitrogen-deprivation resulted in a decline in $^{15}NO_3^-$ influx from its maximal rate. The two inbreds differed significantly in the onset and extent of this decline, although their patterns during initial release from influx suppression were similar. Except for plants of high endogenous nitrogen status, net nitrate uptake was largely attributable to influx, and genetic variation in the regulation of this process is implied.

Depletion of specific nutrients in higher plants is commonly accompanied by an enhanced capacity for uptake of those ions. This response may be viewed as a release from suppression of uptake which occurs in the presence of high endogenous concentrations of the ion and its assimilation products. Influx of phosphate, sulfate, and chloride into roots is subject to this kind of regulation (*e.g.*, Ref. 14).

Some experiments with barley, involving pretreatments with various nitrate concentrations, indicate little effect on subsequent nitrate influx, as measured with $^{36}ClO_3^-$ or $^{13}NO_3^-$ (5–7, 9). In these instances, net uptake was largely influenced by changes in nitrate efflux. In other experiments with barley, however, the stimulation in net nitrate uptake resulting from nitrogen deprivation (15) was accompanied by a stimulation in influx (17). Hence both restricted influx and high efflux (16) were characteristic of plants of high nitrogen status (17). In *Pisum*, both influx and net uptake of nitrate were enhanced by moderate nitrogen deprivation; more severe deprivation was required to restrict efflux (24).

Exposure of roots to highly enriched (~98–99 atom %) $^{15}NO_3^-$ permits direct, simultaneous measurement of the net inward movement of the exogenously supplied $^{15}NO_3^-$ and the net outward movement of previously accumulated endogenous $^{14}NO_3^-$ (22). With wheat plants (12) and decapitated maize roots (19, 20) such experiments have shown that exposure to high nitrate concentrations can suppress net $^{15}NO_3^-$ influx and enhance $^{14}NO_3^-$ efflux, although the relative effects on the two processes may be dissimilar. However, the nitrate influx system also is subject to substrate induction (3, 11, 13, 23), and the induced activity declines upon exposure to nitrate-free media (21). Hence expression of nitrate influx activity during nitrate deprivation initially may reflect the lifting of feedback suppression (derepression or deinhibition), whereas more prolonged deprivation may indicate a decay of the induced system from its fully derepressed or deinhibited state (3). Roots of maize inbreds have been shown to differ in nitrate uptake and assimilation processes (22, 25) and it is possible that the pattern of relief from suppression (15, 17) and/or decay of the induced transport system (21) may exhibit genotypic diversity.

Accordingly, the present investigation was initiated to examine whether (a) increases in nitrate influx and decreases in nitrate efflux occur in parallel as autotrophic corn plants suppressed in net uptake undergo nitrate-deprivation, (b) decay of influx from an initial stimulated rate occurs during prolonged deprivation, and (c) intraspecific differences exist in the nature of those responses.

MATERIALS AND METHODS

Genetic Materials. In a preliminary screening of 19 inbred maize (*Zea mays* L.) lines derived from the open-pollinated population 'Jarvis Golden Prolific,' two lines were identified which differed appreciably in net nitrate uptake and partitioning (27). These two lines, designated as 53 and 71, were selected for comparison in the experiment reported herein.

Plant Culture. Fungicide-treated seed of each genotype were germinated in the dark in paper rolls moistened with 0.1 mM $CaSO_4$ at 30°C and 98% relative humidity. After 84 h, seedlings were selected for uniformity within genotype, seminal roots excised, and the primary roots of two seedlings (a culture) were threaded through holes in hollow polyethylene stoppers (culture cups). The seedlings were grown in aerated nutrient solution containing 1.25 mM K_2SO_4, 1.0 mM $MgSO_4$, 0.25 mM $Ca(H_2PO_4)_2$, 3.72 mM $(CaNO_3)_2$ and micronutrients at two-fifths of the concentration of Hoagland's solution (10). Iron was supplied as ferric diethylenetriaminepentaacetate (3.5 mg Fe L^{-1}) and $FeCl_3$(1.6 mg Fe L^{-1}), and the acidity was adjusted to pH 4.6 with H_2SO_4. Endosperms were packed in cotton and moistened daily with 0.1 mM $CaSO_4$. Plants were maintained in the laboratory under a metal halide light bank which provided a photon flux density of 1000 μmol $m^{-2}s^{-1}$ for 16 h daily. Ambient temperature was 25 ± 2°C, and air exchange was continuously maintained by an electric fan. Solutions were replaced 5 times at progressively shorter intervals as the seedlings grew.

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Beginning 9 d after germination, five replicate cultures of each genotype were transferred first to 1.0 mM $CaSO_4$ for 10 min and then to nitrate-free solutions identical to the above nutrient solution, except that $Ca(NO_3)_2$ was replaced by 1.0 mM $CaSO_4$, and the initial acidity adjusted to pH 5.3. Transfers were made periodically such that at the beginning of the 12th d plants had been in nitrogen-free solution for 0, 6, 12, 24, 36, 48, and 72 h.

The uptake experiment began 5.5 h after the onset of the light period on the 12th d after germination. Roots were exposed for 10 min to aerated, nitrate-free nutrient solution to remove extra cellular nitrate. They were then transferred to individually aerated solutions (325 ml, pH 5.3) containing 0.15 mM $K^{15}NO_3$ (98.7 atom% ^{15}N), 1.0 mM $CaSO_4$, and micronutrients at two-fifths the concentration of Hoagland's solution (10). After 0.5 h, the roots were rinsed with ice-chilled 1.0 mM $CaSO_4$, excised where they emerged from the culture cup, blotted, and weighed. Shoots were excised just below the first node and weighed. Tissues were freeze-dried, reweighed, ground and mixed thoroughly.

Analysis. Nitrate in hot water extracts of the plant material and nitrate depletion of the uptake solutions (net nitrate uptake) were determined by a manual modification of the method of Lowe and Hamilton (18). Atom % ^{15}N of nitrate in the uptake solutions, both before and after the 0.5 h exposure, was determined according to Volk *et al.* (28) on a CEC 21-620 mass spectrometer. The data so obtained permit calculation of the entry of ambient $^{15}NO_3^-$ into the roots ($^{15}NO_3^-$ influx), the exit of endogenous $^{14}NO_3^-$ from the roots to the solution ($^{14}NO_3^-$ efflux), and net uptake (the difference between the two) during the 0.5 h period.

RESULTS

When grown continuously with nitrate, plants of line 53 were larger on d 12 than those of line 71 (Table I). The difference reflected greater shoot growth; root growth was similar for both lines and was restricted to the same extent by nitrate deprivation.

Nitrate concentrations of roots (Fig. 1A) and shoots (Fig. 1B) on d 12 decreased progressively with increasing prior nitrate deprivation. The pattern was similar in both inbreds. Root nitrate concentrations decreased more rapidly than those of shoots, especially during the first 12 h of deprivation. Less than 5 μmol g^{-1} fresh weight were present in both tissues in plants deprived for 72 h.

Nitrate deprivation for up to 24 h increased net nitrate uptake in both inbreds (Fig. 2A). Line 53 maintained higher net uptake than line 71 throughout this period of initial release from nitrate uptake suppression but the pattern was similar for both inbreds. More prolonged deprivation, however, resulted in a pronounced decrease from the maximal nitrate uptake rate with significant differences between inbreds in this response. Line 53 decreased by 50% between 24 and 48 h deprivation, whereas line 71 maintained its maximal rate during this period and declined only after 48 h. The patterns for $^{15}NO_3^-$ influx (Fig. 2B) were similar to those of net nitrate uptake (Fig. 2A).

Table I. *Root and Shoot Growth*

Fresh weight and standard errors of the means (5 replicates) of two inbred maize lines grown with continuous nitrate (7.5 mM) or in nitrogen-free solutions for various periods up to 72 h prior to the 12th d.

Time in N-Free Solution	Roots	Shoots
	g plant^{-1}	
A. Inbred 71		
0–24[a]	2.31 ± 0.09	3.56 ± 0.08
36	2.13 ± 0.10	3.60 ± 0.10
48	1.95 ± 0.06	3.25 ± 0.04
72	1.69 ± 0.08	3.00 ± 0.09
B. Inbred 53		
0–24[a]	2.26 ± 0.06	5.39 ± 0.20
36	1.99 ± 0.38	5.00 ± 0.83
48	1.90 ± 0.22	4.26 ± 0.54
72	1.69 ± 0.19	3.78 ± 0.40

[a] Means of cultures exposed to 0, 6, 12, and 24 h of nitrogen-free solutions; no significant differences in weight occurred among these treatments.

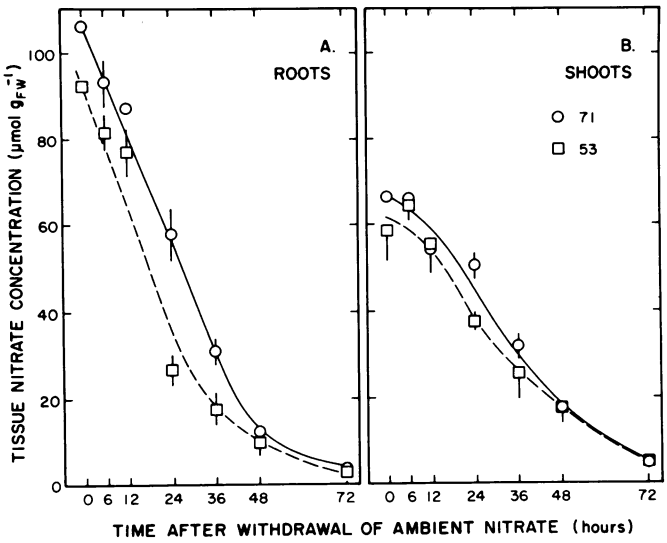

FIG. 1. Root (A) and shoot (B) nitrate concentrations of 12-d-old maize inbreds (71 and 53) grown with continuous nitrate (7.5 mM) or in nitrogen-free solutions for up to 72 h prior to the 12th d. Vertical lines represent the standard error of the means of 5 replicate cultures when they exceed the size of the symbols.

Efflux of $^{14}NO_3^-$ (Fig. 2C) was highest in plants continuously supplied with nitrate and, under those conditions, was 51 and 59% of $^{15}NO_3^-$ influx in lines 53 and 71, respectively. It declined steadily with nitrate deprivation, and no differences in the rate of decline between the two hybrids were evident. The decline in $^{14}NO_3^-$ efflux (Fig. 2C) occurred while $^{15}NO_3^-$ influx underwent an initial increase and subsequent decrease with progressive nitrogen deprivation (Fig. 2B). Within the limits of detection, the relationship between root nitrate concentration and $^{14}NO_3^-$ efflux was linear for both inbreds (Fig. 3) with a reasonable fit (r^2 = 0.98) for line 71. It was less definitive with line 53 (r^2 = 0.95) because of greater variability in the measurement of efflux.

DISCUSSION

The inward flow of $^{15}NO_3^-$ and the outward flow of $^{14}NO_3^-$ in these experiments underestimate the actual plasmalemma influx and efflux, respectively, because of potential recycling outward and inward of the two isotopic forms (3, 17). Nevertheless, with maize root systems exposed to 0.2 mM nitrate under steady state conditions, the net inward movement of $^{15}NO_3^-$ during a 0.5 h exposure period was only marginally less than influx as estimated from the initial maximal rate (13). Loss of $^{14}NO_3^-$ to the ambient solution tended to underestimate efflux to a similar extent. Thus measurement of the reciprocal net flows of each isotopic species during a 0.5 h interval provides a reasonable approximation of influx and efflux in maize roots.

Prior to transfer to nitrate-free solutions, root nitrate concentrations (Fig. 1A) were 106 ± 6 and 93 ± 2 μmol g^{-1} fresh weight for lines 71 and 53, respectively, and their corresponding rates of $^{15}NO_3^-$ influx were 4.0 ± 0.5 and 5.5 ± 0.5 μmol g^{-1} fresh weight h^{-1} (Fig. 2B). Restricted $^{15}NO_3^-$ influx (Fig. 2B) as well as high $^{14}NO_3^-$ efflux (Fig. 2C) therefore contributed to

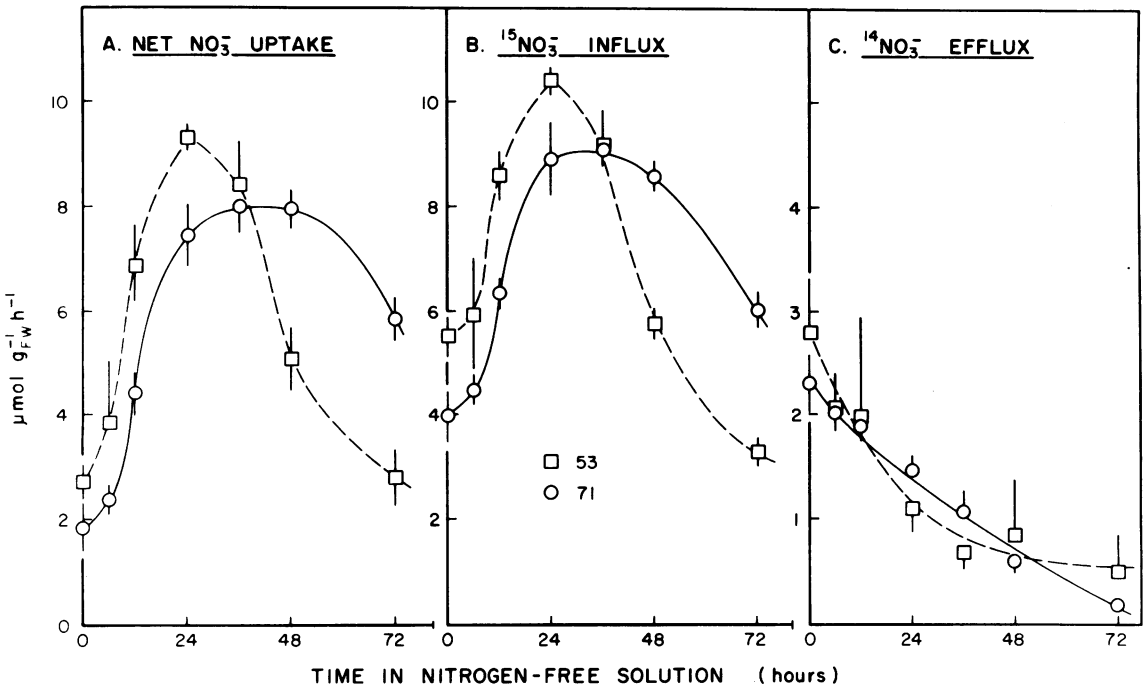

FIG. 2. Effect of nitrate deprivation during prior growth on net nitrate uptake (A), $^{15}NO_3^-$ influx (B), and $^{14}NO_3^-$ efflux (C), by 12-d-old maize inbreds during 0.5 h exposure to 0.15 mM KNO_3 (98.7 atom% ^{15}N). Vertical lines represent the standard error of the means of 5 replicate cultures when they exceed the size of the symbols.

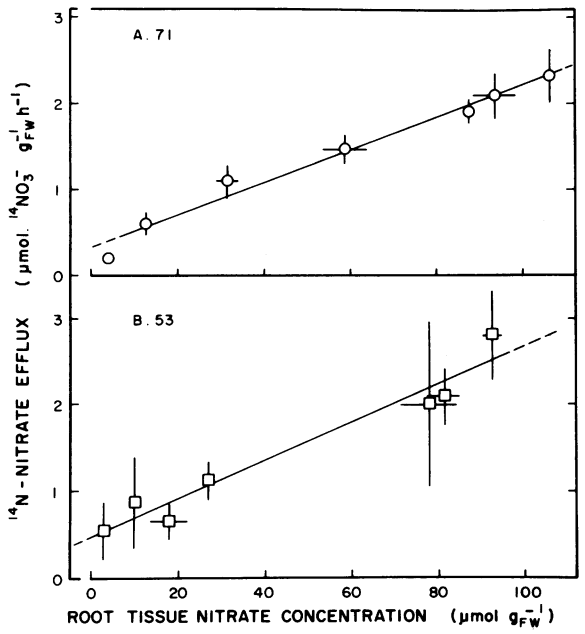

FIG. 3. Relationship between root nitrate concentration, resulting from prior growth for increasing times in nitrogen-free solutions, and $^{14}NO_3^-$ efflux during 0.5 h exposure to 0.15 mM $K^{15}NO_3^-$ (98.7 atom% ^{15}N) for 12-d-old maize inbreds 71 (A) and 53 (B). Vertical and horizontal lines represent the standard error of the means of 5 replicate cultures when they exceed the size of the symbols. The relationship for A is $y = 0.325 + 0.019x$ ($r^2 = 0.98$) and for B is $y = 0.485 + 0.022x$ ($r^2 = 0.95$) where y is $^{14}NO_3^-$ efflux and x is root nitrate concentration.

the suppression of net nitrate uptake (Fig. 2A) with plants continuously supplied with nitrate compared to those deprived of nitrate for 24 h. Upon nitrate-deprivation, $^{14}NO_3^-$ efflux (Fig. 2C) declined in parallel with the decline in root nitrate concentration (Fig. 3). A similar relationship, but with a greater sensitivity of $^{14}NO_3^-$ efflux to the decrease in root nitrate concentration, has been observed in roots of decapitated maize seedlings (21).

The pattern of the initial response of $^{15}NO_3^-$ influx upon nitrate deprivation was similar with both inbreds (Fig. 2B). Increases were clearly evident within 12 h, at which time root nitrate concentrations of each had decreased by 17% (Fig. 1A). Maximal $^{15}NO_3^-$ influx developed with 24 h deprivation, but longer deprivation resulted in a decline of $^{15}NO_3^-$ influx with the onset of the decline differing significantly between the inbreds (Fig. 2B). The general response thus conforms to a pattern suggestive of an initial relief from feedback suppression and a subsequent decay of a preexisting induced transport system (3). In this view the relatively low $^{15}NO_3^-$ influx of plants continuously exposed to nitrate reflects suppression (repression or inhibition) by nitrate (4, 26) or products of its reduction (1, 8, 17). The initial increase upon deprivation would thus result from removal from the cytoplasm of these negative effectors via assimilation and xylem or vacuolar deposition. Because root carbohydrate concentrations increase appreciably during nitrogen deprivation (2), this initial increase also could reflect an increase in the available energy supply which either enhances the influx process directly or facilitates removal of the negative effectors.

The decline in $^{15}NO_3^-$ influx (Fig. 2B) after nitrate deprivation for 24 h (line 53) or 48 h (line 71) occurred while root nitrate concentrations (Fig. 2C), and presumably products of its reduction, continued to decrease and while root carbohydrate concentrations would continue to increase (2). That the decline occurred in spite of conditions expected to optimize nitrate uptake (2, 15) implies a decay of the influx system initiated after (or perhaps during) relief from suppression. A decline in root water content occurred with nitrate-deprivation. In line 53 it decreased linearly at 0.022% h^{-1} from an initial 94.74 ± 0.08% in nitrate-replete plants; corresponding values for line 71 were 0.031% h^{-1} and 95.22 ± 0.05%. Although the rate of decrease in water content was small, it is conceivable that either the initial increase in $^{15}NO_3^-$ influx or its subsequent decay (Fig. 2B), but not both,

may be associated with osmotic adjustments. With our present understanding, however, it seems more straight-forward to view the initial increase as resulting from removal of negative effectors from (3), or enhancement of the energy status for (2), an induced transport system (11), with the subsequent decline being attributed to net degradation of the transport system (21).

Net nitrate uptake by barley decreased following three days deprivation after an initial simulation (15), and Mackown (21) has demonstrated a 50% decay in the 'induced' component of $^{15}NO_3^-$ influx in roots of decapitated maize within 32 h deprivation. This rate of decay is similar to that of line 53 following its maximal stimulation by 24 h (Fig. 2B). The onset of the decline in line 71 after 48 h was associated with a lower root nitrate concentration (12 ± 1 μmol g^{-1} fresh weight) than the onset of the decline after 24 h in line 53 (27 ± 4 μmol g^{-1} fresh weight). The data imply differential turnover of the nitrate influx system during nitrate deprivation, or differential maintenance of nitrate in an inducing pool, and thus indicate the possibility of significant genotypic diversity in maintaining activity of the system.

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