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DISTRIBUTION OF ACID INVERTASE IN THE TOMATO PLANT

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; acid invertase; fruit ripening; wild species.

Abstract—Acid invertase activity in *Lycopersicon esculentum* was highest in the locular wall of ripe fruit and lowest in roots. Activity was greater in leaf laminae than in petiole tissue and increased with leaf age, whereas there was more invertase in the upper part of the stem compared with the older portion. Activity in whole fruit increased with increasing ripeness and was greatest in overripe fruit. Of various tissues from a number of wild tomato species examined, the fruit of *L. pimpinellifolium* were particularly rich in the enzyme, in contrast to the fruit of *L. hirsutum*, *L. hirsutum* var. *glabratum* and *L. peruvianum* which had low activity.

INTRODUCTION

Acid and neutral invertases (β-fructofuranoside fructohydrolase, E.C. 3.2.1.26) are widely distributed in higher plants [1–4]. Attention has recently been focussed on the physiological role of these enzymes, although it has been pointed out [5] that progress is hampered by uncertainties as to their precise location *in vivo*. Some of the reported data on the distribution of soluble and cell wall bound invertases may be inaccurate on account of the marked effects which pH, ionic strength and composition of the extracting medium can exert on the solubilization of the enzyme [3, 5, 6].

High acid invertase activity has been shown to be present in tissues undergoing rapid cell elongation and expansion [3, 4, 7–13], and there is also evidence that increased invertase activity is associated with increased tissue concentrations of hexoses and generally with decreased concentrations of sucrose [3, 4, 12–15]. Furthermore, low invertase activity may accompany low hexose and high sucrose concentrations [4, 11].

A widely accepted view is that invertase catalyses the hydrolysis of sucrose to hexoses when the latter are required for energy processes and other immediate metabolic demands [3, 12, 13], whereas sucrose hydrolysis by the reverse action of sucrose synthetase (E.C. 2.4.1.13) leads to starch synthesis by a more energy conserving pathway [13, 16–18]. Invertase may therefore play a key role in growth by controlling sucrose storage and utilization [3, 12, 19].

Characteristic differences in the distribution of sugars have been found in various parts of the stem and roots of the tomato plant [20], as well as in the fruit at different stages of ripeness [21, 22]. Differences in sugar composition also occur among the fruit of different tomato species [23]. It has not yet been established whether invertase is a mediating factor, but invertase activity has been demonstrated in excised tomato roots [8, 24] and fruit [25], and has been reported to increase during the ripening of fruit [26].

In the present paper the distribution of acid invertase has been examined in the cultivated tomato (*Lycopersicon esculentum* Mill.) and in fruit and tissues of other *Lycopersicon* species in an attempt to assess the role of the enzyme in determining the sugar composition of tomato fruit.

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RESULTS AND DISCUSSION

Distribution of invertase activity in the cultivated tomato

Invertase activity, whether expressed on the basis of tissue fresh weight, dry weight or protein–N content, was low in roots and in the bottom and middle sections of the stem, although young stem tissue had a higher activity (Table 1). On a tissue fresh or dry weight basis, activity was greater in leaf laminae than in petiole tissue, there being a 5·7–8·1-fold difference in terms of fr. wt and a 3·9–5·5-fold difference in terms of the dry weight. However, when expressed in terms of protein–N, these tissues were comparable in activity. Invertase activity was slightly higher in laminae from old as compared to young leaves.

On a dry weight or protein–N basis, the tissue with the highest invertase activity was the locular wall of red fruit. The locular contents and the placenta were also good sources of the enzyme. Less was present in the corresponding tissues of mature green fruit, where the activity of the locular contents was higher than that of the locular wall. The bracts of calyces contained substantial amounts of the enzyme, whereas activities in the stem of the calyx and the peduncle were lower.

Table 2. Invertase activity of whole tomato fruit at different stages of ripeness

Stage of ripeness	Invertase activity* Units g^{-1} fr. wt	Units g^{-1} dry wt	Units mg^{-1} protein–N
Immature green	0·85	12·7	2·22
Mature green	0·89	14·3	2·13
Green–yellow	0·99	17·2	4·60
Yellow–orange	1·41	24·6	6·70
Red	2·30	38·4	9·79
Overripe	4·26	56·9	13·1

* Each value is the mean of at least six determinations.

Invertase activity and fruit ripeness

Table 2 summarizes the data on invertase activity in whole fruit at six stages of ripeness as defined by Winsor *et al.* [21]. There was a progressive increase in activity with ripening, the change being 2·6-fold between the mature green and red stages ($P < 0·01$). Overripe fruit contained 1·3–1·9-times the activity of red fruit, depending on the basis of expressing the activity. The difference in invertase activity between fruit at the green and yellow–orange stages was not statistically significant, but that between fruit at the yellow–orange and red stages was significant

Table 1. Invertase activity in the cultivated tomato plant

Tissue	Invertase activity* Units g^{-1} fr. wt	Units g^{-1} dry wt	Units mg^{-1} protein–N
Mature green fruit—locular wall	1·06	16·7	3·73
locular contents	2·29	32·2	5·11
placenta	1·04	15·4	5·23
Ripe fruit—locular wall	2·35	39·7	11·5
locular contents	1·79	28·0	8·59
placenta	1·44	26·8	9·08
Young leaves—laminae	3·71	24·5	1·58
petiole	0·60	5·41	1·07
Mature leaves—laminae	4·88	29·8	2·37
petiole	0·86	7·68	2·03
Old leaves—laminae	6·15	34·4	2·75
petiole	0·76	6·20	1·48
Unripe calyx—stem	1·20	5·96	1·50
bract	2·37	18·0	2·90
Ripe calyx—stem	1·26	5·24	1·17
bract	2·93	20·6	2·68
Peduncle	0·74	4·39	1·19
Stem—top	0·89	9·58	3·25
middle	0·51	3·45	1·89
bottom	0·41	2·85	1·16
Roots	0·22	1·57	0·57

* Each value is the mean of four determinations.

at $P < 0.1$. This increase in invertase activity with advancing ripeness is in agreement with the findings of Nakagawa *et al.* [26].

Invertase activity in some "wild" tomato species

A comparison of the invertase activity of various tissues of four "wild" tomato species is given in Table 3. All tissues of *L. hirsutum* including the fruit, when assayed on a dry wt basis, were low in activity, as were those of *L. hirsutum* var. *glabratum* with the exception of the calyces and shoot tip, and those of *L. peruvianum* with the exception of leaf laminae.

In contrast, the fruit of *L. pimpinellifolium* were particularly rich in the enzyme. Fruit at the orange stage of ripeness showed the highest activity, which was about 20-times that in fruit of *L. esculentum* at the same stage of ripeness. It is of interest that red fruit of *L. pimpinellifolium* contained less enzyme than green or partially ripe fruit.

Invertase activity and sugar concentrations in tomato tissues

In a study of the distribution of carbohydrates in parts of the cultivated tomato plant, van Die [20] showed that for young plants, var. Ailsa Craig, the concentration of reducing sugars was higher in the top than in the bottom sections of the stem. The reverse was the case for sucrose, and the (glucose + fructose)/(sucrose) ($(G + F)/(S)$) ratios for top and bottom sections of stem

Table 3. Invertase activity in some "wild" tomato species

Species	Tissue	Invertase activity* Units g^{-1} fr. wt	Units g^{-1} dry wt	Units mg^{-1} protein–N
L. pimpinellifolium (Eulycopersicon)	Whole fruit—green	26·7	252	43·6
	orange	60·5	542	133
	red	21·9	178	44·8
	Calyx—unripe	1·98	11·5	1·01
	half ripe	3·00	16·4	1·70
	ripe	4·08	21·0	2·16
	Mature leaves—laminae	2·03	10·9	1·25
	petiole	0·71	6·21	1·70
	Stem, middle	0·63	4·00	1·34
	Roots	0·46	4·01	1·24
L. hirsutum (Eriopersicon)	Whole fruit—unripe	0·76	5·85	0·96
	ripe	0·64	4·07	1·57
	Mature leaves—laminae	0·49	1·82	0·64
	petiole	0·46	2·54	1·23
	Stem, middle	0·39	1·38	1·83
	Roots	0·29	1·63	0·50
L. hirsutum var. *glabratum* (Eriopersicon)	Whole fruit—unripe	0·33	3·63	2·73
	ripe	0·19	2·05	1·83
	Calyx—unripe	1·37	10·3	3·95
	ripe	1·50	10·3	4·95
	Mature leaves—laminae	1·38	9·85	2·05
	petiole	0·47	5·27	6·54
	Stem—tip	1·28	11·6	2·46
	upper	0·28	3·47	1·48
	middle	0·22	1·23	1·11
	bottom	0·11	0·54	0·55
	Roots	0·53	3·81	3·70
L. peruvianum (Eriopersicon)	Whole fruit—unripe	0·68	6·61	0·73
	semi-ripe	0·49	3·14	0·97
	ripe	0·44	3·18	0·84
	Mature leaves—laminae	1·24	10·3	0·94
	petiole	0·93	4·56	1·58
	Stem, middle	0·17	0·59	0·29
	Roots	0·32	1·51	0·73

* Each value is the mean of four determinations.

were 16·8 and 1·9, respectively. As shown in Table 1, the top sections of tomato stems had higher invertase activities than the bottom sections, the difference being 3·4-fold on a dry wt basis or 2·2-fold on a fr. wt basis. In roots, van Die's data show $(G + F)/(S)$ ratios of 0·85–1·1, where in the present experiments low invertase activity was found.

This association of high invertase activity with high $(G + F)/(S)$ ratios, and its converse also extends to the fruit. Sucrose is present only in small amounts in mature fruit [21, 23], and unpublished observations by Davies and Kempton [22] for fruit of varieties Craigella and Kingley Cross show sucrose concentrations of 0·007–0·037% fr. wt of tissue and $(G + F)/(S)$ ratios of 69 and 519 for unripe and ripe fruit, respectively. Tables 1 and 2 indicate that tomato fruit contain appreciable invertase activity and, moreover, during ripening from the mature green to the red stage there was a 2·6-fold increase in activity, which parallels a 7·5-fold increase in the $(G + F)/(S)$ ratio calculated from data of Davies and Kempton [22].

As part of the present study, individual sugars were determined in fruit of the wild species *L. pimpinellifolium* and *L. peruvianum*. The former contained very high levels of invertase activity (Table 3), a low concentration of sucrose (0·025% fr. wt) and a $(G + F)/(S)$ ratio of 168. Fruit of *L. peruvianum*, on the other hand, had a lower invertase activity accompanied by a relatively high sucrose content (1·8% fr. wt) and a low $(G + F)/(S)$ ratio (2·07). These distributions of sucrose and reducing sugars agree qualitatively with the values reported by Davies [23], whose data also indicate low $(G + F)/(S)$ ratios for fruit of the species *L. hirsutum* and *L. hirsutum* var. *glabratum*, in which invertase activity was found to be low (Table 3).

In contrast to other tissues in the tomato, leaves did not show the above invertase–sugar interrelationship. As shown in Table 4, laminar tissue from *L. esculentum*, although having appreciable invertase activity, was also relatively rich in sucrose, with a low $(G + F)/(S)$ ratio. In petioles lower invertase activity was accompanied by a higher $(G + F)/(S)$ ratio. This was also found to apply to leaves from the two wild tomato species examined, and it indicates that in this part of the plant other factors than invertase are exerting a more dominant control on sugar distribution and utilization.

EXPERIMENTAL

Plant material. Tomato plants, *Lycopersicon esculentum* Mill var. "Potella", *L. hirsutum*, *L. hirsutum* var. *glabratum*, *L. peruvianum* and *L. pimpinellifolium* were grown in pots in a heated glasshouse. Bulk samples of each plant part were obtained, except for fruit of "Potella", when individual fruit weighing 50–100 g were selected. The samples were washed with ice-cold tap H_2O, dried with absorbant paper and 5 g sub-samples taken for enzyme assays and other analyses. Samples were also taken for determination of dry wt by heating tissue to constant wt in a forced-draught oven at 80°.

Methods. Tissue samples (5 g), weighed out as soon as possible after picking, were macerated for 2 min in 50 ml of extraction medium consisting of Tris acid maleate buffer, pH 7·0, ionic strength 0·4 (0·246 M), containing added KCl (0·60 M) to give a final ionic strength of 1·0, the temp. being kept below 4°. The macerate was filtered through glass wool and centrifuged at 38000 *g* for 10 min. No significant amounts of invertase remained in the insoluble fraction, as checked by a further extraction of the sediment. The clear supernatant was diluted 5 times and invertase activity assayed by incubating 1 ml aliquots with 2 ml 0·15 M sucrose in 75 mM NaOAc buffer, pH 4·2, at 25°. The reaction was stopped by the addition of 3 ml Miller's modified 3,5-dinitrosalicylic acid reagent [27], and colour developed at 100° for 10 min and read at 560 nm against a reagent blank. The rate of hydrolysis was virtually constant for at least 30 min, and change in extinction was determined over the first 20 min to obtain initial reaction rate.

Table 4. Individual sugars in mature leaves of some tomato species

Species	Tissue	Sugar concentration (g/100 g fr. wt)* Glucose (*G*)	Fructose (*F*)	Sucrose (*S*)	$(G + F)/(S)$
L. esculentum	Laminae	0·11	0·17	0·50	0·56
	Petiole	1·13	0·04	0·39	3·0
L. pimpinellifolium	Laminae	0·62	0·085	0·18	0·81
	Petiole	0·079	0·047	0·13	0·97
L. peruvianum	Laminae	0·030	0·023	0·28	0·19
	Petiole	0·092	0·060	0·16	0·97

* Values are means of duplicate determinations.

A shorter time was used for more active preparations. A standard calibration curve was constructed using 0–6 μmol glucose in the presence of 0·1 M sucrose, 50 mM acetate buffer, pH 4·2 and 5-fold dild extraction medium. The slope of the plot was 0·17 $\Delta E_{560}/\mu$mol reducing sugar. As described by other workers [4, 28] calibration curve showed a "lag" at very low reducing sugar concns, which could be eliminated by addition of 350 μg glucose to each sample. Lag was considerably reduced in the presence of freshly prepared sucrose soln. However, it was completely absent when the sucrose–acetate buffer mixture was allowed to age at room temp. for 2 weeks, and use of the latter reagent proved simpler than the direct addition of glucose to each sample prior to analysis. One unit of invertase activity was defined as the amount of enzyme which liberated 1 μmol of reducing sugar per min at 25° in the presence of 0·1 M aged sucrose and 50 mM NaOAc buffer, pH 4·2. Validity of the colorimetric procedure as a measure of sucrose hydrolysis by invertase was checked by a radioisotopic method, using ^{14}C-labelled substrate, and glucose and fructose formed were separated and assayed individually. An active preparation of invertase was prepared from 50 g of locular walls of ripe fruit of *L. esculentum*, which was macerated in 30 ml extracting medium (as above) and supernatant dialysed and subjected to Me_2CO prptn at −10°. The enzyme preparation (2 ml, containing about 1·8 units/ml) was added to a mixture of 2 ml 0·06 M sucrose in 0·15 M NaOAc buffer, pH 4·2, and 2 ml sucrose-[U-^{14}C] (1 μCi/ml) soln, and the whole mixture incubated at 25° for 1 h. The reaction was then stopped by heating the mixture at 100° for 5 min. Samples (1 ml) were withdrawn for chemical analysis of reducing sugar and for total ^{14}C content by liquid scintillation counting [29]. Samples (0·25 ml) were also taken for chromatographic separation of the labelled sugars present. Each of these samples was loaded on to a 3·5 cm wide strip of Whatman 3MM paper for descending chromatography in *n*-BuOH–HOAc–H_2O (12:3:5) for 51 hr at room temp. After being dried, the chromatograms were scanned with a 4π-radiochromatogram scanner fitted with an integrator, and the ^{14}C activity of each sugar band determined. "Zero time" readings were obtained by withdrawing samples from a mixture of 2 ml boiled enzyme preparation, 2 ml sucrose–acetate buffer and 2 ml labelled sucrose soln after the mixture had been heated at 100° for 5 min.

After 1 h, 40·7% of the ^{14}C in the reaction mixture was present as unchanged sucrose, 30·3% as glucose and 29·0% as fructose, indicating 59·3% hydrolysis of the substrate. Scanning of the chromatograms from the boiled enzyme preparation showed that 7·2% hydrolysis had occurred at "zero time", so that enzymic hydrolysis had accounted for breakdown of 52·1% of substrate. Colorimetric procedure gave a mean extinction change of 0·609 extinction units over the same time interval, corresponding to 48·9% hydrolysis of the sucrose, which compares well with the value obtained by the tracer method.

Protein–N was determined in 20 ml samples of the supernatant from macerated tissues, using a Technicon AutoAnalyser [30], after treatment with TCA and digestion of the precipitate with sulphuric acid in the presence of selenium as catalyst. Sugars in tomato fruit, leaf laminae and petioles were extracted with boiling 80% aq. EtOH and were determined by GLC as their trimethylsilyl ethers [31], after removal of interfering chromogenic material with $CHCl_3$.

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REFERENCES

1. Myrbäck, K. (1960) *The Enzymes* (Boyer, P. D., Lardy, H. and Myrbäck, K., eds.), Vol. 4, pp. 379–396. Academic Press, New York.
2. Straus, J. (1962) *Plant Physiol.* **37,** 342.
3. Ricardo, C. P. P. and Rees, T. ap (1970) *Phytochemistry* **9,** 239.
4. Lyne, R. L. and Rees, T. ap (1971) *Phytochemistry* **10,** 2593.
5. Little, G. and Edelman, J. (1973) *Phytochemistry* **12,** 67.
6. Hawker, J. S. (1969) *Phytochemistry* **8,** 337.
7. Hellebust, J. A. and Forward, D. F. (1962) *Can. J. Botany* **40,** 113.
8. Chin, C. K. and Weston, G. D. (1973) *Phytochemistry* **12,** 1229.
9. Hatch, M. D. and Glasziou, K. T. (1963) *Plant Physiol.* **38,** 344.
10. Seitz, K. and Lang, A. (1968) *Plant Physiol.* **43,** 1075.
11. Cooper, R. A. and Greenshields, R. N. (1964) *Biochem J.* **92,** 357.
12. MacLachlan, G. A., Datko, A. H., Rollit, J. and Stokes, E. (1970) *Phytochemistry* **9,** 1023.
13. Shukla, R. N., Singh, S., Das, N., Baijal, M. and Sanwal, G. G. (1973) *Phytochemistry* **12,** 979.
14. Hawker, J. S. (1969) *Phytochemistry* **8,** 9.
15. Pressey, R. and Shaw, R. (1966) *Plant Physiol.* **41,** 1657.
16. Murata, T., Sugiyama, T. and Akazawa, T. (1964) *Arch. Biochem. Biophys.* **107,** 92.
17. Fekete, M. A. R. de (1969) *Planta* **87,** 311.
18. Pressey, R. (1969) *Plant Physiol.* **44,** 759.
19. Sacher, J. A., Hatch, M. D. and Glasziou, K. T. (1963) *Plant Physiol.* **38,** 348.
20. Die, J. van (1962) *Acta Botanica Neerlandica* **11,** 418.
21. Winsor, G. W., Davies, J. N. and Massey, D. M. (1962) *J. Sci. Food Agr.* **13,** 108.
22. Davies, J. N. and Kempton, R. J. Private communication.
23. Davies, J. N. (1966) *Nature* **209,** 640.
24. Weston, G. D. and Street, H. E. (1968) *Ann. Botany* **32,** 521.
25. Takehana, H. and Nakagawa, H. (1970) *Tech. Bull. Fac. Hortic. Chiba Univ.* **18,** 67.
26. Nakagawa, H., Sakuma, A. and Takehana, H. (1970) *Tech. Bull. Fac. Hortic. Chiba Univ.* **18,** 77.
27. Miller, G. L. (1959) *Anal. Chem.* **31,** 426.
28. Arnold, W. N. (1965) *Biochim. Biophys. Acta* **110,** 134.
29. Davies, J. N. and Maw, G. A. (1972) *J. Sci. Food Agr.* **23,** 969.
30. O'Neill, J. V. and Webb, R. A. (1970) *J. Sci. Food Agr.* **21,** 217.
31. Davies, J. N. and Kempton, R. J. (1972) *Commun. Soil Sci. Plant Anal.* **3,** 305.