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SUCROSE METABOLISM AND ACCUMULATION IN DEVELOPING FRUIT OF *CUCUMIS*

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Key Word Index—*Cucumis melo*; *C. sativus*; Cucurbitaceae; invertase; sucrose synthase; sink metabolism.

Abstract—Sucrose, reducing sugars and starch content were measured in developing cucumber (*C. sativus* cv Delilah), sweet melon (*C. melon* cv Galia and cv Noy Yizre'el) and non-sweet melon (*C. melo* cv Bird's Nest) fruit. Sweet melon were characterized by accumulation of sucrose in the maturing fruit, while cucumber and non-sweet melon had a low sucrose content at all stages studied. Soluble acid invertase activity (EC 3.2.1.26) dramatically decreased in sweet melon, concomitant with the onset of sucrose accumulation. Significant activity of soluble acid invertase was retained in mature cucumber and non-sweet melon. Insoluble acid invertase, determined not to be an artifact of extraction, constituted a significant portion of total invertase activity (*ca* 25% in young sweet melon and *ca* 50% in young cucumber). In sweet melon sucrose synthase activity (EC 2.4.1.13), measured in both the cleavage and synthesis direction, increased during the sucrose accumulation period. The results are discussed in terms of the roles of invertase and sucrose synthase in sucrose accumulation in *Cucumis*.

INTRODUCTION

Fruit quality of sweet melons (*C. melo* var. reticulatus) is primarily determined by sugar content [1] which is principally a phenomenon of sucrose accumulation [2]. Sucrose content in young ovary mesocarp tissue is low, and during maturation dramatically rises and may reach 5% on a fresh weight basis [3] or 50–60% on a dry weight basis [4]. Cucumber mesocarp, on the other hand, has a constant and insignificant sucrose content throughout development [5]. Instead, citric acid content increases throughout development [5, 6], yielding a sourish taste in the ripe cucumber. Many botanical varieties of *C. melo* are also sour at maturity rather than sweet (i.e. *C. melo* var. acidosus [7]).

Although the Cucurbitaceae transport galactosylsucrose (raffinose and stachyose) rather than sucrose [8], the sugar that finally enters the fruit is apparently sucrose [9]. Therefore, the matabolic fate of the imported sucrose may determine the carbohydrate pattern of developing *Cucumis* fruit. Once sucrose enters the sink its metabolic fate is determined by events occurring in the sink tissue, and sucrose can either be metabolized or compartmentalized intact as sucrose. In studies carried out on sucrose storage organs such as carrot root [10], sugar beet root [11], citrus fruit [12] and sugar cane stem [13], it has repeatedly been shown that these organs undergo a metabolic transition from their actively growing stage to their sucrose storage stage. This transition is characterized by changes in sucrose metabolism. The actively growing stage is characterized by relatively high activities of acid invertase while the storage stage is characterized by low levels of acid invertase and increased activity of either alkaline invertase [10, 13] or sucrose synthase [11].

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The significance of these metabolic changes has been explained by ap Rees [14] and more recently by Avigad [15] and Hawker [16]. Soluble acid invertase, presumably compartmentalized in the vacuole [17] functions in efficiently hydrolysing imported sucrose to hexoses which are utilized for necessary growth metabolism. Loss of vacuolar acid invertase activity allows for vacuolar sucrose storage. The two other enzymes implicated in sucrose hydrolysis, alkaline invertase and sucrose synthase, are purportedly compartmentalized in the cytoplasm thereby permitting sucrose accumulation in the vacuole. These two enzymes may fulfil the function of hydrolysing sucrose to meet the metabolic needs of the storage tissue.

The purpose of this study was to determine whether sweet melon undergoes a developmental change in sucrose metabolism, as observed in other sucrose storing tissues, and whether differences between sweet and non-sweet genotypes of *Cucumis* can be related to differences in sucrose metabolism through development.

RESULTS

The sugar and starch content (Fig. 1) of developing sweet melon (cv Galia) and cucumber (cv Delilah) were measured from 17 days after anthesis (daa) until 56 daa, at which stage both melon and cucumber epicarp were yellow. Reducing sugar content did not significantly change in the sweet melon or cucumber during this period. Sucrose level was low throughout the period in cucumber, whereas in sweet melon sucrose content increased from *ca* 30 daa until 55 daa. Starch content was low during the development of both sweet melon and cucumber, although in melon there was a steady decline from 0.41 to 0.13 mg/g fr. wt.

Soluble and insoluble invertase activity of young (23 daa) and mature (55 daa) melons and cucumbers showed peak activities in the acid range (pH 5) with no discernible

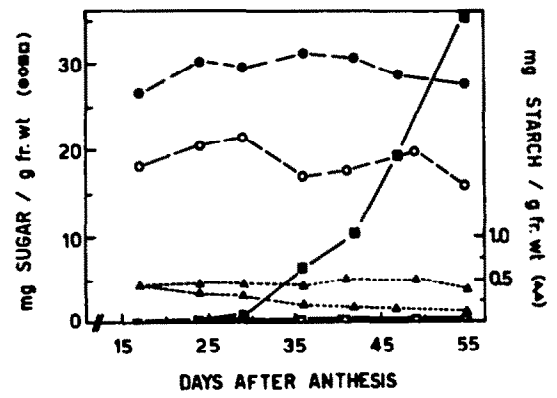

Fig. 1. Carbohydrate content of mesocarp tissue from developing fruit of *Cucumis melo* cv Galia (filled symbols) and *C. sativus* cv. Delilah (empty symbols). □ —□: Sucrose; ○ —○: Reducing sugars; △---△ : Starch. Each point is the average of three samples from individual fruit.

second peaks in the neutral–alkaline range (data not shown). Invertase activity associated with the soluble fraction showed highest activities at the young immature stages in both melon and cucumber (Fig. 2a). Soluble invertase activity declined in melon concomitant with the increase in sucrose. Soluble invertase activity also declined through development in cucumber but to a much lesser extent and at the later stages of maturity cucumber soluble invertase activity was significant.

Mixing experiments between young (23 daa) and mature (56 daa) melon and cucumber (all four combinations) were carried out on the soluble fraction both before and after dialysis, in order to determine whether the decline in soluble invertase activity was due to either a low or high M_r inhibitor. Activity of mixed aliquots in all four combinations were always the average of activities of the two individual aliquots (data not shown), giving no indication that decline in activity was due to soluble inhibitors.

K_m values of soluble invertase from young and mature melon and cucumber were all in the range of 2.4–3.2 mM sucrose. K_m values of insoluble invertase from young and mature cucumber and from young melon were in the range of 2.9–3.6 mM sucrose. The K_m value for insoluble invertase from mature melons was *ca* 10 mM sucrose.

Insoluble invertase activity was significantly higher throughout development in cucumber than in melon (Fig. 2b). There were no significant trends that could be correlated with sucrose content in melons. Insoluble invertase comprised *ca* 50% of invertase activity throughout development of cucumber. In young melon it comprised *ca* 25% of total activity whereas in mature melons, due to the loss of the soluble enzyme activity, all activity was associated with the insoluble fraction.

In order to determine whether the insoluble activity was an artifact of extraction we studied the effect of different extraction media which have been used in previous studies [18, 19] (Table 1). None of the media tested effected a significant decrease in activity associated with the insoluble fraction and a concomitant increase associated with the soluble fraction, suggesting that the insoluble activity we observed is not an artifact.

Sucrose synthase activity measured in both the synthesis and cleavage directions showed a significant increase in activity during the later stages of sucrose accumulation in sweet melon (Fig. 3). In cucumber, activity in the cleavage direction showed no perceptible trend other than the sharp decrease in activity at the final stages. When assayed in the synthesis direction a steady decline in activity was observed in developing cucumbers.

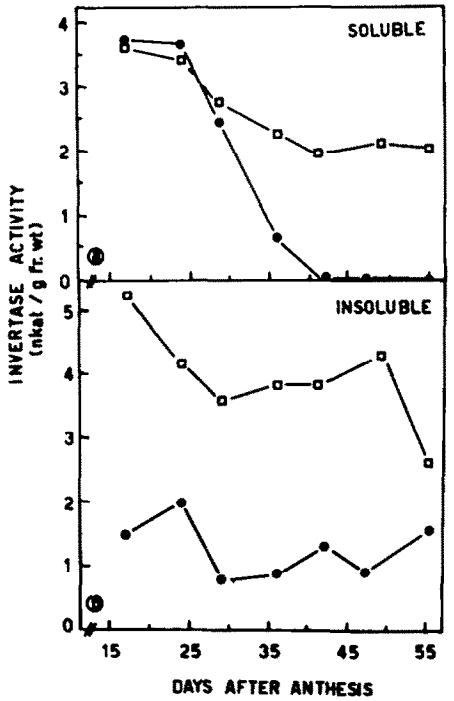

Fig. 2. Invertase activity of mesocarp tissue from developing fruit of *Cucumis melo* cv. Galia (●—●) and *C. sativus* cv. Delilah (□—□). Each point is the average of three samples from individual fruit for soluble invertase (2a) and of two samples from individual fruit for insoluble invertase (2b).

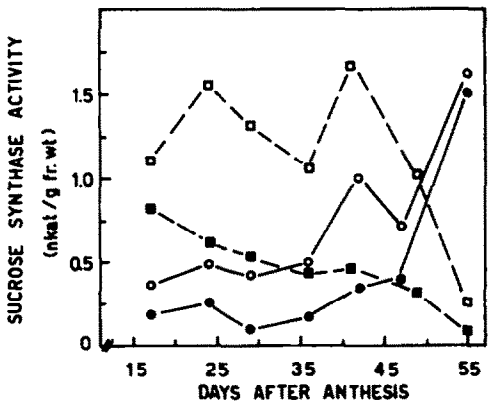

Fig. 3. Sucrose synthase activity of mesocarp tissue from developing fruit of *C. melo* cv Galia (○—○, ●—●) and *C. sativus* cv Delilah (□---□, ■---■). Empty symbols represent sucrose synthase activity in the cleavage direction and filled symbols represent activity in the synthesis direction. Each point is the average of three samples from individual fruit.

Table 1. Soluble and insoluble acid invertase in mesocarp of *C. sativus* cv Delilah and *C. melo* cv Galia as affected by extraction media

Extraction medium	Invertase activity (nkat/g fr. wt) Insoluble	Soluble	Total	Percentage insoluble
C. sativus cv Delilah (23 daa)				
Expt. 1: HEPES 7.2	5.6	4.9	10.5	53.2
+0.5% BSA (50 mg)	4.6	5.3	9.9	46.2
+1.0% PVP (100 mg)	5.8	4.9	10.7	54.3
+1.0% PVP +0.5% BSA	5.0	5.1	10.1	49.5
Expt. 2: HEPES 7.2	4.4	4.0	8.4	52.3
+0.5 M KCl	3.4	3.4	6.8	49.9
Expt. 3: HEPES 7.2	3.8	2.2	6.0	62.8
+6% Carbowax 4000	3.6	3.6	7.2	50.3
+1% Tween 20	4.3	2.8	7.1	60.9
Borate 8.5	3.6	2.1	5.7	63.2
C. melo cv Galia (23 daa)				
Expt. 1: HEPES 7.2	2.8	5.5	8.3	33.9
0.5% BSA	2.6	5.1	7.7	34.1
1.0% PVP	1.9	5.1	7.0	26.8
1.0% PVP +0.5% BSA	1.8	5.6	7.4	24.6
Expt. 2. HEPES 7.2	3.1	6.9	10.0	31.0
+0.5 M KCl	2.9	6.2	9.1	31.9
Expt. 3: HEPES 7.2	2.4	4.5	6.9	35.2
+6% Carbowax 4000	2.5	5.0	7.5	33.1
+1% Tween 20	2.4	5.0	7.4	32.4
Borate 8.5	2.4	4.5	6.9	34.4

Table 2. Sugar content, invertase and sucrose synthase (cleavage direction) activity of immature and mature, non-sweet (cv Bird's Nest) and sweet (cv Noy Yizre'el) *Cucumis melo*. Data are means ± SEM of a minimum of four replications for enzyme activity and three replications for sugar content

Fruit description	Sugar (mg/g fr. wt) Reducing	Sucrose	Enzyme activity (nkat/g fr. wt) Acid invertase Soluble	Insoluble	Sucrose synthase
C. melo cv Bird's Nest (*ca* 20 daa)	34.4 ± 4.9	n.d.*	2.89 ± 0.91	2.73 ± 0.87	n.d.
C. melo cv Bird's Nest (*ca* 40 daa)	25.3 ± 4.2	1.0 ± 1.5	1.03 ± 0.41	1.93 ± 0.28	n.d.
C. melo cv Noy Yizre'el (*ca* 20 daa)	36.0 ± 1.6	n.d.	2.52 ± 1.22	2.14 ± 0.66	n.d.
C. melo cv Noy Yizre'el (*ca* 40 daa)	24.7 ± 1.0	38.1 ± 7.2	0.05 ± 0.10	2.12 ± 0.57	0.98 ± 0.53

*n.d. = Not detected.

Analogous results were obtained when we compared two varieties of *C. melo*, cv. Noy Yizre'el (sweet melon) and cv Bird's Nest (non-sweet). Sucrose content in cv Noy Yizre'el was high at maturity whereas in cv Bird's Nest sucrose content was similar to that observed in cucumber. Reducing sugar content was not different at each stage between the two cvs although content was lower in mature than in young fruit.

Insoluble invertase did not show large differences between mature and young fruit in either cv. However, soluble invertase activity declined in Bird's Nest but was still high, similar to that observed in cucumber, as compared to Noy Yizre'el which declined to low levels, similar to levels observed in sweet melon cv Galia. In addition, sucrose synthase activity was observed only in the mature sweet melon cv Noy Yizre'el.

DISCUSSION

Our results with respect to the carbohydrate content of developing cucumber and melon fruit, and in particular patterns of sucrose accumulation, are in general agreement with previously published results for cucumber [5] and sweet melon [2–4]. Sweet melon initially stores little sucrose and sometime after the period of active fruit growth, determined presumably by both genetic and environmental factors, undergoes a metabolic transition to a sucrose storage stage.

Imported sucrose is presumably compartmentalized in the vacuole [17] where its storage is made possible by the decline in soluble acid invertase activity (Fig. 2), also presumably compartmentalized in the vacuole [17]. In non-sweet *Cucumis* imported sucrose may be compartmentalized; however, upon compartmentation, hydrolysis would take place. Resultant hexoses may then be further metabolized to, for example, citric acid in cucumbers [5, 6] and presumably also in sour botanical varieties of *C. melo* [7]. This would indicate that the difference between sweet and non-sweet genotypes of *Cucumis* is not primarily the ability to import and compartmentalize sucrose but rather the ability to further metabolize imported sucrose. The loss of soluble acid invertase activity effectively blocks further sucrose metabolism, subsequently permitting sucrose to be stored.

The loss of soluble acid invertase activity appears to be a general prerequisite for sucrose storage. Other tissues that store sucrose (i.e., sugar beet [11], citrus fruit [12], carrot root [10], sugar cane stem [13]) are all characterized by such a loss of activity. On the other hand, storage tissues that do not store sucrose retain their soluble acid invertase activity as, for example, tomato fruit [20], radish and turnip roots [21] and botanical relatives of sugar cane that do not store sucrose [22]. Our results comparing sucrose storing and non-storing genotypes of *Cucumis* support the hypothesis that, as a rule, loss of soluble acid invertase is required for sucrose storage to take place.

The cause of the loss of soluble acid invertase activity remains to be determined. Our results from mixing experiments do not support the presence of an invertase inhibitor as the cause for loss of activity but they do not rule it out, either. Invertase inhibitors have been reported in the sucrose storing red beet, sugar beet and sweet potatoes [23]. Similarly, Nakagawa *et al.* [24] showed that in senescent tomato fruit loss of invertase activity was due to catalytically inactive but immunologically active enzyme molecules, suggesting the presence of an inhibitor. Alternatively, loss of activity could be due to turnover and loss of synthesis ability.

The role of the insoluble acid invertase is not clear. High activity in cucumber may indicate significant apoplastic hydrolysis which could also be a factor in the lack of sucrose accumulation. In sweet melon apoplastic hydrolysis may also occur and resynthesis of sucrose, via sucrose synthase, after transfer into the cytoplasm cannot be ruled out.

The significant increase in sucrose synthase activity, measured in either the sucrose cleavage or sucrose synthesis direction in sweet melon during the latter portion of the sucrose storing period may be related to sucrose accumulation. Giaquinta [11] reported similar results for sugar beet taproots. Although sucrose synthase is often considered to be involved in sucrose cleavage [see 14–16], Gross and Pharr [9] have reported that in cucumber fruit peduncles the enzyme may play a synthetic role in the conversion of the galactosyl moieties of translocated stachyose into sucrose.

It is tempting to speculate that the increase in sucrose synthase activity we observed represents an increase in sucrose synthesizing capacity in the sweetening fruit. Thom *et al.* [25] have recently reported that a UDP-glucose dependent translocator participates in sucrose synthesis and transport across the tonoplast in beet root, a tissue presumed to compartmentalize imported sucrose intact via a sucrose/H^+ cotransporter [26]. Their results implicate sucrose phosphate synthase in the synthesis. Similarly, Brown and Coombe [27] hypothesized that in ripening grape berries there is a vectorial synthesis of sucrose phosphate at the tonoplast by an enzyme complex containing sucrose phosphate synthase. However, a synthetic role for sucrose synthase in such a translocator is also feasible, Goldschmidt and Branton [28] have reported sucrose synthesis from UDP-glucose and fructose via sucrose synthase in red beet tonoplasts and suggested that sucrose synthase is loosely attached to the tonoplast.

Such a translocator, together with a sucrose/H^+ transporter, could increase the efficiency of sucrose accumulation and compartmentation. With respect to substrates, UDP-glucose and fructose could be supplied by apoplastic sucrose hydrolysis (insoluble invertase) and subsequent conversion of glucose to UDP-glucose by a series of enzymatic reactions, or complex, consisting of hexokinase, phosphoglucomutase and UDPG pyrophosphorylase [27]. Alternatively, galactose moieties from translocated stachyose may be converted to UDP-glucose by a series of enzymatic reactions including galactokinase, UDP-gal pyrophosphorylase and UDP-gal-4'-epimerase [9 and D. M. Pharr, personal communication].

In conclusion, our results point to two factors involved in the metabolic phenomenon of sucrose accumulation in *Cucumis*. The first factor is the loss of soluble acid invertase activity which effectively blocks further metabolism of sucrose. The cause of this loss of activity remains to be determined. The second factor is the increase in sucrose synthase activity in sweetening *Cucumis* which may be related to the increase in sucrose content.

EXPERIMENTAL

Plant material. Plants of *C. melo* cv Galia and *C. sativus* cv Delilah were grown in the greenhouse and ovaries were pollinated during the 2nd week of March 1986. Fruit were harvested at *ca* weekly intervals starting at 17 daa, when fruits were *ca* 60% fr. wt. Fruit of *C. melo* cv Bird's Nest and cv Noy Yizre'el were taken from field grown, open pollinated plants. Ovaries were open-pollinated during the 2nd week of June, 1986. Fruit mesocarp, after removal of epicarp and endocarp, was frozen, lyophilized and stored at −20°.

Carbohydrate determinations. Lyophilized mesocarp of *C. melo* cv Galia and *C. sativus* cv Delilah tissue was extracted in hot 80% EtOH (3 ×) and reducing sugars, sucrose and starch were measured according to the methods of refs [29–31], as recently described in ref. [32]. Reducing sugars and sucrose from samples of *C. melo* cv Bird's Nest and cv Noy Yizre'el were measured on aliquots of supernatant from the enzyme preparation (below).

Enzyme preparation. 200 mg lyophilized material was extracted in 10 ml buffer containing 50 mM HEPES–NaOH (pH 7.2), 0.5 mM EDTA, 0.5 mM $MgCl_2$, 3 mM DTT and 3 mM diethyldithiocarbamate in a Kinematica homogenizer, as modified from ref. [32, 33]. The crude homogenate was centrifuged at 20 000 *g* for 15 min. The resulting supernatant was dialysed overnight against the above buffer (2 × dilution) and used as the crude soluble enzyme extract. For measurement of enzyme activity associated with the insoluble fraction, the pellet obtained from the 20 000 *g* centrifugation was washed 3 × in dialysis buffer and suspended in 3 ml dialysis buffer. The suspension was used as the insoluble crude enzyme extract according to ref. [34].

Attempts to solubilize 'insoluble' enzyme activity. The following additives to the above extraction medium were used: 0.5 M KCl,

0.5% BSA, 1% insoluble polyvinylpyrrolidone (Polyclar AT), 6% Carbowax 4000 and 1% Tween. In addition, 0.1 M borate buffer (pH 8.5) was also used. The resultant supernatant and pellet were considered as the soluble and insoluble fractions, respectively, as above.

Enzyme activity. Invertase activity was determined as previously described [32, 33] with enzyme blanks containing boiled enzyme. Sucrose synthase activity was assayed in the cleavage direction according to a modification of ref. [35], using dinitrosalicylic acid to measure the released reducing sugar [29], as recently described [32]. The additional fructose produced at pH 7 in the presence of 5 mM UDP, as compared with reaction mixtures without UDP was attributed to sucrose synthase activity. Sucrose synthase in the synthesis direction was assayed in 200 μl final reaction vol. (pH 7.5) containing 16 mM UDP-glucose, 16 mM fructose-6-phosphate, 7.5 mM $MgCl_2$, 20 mM Hepes, 1 mM NaF, 0.5 mM sodium molybdate, 25 mM Tris and enzyme extract, according to J. Daie (personal communication) as recently described [32]. Incubation was for 30 min at 37°. The reaction was stopped and remaining hexoses destroyed by boiling for 10 min with 200 μl 30% KOH. Sucrose was assayed according to ref. [30]. Enzyme blanks contained boiled enzyme or reaction mixtures incubated with KOH.

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