

pH CONTROL IN NITRATE UPTAKE STUDIES WITH EXCISED ROOTS *

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INTRODUCTION

More attention has been given to cation uptake mechanisms of root systems than to the concurrent process of anion uptake, even though such major nutrient elements as nitrogen, phosphorus, and sulfur ordinarily enter the root as anions. Short-term experiments with excised roots provide valuable information about ion uptake and accumulation by plant roots². Excised roots often lend themselves better to the determination of uptake rates than when intact plants are employed, since the complicating influence of stems and leaves is eliminated.

When excised roots are placed in a single salt solution, the cationic and anionic components are usually absorbed at different rates resulting in rapid pH changes which, if not controlled, could greatly influence the uptake mechanisms⁵. Hoagland and Broyer² observed that anion absorption may be markedly accelerated by a lowering of the pH of the medium and cation absorption by increasing the pH. Van den Honert and Hooymans³ clearly demonstrated the profound influence of pH on the rate of NO_3^- absorption by maize roots, and this has been confirmed by other workers.

A procedure for close control of pH in cation-uptake studies, involving periodic additions of an anionic exchange resin in the

* Contribution from the Plant Nutrition Laboratory, Department of Botany, The University of Michigan, paper number 39. This work was supported by research grant GM-07339-02 from the National Institutes of Health, U. S. Public Health Service.

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OH⁻ form, was recently reported by Parr and Norman⁷. This paper is an extension of this principle to studies on the uptake of an anion such as NO₃⁻ by excised barley roots.

METHODS

Preparation and treatment of excised roots

Excised barley roots (*Hordeum Vulgare* L., var. Atlas 54) from 6-day-old seedlings were used throughout this study. The method of culture and treatment was exactly as described by Parr and Norman⁷ in which barley seedlings were grown in mass culture in an aerated solution of 7.5×10^{-5} M CaSO₄ and 2.5×10^{-5} MgSO₄. After excision, the roots were rinsed several times with cold distilled water and the excess water removed in a basket centrifuge. Fresh weight samples (7.5 g) of root material were placed in polystyrene vessels containing 200 ml of either KNO₃ or Ca(NO₃)₂ at concentrations ranging from 5×10^{-4} N to 2×10^{-3} N. Solutions were aerated continuously through special manifolds fitted with 0.015-inch I. D. polyethylene tubing.

In one series of experiments involving the absorption of NO₃⁻ from Ca(NO₃)₂ solutions, the exchange sites on the roots were initially saturated with Ca⁺⁺ by pretreatment for 10 minutes in a solution of 5×10^{-4} M CaSO₄. After being allowed to drain for one minute, and without rinsing, the root samples were transferred to the Ca(NO₃)₂ solutions to begin the NO₃⁻ absorption study. The justification for this pretreatment will be discussed later.

Small samples of the ambient solutions were withdrawn periodically during the absorption period to determine the rate of NO₃⁻ uptake. All experiments were conducted at 25°C.

Preparation and use of resin for control of pH

The pH of salt solutions in these experiments, unless otherwise specified, were maintained close to 5.5 during the absorption period by periodic additions of small measured volumes of analytical grade Amberlite IR-120 (sulfonic acid type), supplied in the H⁺ form by the Rohm and Haas Company Philadelphia, Pennsylvania. Prior to use the resin was thoroughly washed with distilled water and, after repeated washing and settling to remove finer particles, the bead diameter of the resin ranged from 0.45 to 0.60 mm.

The resin was used in a moist state after excess water had been removed on a Buchner funnel. The capacity of the resin to lower the pH of various nitrate salt solutions ranging in concentration from 5×10^{-4} N to 2×10^{-3} N, in the absence of roots, was determined by observing the shift in pH after successive additions of 0.05 cc moist resin. After each addition an equilibration period of 30 minutes was allowed. All pH changes were followed with a Beckman Zeromatic pH meter.

Throughout the absorption period pH was maintained near 5.5 by addition

of small increments of resin directly to the bottoms of the containers after gently pulling the roots to one side. With 7.5 g of roots in 200 ml of $\text{Ca}(\text{NO}_3)_2$ solution 2 or 3 resin additions, each about 0.015 g dry weight, were effective in providing pH control during a 6-hour absorption period.

Nitrate determinations

The uptake of NO_3^- by excised roots was determined by the difference between the initial and final NO_3^- concentration of the ambient solutions using the phenoldisulfonic acid method ⁴. Color densities were read in an Evelyn colorimeter at 420 $m\mu$.

RESULTS AND DISCUSSION

The initial experiments were conducted with different nitrate salts, involving both monovalent and divalent cations, in order to determine the extent of the pH changes in the presence of excised roots, without pH control. In the case of four divalent salts (Ca, Mg, Sr, Ba) the pH trend was quite similar and is typified by the Ca $(\text{NO}_3)_2$ curve in the upper portion of Figure 1. On placing the excised roots in the salt solutions, an initial rapid downward deflection in pH occurred, due mainly to an immediate adsorption of the cationic component by exchange sites on the roots ⁶. The downward deflection from the initial solution pH of 5.5 was complete in 15 to 20 minutes and after a short stationary period lasting for 5 to 10 minutes a steady increase in pH was observed. This increase in pH continued for all the divalent salts throughout the rest of the absorption period, suggesting a more rapid rate of NO_3^- uptake than of the cationic component. Similar observations have been reported by Hoagland and Broyer ².

In the case of the monovalent salts (Na, K, NH_4), the trend was somewhat different and is typified by the KNO_3 curve in the upper portion of Figure 1. The rapid initial downward pH deflection was somewhat greater than in the divalent salt solutions and continued longer. In the monovalent salt solutions a minimal pH value of 4.3 was attained after about one hour and maintained at that level, indicating approximately equal rates of absorption of the anionic and cationic components at that pH.

The pH changes in these systems can be overcome to a large extent by using small root samples in large volumes of salt solution, *i.e.* smaller root-to-solution ratios ⁵; however, this necessitates root

ashing to determine ion uptake. A higher root-to-solution ratio allows the determination of ion uptake directly by following the depletion of the ion in solution.

These observations of pH trends made it evident that divalent nitrate salts would be preferable in controlled pH experiments at rather high root-to-solution ratios, because after the initial downward deflection due to ion exchange adsorption on roots, there was a progressive upward trend with time. An effective control procedure would then allow experiments to be carried out within physiologically desirable pH limits. When $\text{Ca}(\text{NO}_3)_2$ was used during the absorption period, the initial downward shift in pH was effectively minimized by a 10-minute pretreatment of the excised roots with $5 \times 10^{-4} M$ CaSO_4 . Although the exchange sites on the root are at least partially satisfied by Ca^{++} and Mg^{++} from the salt solutions used in the root-culture procedure, without this pretreatment there was an initial deflection from pH 5.5 to 4.8 (Fig. 1, upper curve);

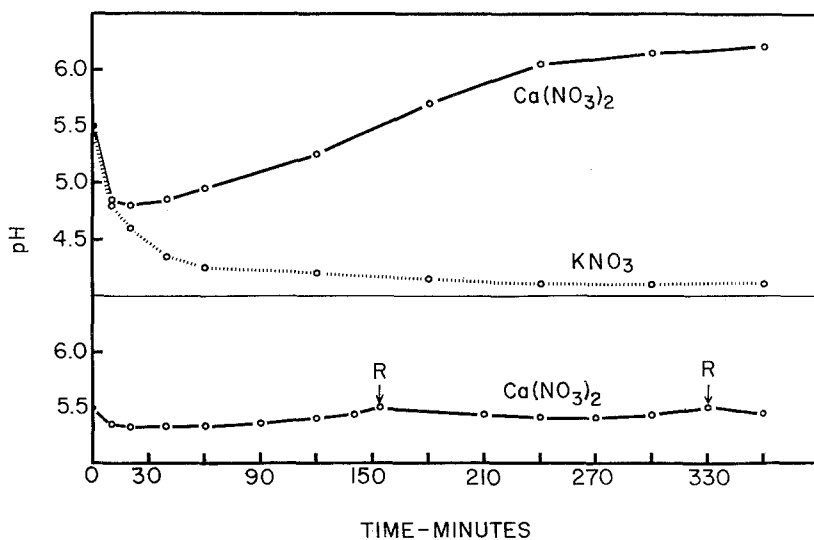


Fig. 1. *Upper portion:* Changes in pH as a result of unequal rates of absorption of the anionic and cationic components of two nitrate salts by excised barley roots. *Lower portion:* Control of pH during salt absorption by excised barley roots. Roots pretreated with $5 \times 10^{-4} M$ CaSO_4 for 10 minutes, and pH of solutions maintained close to 5.5 by two additions (R) of Amberlite exchange resin IR-120 H^+ .

All experiments involved 7.5 g roots, fresh weight, in 200 ml $2 \times 10^{-3} N$ nitrate solution at 25°C for 6 hours. Each point is the mean of 4 replicates.

but if the exchange sites were virtually saturated with Ca^{++} , the minimum reached was only 5.35 (Fig. 1, lower curve). Since $\text{SO}_4^{=}$ apparently does not compete with NO_3^- for specific active sites during the absorption process¹, the small amount of residual $\text{SO}_4^{=}$ left on the roots after pretreatment does not interfere with subsequent NO_3^- absorption.

In controlled-pH studies, therefore, pretreatment of excised roots with CaSO_4 was practiced, followed by periodic additions of Amberlite IR-120 H^+ to check the upward shift in pH. In our studies we have used the resin procedure successfully for control of pH of solutions ranging from 5 to 7. The lower curve of Figure 1 shows the effectiveness of this resin to control pH within rather narrow limits. Differences in pH between replicates controlled by resin did not exceed 0.2 pH units at any time. In the NO_3^- -uptake studies reported here with $2 \times 10^{-3} N \text{Ca}(\text{NO}_3)_2$, the pH was maintained near 5.5. using the resin procedure and excellent reproducibility was observed between replicates as shown in Table 1. In these experiments pH was effectively controlled at 5.5 with two small resin additions, each about 0.015 g dry weight, during a six-hour period. The lower curve of Figure 1 illustrates that the resin functions as an effective buffer in which the exchange between the H^+ of the resin and the excess Ca^{++} of the solution extends over a substantial period of time.

The effectiveness of pH control on the course of nitrate uptake by excised barley roots from a $2 \times 10^{-3} N \text{Ca}(\text{NO}_3)_2$ solution is shown in Table 1. As the pH increased in the uncontrolled solutions the uptake rate declined so that at 6 hours the cumulative uptake was only 77 per cent of that from solutions that were maintained near pH 5.5. This difference developed largely in the final two hours during which period the pH of the two series diverged markedly.

TABLE 1

Effect of pH control on nitrate uptake by excised barley roots. 7.5 g roots, fresh weight, in 200 ml $2 \times 10^{-3} N \text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ at 25°C for 6 hours		
Hours after start of uptake	Milligrams of nitrate absorbed * and pH reading	
	pH controlled with resin	No pH control
2	0.86 ± 0.04 (5.40)	0.85 ± 0.02 (5.65)
4	1.70 ± 0.10 (5.40)	1.58 ± 0.06 (5.90)
6	3.70 ± 0.08 (5.35)	2.86 ± 0.02 (6.40)

* Mean and standard deviation of 4 replicates, with pH values in parentheses.

There are several advantages in the use of resin for the control of pH. It provides for the control of pH by addition of H^+ without an accompanying anion which might interfere with the absorption of the anion under study. In the present studies attempts to control pH with H_2SO_4 were rather unsuccessful since very dilute solutions had the effect of diluting the NO_3^- -concentration, while more concentrated H_2SO_4 caused very abrupt deflections. The resin method allows anion uptake studies to be conducted at high root-to-solution ratios with the distinct advantage of permitting a direct determination of anion absorption by noting differences in anion concentration between initial and final ambient solutions.

SUMMARY

In the investigation of NO_3^- uptake by excised roots, pH of salt solutions can be effectively controlled by periodic additions of a cation exchange resin, Amberlite IR-120, in the H^+ form. This method of pH control provides for the addition of H^+ to systems without an accompanying anion which might influence the absorption of NO_3^- . The resin appeared to provide a favorable buffering capacity during an extended period and the method was especially useful for NO_3^- uptake studies conducted at high root-to-solution ratios.

Received November 11, 1963

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