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SHORT COMMUNICATION

Calcium complexes in the xylem sap of apple shoots

Summary

Concentrations of total calcium, ionic calcium, citric and malic acids have been measured in xylem sap extracted from apple shoots. Ionic calcium, as measured by an ion selective electrode, was about 50 per cent of the total calcium. The remainder of the soluble calcium was present as complexes with citric and malic acids. The implication of these findings on the mobility of calcium in the xylem is discussed.

Introduction

Calcium is strongly adsorbed in the xylem, initially by a reversible ion exchange process followed by an irreversible accumulation phase[1]. It has been shown[2] that movement of calcium through bean shoots is increased in the presence of root exudates and it was concluded that this was due to competition between calcium and other cations in the exudate for exchange sites in the xylem. However, it is also possible that the mobility of calcium may be increased by the presence of compounds in the exudate, for example organic acids, with which it might form either uncharged or negatively charged complexes thus reducing the degree of adsorption at the negative exchange sites in the xylem vessels. Iron has been shown to be translocated as a negatively charged citrate complex in the xylem exudate of soybean[3] but no similar relationship could be shown between calcium and citrate[4]. The total concentration of cations present in xylem sap, extracted from the terminal shoots of apple trees, has previously been reported[5] but no work appears to have been carried out on the measurement of the fraction of the total calcium which may be present as Ca^{2+} ions. Also, little information is available on the concentration and the nature of organic acids which might be present, although succinic, malic and citric acids have been found in the exudate from excised maize roots[6]. The object of the present study was to measure the concentration of Ca^{2+} in xylem sap using a calcium ion selective electrode and to relate these measurements to the concentration of total calcium and of organic acids in the sap.

Materials and methods

Shoots, approximately 1 metre long with a base diameter of 10 mm, were cut from 2-year-old trees, cv. Cox on MM 106 rootstock, which had been grown in potting compost in pots of 240 mm diameter. Samples were taken at weekly intervals from bud break until 7 weeks after petal fall and xylem sap was extracted using the method described by Bollard[5]. Each sample consisted of 4 shoots; the extracted sap was bulked and yielded a sample volume of 15–20 ml. Samples of sap were stored in deep freeze until required for analysis. Total calcium and magnesium concentrations were determined by atomic absorption spectroscopy and ionic calcium (Ca^{2+}) measured by a PVC membrane calcium ion selective electrode[7]. Citric and malic acids were determined by enzymatic methods[8].

Results and discussion

Ionic calcium, as measured by the electrode, was about 50 per cent of the total calcium in all the samples of apple xylem sap examined. The coincidence of the curves for total calcium concentration and total citric acid concentration for the first 4 samples suggests that the remainder of the soluble calcium may be present as citrate complexes with a small amount present as the malate complex; in the later samples, malic acid appears to be the predominant acid (Fig. 1). At the pH of the xylem sap (5.5 to 5.7) the predominant

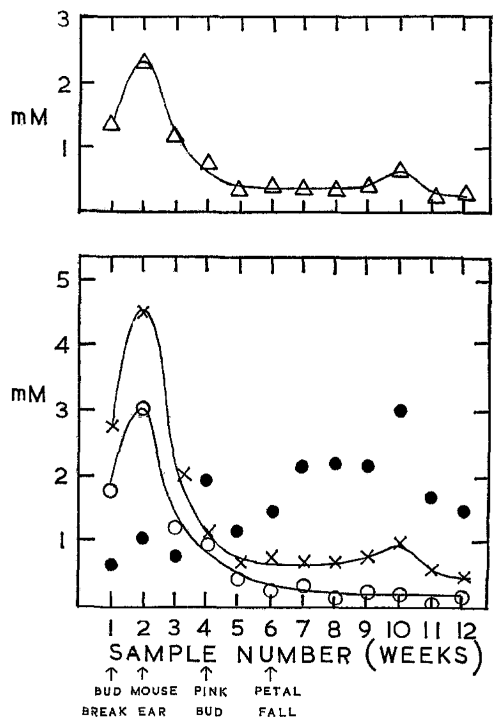

Fig. 1. Concentrations of total calcium (×), Ca^{2+} (△), total citrate (○) and total malate (●) in the xylem sap of apple shoots at weekly sampling dates from bud break.

forms of citric acid present will be HC^{2-} and C^{3-}, where H_3C represents undissociated citric acid and possible calcium complexes are CaHC and CaC^-. The predominant form of malic acid is M^{2-} giving a calcium complex CaM. Magnesium would form similar complexes. From a knowledge of the stability constants of these complexes at the ionic strength of the xylem sap, the dissociation constants of citric and malic acids, the pH of the sap and the total concentrations of calcium, magnesium, citric and malic acids, it is possible to calculate expected values for the concentrations of Ca^{2+}, Mg^{2+} and of various complexes of calcium and magnesium with citric and malic acids. (see Appendix) This has been carried out for sap sample No. 2 (mouse ear stage of bud break), when the concentrations of calcium and citric acid were at their maximum, and the results are shown in Table 1. The reasonably good agreement between the calculated value of the proportion of calcium present as Ca^{2+} (42 per cent) and that measured by the electrode (50 per cent) support the suggestion that about half of the calcium in the xylem sap extracted from these apple shoots may be present as citrate and malate complexes.

TABLE 1

Forms of calcium and magnesium (as per cent of total) present in apple xylem sap calculated from measurements of sap composition and known values of stability constants of complexes and acid dissociation constants

Ca^{2+}	42	(50% measured by electrode)	Mg^{2+}	70
CaC^-	34		MgC^-	20
CaHC	16		MgHC	2
CaM	8		MgM	8

H_3C = citric acid; H_2M = malic acid.

The presence of uncharged or negatively charged complexes of calcium in the sap could reduce the degree of adsorption of calcium at negatively charged sites and promote its mobility in the xylem. The concentration of organic acids in plants is influenced by nutritional treatments. For example, it has been shown [6] that the exudate from maize roots grown in a sulphate nutrient medium contained much higher concentrations of organic acids than those grown in a chloride medium. It has also been shown [9] that tomato plants grown with nitrogen supplied as nitrate had higher organic acid contents than those supplied with nitrogen as ammonium. Therefore, it seems possible that the mobility of calcium in the xylem might be promoted by nutritional treatments which increase the concentration of organic acids in the sap and this field of investigation is being explored.

E. G. Bradfield

Department of Agriculture and Horticulture,
University of Bristol, Research Station,
Long Ashton, Bristol, BS18 9AF, England

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Appendix

$[Ca_T]$ = concentration of total calcium.
$[Mg_T]$ = concentration of total magnesium.
$[C_T]$ = total concentration of citric acid.
$[M_T]$ = total concentration of malic acid.
$[Ca^{2+}]$ = concentration of ionic calcium.
$[CaC^-]$ and $[CaHC]$ = concentrations of calcium citrate complexes.
$[CaM]$ = concentration of calcium malate complex.
$[Mg^{2+}]$ = concentration of ionic magnesium.
$[MgC^-]$ and $[MgHC]$ = concentrations of magnesium citrate complexes.
$[MgM]$ = concentration of magnesium malate complex.
$[C^{3-}]$, $[HC^{2-}]$ and $[H_2C^-]$ = concentrations of predominant forms of citric acid present at the pH of xylem sap.
$[M^{2-}]$ and $[HM^-]$ = concentrations of forms of malic acid present.
K_{CaC^-}, K_{CaHC}, K_{MgC^-}, K_{MgHC} = stability constants of calcium and magnesium complexes with citric acid.
K_{CaM} and K_{MgM} = stability constants of calcium and magnesium complexes with malic acid.
K_{2C} and K_{3C} = acid dissociation constants of citric acid.
K_{2M} = acid dissociation constant of malic acid.
The following equations may then be written:

$$[Ca_T] = [Ca^{2+}] + [CaC^-] + [CaHC] + [CaM]$$
$$[Mg_T] = [Mg^{2+}] + [MgC^-] + [MgHC] + [MgM]$$
$$[C_T] = [C^{3-}] + [HC^{2-}] + [H_2C^-] + [CaC^-] + [CaHC] + [MgC^-] + [MgHC]$$
$$[M_T] = [M^{2-}] + [HM^-] + [CaM] + [MgM]$$

$$K_{CaC^-} = \frac{[CaC^-]}{[Ca^{2+}][C^{3-}]}$$

$$K_{CaHC} = \frac{[CaHC]}{[Ca^{2+}][HC^{2-}]}$$

$$K_{CaM} = \frac{[CaM]}{[Ca^{2+}][M^{2-}]}$$

$$K_{MgC^-} = \frac{[MgC^-]}{[Mg^{2+}][C^{3-}]}$$

$$K_{MgHC} = \frac{[MgHC]}{[Mg^{2+}][HC^{2-}]}$$

$$K_{MgM} = \frac{[MgM]}{[Mg^{2+}][M^{2-}]}$$

$$K_{2C} = \frac{[HC^{2-}][H^+]}{[H_2C^-]}$$

$$K_{3C} = \frac{[C^{3-}][H^+]}{[HC^{2-}]}$$

$$K_{2M} = \frac{[M^{2-}][H^+]}{[HM^-]}$$

From these thirteen equations, the following thirteen unknown values were calculated: $[Ca^{2+}]$, $[CaC^-]$, $[CaHC]$, $[CaM]$, $[Mg^{2+}]$, $[MgC^-]$, $[MgHC]$, $[MgM]$, $[C^{3-}]$, $[HC^{2-}]$, $[H_2C^-]$, $[M^{2-}]$, $[HM^-]$, since all other terms in the equations were known. For sap sample No. 2 these were:
$[Ca_T] = 4.5 \times 10^{-3}\,M$, $[Mg_T] = 1.0 \times 10^{-3}\,M$, $[C_T] = 3.0 \times 10^{-3}\,M$,
$[M_T] = 1.1 \times 10^{-3}\,M$, $[H^+] = 3.73 \times 10^{-6}\,M$, $K_{CaC-} = 7.08 \times 10^3$,
$K_{CaHC} = 1.00 \times 10^3$, $K_{CaM} = 4.17 \times 10^2$, $K_{MgC-} = 2.51 \times 10^3$,
$K_{MgHC} = 91$, $K_{MgM} = 2.33 \times 10^2$, $K_{2C} = 2.75 \times 10^{-5}$,
$K_{3C} = 1.10 \times 10^{-6}$, $K_{2M} = 1.26 \times 10^{-5}$.