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SUCROSE ABSORPTION AND SYNTHESIS BY EXCISED *LYCOPERSICON ESCULENTUM* ROOTS

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; root growth; asymmetrically-labelled sucrose; sucrose synthetase; sucrose phosphate synthetase.

Abstract—Asymmetrically-labelled sucrose was absorbed intact by excised roots of tomato, grown in sucrose. Glucose-grown roots possessed sucrose synthetase and sucrose phosphate synthetase activity.

INTRODUCTION

Sucrose is the best carbon source for the growth of excised roots of tomato [1, 2]. Despite continued investigation [3–6], no explanation for this preference has been found. The view has been expressed that these roots require a critical level of sucrose in their meristems and that this is established and maintained only by the provision of exogenous sucrose [7]. This presupposes the absorption of the intact sucrose molecule and the inability to synthesize sucrose adequately. Bean endocarp [8], tobacco leaves [9] and castor bean endosperm [10] absorb sucrose without inversion, whereas sugar cane [11] absorbs only the inverted products. Sucrose is synthesized by two enzymes [12, 13]: sucrose synthetase (UDP glucose: D-fructose 2-glucosyl transferase, E.C. 2.4.1.13) and, coupled with phosphatase [14], sucrose phosphate synthetase (UDP glucose: D-fructose 6-phosphate 2-glucosyl transferase, E.C. 2.4.1.7). Neither of these enzymes is detectable in sucrose-grown roots of tomato [15]. This paper reports experiments on the absorption of asymmetrically-labelled sucrose by sucrose-grown roots and on the levels of these two enzymes in glucose-grown roots.

RESULTS AND DISCUSSION

Excised roots were fed 10 μCi of sucrose ^{14}C-labelled in the fructosyl moiety (242·6 mCi/mmol) and the ratio of label in the glucose and fructose moieties of endogenous sucrose determined by the technique of Edelman and Hanson [16]. This ratio was 0·04 after 4 hr and 0·05 after 6 hr, demonstrating the absorption of the intact sucrose molecule. This was further tested by feeding 25 μCi of ^{14}C-U sucrose (32 mCi/mmol) for 4 hr in the presence of 0·027 M glucose or fructose. The glucose:fructose ratio of label in endogenous sucrose was 0·92 and 1·12 respectively, demonstrating that endogenous sucrose was not synthesized *de novo* but absorbed intact from the surrounding medium.

Glucose-grown roots contain sucrose synthetase and sucrose phosphate synthetase activity. Sucrose synthetase was purified 66-fold, to a specific activity of 7·2 μmol sucrose formed/hr/mg protein. The K_m for fructose was 3·8 mM, which is slightly higher than that reported in wheat germ [12], mung bean [17] and artichoke tubers [18]. Sucrose phosphate synthetase was purified 82-fold, to a specific activity of 14·8 μmol of sucrose phosphate formed/hr/mg protein. K_m for fructose-6-phosphate was 4 mM, which is slightly higher than that of wheat germ [13]. The absence of the two enzymes in sucrose-grown roots raises the possibility that their synthesis is repressed by sucrose. The above results demonstrate that glucose-grown roots possess sucrose-synthesizing enzymes at activities comparable to those of other tissues. Since glucose-grown roots consistently contain lower levels of sucrose than do sucrose-grown roots [5,7], it appears that these levels of enzymes are not sufficient to support the synthesis of sucrose to levels which permit maximum growth of roots. Since the main axis meristems of seedling roots obtain sucrose by trans-location from the shoot, seedling root growth is not necessarily limited by its ability to synthesize sucrose. Since roots

absorb [6], respire [19], and incorporate [15] glucose into cell fractions at comparable rates to sucrose and yet support less than 40% as much growth, sucrose must be performing critical morphogenetic roles not carried out by glucose. The nature of these roles is not known at present.

EXPERIMENTAL

The clone described earlier [15] was used throughout. Crude extracts were obtained by homogenizing in 0·05 M phosphate citrate buffer, pH 7·0, followed by centrifugation at 12000 *g*. Sucrose synthetase and sucrose phosphate synthetase were partially purified, using a method similar to that of Mendicino [20]. The two enzymes were precipitated in the 0–60% $(NH_4)_2SO_4$ fraction and separated by 1% protamine sulphate treatment at pH 7·3; sucrose phosphate synthetase is insoluble in 1% protamine sulphate. The enzyme activities were measured by the method of Lavintman and Cardini [21]. Protein was determined by the method of Lowry *et al.*[22].

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