METABOLIC REGULATION OF NITRATE EFFLUX AND NET UPTAKE IN ACALA AND PIMA COTTON

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Abstract

The effect of temperature and several metabolic inhibitors on NO_3^- fluxes in intact roots of 8-day-old seedlings of Pima (S-7) (*Gossypium Barbadense* L.) and Acala (Maxxa) (*Gossypium hirsutum* L.) cotton was studied. Fluxes were induced with 0.1 mM NO_2^- (influx) or 0.1 mM NO_3^- (net uptake and efflux) for 24 h. Absorption (influx and net uptake) and efflux, respectively, were determined by following NO_3^- depletion from, and accumulation in, the external solution. Both NO_3^- uptake and efflux were sensitive to temperature extremes. Net NO_3^- uptake decreased both at lower and higher temperatures, whereas efflux was stimulated. Azide and arsenate inhibited net NO_3^- uptake; however, arsenate had no effect on efflux, while azide stimulated that system. Thus, azide had a greater inhibitory effect on net uptake than on influx. Dinitrophenol also inhibited net NO_3^- uptake and influx and had little effect on efflux. Carbonyl-*m*-chlorophenyl hydrazone and diethylstilbestrol inhibited net uptake and stimulated efflux. The responses of the metabolic modifiers were similar in both Pima and Acala cotton.

Introduction

Net NO_3^- uptake by plant roots involves simultaneous influx and efflux between roots and the external solution (Morgan et al. 1973; Jackson et al. 1976). Hence, net NO_3^- uptake is equal to the difference between influx and efflux (Morgan et al. 1973, Jackson et al. 1976). The metabolic nature of influx has been clearly defined. For example, Glass et al. (1990, 1992) reported that NO_3^- absorption by plant roots is a thermodynamically active process that requires metabolic energy. Thus, it is not surprising that NO_3^- uptake is inhibited by low temperature (Hallmark and Huffaker 1978), anaerobiosis (Rao and Rains 1976) and metabolic inhibitors (Rao and Rains 1976; McClure et al. 1990). On the other hand, little is known regarding the metabolic nature of NO_3^- efflux. We recently reported that in barley roots metabolic inhibitors which reduced net NO_3^- uptake more than influx, did so by stimulating its efflux (Aslam et al., 1997a). In the same study both efflux and net uptake were inhibited when the solution temperature was lowered. In contrast, this study shows that while lower temperature inhibited net NO_3^- uptake in cotton roots, efflux was stimulated. This study also shows that influx and efflux are separate processes and are independently regulated by metabolic inhibitors.

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Abbreviations

CCCP = carbonyl cyanide-*m*-chlorophenyl hydrazone; DES = diethylstilbestrol; DNP = 2,4-dinitrophenol.

Materials and Methods

Seedlings Culture

Pima (S-7) and Acala (Maxxa) cultivars were used for this study. Seeds were rinsed with diluted commercial bleach solution prior to germination. The washed seeds were placed on moist germination paper and maintained at room temperature in darkness for five days (Aslam et al., 1997b). The seedlings were then 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 0.1 mM NO_2^- or 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^- Fluxes

Influx and net uptake were determined by following NO_3^- depletion from the external solution as described before (Aslam et al., 1997b). Since roots of NO_2^--induced seedlings contain little NO_3^-, initial uptake rates are equivalent to influx. NO_3^- efflux was determined by following its accumulation in the efflux solution. 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). All measurements were made at 25°C unless otherwise noted. In the temperature study flux solution temperatures were varied from 10° to 50°C during the assay period, while the ambient temperature was kept at 25°C. Inhibitors were supplied directly to the flux solutions (for concentrations see Fig. 3 to 5 legends) and were present during flux measurements.

NO_3^- Determination

NO_3^- from the flux solutions 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

Effect of Temperature

In both cultivars net NO_3^- uptake increased as the temperature of the uptake solution was increased from 10° to 35°C, uptake then decreased abruptly as the temperature

was further increased (Fig. 1). In fact, at extreme temperatures net uptake was negative, more so at higher than at lower temperature (Fig. 1). In contrast, NO_3^- efflux increased at extremes temperatures (Fig. 2). The increase in efflux was more abrupt at higher temperature than at lower temperature. Thus, the negative net uptake at extreme temperatures was due to a stimulation of efflux. The response of efflux to lower temperatures in cotton roots is different from that in barley roots where, similar to NO_3^- uptake, efflux was also inhibited at lower temperatures (Aslam et al., 1997a). At higher temperature net uptake and efflux were affected more in the Pima than in the Acala cultivar.

Effect of Azide and Arsenate

Both azide and arsenate inhibited influx and net uptake; however, azide inhibited net uptake more than influx (Fig. 3). The azide response was due to a stimulation of efflux and was similar in both cultivars. In contrast, arsenate had no effect on efflux. Thus, both influx and net uptake were reduced to the same extent by this inhibitor. The effect of arsenate on cotton roots differed from that in barley roots where NO_3^- efflux was also stimulated (Aslam et al., 1997a).

Effect of DNP and CCCP

DNP inhibited NO_3^- influx but had little effect on efflux; Thus, its effect on of net uptake and influx were similar (Fig. 4). The lack of an effect on efflux by DNP suggests that either the efflux system is not sensitive to this inhibitor, or that the metabolic energy produced by substrate level phosphorylation is sufficient to maintain efflux. CCCP, which also uncouples oxidative phosphorylation, stimulated NO_3^- efflux . As a result, net NO_3^- uptake was inhibited more than efflux (Fig. 4).

Effect of DES

DES inhibited net NO_3^- uptake more than influx (Fig. 5). Again, this was the result of the stimulation of efflux Similar results were obtained with barley roots (Aslam et al., 1997a). These results indicate that NO_3^- transport across the plasma membrane may be regulated by the H^+-ATPase activity.

Conclusions

In summary, the evidence supports the interpretation that influx and efflux are separate processes and are under independent metabolic control in cotton. Earlier studies have clearly defined influx as an active, energy requiring process. These results support that conclusion. The nature of the efflux process is less well defined. We have previously shown that the efflux process in barley has a Q_{10} of 2.0 suggesting that it may also be an active process. However, it is also possible that an energy barrier to outward diffusion of NO_3^- exists which is overcome at higher temperatures. (Nobel, 1983). If so then efflux is likely a passive process. The results of anion channel blockers in the study with barley roots support the latter interpretation. Channel blocker experiments have not yet been done with cotton.

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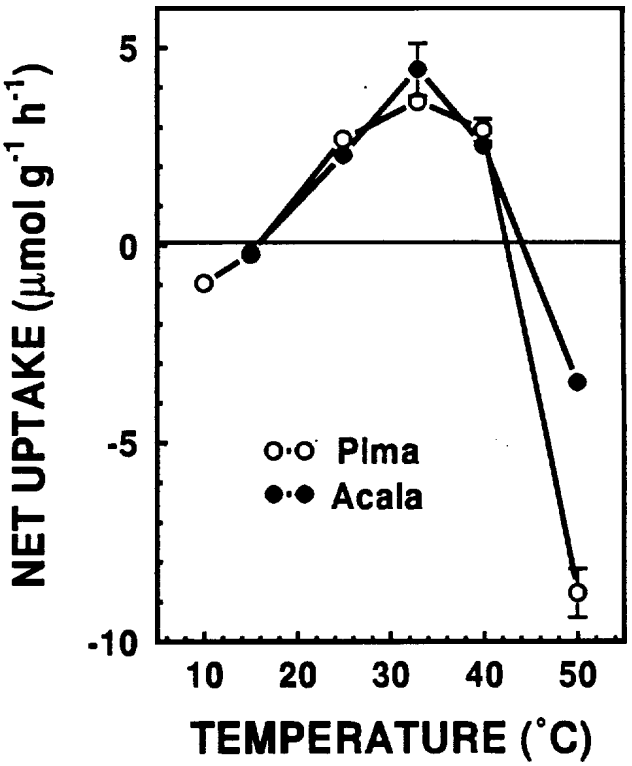

Figure 1. Effect of temperature on net NO_3^- uptake in roots of Pima and Acala cotton. The seedlings were induced with 0.1 mM NO_3^- in continuous light. After 24 h of induction, net uptake was determined by following NO_3^- depletion from the external solution. The assay period was 15 min with sampling every 3 min. The solution temperature was varied from 10° to 50°C, while the ambient temperature was kept at 25°C. Uptake rates were calculated by linear regression analysis of the cumulative uptake data.

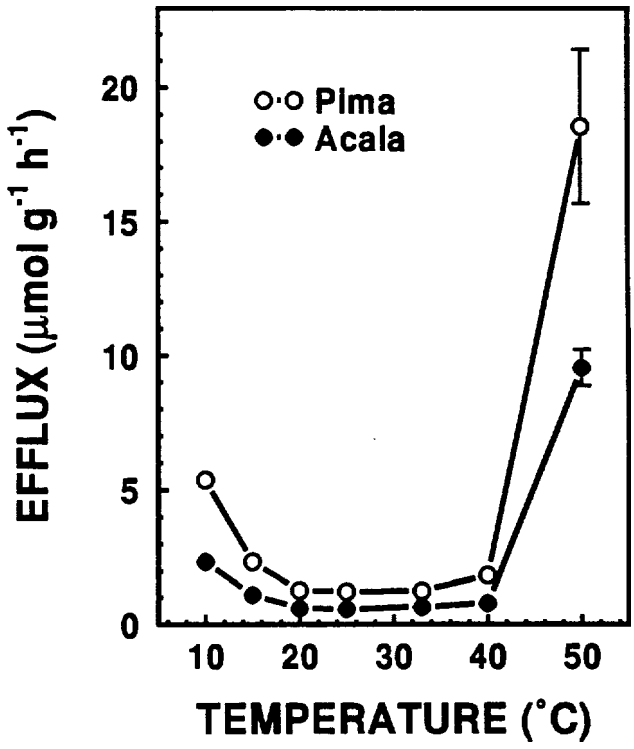

Figure 2. Effect of temperature on NO_3^- efflux from roots of Pima and Acala cotton. The seedlings were induced with 0.1 mM NO_3^- in continuous light. After 24 h of induction, efflux was determined by following NO_3^- accumulation in the external solutions. The assay period was 15 min with sampling at 1.5 min. intervals. The solution temperature was varied from 10° to 50°C, while the ambient temperature was kept at 25°C. Efflux rates were calculated by linear regression analysis of the cumulative efflux data.

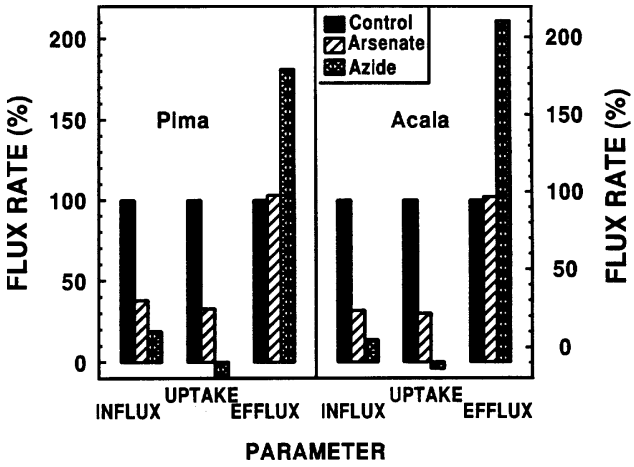

Figure 3. Effect of arsenate and azide on NO_3^- fluxes in roots of Pima and Acala cotton seedlings induced with 0.1 mM NO_2^- (influx) or NO_3^- (net uptake and efflux) for 24h. Experimental procedures were the same as described in figures 1 and 2, except that flux solution temperature was 25°C. The flux solutions contained 1.0 mM sodium arsenate or 0.1 mM sodium azide.

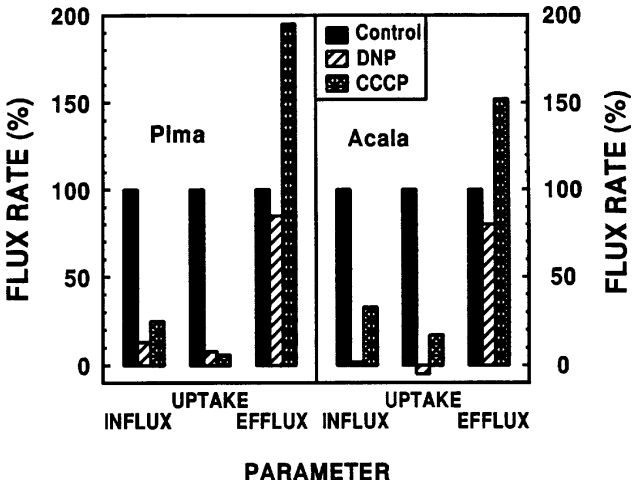

Figure 4. Effect of DNP and CCCP on NO_3^- fluxes in roots of Pima and Acala cotton seedlings induced with 0.1 mM NO_2^- (influx) or NO_3^- (net uptake and efflux) for 24h. Experimental procedures were the same as described in figures 1 and 2, except that flux solution temperature was 25°C. The flux solutions contained 0.05 mM DNP or 0.02 mM CCCP.

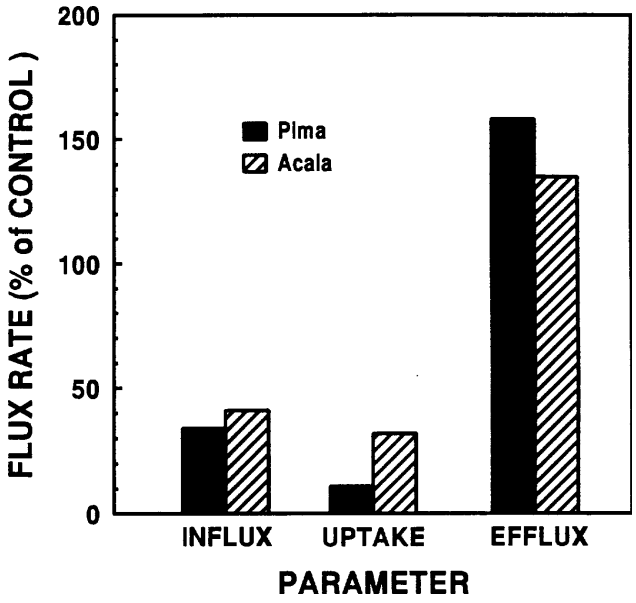

Figure 5. Effect of DES on NO_3^- fluxes in roots of Pima and Acala cotton seedlings induced with 0.1 mM NO_2^- (influx) or NO_3^- (net uptake and efflux) for 24h. Experimental procedures were the same as described in figures 1 and 2, except that the flux solution temperature was 25°C. DES was supplied at 0.05 mM concentration.