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p-Fluorophenylalanine-Induced Restriction of Ion Uptake and Assimilation by Maize Roots[1]

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ABSTRACT

Roots of decapitated maize seedlings (*Zea mays* L.) were exposed for 12 hours to 1.0 millimolar KNO_3 (98.5 atom per cent ^{15}N) in the presence and absence (control) of 0.1 millimolar *p*-fluorophenylalanine (FPA), an analog of the amino acid phenylalanine. FPA decreased nitrate uptake but had little effect on potassium uptake. In contrast, accumulation of both ions in the xylem exudate was greatly restricted. The proportion of reduced ^{15}N-nitrogen that was translocated at each time was also restricted by FPA. These observations are interpreted as indicating that synthesis of functional protein(s) is required for nitrate uptake and for transport of potassium, nitrate, and reduced-^{15}N from xylem parenchyma cells into xylem elements. The effect of FPA on nitrate reduction is less clear. Initially, FPA limited nitrate reduction more than nitrate uptake, but by 8 hours the cumulative reduction of entering nitrate was similar (~35%) in both control and FPA-treated roots. A relationship between nitrate uptake and nitrate reduction is implied. It is suggested that nitrate influx regulates the proportion of nitrate reductase in the active state, and thereby regulates concurrent nitrate reduction in decapitated maize seedlings.

Utilization of nitrate by higher plants involves the basic processes of uptake, reduction and translocation. Nitrate reductase, the enzyme which catalyzes the rate-limiting step in nitrate assimilation, requires continued protein synthesis to maintain its activity (10, 11, 16, 23, 24). There is also evidence that uptake and translocation of nitrate are dependent on protein synthesis (9, 19, 26). This latter evidence, however, is based primarily on studies with inhibitors of RNA and protein synthesis, some of which are known to affect cellular processes other than protein synthesis (3, 7, 8). For example, cycloheximide has been reported to stimulate respiration and inhibit chloride uptake by beet root discs within 1 h of its application (8). Because of this lack of specificity, we have reexamined the effect of impaired protein synthesis on nitrate uptake and assimilation, using FPA,[3] an analog of phenylalanine, to produce ineffective protein (18, 21). Potassium fluxes were also quantified, since this cation is the common counterion associated with nitrate uptake and translocation.

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[3] Abbreviations: FPA, *p*-fluorophenylalanine; NR, nitrate reductase; A % ^{15}N, atom per cent ^{15}N.

MATERIALS AND METHODS

Growth of Maize Seedlings. DeKalb XL-45 maize seeds (*Zea mays* L.) were germinated on paper towels moistened with 0.1 mM $CaSO_4$ in an incubator maintained in darkness at 30°C and 98% RH. After 3 d, all roots except the primary roots were excised, and the seedlings were assembled into cultures of five seedlings each. The cultures were placed into aerated, nitrogen-free nutrient solution and returned to the incubator. The solution (pH 6.0) contained 0.05 mM K_2SO_4, 0.4 mM KH_2PO_4, 0.25 mM $MgSO_4$, 1.0 mM $CaSO_4$, trace elements at 0.4 Hoagland concentration (12) and FeEDTA at 1.0 mg Fe l^{-1}. On the 4th d, the shoots were excised below the first node, and exudate collectors, small plastic pipet tips, were sealed to the mesocotyls with silicone grease. The cultures were placed in fresh, nitrogen-free nutrient solution and returned to the incubator. The experiment was run 20 h later, when the decapitated seedlings were 5-d old.

Experimental Procedure. Just prior to the experiment, exudate was removed from the collectors, and the roots were rinsed thoroughly in 1.0 mM $CaSO_4$ (30°C). The roots of each culture were then exposed to 250 ml of uptake solution containing 1.0 mM $K^{15}NO_3$ (98.5 A % ^{15}N) and O (control) or 0.1 mM FPA. Otherwise, the composition of the uptake solution was identical to that of the nitrogen-free, nutrient solution. During the 12-h exposure period, the uptake solutions (pH 6.0) were maintained at 30°C, aerated continuously, and replaced with fresh solution after 8 h. Exudate was collected periodically and kept frozen prior to analysis. Four replicate cultures per treatment were harvested after 0, 2, 4, 8, and 12 h. The roots were washed in distilled H_2O and then held for 15 min in ice-cold 'rinse' solution which was identical to the control uptake solution except for substitution of 1.0 mM KCl for 1.0 mM $K^{15}NO_3$. Roots were then washed in ice-cold, 1.0 mM $CaSO_4$ and separated from the seed/mesocotyl. Both tissues were blotted dry, weighed, freeze-dried, and reweighed. Prior to analysis, each root was recombined with its seed/mesocotyl.

Analytical Procedures. Potassium in the uptake solution and xylem exudate was measured by atomic absorption. Nitrate-nitrogen in the xylem exudate was determined by a manual adaptation of the Lowe and Hamilton procedure (14). Following treatment with H_2SO_4-H_2O_2 to remove nitrate (17), reduced nitrogen in the exudate was digested (15), and ammonium nitrogen in the digest was measured colorimetrically (2). Nitrogen in the tissue was partitioned into soluble and insoluble fractions (17). Nitrate and reduced nitrogen in the soluble fraction were determined by the same procedures used for the exudate. Insoluble reduced nitrogen was determined colorimetrically (2) after Kjeldahl digestion (15). Enrichment of ^{15}N in all reduced fractions was determined mass spectrometrically, using the freeze-layer procedure (27). Enrichment of ^{15}N in nitrate from both tissue and exudate was assumed to be identical to that supplied to the roots (98.5 A % ^{15}N), since the plants were grown without nitrate prior to the experiment.

Uptake of $^{15}NO_3^-$ was computed as the sum of the individual ^{15}N-nitrogen constituents in the tissue and exudate. Reduction of $^{15}NO_3^-$ was calculated as the sum of the soluble and insoluble reduced-^{15}N in tissue and exudate.

During the course of this experiment, the fresh weight of roots increased from 240 ± 5 to 270 ± 8 mg plant^{-1} for the control plants, whereas no significant increase was detectable for the FPA-treated plants. All data are presented on a per plant basis.

RESULTS

Except for the anomalous data at 2 h, FPA had little effect on uptake of K^+ (Fig. 1). In contrast, FPA severely restricted K^+ translocation. The restriction was evident at 4 h, and by 12 h cumulative translocation of K^+ by FPA-treated seedlings was only 40% of the control.

Unlike K^+ uptake, the uptake of $^{15}NO_3^-$ was appreciably less in the presence of FPA (Fig. 2). The effect of FPA was evident within 2 h. By this time the control plants had taken up 30% more nitrate than FPA-treated plants.

Upon entering the root, nitrate is subject to three processes: reduction, translocation to the xylem, and accumulation by the tissue. The restrictive effect of FPA on each of these processes is presented in Figure 2, B, C, and D, respectively. During the first 4 h, accumulation of nitrate by tissue exceeded the other two processes in both control and FPA-treated seedlings (Fig. 2D). Thereafter, reduction and translocation became dominant in control seedlings, whereas accumulation of nitrate still exceeded reduction and translocation in FPA-treated seedlings. At 8 and 12 h, FPA had no significant effect on the percentage of incoming nitrate that was reduced, 33 to 36% (Fig. 2B). FPA did, however, restrict the percentage that was translocated (Fig. 2C).

Nitrate which is reduced by the root is also subject to three processes. It can be translocated to xylem, retained by root cells, or incorporated into protein and other insoluble forms. The suppressive effect of FPA on each of these processes is presented in Figure 3. During the first 4 h, the dominant process in both control and FPA-treated seedlings was the accumulation of soluble reduced-^{15}N (Fig. 3B). During the following 8 h, however, translocation increased markedly in control plants (Fig. 3A). Thus, by the end of the experiment, translocation accounted for half of the $^{15}NO_3^-$ reduced by control plants. In contrast, FPA severely curtailed translocation of reduced-^{15}N, both in absolute amount and when expressed as a percentage of nitrate reduction (Fig. 3A). Concurrently, a higher percentage of the reduced-^{15}N accumulated in tissue (Fig. 3B), and a greater proportion was incorporated into protein and other insoluble forms (Fig. 3C).

The marked effect of FPA on solute translocation (Figs. 1B, 2C, 3A) was associated with a pronounced restriction in the xylem exudation rate. During the 4- to 12-h period, this remained constant at 115 ± 6 μl plant^{-1} h^{-1} for the control plants but was only 38 ± 3 μl plant^{-1} h^{-1} for seedlings exposed to FPA.

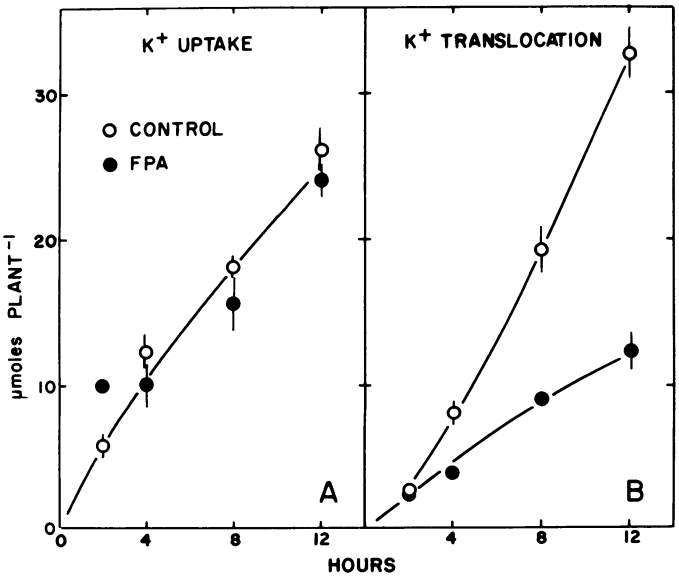

FIG. 1. Cumulative uptake and translocation of K^+ by decapitated maize seedlings exposed to 1.0 mM KNO_3 in the presence and absence (control) of 0.1 mM FPA. Each symbol is the mean of four replicates ± SE, represented by a vertical line when larger than the symbol.

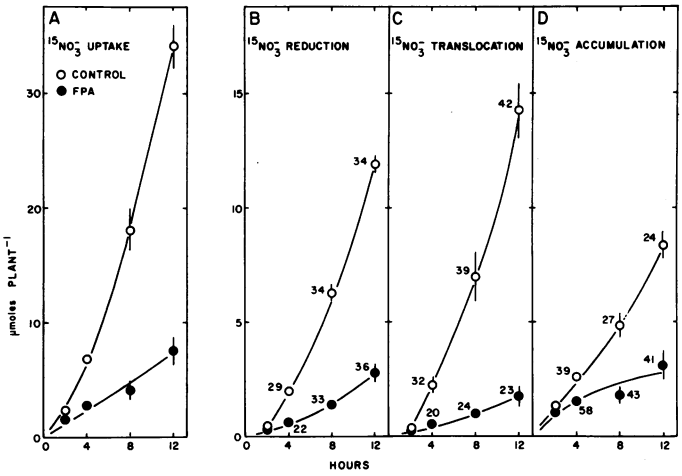

FIG. 2. Cumulative uptake (A), reduction (B), translocation (recovery in xylem exudate) (C), and accumulation (retention by tissue) (D) of $^{15}NO_3^-$ by decapitated maize seedlings exposed to 1.0 mM $K^{15}NO_3$ (98.5 A % ^{15}N) in the presence and absence (control) of 0.1 mM FPA. Cumulative reduction, translocation, and accumulation as percentages of cumulative uptake are listed by the symbols. Each symbol is the mean of four replicates ± SE, represented by a vertical line when larger than the symbol.

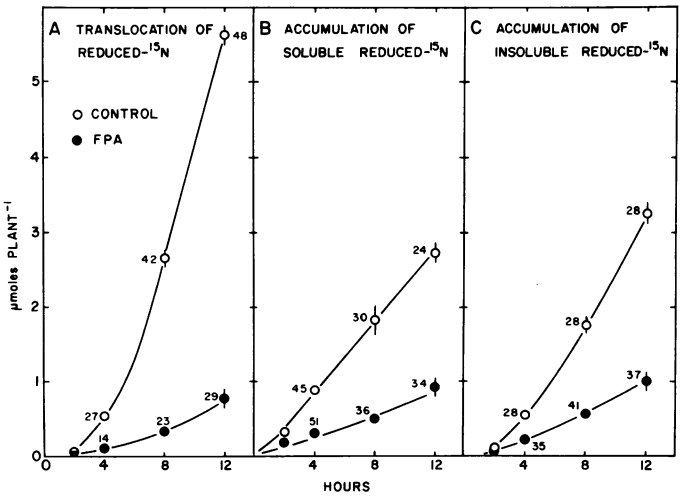

FIG. 3. Cumulative translocation of reduced-^{15}N (A), accumulation (retention by tissue) of soluble reduced-^{15}N (B), and accumulation of insoluble reduced-^{15}N (C) by decapitated maize seedlings exposed to 1.0 mM $K^{15}NO_3$ (98.5 A % ^{15}N) in the presence and absence (control) of 0.1 mM FPA. The numbers by symbols represent cumulative reduced-^{15}N (soluble + insoluble). Each symbol is the mean of four replicates ± SE, represented by a vertical line when larger than the symbol.

DISCUSSION

Potassium Uptake and Translocation. Although FPA restricted K^+ translocation by decapitated maize seedlings, it had little

effect on K^+ uptake. Similar results were reported for Cl^- uptake and translocation by barley roots, in which protein integrity had been altered by exposure to FPA (21) or azetidine 2-carboxylic acid (18), an analog of proline. Since root respiration is unaffected by FPA (21), and since ineffective protein results when FPA replaces phenylalanine in protein (4, 28), the potassium data reported here indicate that continued synthesis of a transport protein, possibly in the plasmalemma of xylem parenchyma cells, is required to sustain deposition of K^+ into xylem elements. In contrast, the insensitivity of K^+ uptake to FPA may indicate that protein synthesis is not involved in this process. Alternatively, a K^+ transport protein of rather long half-life might not have been impaired during the 12-h exposure period. Finally, the dissimilar effects of FPA on K^+ uptake and translocation may have been associated with differential effects on membrane potential. This possibility is based on the observation (6) that FPA decreased the membrane potential measured in xylem vessels of clover roots by 50%, whereas it had little effect on potentials measured in cortical cells.

Nitrate Uptake. Unlike K^+ uptake, $^{15}NO_3^-$ uptake was severely restricted by FPA, the effect being evident within 2 h (Fig. 2A). By 12 h, the cumulative NO_3^-/K^+ uptake ratio had dropped from 1.3 in control plants to 0.3 in FPA-treated plants. As a consequence, an inordinate change in cytoplasmic pH or marked organic acid synthesis must have occurred in FPA-treated roots in order to have maintained ionic charge balance in the tissue.

Exposure to FPA prevented development of the accelerated phase of nitrate uptake, virtually eliminating the 4-fold increase which occurred in control plants (Fig. 2A). The restriction probably cannot be attributed to transinhibition (5, 22), because the nitrate concentration in FPA-treated plants was only half that in control plants (Fig. 2D). The validity of this conclusion, however, depends on the assumption that tissue nitrate concentration reflects that in the cytoplasm, where transinhibitory effects occur.

Since the antiport of hydroxyl ion constitutes part of the driving force for nitrate uptake (20, 22), it is conceivable that FPA limited nitrate uptake indirectly by restricting NR activity, nitrate reduction (Fig. 2B), and associated hydroxyl ion generation. Nitrate reduction and hydroxyl ion generation occur in a 1:1 molar ratio. If the antiport of one hydroxyl ion is assumed to accompany the influx of one nitrate ion (20), the decrease (control-FPA) in nitrate reduction, 8.9 μmol plant^{-1} (Fig. 2B), and hence in hydroxyl generation, can account for only one-third of the decrease in nitrate uptake, 26.7 μmol plant^{-1} (Fig. 2A). Even if the NO_3^-/OH^- antiport stoichiometry is 2:1 (25), the decrease in nitrate reduction cannot account for the decrease in nitrate uptake. We conclude that a significant component of the nitrate uptake process was restricted by the synthesis of abnormal protein in which FPA replaced phenylalanine (18, 21). Concurrent synthesis of a specific transport protein thus appears to be necessary to initiate and sustain nitrate influx. Previous studies with inhibitors (9, 13, 19, 25) also indicate that protein synthesis is involved in nitrate uptake. Whether this protein synthesis is derived from entering nitrate or from turnover of endogenous nitrogen cannot be distinguished from the present data.

Nitrate Assimilation. The presence of FPA restricted all three aspects of the nitrate assimilation pathway: reduction, accumulation by the root, and translocation to the xylem (Fig. 2). Interpretation of these results, however, depends on the extent to which they can be attributed to FPA-restricted nitrate uptake. An uptake-independent comparison of FPA effects can be obtained by calculating each process as a percentage of cumulative nitrate uptake. A summary of these percentages for the 12-h period is presented in Figure 4, and intermediate values are shown in Figure 2. It is apparent that FPA had little effect on nitrate reduction beyond its effect on nitrate uptake, since about

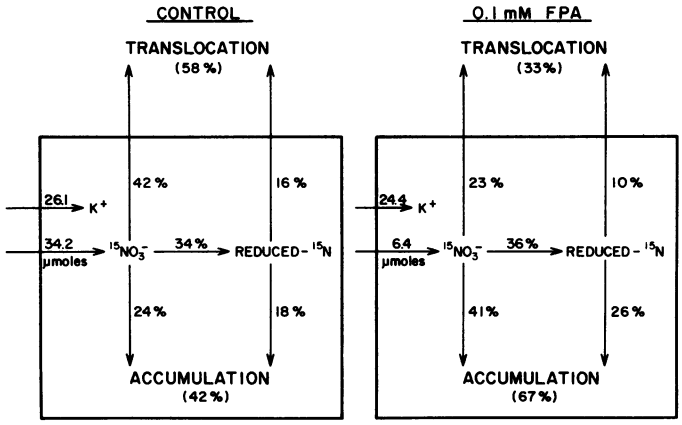

FIG. 4. Uptake and partitioning of $^{15}NO_3^-$ by decapitated maize seedlings exposed for 12 h to 1.0 mM $K^{15}NO_3$ in the presence and absence (control) of 0.1 mM FPA. Partitioning values are given as percentages of total $^{15}NO_3^-$ absorbed.

35% of the incoming nitrate was reduced by both the control and FPA-treated plants (Fig. 4). Nitrate translocation, however, was restricted more than was nitrate uptake: only 23% of the incoming nitrate was translocated in FPA-treated roots compared to 42% in control roots. As a consequence, a greater percentage of the incoming nitrate was accumulated by FPA-treated roots (41%) than by control roots (24%). These effects on translocation and accumulation were apparent by 4 h (Fig. 2).

Since FPA restricted translocation more than uptake, and since inhibitors of RNA synthesis also restrict nitrate translocation (9, 26), it appears plausible that continued synthesis of a short half-life protein is required for transport of nitrate into the xylem. This conclusion remains tentative, however, because as indicated previously, it is possible that FPA may have altered membrane potentials (6).

The similar percentage reduction of incoming nitrate by control and FPA-treated roots (Figs. 2B and 4) raises a question as to whether *de novo* synthesis of ineffective NR was in fact involved in the observed restriction in the actual amounts of nitrate reduced (Fig. 2B). We suggest as an alternative possibility that nitrate reduction was regulated by nitrate influx, the incoming nitrate being required to maintain NR in an active state. This activation is viewed as responding quickly to alterations in nitrate influx. Thus, as FPA restricted nitrate uptake, it would concurrently limit nitrate reduction by altering the proportion of NR in the active state. This hypothesis is in accord with the observation that when wheat is grown under nitrate stress, part of the NR isolated from leaves is in the inactive state (1).

Utilization of Reduced-^{15}N. The products of $^{15}NO_3^-$ reduction, primarily amino acids and amides, can be utilized by the decapitated maize root in three ways; they can be translocated to the xylem, accumulated by the root cells, or incorporated into insoluble forms such as proteins and nucleic acids. Each of these processes was diminished in the presence of FPA (Fig. 3). The question again arises, however, as to what extent these restrictions can be accounted for by the slower reduction of nitrate in the presence of FPA. Expressing each process as a percentage of nitrate reduction at the different experimental times (Fig. 3, numbers adjacent to symbols) reveals that only translocation of reduced-^{15}N was restricted by FPA. This is also evident when translocation of reduced-^{15}N is expressed as a percentage of nitrate uptake (Fig. 4). Associated with the restriction in translocation, a greater proportion of the reduced-^{15}N accumulated in FPA-treated roots than in control roots, both as soluble reduced-^{15}N (Fig. 3B) and as insoluble-^{15}N (Fig. 3C). The latter, which consists largely of protein, indicates that protein synthesis *per se*

was not adversely affected by FPA. Moreover, the rather constant percentage of reduced-^{15}N which accumulated in the insoluble nitrogen fraction (Fig. 3C, numbers adjacent to symbols) indicates a preferential utilization of the products of nitrate reduction for protein synthesis rather than for translocation.

The effect of FPA on the translocation of soluble reduced-^{15}N is consistent with its effect on the translocation of both K^+ and NO_3^-. Again the requirement for continued synthesis of a transport protein in the plasmalemma of xylem parenchyma cells seems the most logical explanation. Whether the same or different proteins are involved in the transport of the three species cannot be ascertained from the present data.

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