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To cite this article: J. V. de Magalhães , V. M. C. Alves , R. F. de Novais , P. R. Mosquim , J. R. Magalhães , A. F. C. Bahia Filho & D.M. Huber (2000) Influence of phosphorus stress on ammonium uptake by maize, Journal of Plant Nutrition, 23:2, 263-273, DOI: [10.1080/01904160009382013](https://doi.org/10.1080/01904160009382013)

To link to this article: <https://doi.org/10.1080/01904160009382013>



Published online: 21 Nov 2008.



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Influence of Phosphorus Stress on Ammonium Uptake by Maize¹

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ABSTRACT

The effect of phosphorus (P) starvation on ammonium (NH₄) uptake was evaluated by growing single-cross seedlings of the male progenitor of the maize double-cross hybrid BR 201 in nutrient solution. The kinetics of NH₄ uptake were measured after P starvation and non-starvation periods of 2, 4, 6, 8, and 10 days. The effect of P addition during the study period (resupply) was also tested. Ammonium uptake decreased 45.7% after two days of P

¹This research was supported by the Maize and Sorghum Research Center, EMBRAPA, and by a scholarship from CNPq-Brazil.

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stress compared to the fully P-sufficient control. Ammonium uptake decreased 83.0% when P was withheld for 10 days. The decline in NH_4 uptake was partially reversed when P was resupplied during the early periods of P deficiency, but this effect diminished as the P stress increased. These results suggest that maize plants are physiologically dependent on NH_4 rather than nitrate (NO_3) when under P stress.

INTRODUCTION

The general chemical characteristics of acid tropical soils are conducive for periods of P stress for crops. Since visual symptoms of nitrogen (N) deficiency are not always corrected by the application of N, but may be corrected by the application of phosphate fertilizers, the possibility of a limiting P supply disturbing N uptake was indicated. Reduced NH_4 uptake as a consequence of P deficiency has been described in buckwheat, barley, rape (Schhørring, 1986), and algae (Vona et al., 1992).

The first detailed characterization of NH_4 uptake was provided in rice by Wang et al. (1993). Ammonium influx is biphasic and mediated by two discrete transport systems. In the low NH_4 external concentration range (below 1 mmol L^{-1}), influx occurs via a saturable high affinity transport system (HATS), whereas from 1 to 40 mmol L^{-1} of external NH_4 , a second, non-saturable low affinity transport system (LATS) becomes operational. A saturable and high affinity transport system across the plasmatic membrane has been described in maize (Glass and Siddiqi, 1995). Results of inhibitor studies on the transmembrane electrical potential difference (Wang et al., 1994) as well as on $^{13}\text{NH}_4$ influx (Wang et al., 1993), provide evidence for NH_4 transport to be dependent on the proton motive force.

The two transport systems (HATS and LATS) have different mechanisms for energy coupling with the HATS system being more electrogenic than LATS (Wang et al., 1994). Considering the occurrence of P stress in acid tropical soils and the physiological evidence for NH_4 uptake being dependent on metabolic energy, it was proposed that an inadequate P supply may be one of the most important factors disturbing N uptake in these areas. As a consequence of an inadequate supply of P, N utilization could be severely limited in tropical areas. The aim of this work was to identify the main changes in NH_4 uptake by maize plants caused by increasing periods of P starvation.

MATERIALS AND METHODS

Seeds of the male progenitor of the maize double-cross hybrid BR 201 were germinated in paper rolls in pots containing deionized water. After emergence, 30 uniform seedlings were transplanted to each of eight plastic receptacles containing 13 L of Steinberg's Nutrient Solution, pH 5.5, as modified by Foy et al. (1967).

Aeration was kept constant and the solution was changed every two days. Seven days after the plants had grown in a complete nutrient solution in the greenhouse, P was omitted from half of the solutions while a normal P supply was maintained in the other half. Plants were transferred to a growth chamber with $25.3 \pm 0.5^\circ\text{C}$ average day temperature, $19.3 \pm 0.9^\circ\text{C}$ average night temperature, $71.7 \pm 2.3\%$ relative humidity, and $540 \mu\text{E m}^{-2}\text{s}^{-1}$ light intensity. The effects of P withdrawal for 2, 4, 6, 8, and 10 days on the kinetics of NH_4 uptake were measured. Phosphorus resupply during the study period was also evaluated. The treatments were arranged as a 2×2 factorial, random block design with four replications. Plants were divided into four treatments: i) plants deficient in P prior to and during the study period (-P-P), ii) plants deficient in P prior to the study, but supplied P during the study period (-P+P), iii) plants initially supplied P but P withheld during the study period (+P-P), and iv) plants provided P both before and during the study period (+P+P). Two days before initiation of the kinetic studies, two uniform plants were transplanted to each individual pot containing the respective nutrient solution. The kinetic solution was composed of $100 \mu\text{mol}$ ammonium nitrate (NH_4NO_3) L^{-1} and $50 \mu\text{mol}$ calcium chloride (CaCl_2) L^{-1} for all treatments. Treatments receiving P during the kinetic period (+P+P and -P+P), received $100 \mu\text{mol}$ potassium dihydrogen phosphate (KH_2PO_4) L^{-1} . After one hour (time to reach the steady state), the treatment solution was renewed and 10 mL of this solution were withdrawn every 30 minutes for nine hours and immediately frozen for later NH_4 analysis. The plants were harvested at the end of the experiment, split into roots and shoots, dried at 70°C , weighed, and ground.

Ammonium in the treatment solution was determined by flow injection analysis as described by Alves et al. (1993). Data from the depletion curves were corrected for NH_4 lost due to sampling. The solution volume was corrected for evapotranspiration and sampling loss, and the results are presented as NH_4 uptake curves (Figure 1).

RESULTS

Dry Matter Accumulation

Dry matter accumulation in shoots was significantly reduced after 6, 8, and 10 days of P starvation (Table 1). Although not significant, shoot dry matter tended to be lower after four days of P stress. Root dry matter accumulation was not significantly decreased by any period of P starvation.

Ammonium Uptake

Ammonium uptake was based on the slope of the linear portion of the curves (Figure 1) as estimated by linear regression coefficients. The average rates of NH_4 uptake for the four treatments are shown in Table 2. To evaluate an observed slope change due to P resupply (-P+P treatment), the break-point time was the time where

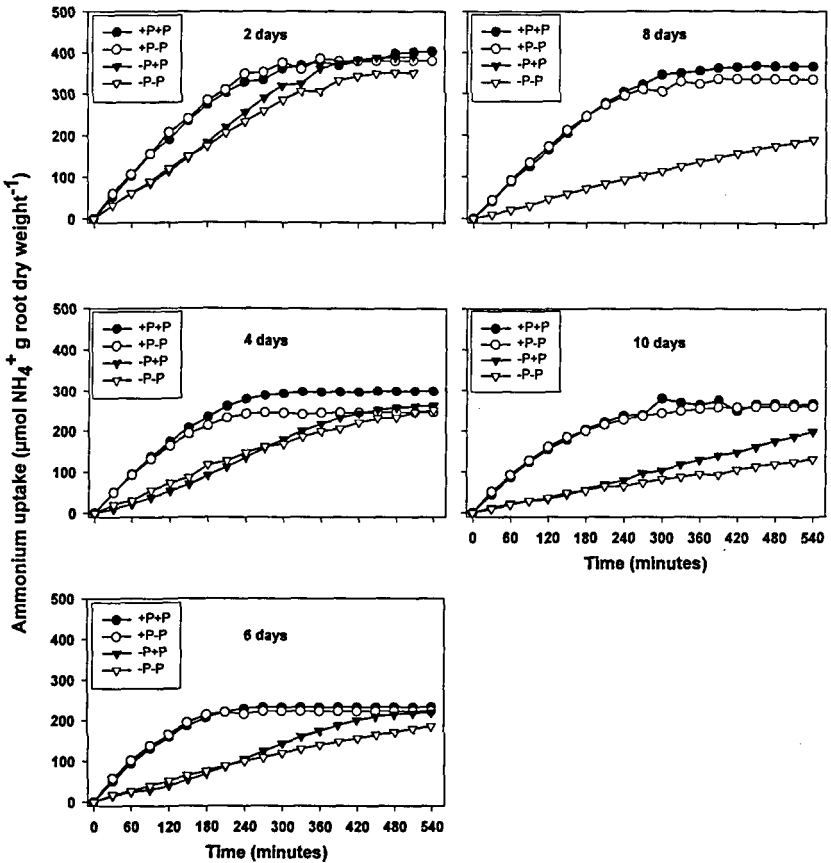


FIGURE 1. Ammonium uptake by maize after 2, 4, 6, 8, and 10 days. Treatments: -P-P=plants without phosphorus before and during the study period, -P+P=plants without phosphorus before but supplied phosphorus during the study period, +P-P=plants receiving phosphorus prior to but not during the study period, and +P+P=plants supplied phosphorus prior to and during the study period. Values are the averages of four replications (the -P+P: 8 days sample was lost).

the -P+P curve exceeded the -P-P curve at each period (Figure 1). The break-point for non-P stress curves (+P+P and +P-P) and -P-P curves, was the one corresponding to the best r^2 calculated by linear regression. Slope statistical significance and statistical homogeneity between slopes were tested by Student's-t test (Steel and Torrie, 1960). Pair-wise comparisons between rates are shown in Table 2.

TABLE 1. Dry matter accumulation in maize shoots and roots.

Treatment ^a	Sampling period (days)				
	2	4	6	8	10
-----Shoot tissue (g per pot)-----					
+P+P	1.37 A ^b	2.01 B	3.05 A	4.14 A	5.87 A
+P-P	1.34 A	2.35 A	3.26 A	4.27 A	5.92 A
-P+P	1.37 A	1.91 B	2.37 B	2.40 B	2.70 B
-P-P	1.40 A	1.86 B	2.28 B	2.47 B	2.69 B
*C.V.(%)	14.48	4.71	8.68	12.84	16.07
-----Root tissue (g per pot)-----					
+P+P	0.41 A	0.55 A	0.72 A	0.80 A	1.12 A
+P-P	0.43 A	0.68 A	0.75 A	0.85 A	1.14 A
-P+P	0.44 A	0.63 A	0.77 A	0.83 A	1.07 A
-P-P	0.46 A	0.64 A	0.78 A	0.91 A	1.24 A
*C.V.(%)	16.72	10.0	10.56	9.16	17.39

^aTreatments: -P-P=plants without phosphorus before and during the study period, -P+P=plants without phosphorus before, but supplied phosphorus during the study period, +P-P=plants receiving phosphorus prior to, but not during the study period, and +P+P=plants supplied phosphorus prior to and during the study period.

^bDry matter averages (four replications) followed by the same letter in the columns are not statistically different at the 1% level of probability (Duncan's test).

*C.V.=Coefficient of variation.

Two days of P starvation (-P-P) reduced NH_4 uptake by 45.3% compared with non-P stressed plants (+P-P, Figure 1 and Table 2). Resupplying P activated NH_4 absorption (-P+P 150-270 minutes), however, a complete recovery of NH_4 uptake was not observed. Ammonium absorption due to early P stress (-P-P) decreased as the length of the P stress increased and achieved a rate of only 16.5% of the control (+P-P) after 10 days of P starvation (Figure 1). Ammonium uptake, as a function of P resupply (-P+P), was 69% of the control (+P+P) in plants two days P stressed and only 28% of the control in plants stressed for ten days.

TABLE 2. Ammonium uptake rates by maize after phosphorus stress.

Treatments ^a	Sampling period (days)				
	2	4	6	8	10
+P+P	1.724**A (0-90) ^b	1.516**A (0-90)	1.331**A (0-120)	1.325**A (0-210)	1.382**A (0-90)
+P-P	1.709**A (0-120)	1.461**A (0-90)	1.521**A (0-90)	1.420**A (0-150)	1.415**A (0-90)
-P+P	0.951**B (0-150)	0.593**B (0-270)	0.386**B (0-210)	ND	0.289**B (0-180)
-P-P	1.200**C (150-270)	0.661**B (270-360)	0.626**C (210-310)	ND	0.385**C (180-480)
-P-P	0.935**B (0-330)	0.565**B (0-360)	0.377**B (0-390)	0.381**B (0-420)	0.234**B (0-540)

^aTreatments: -P-P=plants without phosphorus before and during the study period, -P+P=plants without phosphorus before, but supplied phosphorus during the study period, +P-P=plants receiving phosphorus prior to, but not during the study period, and +P+P=plants supplied phosphorus prior to and during the study period. Data is $\mu\text{mol NH}_4^+$ g root dry weight⁻¹ minute⁻¹.

**Statistically significant at the 1% probability level by Student's-t test. Rates followed by the same letter in the columns are not statistically different by Student's-t test (pair-wise comparisons).

^bTime period (minutes) considered for linear regressions are shown in parenthesis. Values are averages of four replicaitons.

ND=Not determined.

Comparison of Ammonium with Nitrate Uptake

Ammonium uptake averaged 3-fold higher than NO_3 throughout the 10-day study period (Figure 2). Nitrate uptake of P stressed (-P-P) plants decreased 61% while NH_4 uptake decreased 46% after two days compared to the P-sufficient control plants (+P+P, Figure 2). Although NH_4 uptake of P-stressed plants decreased 72% after six days, NO_3 uptake dropped 87.5%. Nitrate uptake stopped 10 days after withholding P while NH_4 uptake was still 17% of the P-sufficient control.

DISCUSSION

Ammonium absorption rates are commonly observed to be higher than NO_3 (Macklon et al., 1990; Glass and Siddiqi, 1995). When supplied singly, NH_4 uptake

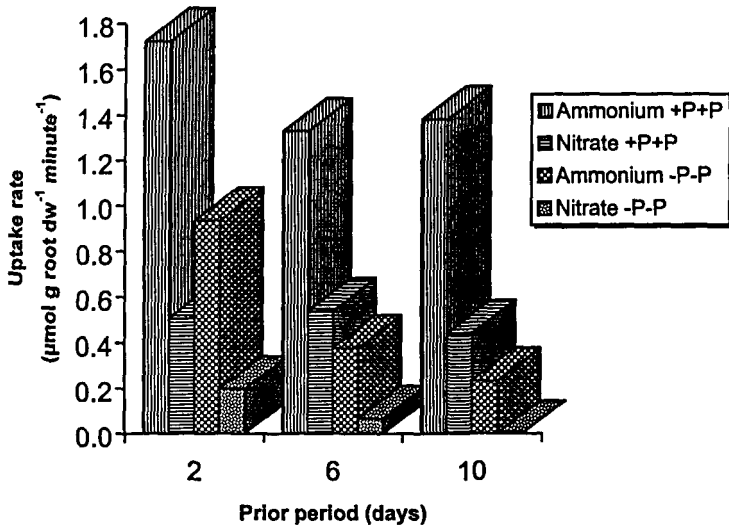


FIGURE 2. Ammonium and nitrate uptake by maize after 2, 6, and 10 days of phosphorus stress. Treatments: -P-P=plants without phosphorus before and during the study period; +P+P=plants supplied phosphorus prior to and during the study period (dw: dry weight).

was much faster than NO_3 in *Allium cepa* L. and this difference became even greater when the two ions were present together in equimolar solutions (Macklon et al., 1990). Although various reasons are given to explain these differences, this research indicates that these preferences may be influenced by the prior nutritional condition of the plants. The genotype used in this experiment (male progenitor of the maize double cross hybrid, BR 201) is a component of a hybrid showing high adaptation to acid soil conditions. Seminal root length in a 6 mg aluminum (Al) L^{-1} nutrient solution as well as Al induced callose (S.N. Parentoni, personal information), indicate that this male single-cross is the source of Al tolerance of the hybrid BR 201. Adaptation to strongly acid soils and Al resistance for some tropical grasses involve tolerance to high NH_4 concentrations that are toxic to susceptible plants (Blum, 1988). Thus, selection of the BR 201 genotype in acid soils may be a factor related to the observed preference for NH_4 since NH_4 rather than NO_3 may be the predominant form of N under these conditions.

The higher NH_4 than NO_3 uptake also may result from a possible inhibition of NO_3 influx by NH_4 . Lee and Drew (1989) reported that NO_3 influx in barley was progressively inhibited as the NH_4 concentration increased above 0.005 mol NH_4 m^{-3} . The magnitude of NO_3 suppression reported by these authors would only partially explain the lower NO_3 than NH_4 uptake observed in this study (Figure 2). A genotype effect per se would probably account for the kinetic data obtained.

Although there were differences in NH_4 and NO_3 uptake between the P stress treatment and the control (-P-P and +P+P), NH_4 uptake tended to decrease somewhat less than NO_3 and the rates observed for NH_4 uptake were still high even when the plants were submitted to P stress (Table 2, Figure 2). Therefore, NH_4 was the main source of N absorbed by maize plants under P stress. A significant increase in NH_4 uptake of P-stressed plants was observed when they were subsequently supplied P (-P+P). Full recovery of NH_4 uptake was not observed after resupplying P even after the shortest period (Table 2, 2 days) of P stress (-P+P=1.200) compared with non-P stressed plants (+P+P=1.724). Compared to NO_3 , NH_4 accounted for most of the total N absorbed after resupplying P to P-starved plants. A comparison of the effect of P resupply on NH_4 (1.200) and NO_3 (0.479) uptake after two days of P stress (-P+P) clearly shows the higher NH_4 absorption rate. Similar results were obtained with plants submitted to the other stress periods and then resupplied P.

One hypothetical explanation of these results is the relative energy cost for uptake and assimilation of the two forms of N. Bloom et al. (1992) reported that 14% of total root carbon catabolism of barley was expended on N absorption and assimilation under NH_4 nutrition and 23% under NO_3 nutrition. The additional energy required for NO_3 assimilation could limit growth since energy may be more limited under NO_3 than NH_4 nutrition. The lower "energy" requirement for NH_4 metabolism also is indicated since Q10 values for NO_3 uptake are significantly higher than for NH_4 uptake (Glass and Siddiqi, 1995). Ammonium nutrition could be most beneficial under conditions of "energy" shortage as probably caused by P stress in maize plants.

Under NH_4NO_3 nutrition, the root is the major site of N assimilation in maize (Murphy and Lewis, 1987). When nutrient elements limit growth, especially N and P, the roots become stronger carbohydrate sinks than shoots (Clarkson, 1985). The maintenance of root dry matter under P stress conditions in contrast to a decrease in shoots (Table 1), may result from this root carbohydrate sink. Under NH_4 nutrition, as a consequence of its complete conversion to organic N in roots prior to translocation (Ivanko and Ingversen, 1971), an induced carbohydrate sink is established in the root which could create an energy deficiency in other tissues (Magalhaes et al., 1995). In contrast to the rapid assimilation of NH_4 in roots, absorbed NO_3 is translocated to aerial parts for later reduction and assimilation. This root sink, possibly enhanced by the combination of P stress and NH_4 nutrition, could be an energy disadvantage for NO_3 , and thereby favor NH_4 metabolism. The BR 201 double-cross hybrid maintains high root sugar concentrations (Alves et al., 1995) under P stress conditions and reinforces the root carbohydrate sink theory. The compartmentalization of NH_4 metabolism in roots which are possibly better supplied with energy than shoots, could lead to increased energy utilization efficiency under NH_4 nutrition and favor its uptake over NO_3 .

While NO_3 uptake seems to be entirely active at an external concentration range up to $100 \mu\text{mol L}^{-1}$ (Glass et al., 1992; King et al., 1992), transport of NH_4 above an external concentration of $42 \mu\text{mol L}^{-1}$ could be predicted as a passive process in

rice (Wang et al., 1994). Although certain conditions, such as the external concentration of NH_4 can increase the concentration limit, the occurrence of a partially passive transport can be considered during this study period (Figure 1). Accordingly, Ullrich et al. (1984) reported an inward-directed driving force acted at all external concentrations down to $67 \mu\text{mol L}^{-1}$. Wang et al. (1994) cautioned that free energy estimations provide only a prediction of the feasibility of the uptake process occurring under the prescribed conditions; however, even with this consideration, a partially passive transport mechanism could contribute to the lower sensitivity for NH_4 uptake with P stress compared to NO_3 uptake.

The marked predominance of NH_4 in savanna soils after urea application (Coelho, 1995) and the lower sensitivity of NH_4 uptake than NO_3 uptake to P stress, suggests that that this single-cross may be physiologically dependent on NH_4 rather than NO_3 under these conditions. Therefore, this apparent dependence should be considered when practices that affect the NO_3 to NH_4 ratio in soil, as well as breeding strategies towards the development of genotypes adapted to low pH conditions, are adopted when aiming for high yield of crops in acid soils.

CONCLUSIONS

The male progenitor of the BR 201 hybrid showed NH_4 uptake rates markedly greater than NO_3 . These rates remained greater even under P stress conditions.

Ammonium uptake was decreased by P stress and this reduction was intensified as the length of the stress period increased.

Resupply of P to plants previously P stressed activated NH_4 uptake. The magnitude of this recovery was dependent on the stress severity. Ammonium uptake was less sensitive to P stress than NO_3 uptake.

ACKNOWLEDGMENTS

This research was supported by the Maize and Sorghum Research Center, EMBRAPA and a scholarship from CNPq-Brazil. The authors thank Wagner Geraldo S. Campos, Gislene Braga Cristeli, and Carla Cristina Moura França for their technical help and friendship.

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