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DISTRIBUTION IN EXCISED *LYCOPERSICUM ESCULENTUM* ROOTS OF THE PRINCIPAL ENZYMES INVOLVED IN SUCROSE METABOLISM

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Abstract—Sucrose synthetase and sucrose phosphate synthetase could not be detected in 7-day-old excised tomato roots grown in sucrose. These roots, however, possessed a highly active acid invertase and a neutral invertase of low activity. The distribution of the cell wall-located acid invertase along the root axis appeared to be related to growth. This was not the case for the soluble enzyme. The possible functions of these two enzymes are discussed.

INTRODUCTION

EXCISED tomato roots have a unique carbon source requirement; they grow well on medium containing sucrose as carbon source but not with any of a wide range of other sugars.[1,2] In view of this apparent specificity it might be expected that the distribution of enzymes concerned with sucrose metabolism might show a relationship to growth. Several investigators have studied the relation between the spatial distribution of invertase along the main axis of roots and the position of the growth zone.[3–6] They have all reported a peak of invertase activity in the region of cell elongation. In their experiments they measured the total invertase activity of homogenates, or the soluble invertase activity. However, in many plant tissues invertase activity was found in the cell wall as well as in the soluble fraction.[7,8] The purpose of the work described here was to determine the presence and distribution of several enzymes of sucrose metabolism, namely sucrose synthetase (UDPglucose; D-fructose 2-glucosyltransferase, E.C. 2.4.1.13), sucrose phosphate synthetase (UDPglucose; D-fructose-6-phosphate 2-glucosyltransferase, E.C. 2.4.1.14), and invertase (β-D-fructofuranoside fructohydrolase, E.C. 3.2.1.26), paying particular attention to the role of soluble and insoluble invertases and their localization in relation to the zone of cell elongation.

RESULTS

Sucrose Synthetase and Sucrose Phosphate Synthetase

Using three commonly used methods, in triplicate determinations, neither sucrose

[1] STREET, H. E. and LOWE, J. S. (1950) *Ann. Bot.* **14**, 307.
[2] FERGUSON, J. D., STREET, H. E. and DAVID, S. B. (1958) *Ann. Botany* **22**, 5512.
[3] ROBINSON, E. and BROWN, R. (1952) *J. Exp. Botany* **3**, 356.
[4] HELLEBUST, J. A. and FORWARD, D. F. (1962) *Can. J. Botany* **40**, 113.
[5] SEXTON, R. and SUTCLIFFE, J. F. (1969) *Ann. Botany* **33**, 407.
[6] LYNE, R. L. and AP REES, T. (1971) *Phytochemistry* **10**, 2593.
[7] VAUGHAN, D. and MACDONALD, I. R. (1967) *J. Exp. Botany* **18**, 578.
[8] STRAUS, J. (1962) *Plant Physiol.* **37**, 342.

synthetase nor sucrose phosphate synthetase could be detected. To check our use of these methods the two enzymes were extracted from wheat germ and assayed; levels of activity similar to those reported in the literature were obtained with all three methods.[9]

TABLE 1. ATTEMPTED SEPARATION OF pH 4·8 AND pH 7·0 INVERTASE BY AMMONIUM SULPHATE PRECIPITATION

Ammonium sulphate saturation (%)	Invertase activity mg hexose/g fr. wt/2 hr	
	pH 4·8	pH 7·0
0–30	0·73	0·45
30–45	2·95	1·18
45–70	14·65	3·90
70–100	3·20	0·64

Distribution of Invertase in Soluble and Cell Wall Fractions

Invertase activity was found in both soluble and cell wall fractions. The effect of pH on the soluble and cell wall enzymes was very similar. There was a major peak of activity at pH 4·8, the so-called acid invertase, and a shoulder in the region of pH 7·0, possibly caused by a neutral invertase of low activity. Ricardo and ap Rees[10] were able to separate the acid and neutral invertases of carrot roots by ammonium sulphate precipitation, but this method was not successful with tomato root invertases (Table 1). Therefore, owing to the low level of activity at pH 7·0, and the inability to easily separate it from the pH 4·8 enzyme, no further experiments were conducted on the neutral invertase.

TABLE 2 EFFECT OF CARBOWAX 4000, TWEEN 20 AND BORATE BUFFER ON CELL WALL INVERTASE

Treatment	Invertase activity* mg hexose/g fr. wt/2 hr	Change (%)
Control	8·24	
6% Carbowax 4000	8·88	+8
10% Carbowax 4000	8·68	+5
1% Tween 20	8·44	+2
5% Tween 20	8·24	0
0·1 M Borate buffer (pH 7·0)	8·2	−1

The cell wall suspension was treated for 1 hr centrifuged at 12 000 *g* for 10 min, and the pellet washed twice with 0·5 M citrate–phosphate buffer (pH 7·0). The final sediment was resuspended in 0·05 M citrate–phosphate buffer (pH 7·0)

* Assayed at pH 4·8.

Hawker[11] has reported that Carbowax 4000, Tween 20 and borate buffer could solubilize most of the invertase of the cell wall of grape berries. He considered that this occurred by the disruption of either a tannin–protein complex or a protein–tannin-cell wall complex.

[9] MENDICINO, J. (1960) *J. Biol. Chem.* **235**, 3347.
[10] RICARDO, C. P. P. and AP REES, T. (1970) *Phytochemistry* **9**, 239.
[11] HAWKER, J. S. (1969) *Phytochemistry* **8**, 337

The invertase in the cell wall fraction of tomato roots could not be solubilized by such treatments (Table 2) and it is concluded that the cell wall activity is not due to the formation of such complexes, but to a firmly bound enzyme.

TABLE 3. EFFECT OF pH OF EXTRACTION BUFFER ON DISTRIBUTION OF TOMATO ROOT INVERTASE

pH of extraction medium	Invertase activity* mg hexose/g fr. wt/2 hr Soluble	Cell wall	Total activity	Cell wall activity as % of total
4·8	15·24	9·33	24·57	38·0
6	18·00	7·80	25·80	30·2
7	24·48	6·28	30·76	20·6
8	25·60	6·02	31·62	19·0

* Assayed at pH 4·8.

Ricardo and ap Rees[10] showed that the distribution of acid invertase activity in homogenates of aged carrot disks could be drastically altered by changing the pH of the extraction medium. They showed that the cell wall activity was increased from 35% of total activity at pH 7·0–98% of total activity at pH 4·5 (calculated from their Table 3). A similar, but much smaller change in distribution of activity occurred when excised tomato roots were homogenized at pH 4·8 as compared with pH 7·0 (Table 3). Here the increase in cell wall activity was from 20·6 to 38% of the total activity. A reasonable interpretation of the above results is that fractionation at pH 4·8 leaves a moiety of soluble invertase adsorbed to the wall.

TABLE 4. EFFECT OF ALTERING pH AFTER EXTRACTION ON DISTRIBUTION OF INVERTASE ACTIVITY IN TOMATO ROOT HOMOGENATE

Treatment of homogenate	Invertase activity* mg hexose/g fr. wt/2 hr Homogenized at pH 4·8 Soluble	Cell wall	Homogenized at pH 7·0 Soluble	Cell	Cell wall activity as % of total
Control	15·24	9·33			38
			24·48	6·28	20
pH changed 4·8–7·0 with Na_2PO_4	18·09	3·48			16
pH changed 7·0–4·8 with citric acid			16·64	8·84	35

* Assayed at pH 4·8.

This adsorption does not occur at pH 7·0 or higher. If this is the case then alteration of the pH of the extraction medium after extraction but before centrifugation should alter the distribution of the enzyme. Table 4 shows that this is indeed the case, and confirms the findings of Ricardo and ap Rees. Once again, however, there was a large quantitative difference between carrot roots and excised tomato roots. In the former, alteration of the pH from 7·0 to 4·5 caused the cell wall activity to be increased from 28 to 98% of total activity, whereas with tomato roots alteration of the pH from 7·0 to 4·8 caused an increase from 20 to 35%. These quantitative differences point to marked differences in the ionic

properties of the cell wall and/or invertase protein of carrot roots as compared with tomato roots. The invertase activity adsorbed to the wall at low pH may be equivalent to the loosely bound fraction of wall invertase reported by Copping and Street.[12] In the experiments described below extraction and fractionation were carried out at pH 7·0.

Distribution of Soluble and Cell Wall Invertase along the Root Axis

Figure 1 shows the distribution of soluble and cell wall invertase activity and cell number in successive 1·5 mm segments of the main axis of the root. The cell number was high in the first segment and dropped markedly in the second segment indicating that the main elongation region was between 0·75 and 2·25 mm from the tip. From the second to the fourth segment the cell number fell slowly and from the fourth segment onwards the cell number remained almost constant. This showed that the cells enlarged slowly in the region 2·25–5·25 mm from the tip and from here onwards no further enlargement occurred. When expressed on a per segment basis both soluble and cell wall activities were highest in the actively growing zone. Activity declined as the enlargement process slowed down but nevertheless remained high in the region where there was no apparent cell enlargement. This contrasts markedly with the previously reported work where activity fell to a very low level once growth had ceased.[3–6]

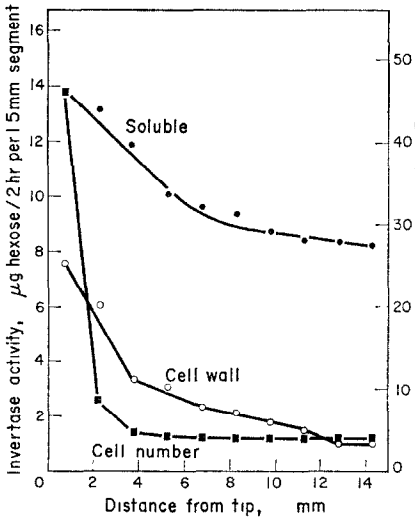

FIG. 1. SPATIAL DISTRIBUTION OF NUMBERS OF CELLS AND INVERTASE ACTIVITY, EXPRESSED AS PER 1·5 mm SEGMENT.

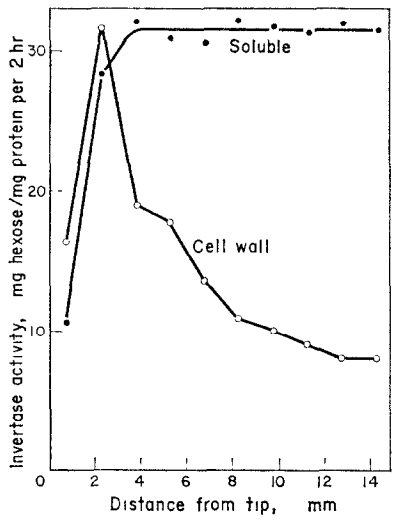

FIG. 2. SPATIAL DISTRIBUTION OF SOLUBLE AND CELL WALL INVERTASES, EXPRESSED AS PER UNIT PROTEIN.

When the invertase activity was expressed on a per unit protein basis the distribution patterns of soluble and cell wall invertase were shown to be entirely different (Fig. 2). The soluble invertase activity rose rapidly in the growing region but remained relatively uniform thereafter. The cell wall invertase activity also rose rapidly in the growing region but unlike the soluble invertase it fell rapidly in the region where growth slowed down. Thus only the cell wall invertase showed a peak of activity in the growth zone.

[12] COPPING, L. G. and STREET, H. E. (1972) *Physiol. Plant* **26**, 346.

The rapid rate of decline of specific activity in Fig. 2 was reduced from the third segment onwards, thus producing a pronounced tail to the peak. The sharpness of a peak depends upon the rate of synthesis and breakdown of the enzyme and the rate of growth of the tissue. When protein synthesis was inhibited by cycloheximide the reduction of invertase activity had a halftime longer than 48 hr (Fig. 3). This is much longer than the turnover rate of sugarcane invertase which has a half-life of only 2 hr.[13] Figure 3 also shows that the growth rate of 5-day-old excised tomato roots from the 5th to the 7th day was *ca.* 3 cm per day. This growth rate is 3 times faster than the growth rate of the pea roots used by Sutcliffe and Sexton.[5] The slower rate of breakdown of the enzyme together with the faster growth rate of the tissue were probably the factors causing the tail to the peak.

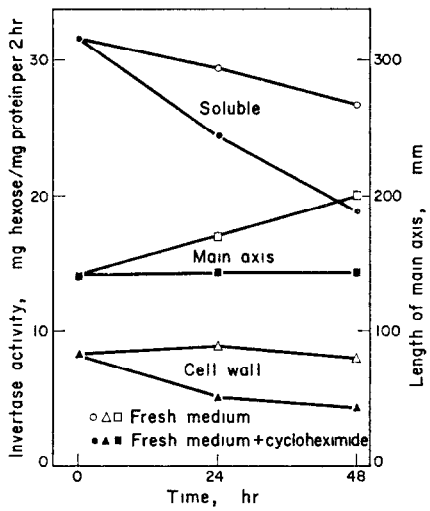

FIG. 3. EFFECT OF CYCLOHEXIMIDE (2 mg/l.) ON GROWTH AND INVERTASE ACTIVITY.
At zero time 5-day-old roots were transferred either to fresh medium or fresh medium containing cycloheximide.

DISCUSSION

Though the actions of both sucrose synthetase and sucrose phosphate synthetase are reversible, there is evidence indicating that the main function of sucrose synthetase is the utilization of sucrose, thus retaining in UDPG part of the energy of the glucose–fructose bond, whereas sucrose phosphate synthetase is involved in sucrose synthesis.[14–16] Using methods which were successful in assaying these two enzymes in wheat germ, their presence could not be demonstrated in tomato roots. It is possible that the enzymes from tomato roots are inhibited *in vitro* by a compound released during grinding. The use of enzymes partially purified by ammonium sulphate precipitation precludes the possibility of inhibition by a low MW compound, but not the possibility of a protein operating in this manner. However, there has been no report in the literature of such an inhibitor.

[13] GLASZIOU, K. T., GAYLER, D. R. and WALDRON, J. C. (1968) in *Biochemistry and Physiology of Plant Growth Substance* (WIGHTMAN, F. and SETTERFIELD, G., eds.), p. 433, Runge Press, Ottawa.
[14] DE FEKETE, M. A. R. and CARDINI, C. E. (1964) *Arch. Biochem. Biophys.* **104,** 173.
[15] MURATA, T., SUGIYAMA, T. and AKAZAWA, T. (1964) *Arch. Biochem. Biophys.* **107,** 92.
[16] PRESSEY, R. (1969) *Plant Physiol.* **44,** 759.

Hatch[16] has shown that sucrose phosphate synthetase is coupled to sucrose phosphate phosphatase in the synthesis of sucrose by sugar cane. If these two enzymes are regulatory enzymes they would be expected to be present in low quantities when the endogenous sucrose level is high. If the same system operates in tomato roots then the inability to detect these two enzymes is not unexpected since they were grown in a sucrose medium. Roots grown in glucose are capable of synthesizing sucrose but to a level of concentration which suggests that the rate of sucrose synthesis is the limiting factor in the growth of roots in glucose.[17,18] The conclusion is therefore drawn that sucrose synthetase is not involved in the utilization of sucrose by excised tomato roots cultured in sucrose.

Excised tomato roots possess a highly active acid invertase and a less active neutral invertase situated in both the soluble and the cell wall fractions. Temperature inactivation studies suggested that the soluble and wall-located enzymes are the same protein species. When the distribution of the acid enzyme was expressed on a per segment basis both soluble and cell wall invertase had their highest activity in the rapidly growing region. Hence there is a close association between invertase activity and rapidly growing cells. However, the high invertase activity in the growing region could merely reflect the high cell number and high protein content of the growing region. Expressed on a per unit protein basis the distribution patterns of the soluble and cell wall-located enzyme showed a marked difference. The soluble invertase rose rapidly in the growing region but was maintained at a high level in the region that had ceased to grow. It therefore did not show a peak of activity. The cell wall invertase, however, clearly showed a peak of activity in the growing region. The close association of cell wall invertase activity with growth rate suggests that the enzyme in this location may have a role in the growth process.

Several possible functions in growth have been suggested. It has been proposed that invertase by the inversion of sucrose could be involved in helping to maintain the osmotic pressure of the cell sap during rapid water uptake.[4] While the soluble invertase could have such a function the cell wall invertase due to its location clearly could not play such a role. Indeed the action of the cell wall-located enzyme on apparent free space sucrose would tend to reduce the water potential gradient and thus to reduce growth. It has also been suggested that the inversion of sucrose in the apparent free space may facilitate its absorption.[19] Hatch *et al.* have produced evidence to show that inversion of sucrose in the apparent free space is essential for sucrose uptake in sugar cane. However, with excised tomato roots, Weston and Street[20] reported that alterations of external pH drastically altered the levels of glucose and fructose appearing in the medium but did not affect sucrose absorption. If the cell wall invertase is involved in sugar absorption one might expect that absorption would increase at pH 4·8 which is the optimal pH of the enzyme. This was found not to be the case and it is considered unlikely that the enzyme is involved in sugar absorption in excised tomato roots.

The hydrolysis of sucrose by the soluble invertase would provide respiratory substrates and in view of the inability to demonstrate sucrose synthetase activity this could be an important function of the soluble enzyme. However, since there is no report of the presence of mitochondria in the cell wall, the cell wall-located enzyme does not appear to have such a

[17] THOMAS, D. R., CRAIGIE, J. S. and STREET, H E. (1963) in *Plant Tissue and Organ Culture—a Symposium* (MAHESHWARI, P. and RANGA SWAMY, N. S eds), p. 26, The International Society of Plant Morphologists, Delhi.

[18] THOMAS, D R. and RIER, M. (1967) *New Phytologist* **66,** 125.

[19] HATCH, M. D., SACHER, J. A. and GLASZIOU, K. T (1963) *Plant Physiol.* **38,** 338.

[20] WESTON, G. D and STREET, H E (1968) *Ann. Botany* **32,** 521.

function. It is possible that the cell wall invertase may be involved in providing substrates for the biosynthesis of the cell wall. There is, however, evidence indicating that the cell wall itself may not be involved in cell wall synthesis. Thus Ray *et al.*[21] isolated particles containing polysaccharide synthetase activity which appear to be Golgi material. Further protoplasts isolated from tomato fruit,[22] and *Convolvulus* tissue cultures[23] could regenerate wall-like structures. This evidence shows that the cell wall and therefore cell wall invertase is not essential in the synthesis of cell wall materials. However, the final stages of cell wall manufacture occur external to the plasmalemma and presumably these reactions are enzyme catalysed. It is theoretically possible that cell wall invertase plays a part in this process.[12] Invertase attacks not only sucrose but also other substrates possessing a terminal unsubstituted β-D-fructofuranosyl residue, causing either hydrolysis or transfer. This enzyme may therefore transfer a β-fructofuranosyl unit to a molecule with a terminal primary alcohol, thus protecting it, although this is unlikely since in cell free systems the equilibrium is very heavily in favor of hydrolysis. Another interesting possibility is that invertase removes a fructose molecule from an oligo or polysaccharide terminated in sucrose, thus exposing the C1 position of the glucose molecule, and facilitating the addition of further oligosaccharides. Since there is no evidence for terminal fructofuranosyl residues either on wall-located polysaccharides or on cytoplasm-located but wall-destined oligosaccharides this role must be considered highly speculative. Thus although high cell wall invertase activity is found associated with the rapidly growing region of excised tomato roots no clear function can be satisfactorily attributed to it at the present time.

EXPERIMENTAL

Material. A clone of excised roots was established from a single seed of *Lycopersicum esculentum*, Sutton's 'Best of All', using the techniques of Dormer and Street[24] and Street and Lowe.[1] 7-day-old roots were used. In enzyme distribution experiments roots were sectioned into 1·5 mm segments with a specially constructed metal rig similar to that described by Hellebust and Forward.[4] Roots or root segments were incubated in dist. H_2O for 2 hr prior to extraction.

Methods. The root segments were homogenized in 0·5 M citrate–phosphate buffer (pH 7·0) with a glass homogenizer. The homogenate was centrifuged at 12 000 *g* for 10 min. The pellet was washed with the same buffer and centrifuged at 12 000 *g* for 10 min. The process was repeated 2 × and the sediment homogenized with 0·05 M citrate–phosphate buffer (pH 7) with a glass homogenizer. This suspension and the original supernatant were used as cell wall and soluble enzyme respectively. All operations were carried out at 4°. The enzyme preparations were stored at 4° until assayed. The invertase assay system contained 1 ml 10% sucrose, 0·9 ml 0·1 M citrate–phosphate buffer (pH 4·8) (for the pH study, buffers of different pH were used) and 0·1 ml enzyme preparation. The reaction mixture was incubated at 28° for 2 hr, and the reaction stopped by rapidly pipetting the sample into Somogyi's solution. Invertase activity was measured by the production of reducing sugars by the method of Somogyi.[22] Cell counts were made by the technique of Brown and Rickless.[23] For the assay of sucrose synthetase and sucrose phosphate synthetase the soluble fraction was fractionated with ammonium sulphate, the 0–40% precipitate being used as the enzyme preparation. Three methods were used; those of Grimes *et al.*,[27] Lavintman and Cardini,[28] and Pressey.[16] Protein determinations were made by the Folin phenol method of Lowry *et al.*[29,30]

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