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 roots 2. 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 mechanisms 5. Hoagland and Broyer 2 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 Hooymans 3 clearly demonstrated the profound influence of pH on the rate of NO₃⁻ 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

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OH\(^{-}\) form, was recently reported by Parr and Norman\(^7\). This paper is an extension of this principle to studies on the uptake of an anion such as NO\(_3^{-}\) 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 Norman\(^7\) in which barley seedlings were grown in mass culture in an aerated solution of 7.5 \(\times\) 10\(^{-5}\) M CaSO\(_4\) and 2.5 \(\times\) 10\(^{-5}\) MgSO\(_4\). 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 KNO\(_3\) or Ca(NO\(_3\))\(_2\) at concentrations ranging from 5 \(\times\) 10\(^{-4}\) N to 2 \(\times\) 10\(^{-8}\) 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 NO\(_3^{-}\) from Ca(NO\(_3\))\(_2\) solutions, the exchange sites on the roots were initially saturated with Ca\(^{++}\) by pretreatment for 10 minutes in a solution of 5 \(\times\) 10\(^{-4}\) M CaSO\(_4\). After being allowed to drain for one minute, and without rinsing, the root samples were transferred to the Ca(NO\(_3\))\(_2\) solutions to begin the NO\(_3^{-}\)-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 NO\(_3^{-}\)-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 \(\times\) 10\(^{-4}\) N to 2 \(\times\) 10\(^{-8}\) 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