Chemical Composition of the Exudate from Excised Maize Roots

J. C. COLLINS and E. J. REILLY
University of East Anglia, School of Biological Sciences, Norwich, U.K.

Received July 19, 1968

Summary. The concentrations of inorganic ions in the exudate from excised roots of *Zea mays* have been measured for roots grown in both chloride and sulphate media. The results indicated that in roots exuded from sulphate media there was a significant inorganic anion deficit. Using the methods of thin layer chromatography and high voltage electrophoresis some organic constituents of the exudate have been identified. Exudate from sulphate-grown roots was found to contain 3 organic acids and a complex mixture of amino acids. Exudate from chloride-grown roots contained similar organic solutes, but at much lower concentrations than in the sulphate-grown root exudate.

Introduction

It has long been known that plant roots may absorb cations and anions at unequal rates from simple ionic media, and it has been demonstrated that the apparent discrepancy can be accounted for by organic acid synthesis. BURSTROM (1945) working with wheat roots, concluded that the organic acid fraction of the sap was mainly malic acid with a small amount of citric acid; further evidence for the involvement of malate during excess cation absorption was found by JACOBSON and ORDIN (1954) in barley roots. Recently, the work of TORI and LATTES (1966) showed that excised maize roots synthesize transaconitic acid when sulphate is present in the bathing medium.

Recent work from this laboratory has treated the exudate from excised maize roots as a simple ionic solution when the bathing medium is a solution of potassium and calcium chlorides (see for example HOUSE and FINDLAY, 1966). However, when chloride in the bathing medium is replaced by sulphate, ANDERSON and COLLINS (1968) have shown that the exudate is not a simple ionic solution, but contains significant quantities of organic solute. This work describes a rigorous investigation of the ionic composition of the exudate from roots grown in two different media: one with sulphate as the anion and the other with chloride.

Materials and Methods

Excised roots of 3 day old *Zea mays* (cv. White Horse Tooth) seedlings were used. They were grown and exuded as described by HOUSE and FINDLAY (1966); the roots to be exuded from sulphate media were grown under a complete sulphate regime where chloride was totally replaced by sulphate of the same molarity. The
two bathing media used were: 10 mM KCl + 0.1 mM CaCl₂ and 10 mM K₂SO₄ + 0.1 mM CaSO₄. About 10 ml of exudate was collected over 24 hours, from roots in each bathing medium and immediately stored at -14°C.

Inorganic ions were determined by the following methods:
- K and Na on a Unicam SP 900 flame photometer;
- Ca and Mg on a Unicam SP 90 A absorption spectrophotometer;
- Chloride by potentiometric titration with AgNO₃;
- Phosphate by the ammonium molybdate method of Bartlett (1959);
- Nitrate by the phenol disulphonic acid method;
- Sulphate was determined turbidometrically using an EEL nephelometer.

For all the above ion determinations internal standards were run, only in the phosphate determination was a correction necessary.

Osmolalities were determined with a freezing point osmometer. (Advanced Instruments Inc., Model 31 LAS). pH of the exudate was measured with a Radiometer Type 22 pH meter and a Radiometer pH micro electrode.

Three major groups of organic solute were investigated: organic acids; amino acids; sugars. Analysis was accomplished by thin layer chromatography for all three groups.

Initially, a desalting technique using ion-exchange resins was employed (Torr and Latties, 1966) but recoveries of known concentrations of organic solutes were poor. Subsequently, high voltage electrophoresis apparatus (10,000 V capacity by Locarte, London) was used to desalt the exudate samples before analysis. 1 ml samples of exudate were streaked across the centre of 2″ wide strips of Whatman No. 1 paper and subjected to 5 kV for 5 min using formic acid-acetic acid buffer pH 2 (78:148, v/v). This time was found to be sufficient for removing inorganic ions, and gave very little migration of organic ions. The 2″ strip was dried by hot air and a ¼″ wide strip detached from one edge. This was developed to determine the position of the organic constituents in the main strip. These were cut out from the strip and eluted with water for subsequent identification by thin layer chromatography.

The separation of the amino acids was effected by ascending chromatography using pre-coated Silica Gel G plates (Merck, Darmstadt) in closed glass tanks lined with Whatman No. 4 paper saturated with the developing solvent. The plates were activated at 100°C for 30 min before use. Amino acid markers and aliquots of desalted sample were applied in 1 μl amounts under a stream of warm air to facilitate rapid drying and therefore, minimise spot diffusion, up to 40 μl of sample was applied. The plates were run to a distance of 10 cm from the origin in the following solvent systems:

1. Ethanol-water (70:30, v/v).
2. Phenol-water (75:25, w/w, 20 mg NaCN added as antioxidant per 100 g mixture).

The chromatograms were dried and then developed by spraying with ninhydrin (0.5% in butan-1-ol) and heated for 10 min at 110°C.

Organic acids were separated by ascending chromatography on cellulose plates (Merck, Darmstadt) using phenol-water-formic acid (75:25:1, w/v/v) and run to a distance of 10 cm from the origin. The plates were dried in hot air and developed with bromocresol green indicator (0.1% aq.).

Sugars were separated by ascending chromatography on cellulose plates (Merck, Darmstadt) using ethyl acetate-pyridine-water (40:20:20, v/v/v) and run to a distance of 10 cm from the origin. The plates were dried in hot air and developed by spraying with aniline phthalate (aerosol by Merck, Darmstadt) and heated at 110°C for 3—4 min.