Ammonium Does Not Induce Ammonium Absorption in Tomatoes

Bernard A. L. Nicoulaud¹ and Arnold J. Bloom²

Department of Vegetable Crops, University of California, Davis, CA 95616

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ABSTRACT. We examined root ammonium absorption by tomato seedlings (*Lycopersicon esculentum* Mill. 'T-5') after first exposure of the roots to ammonium. Some plants received a nutrient medium containing nitrate as the sole N source. In a second treatment, the leaves were sprayed daily with a urea solution, while the roots were in N-free medium. The last two treatments were initially grown in medium that contained ammonium nitrate, but then either were shifted to a N-free medium for 10 days or had their roots excised and were rerooted in N-free medium for 21 days. Root ammonium absorption remained constant after first exposure to ammonium for the plants exposed to nitrate alone, whereas root ammonium absorption declined with time for the other three treatments. These results indicate that for tomato a) ammonium in the rhizosphere does not induce root ammonium absorption and b) some product of ammonium metabolism represses root ammonium absorption.

Nitrogen transport systems in plant roots appear to be substrate inducible. Extensive study of root NO_3^- absorption indicates that N-deprived plants show a 2- to 10-fold increase in NO_3^- absorption after first exposure to NO_3^- in the rhizosphere (Breteler and Nissen, 1982; Henriksen et al., 1992; Hole et al., 1990; Jackson et al., 1972; McClure et al., 1990a, 1990b; Tompkins et al., 1978). In barley (*Hordeum vulgare* L.), exposure to NH_4^+ (Bloom and Sukrapanna, 1990) or NO_2^- (Aslam et al., 1993) may also induce NO_3^- absorption. By contrast, substrate induction of root NH_4^+ absorption has received relatively little attention. Wheat seedlings (*Triticum aestivum* L.) that required 10 and 6 h to achieve full induction of root NO_3^- and NO_2^- absorption, respectively, required 5 h to achieve full induction of NH_4^+ absorption (Goyal and Huffaker, 1986). In N-deprived wheat and oat (*Avena sativa* L.) seedlings, NH_4^+ influx and efflux increased during the first 5 h of exposure to NH_4^+ so that net NH_4^+ uptake actually declined (Morgan and Jackson, 1988a). Barley seems to have a single transport system for NH_4^+ absorption at low concentrations, and the capacity of this transport system increases with time of exposure to NH_4^+ (Mäck and Tischner, 1994). In soybean (*Glycine max* Merr.), NO_3^- induced NH_4^+ absorption (Saravitz et al., 1994), whereas Ca^{2+} also stimulated NH_4^+ absorption in onion (*Allium cepa* L.) and radish (*Raphanus sativus* L.) (Fenn and Taylor, 1990; Fenn et al., 1991).

Previous studies on the induction of NH_4^+ absorption have focused almost exclusively upon N-deprived plants. Nitrogen deprivation may alter many metabolic processes that may affect NH_4^+ absorption. For example, N deprivation limits energy production at many levels, and NH_4^+ absorption and assimilation are energy intensive processes (Bloom et al., 1992). Net NH_4^+ uptake was low in barley, wheat, and oat plants grown under NO_3^- nutrition, and increased 3- to 4-fold under N deprivation (Morgan and Jackson, 1988a, 1988b, 1989). These authors suggest that a product of NH_4^+ assimilation serves as a negative effector of NH_4^+

influx. Nitrogen deprivation in barley increased the plants' capacity to absorb either NH_4^+ or NO_3^- by 3-fold (Lee and Rudge, 1986). Therefore, the use of N-deprived plants does not permit one to distinguish between the specific effects of the substrate NH_4^+ on the induction of the NH_4^+ transport system and the general effects of recovery from N deprivation on plant metabolism.

In this investigation, we designed several treatments to generate tomato plants that were N replete, but whose roots had never been exposed to NH_4^+ . One treatment was the exposure of plants to NO_3^- as the sole N source. Another was to spray urea on the leaves as a sole N source (Nicoulaud and Bloom, 1996). The last was to reroot plants that had been grown on adequate N in a medium devoid of N. Through such treatments, we were able to test whether short-term exposure to NH_4^+ induced root NH_4^+ absorption in N-sufficient plants.

Materials and Methods

Tomato seeds (*Lycopersicon esculentum* Mill. 'T-5') were germi-

Fig. 1. A schematic of the nutrient flow system to monitor net NH_4^+ and NO_3^- uptake from the roots of intact plant. The arrows indicate the direction of the fluid flow. Pumps 1-3 are piston metering pumps. Pump 4 is a syringe pump that adds KOH to convert NH_4^+ to NH_3 . The top three-way valve selects whether the root cuvette receives a solution with or without NH_4^+ ; the lower three-way valve selects whether the ion-selective electrodes monitor the solution before or after it enters the root cuvette.

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¹Current Address: Fac. De Agronomia-UFRGS, Cx.P. 776, Porto Alegre-RS, 91.501-970, Brazil.

²To whom reprint requests should be addressed.

nated at 25 °C in rolled germination paper standing in 2 mmol·L⁻¹ CaSO₄. After 1 week, the seedlings were transplanted to 1.7-L plastic containers, six per container. The containers were filled with a modified Hoagland's solution consisting of (in mmol·L⁻¹) 2.0 CaSO₄, 1.0 MgSO₄, 0.6 K₂SO₄, 1.0 K₂HPO₄, and either 0.05 KNO₃, no N in the medium, or 0.05 NH₄NO₃. Micronutrients and iron were as described in Epstein (1972). The nutrient solution was continuously bubbled with air. Plants were grown in the laboratory for two d at 20 °C and with a PPFD (photosynthetic photon flux density) of 50 to 150 μmol·m⁻²·s⁻¹ at plant height during a 10-h day before they were transferred to a greenhouse. In the greenhouse, the containers were immersed in a refrigerated water bath (Forma 2425, Marietta, Ohio) to maintain root temperature at 20 ± 2.0 °C. Cooling pads, fans, and heaters in the greenhouse held shoot temperatures within 30 ± 7 °C d and 20 ± 5 °C nights. We supplemented natural lighting with metal halide HID lamps to sustain at least 350 μmol·m⁻²·s⁻¹ PPFD for a 14-h day; at midday, the light levels were above 1200 μmol·m⁻²·s⁻¹ PPFD.

Four treatments were used to obtain plants that were N sufficient but whose roots had either limited or no exposure to NH₄⁺. First, some plants were grown for 3 weeks in a medium where the sole N source was 50 μmol·L⁻¹ KNO₃, and the medium was replaced daily (KNO₃ treatment). Second, plants were exposed to a N-free medium and received foliar urea (urea treatment). Their leaves were sprayed to drip point once a day for 5 weeks with a handheld sprayer containing 0.2% (w/w) urea and 0.3% (v/v) of the surfactant α-alkylaryl-Ω-hydroxypoly(oxyethylene)glycol (Spray-Mate, National Chelating Corporation, Orange, Calif.). To avoid urea leakage into the nutrient solution, a closed-cell foam plug was placed tightly around each stem and the N-free nutrient solution was replaced daily. In the third and fourth treatments, seedlings receiving 50 μmol·L⁻¹ NH₄NO₃ were grown for 2 weeks with the medium replaced every 3 d. They then were either transferred to a medium without N for 10 more days (N-deprived treatment) or had their roots excised and their shoots were rerooted in a medium without N for 3 weeks (rerooted treatment). Although the various treatments resulted in plants of different ages, all the plants had root systems that were ≈25 mg dry mass. This amount of roots provided adequate NH₄⁺ depletion rates and fit nicely within our root cuvette.

The protocol for the NH₄⁺ uptake experiments was similar to that of Bloom and Sukrapanna (1990). The night before an experiment was to be performed, a plant was transferred to a nutrient flow system (Fig. 1). A slotted rubber stopper was fit around the stem and the roots of the intact plant sealed within a 30-mL root cuvette. Aerated nutrient solution made a single pass through the cuvette at 2.3 mL·min⁻¹. To analyze for NH₄⁺, 1.25 N KOH was added to a portion of the solution stream, converting NH₄⁺ to NH₃, and NH₃ was detected with a NH₃-selective electrode (Orion Research model 95-12, Boston). The flow system allowed the cuvette and the electrode analysis loop to receive different solutions. The night before the experiment the root cuvette received nutrient solution that consisted of 0.5 mmol·L⁻¹ CaSO₄ and 0.5 μmol·L⁻¹ K₂HPO₄, while the electrode analysis loop received nutrient solution that consisted of 0.5 mmol·L⁻¹ CaSO₄, 0.5 μmol·L⁻¹ K₂HPO₄, and 50 μmol·L⁻¹ NH₄Cl. In this way, the electrode loop was always receiving a solution that contained NH₄⁺, while the plant was receiving a NH₄⁺-free solution. This was important in order for the electrode to respond quickly enough to measure NH₄⁺ depletion within 1 h of first addition

of NH₄⁺ to the root cuvette. During the experiment, the light level at the plant height was 1000 μmol·m⁻²·s⁻¹ PPFD and root temperature was 20 °C. At the beginning of the experiment, the root cuvette was switched to the solution containing NH₄⁺.

Normally, the electrode analysis loop monitored the solution leaving the plant cuvette. Every 4 h, we would determine the zero drift of the electrode by switching the electrode analysis loop for a brief period to the solution entering the cuvette. The net uptake rate was calculated from the differences in the solution NH₄⁺ concentrations before and after it passed through the root cuvette, multiplied by the flow rate through the cuvette, and divided by the root dry mass. Four plants were monitored for each treatment.

We conducted an analysis of variance with repeated measures (GLM Procedure, SAS Institute, Cary, N.C.) to test the influence of time during the first 8 h of exposure to NH₄⁺ upon the logarithm of net NH₄⁺ uptake. For each plant, we calculated a linear regression of the logarithm of net NH₄⁺ uptake against the first 8 h and averaged the slope, intercept, and correlation over each treatment.

Results and Discussion

The net NH₄⁺ uptake rate of plants grown with NO₃⁻ as the sole N source remained constant at 0.63 ± 0.01 μmol·g⁻¹ root dry mass/min (mean ± SE) during the 30-h observation period after first exposure to NH₄⁺ (Fig. 2). During the first 8 h, time had no significant effect upon the logarithm of net NH₄⁺ uptake rate, and the slope of the linear regression was not significantly different from zero (Table 1). For each of the three remaining treatments, net NH₄⁺ uptake declined significantly after first exposure to NH₄⁺, and the slopes of the log-linear regressions differed from zero (Fig. 3, Table 1).

Our results argue that the presence of NH₄⁺ in the rhizosphere does not induce root NH₄⁺ absorption in tomato. This contrasts with previous reports that exposure to NH₄⁺ induces the NH₄⁺ transport system in wheat and barley (Goyal and Huffaker, 1986; Mäck and Tischner, 1994; Morgan and Jackson, 1988a). The protocols used here differ from those of previous studies in several respects: a) we used predominately N-sufficient plants, whereas the previous studies used predominately N-deficient plants; b) the NH₄⁺ concentrations in the uptake solution were 50 μmol·L⁻¹ in this study versus 400 μmol·L⁻¹ or more in the previous studies; c) we

Fig. 2. The time course of net NH₄⁺ uptake per gram root dry mass per minute for 3-week old tomato plants grown with NO₃⁻ as the sole N source and first receiving NH₄⁺ at time zero. The NH₄⁺ concentration in the medium was 50 μmol·L⁻¹. Shown are the means ± SE for four plants.

Fig. 3. The time course of net NH_4^+ uptake per gram root dry mass per minute for tomato plants that were either N deprived for 2 weeks, rerooted in N-free medium, or grown with urea applied foliarly as the sole N source. These plants were exposed to a medium containing $50 \mu\text{mol}\cdot\text{L}^{-1}$ NH_4^+ at time zero. Shown are the means $\pm$ SE for four plants. Small error bars are incorporated into the symbols.

transferred plants into the measurement cuvette the night before uptake measurements and, thus, avoided transplant shock (Aslam et al., 1996; Bloom and Sukrapanna, 1990), whereas the other studies monitored uptake immediately after transferring plants to a new cuvette; and d) we examined tomato, whereas the other studies examined wheat and barley.

NITROGEN SUFFICIENT VERSUS NITROGEN DEFICIENT. Several of our growth regimes were designed to generate N-sufficient plants whose roots had limited or no exposure to NH_4^+ . We have found in previous studies that N does not limit the growth of tomato when nutrient solutions contain $50 \mu\text{mol}\cdot\text{L}^{-1}$ KNO_3 , NH_4NO_3 , or NH_4Cl (Smart and Bloom, 1991, 1993). Here, the plants received such N levels and, thus, were probably N-sufficient.

Net NH_4^+ uptake in the NO_3^- treatment did not vary with time after first exposure to NH_4^+ (Fig. 2). Although we never detected NH_4^+ in the medium of this treatment, the roots of these plants may still have been exposed to NH_4^+ if a) the plants absorbed NO_3^- in the medium, reduced it to NH_4^+ , excreted the NH_4^+ , and later reabsorbed it or b) dissimilatory NO_3^- respiring microbes in the rhizosphere reduced NO_3^- in the medium to NH_4^+ that was subsequently released into the rhizosphere and absorbed by the plants. To minimize these possibilities, we changed the nutrient solution daily.

The foliar urea treatment also did not show any induction of net

NH_4^+ uptake after first exposure of the roots to NH_4^+ (Fig. 3). Rather, there was a slight, but significant, decline in net NH_4^+ uptake with time of exposure (Fig. 3). In a previous study, plants receiving foliar applications of urea grew at a slower pace than plants grown with NH_4NO_3 , although the urea plants were N sufficient as evidenced by root NH_4^+ contents of $30 \mu\text{mol}\cdot\text{g}^{-1}$ dry mass (Nicoulaud and Bloom, 1996). In the current study, we did not detect any NH_4^+ in the nutrient solutions of urea treatment, but still changed the solutions every day to minimize the possibility that urea converted into NH_4^+ within a plant would be excreted into the nutrient solution in amounts sufficient to induce NH_4^+ absorption.

We also examined plants that were deprived of N for 10 d or were rerooted in a N-free medium to test whether N starvation can be responsible for induction. Net NH_4^+ uptake in these plants halved during the 8 h after first exposure to NH_4^+ (Fig. 3). These results are consistent with previous studies in which NH_4^+ uptake was high in N-starved plants and suppressed by pretreatment with N (Lee and Rudge, 1986; Mäck and Tischner, 1994; Morgan and Jackson, 1988a, 1988b, 1989). The high initial

NH_4^+ depletion rates and subsequent decline may derive, in part, from equilibration of NH_4^+ in the medium with that in the apparent free spaces of root cells (Mäck and Tischner, 1994). The decline that we observed, however, was much slower than would be expected for such processes (6 h vs. 40 min) (Mäck and Tischner, 1994). Moreover, the slopes of the log-linear regressions were similar among the urea, N-deprived, and rerooted treatments (Table 1). These results suggest that a product of NH_4^+ assimilation that takes several hours to accumulate after first exposure to NH_4^+ inhibits NH_4^+ influx (Morgan and Jackson, 1988a).

The intercepts of the log-linear regressions differed among the treatments (Table 1). Several factors may be responsible. The plants in the different treatments varied in age and root to shoot ratio. They also varied in growth rates and tissue NH_4^+ levels (Nicoulaud and Bloom, 1996).

LOW VERSUS HIGH NH_4^+ LEVELS. The NH_4^+ concentration during the induction experiment was $50 \mu\text{mol}\cdot\text{L}^{-1}$, whereas previous studies have used NH_4^+ concentrations in the $\text{mmol}\cdot\text{L}^{-1}$ range. Concentrations of NH_4^+ below $100 \mu\text{mol}\cdot\text{L}^{-1}$ do not inhibit root NH_4^+ uptake (Smart and Bloom, 1998), whereas concentrations in the $\text{mmol}\cdot\text{L}^{-1}$ range can prove toxic (Roberts and Pang, 1992). An initial toxic reaction followed by recovery would appear similar to an induction response. Alternatively, NH_4^+ uptake may depend on the operation of two influx systems (Joseph et al., 1975; Mäck and Tischner, 1994; Morgan and Jackson, 1988a; Wang et al., 1993, 1994): a high affinity system (Mechanism I) that operates at lower concentration and a low affinity one (Mechanism II) that operates at higher concentrations. The low affinity NH_4^+ transport system may be inducible, whereas the high affinity one is not. This seems to be the case for the NO_3^- transport system in maize (Hole et al.,

Table 1. The response of log (net NH_4^+ uptake) during the first 8 h of exposure to NH_4^+ for the various growth conditions. The probability is for the effect of time upon log (net NH_4^+ uptake) from a repeated measures analysis of variance. The slope, intercept, and correlation are from a log-linear regression for each plant. Given are the mean $\pm$ SE (n = 4) for the treatments.

Treatment	Probability	Slope	Intercept	Correlation
NO_3^-	0.18	0.00 ± 0.29	-0.21 ± 0.10	-44%
Urea	<0.0001	-0.027 ± 0.011	-0.11 ± 0.04	-95%
N-deprived	<0.0001	-0.049 ± 0.016	0.35 ± 0.03	-92%
Rerooted	<0.0001	-0.059 ± 0.022	0.14 ± 0.01	-89%

1990) and barley (Aslam et al., 1992) and for the NH_4^+ transport in barley (Mäck and Tischner, 1994).

TRANSPLANT SHOCK. The plants in this study were allowed to recover from transplant shock for at least 10 h before an experiment began. In contrast to earlier studies, we first exposed plants to NH_4^+ by switching solutions flowing through the root cuvette rather than by transferring plants from one container to another. Transferring plants from one container to another depressed net NO_3^- uptake in barley for several hours and mimicked an induction phase (Bloom and Sukrapanna, 1990).

In summary, we found in tomato that NH_4^+ does not induce its own high affinity transport system and some product of NH_4^+ assimilation seems to suppress NH_4^+ uptake. As a constitutive system, the high affinity NH_4^+ transport system in tomato roots is always prepared to take advantage of any NH_4^+ that becomes available in the soil. This rapid uptake of NH_4^+ may also explain why tomatoes may quickly show toxicity symptoms when first exposed to high levels of NH_4^+ (Barker et al., 1967).

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