EFFICIENCY OF NITRATE UPTAKE AND REDUCTION BY COTTON CULTIVARS INDUCED AT DIFFERENT NITRATE LEVELS

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Abstract

The efficiency of nitrate assimilation by 10-day-old seedlings of three Pima, S-7, O.B, Conq, (*Gossypium barbadense* L.) and four Acala, Maxxa, GC-510, Royale, SJ-2, (*Gossypium hirsutum* L.) cotton cultivars was compared. The seedlings were induced with 0.05, 0.1 or 1.0 mM NO_3^- for 24 h. Nitrate uptake, efflux and *in vivo* reduction were determined. Nitrate uptake rates of Pima cultivars were about 50 percent higher when induced at 0.05 mM NO_3^- as compared to the Acala cultivars. The differences in uptake rates narrowed as NO_3^- concentration of the uptake solutions was increased. In contrast, NO_3^- efflux from roots of Pima cotton was about two-fold higher at all levels of NO_3^-. Nitrate concentration in roots induced with 1.0 mM NO_3^- was three-fold higher than in those induced with 0.05 mM NO_3^-; however NO_3^- efflux increased only slightly. *In vivo* NO_3^- reduction by Pima cotton was also higher than that in Acala. The results indicate that Pima cultivars are more efficient in NO_3^- assimilation at lower than at higher NO_3^- concentrations.

Introduction

Physiological and biochemical traits have often been the focus of yield improvement programs in agronomic crops (Deckard and Busch, 1978; Messmer et al., 1984). Since N influences many physiological and developmental processes, its assimilation by young seedlings is an early indicator of subsequent plant growth and development. Thus, the identification of genotypes that are better able to utilize N fertilizer to produce higher yields would be highly desirable. The rate-limiting step in the assimilation of N is the enzyme nitrate reductase which catalyses the reduction of NO_3^- to NO_2^-. This enzyme has been used as an indicator of N utilization by cereals (Deckard and Busch, 1978); however, other studies have shown that correlations between nitrate reductase activity and yield components are realized only when adequate NO_3^- is available (Eilrich and Hageman, 1973). Since soil N is often in short supply, and since nitrate reductase activity in both roots and leaves is regulated by NO_3^- influx (Shaner and Boyer, 1976), absorption rather than assimilation may be a better indicator of the efficiency of NO_3^- utilization and its subsequent impact on yield.

The results presented here summarize experiments in which net NO_3^- uptake, efflux and *in vivo* reduction by three Pima (*Gossypium barbadense* L.) and four Acala (*Gossypium hirsutum* L.) cultivars were monitored over a range of NO_3^- concentrations. The results show that Pima is more efficient in utilizing NO_3^- when it is present in limiting concentrations; however, this advantage disappears at higher NO_3^- concentrations.

Materials and Methods

Seedling Culture: Three Pima (S-7, O. B., Conq) and four Acala (Maxxa, GC-510, Royale, SJ-2) cultivars were used for this study. Seeds were rinsed with bleach and germinated at room temperature in the dark on moist germination paper. After 5 days the seedlings were transferred to a stainless steel screen suspended above the surface of 5 L aerated N-free one-quarter strength Hoagland solution contained in a plastic beaker. After 48 h in light the nutrient solution was replaced with one-quarter strength Hoagland solution containing 0.05, 0.1 or 1.0 mM NO_3^- to induce NO_3^- uptake and efflux systems. Uptake and efflux were determined after an additional 24 h as described below.

Measurement of NO_3^- uptake and Efflux: Uptake and efflux, respectively, were determined by following NO_3^- depletion from, or accumulation in, the external solution as described before (Aslam et al., 1994). Cumulative uptake and efflux were calculated from NO_3^- depletion or accumulation data as described by Goyal and Huffaker (1986). Uptake rates were then calculated by linear regression analysis of the cumulative uptake curves over a short period (12 to 15 min).

Measurement of *In Vivo* NO_3^- reduction: Seedlings were transferred to Erlenmeyer flasks containing 550 mL of aerated uptake solution (0.05 or 1.0 mM NO_3^-) and placed in a growth chamber at 25° C under continuous light. Uptake and accumulation of NO_3^- were measured after 24 h. *In vivo* NO_3^- reduction by intact seedlings was determined by subtracting the total amount of NO_3^- accumulated from that absorbed (Aslam and Huffaker, 1982).

NO_3^- Determination: Roots, leaves and stems were homogenized with a chilled mortar and pestle in 4 mL distilled deionized water per g of tissue, and a small amount of acid washed sand. The extracts were centrifuged at 30,000g at 4°C for 15 min and the supernatants were used for NO_3^- determination. NO_3^- from the uptake solutions and the tissue extracts was determined by measuring its A_{210} after separation by HPLC on a partisil-10 SAX anion-exchange column (Thayer and Huffaker, 1980).

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Results and Discussion

Time Course of Induction of NO_3^- Uptake System: Figure 1 shows the time course of induction of the NO_3^- uptake system in roots of one Pima (S-7) and one Acala (Maxxa) cultivar. Both cultivars exhibited similar low levels of constitutive uptake activity before exposure to exogenous NO_3^-. When NO_3^- was supplied induction proceeded at a relatively rapid rate for the initial 4 h and was similar in both cultivars; thereafter, induction continued at a reduced rate for an additional 8 h. Full induction in both cultivars occurred at about 12 h at which time NO_3^- uptake activity in Pima was 20 to 25% higher than in Maxxa (Fig. 1). The time course of induction of the uptake system in cotton was similar to that reported for cereals (Aslam et al., 1993).

Comparison of NO_3^- Uptake Rates: To compare NO_3^- uptake capacity among cultivars, the seedlings were induced with 0.05, 0.1 or 1.0 mM NO_3^- for 24 h and the uptake rates were determined at the same NO_3^- concentration at which they were induced. Net uptake rates by roots of the Acala cultivars were about half of those of the Pima cultivars when the seedlings were grown in 0.05 mM NO_3^- (Fig. 2). At 0.1 mM NO_3^-, uptake rates in all Acala cultivars were only 20 to 30% lower than those in the Pima cultivars. However; when the uptake system was induced with 1.0 mM NO_3^-, and the rates determined at the same concentration, no differences were observed in the NO_3^- uptake capacity among the cultivars. These results indicate that Pima cultivars may be more efficient in absorbing NO_3^- from the growth medium when NO_3^- is limiting. Why Pima cultivars are more efficient in NO_3^- absorption at lower NO_3^- concentrations is not clear; however, this may relate to morphological differences in the root systems. Pima cultivars have a more cylindrical root system than do the Acala cultivars, which results in greater surface area per unit mass. This likely provides the Pima cultivars with additional uptake sites. It is also possible that the uptake system is fully induced at lower NO_3^- concentration in the Pima cultivars; however, this seems unlikely since when the system was induced at 1.0 mM NO_3^- and the rates determined from solutions initially containing 0.1 mM NO_3^-, net uptake by the Pima cultivars was still 20 to 30% higher than that of the Acala cultivars (data not shown).

Comparison of NO_3^- Efflux: Figures 3, 4 and 5 show NO_3^- efflux from roots of Pima and Acala cultivars induced with 0.05, 0.1 or 1.0 mM NO_3^-, respectively. Efflux from Pima roots was considerably higher than from Acala roots at all NO_3^- concentrations. As is the case with uptake, efflux was about the same for all Acala cultivars; however, it did increase slightly when the concentration of NO_3^- in the induction solution was increased to
1.0 mM. In contrast, efflux from from Pima roots increased when the induction concentration NO_3^- was increased from 0.05 to 0.1 mM, but there was no further increase in efflux as the NO_3^- concentration was increased to 1.0 mM (Figs. 3 and 4). Since both influx and efflux are active, energy requiring processes (Glass et al., 1990; Aslam et al., 1996b), the increased use of metabolic energy to support these processes in the Pima cultivars may be counter productive to growth and development.

Although root NO_3^- concentrations were similar in both groups of cultivars at each external NO_3^- concentration (Table 1), NO_3^- efflux from the Pima cultivars was considerably higher than from the Acala cultivars (Figs. 3, 4, 5). Likewise, whereas NO_3^- accumulation in roots increased three to four fold, there was only 20 to 30% increase in efflux. This indicates that efflux was not entirely dependent upon root NO_3^- concentration. This is in contrast to earlier studies which showed that NO_3^- efflux was strongly correlated with root NO_3^- concentration (Deane-Drummond and Glass, 1983). However; our recent work with barley roots has shown that NO_3^- efflux is an inducible system and after full "induction" there is no increase in NO_3^- efflux even when the root NO_3^- concentration is increased (Aslam et al., 1996a).

Comparison of *In Vivo* NO_3^- Reduction: Nitrate uptake and *in vivo* reduction were similar in Pima and Acala cultivars when the seedlings were induced with 1.0 mM NO_3^- (Table 2). About 45 to 50% of the absorbed NO_3^- was reduced during the 24 h period. However, at 0.05 mM NO_3^- the Pima cultivars were more efficient in NO_3^- assimilation than were the Acala cultivars. Pima not only absorbed more NO_3^-, but *in vivo* reduction was also 50 to 60 % higher (Table 2). *In vivo* NO_3^- reduction is dependent upon nitrate reductase activity, the generation and supply of reductant as well as the availability of NO_3^-. From our results it is not possible to determine which factor was limiting *in vivo* NO_3^- reduction in the Acala cultivars at the lower NO_3^- concentration. However, since there were no differences in *in vivo* reduction at the higher NO_3^- concentrations, it is likely that the decrease in reduction in Acala cultivars at the lower NO_3^- concentration was due to deceased NO_3^- availability (Table 2). Likewise, it is not possible to determine the relative contribution of roots and leaves to NO_3^- reduction from this study; however, Radin (1977) reporteded that cotton root exudate contained approximately 95% of the N as NO_3^-, indicating that most of the absorbed NO_3^- was assimilated in the green tissue.

Summary

Pima cultivars differ from Acala cultivars in the efficiency of NO_3^- utilization under conditions of limiting NO_3^- availability. At limiting NO_3^-, assimilation, including both uptake and *in vivo* reduction, was more efficient in the Pima cultivars. However, at higher NO_3^- concentrations its assimilation was similar in both groups of cultivars. Although, roots of all cultivars accumulated similar concentrations of NO_3^-, efflux from roots of the Pima cultivars was much higher than from roots of Acala cultivars. Whereas, NO_3^- root concentration increased three

to four fold when exogenous NO_3^- concentration was increased from 0.05 to 1.0 mM, NO_3^- efflux increased only 20 to 30%, indicating that efflux may not be a function of root NO_3^- concentration.

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Table 1. Nitrate concentration in roots, stem and leaves of cotton cultivars induced with 0.05, 0.1 or 1.0 mM NO_3^- for 24 h. Data are mean ±SD (*n* = 3). NO_3^- concentration in roots, stem and leaves of cultivars O.B. and ConQ was similar to that in S-7 and in Royale and SJ-2 was similar to that in Maxxa and GC-510.

Cultivar	Roots	Stem	Leaves
	µmol g^{-1} Fresh Weight		
	0.05 mM NO_3^--Induced		
S-7	12.2±0.3	9.8±0.8	11.8±1.5
Maxxa	12.9±0.3	9.2±0.4	6.6±0.4
GC-510	11.2±1.2	6.5±0.8	6.0±1.3
	0.10 mM NO_3^- -Induced		
S-7	16.2±2.2	12.0±0.7	15.4±2.3
Maxxa	21.4±2.1	14.3±0.5	12.0±0.9
GC-510	16.9±1.6	11.5±0.3	11.5±1.1
	1.0 mM NO_3^- -Induced		
S-7	39.6±0.9	37.2±1.1	45.9±2.2
Maxxa	43.2±1.6	34.1±1.3	42.3±2.8
GC-510	39.9±1.8	38.7±0.9	40.2±1.3

Table 2. Comparison of NO_3^- uptake, concn and *in vivo* reduction in Pima (S-7) and Acala (Maxxa, GC-510) cultivars. Data are mean ±SD (n = 3). The values in parenthesis are percent in vivo reduction of the absorbed NO_3^- .

Cultivar	Uptake	Accumulation	Reduction
	µmol g^{-1} Fresh weight $(24\ h)^{-1}$		
	From 0.05 mM NO_3^-		
S-7	12.8±0.2	5.1±0.8	7.7±0.4(60)
Maxxa	7.9±0.5	5.2±0.2	2.7±0.4(34)
GC-510	8.8±0.8	5.2±0.8	3.6±0.3(41)
	From 1.0 mM NO_3^-		
S-7	48.0±0.3	23.3±0.1	24.7±0.2(51)
Maxxa	49.9±0.5	28.0±0.3	21.9±0.3(44)
GC-510	54.6±0.8	30.6±0.4	24.0±0.4(44)

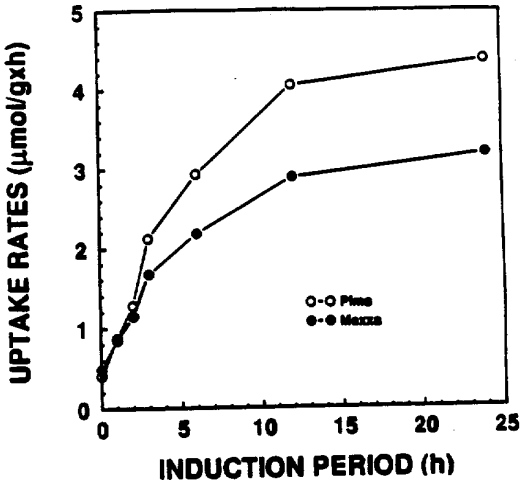

Figure 1. Time-course of the induction of NO_3 uptake system in roots of two cotton cultivars. Seedlings were grown hydroponically in N-free nutrient solution for 6 days in continuous darkness followed by 2 days in continuous light . The seedlings were then transferred into solutions containing 0.1 mM NO_3 while still in light and uptake rates were determined at different intervals. The uptake solutions contained 0.1 mM NO_3.

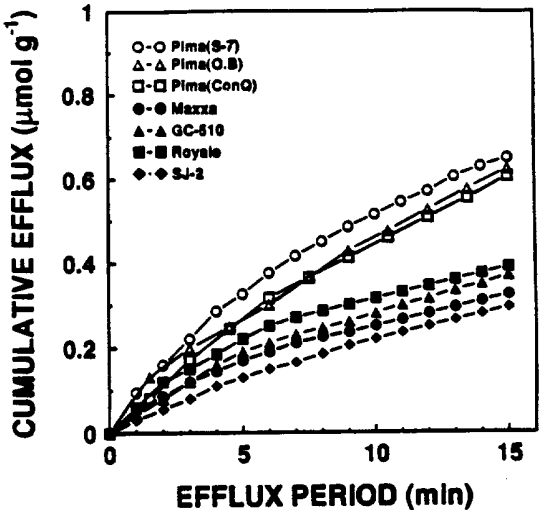

Figure 3. Cumulative efflux of NO_3 from roots of cotton cultivars induced with 0.05 mM NO_3 for 24 h. The seedlings were grown and induced as described in figure 1. NO_3 efflux was determined by following its accumulation in the external solution containing 1 mM NO_3.

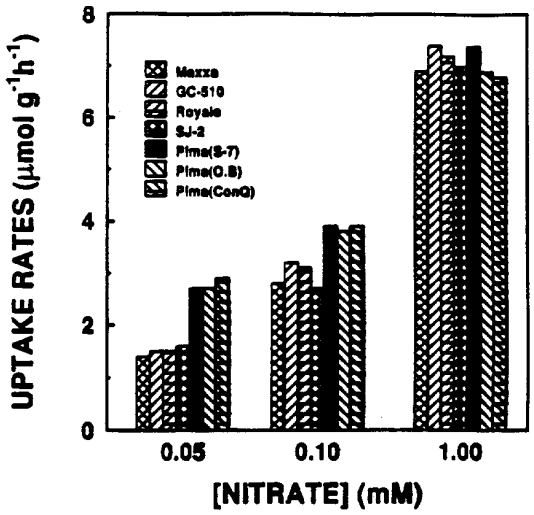

Figure 2. Net NO_3 uptake rates in cotton cultivars induced with 0.05, 0.1 or 1.0mM NO_3 for 24 h. Net uptake rates were determined by following NO_3 depletion from the uptake solutions initially containing the same NO_3 concentration as the induction solution.

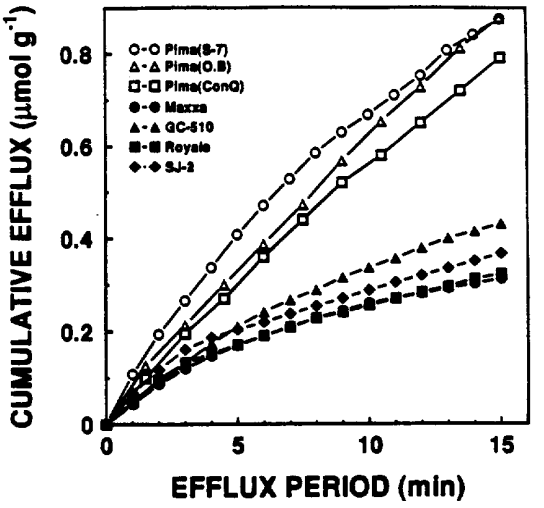

Figure 4. Cumulative efflux of NO_3 from roots of cotton cultivars induced with 0.1 mM NO_3 for for 24h. The seedlings were grown and induced as described in figures 1 and 2. NO_3 efflux was determined by following its accumulation in the external solution containing 1 mM NO_2.

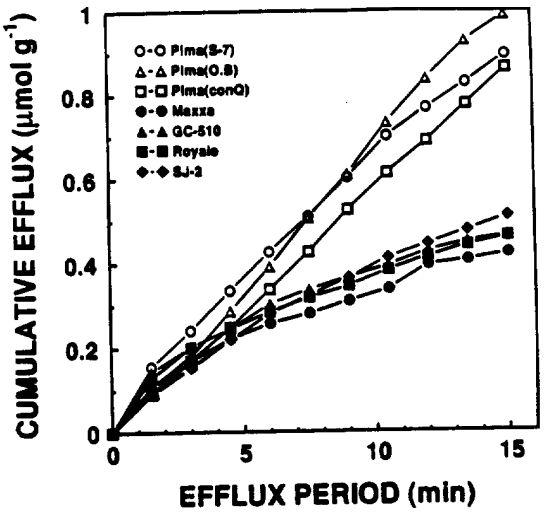

Figure 5. Cumulative efflux of NO_3 from roots of cotton cultivars induced with 1 mM NO_3 for 24 h as in figure 5. NO_3 efflux was determined by following its accumulation in the external solution cotaining 1mM NO_2.