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CARBOHYDRATE CONTENT AND SUCROSE METABOLISM IN DEVELOPING *SOLANUM MURICATUM* FRUITS

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Key Word Index—*Solanum muricatum*; Solanaceae; pepino fruit; invertase; sucrose synthase; sink metabolism.

Abstract—Soluble sugars, starch, invertase and sucrose synthase activities were measured in developing fruit of pepino (*Solanum muricatum*). Sucrose was present in low amounts during the early stages of growth and its accumulation began when the fruit neared full size. Starch, present in the immature fruit, was nearly absent in the mature fruit Soluble acid invertase (E.C. 3.2.1.26) activity was high in the young developing fruit but decreased to low levels, concomitant with the onset of sucrose accumulation. Sucrose synthase (E.C. 2.4.1.13) activity which was low in the young fruit, rose to high levels in the mature fruit. Acid invertase of young fruits showed an apparent K_m of 3 mM sucrose while sucrose synthase activity showed an apparent K_m of 49 mM. The results are discussed in terms of the metabolic events associated with sucrose accumulation.

INTRODUCTION

The pepino (*Solanum muricatum*) is a native of the Northern Andes region of South America and is presently being cultivated in various parts of the world for its ripe, sweet fruits. Redgewell and Turner [1] recently reported that sucrose accounted for half of the total soluble sugars in the ripe fruit and observed that a major change during maturation was the increase in sucrose content. Many sucrose storing tissues are characterized by a metabolic transition in sucrose metabolism during development. Soluble acid invertase activity, which is generally high during early stages of tissue development [2–8] declines concomitant with the increase in sucrose content. In sugar beet tap roots [2] and sweet melon fruit [7, 8] there is, in addition, a rise in sucrose synthase activity at the sucrose storing stage, suggesting a possible role for this enzyme in sucrose metabolism.

The purpose of the present study was to characterize the developing pepino fruit with respect to sugar content and sucrose metabolism in order to shed some light on the metabolic events accompanying sucrose accumulation in this fruit.

RESULTS AND DISCUSSION

Growth of the pepino fruit follows a sigmoidal pattern and increase in fruit size is essentially complete *ca* 60 days post anthesis (dpa) (Fig. 1). Sucrose was present in low amounts (Fig. 2) during the period of fruit growth and expansion and began to accumulate only as the fruit approached full size. Sucrose content decreased in the soft, 82 dpa fruit. Throughout development there were no significant changes in the ratio of glucose to fructose (Fig. 2). The only major change in these sugars was a significant decrease observed at 75 dpa, after which the glucose and fructose content increased. Starch was present in significant amounts in the young fruit until at least 67 dpa at concentrations ranging from 10–30% of the dry wt. In the mature 75 and 82 dpa fruit starch content had declined to low values of 1–4%.

Soluble acid invertase activity was high in young, actively growing fruit and progressively declined, concomitant with the increase in sucrose content (Fig. 3). Sucrose synthase activity declined from 24 to 34 dpa and remained low until it rose sharply at 75 dpa and remained high at 82 dpa. Apparent K_m for sucrose for acid invertase and sucrose synthase, calculated from Lineweaver–Burk plots, were 3 and 49 mM, respectively. Acid invertase activity associated with the insoluble fraction remained

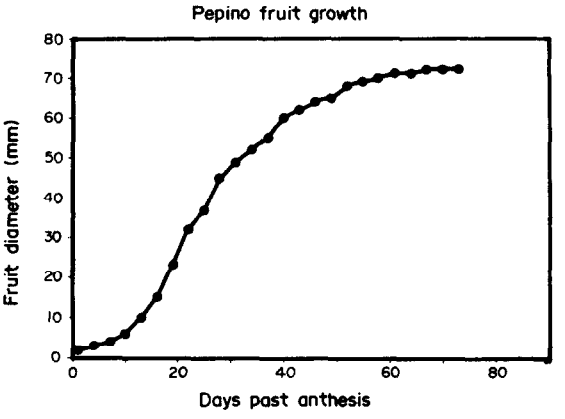

Fig. 1. Growth of developing pepino fruit. Fruit diameter was measured at three day intervals and each data point is the mean value from 10 fruits.

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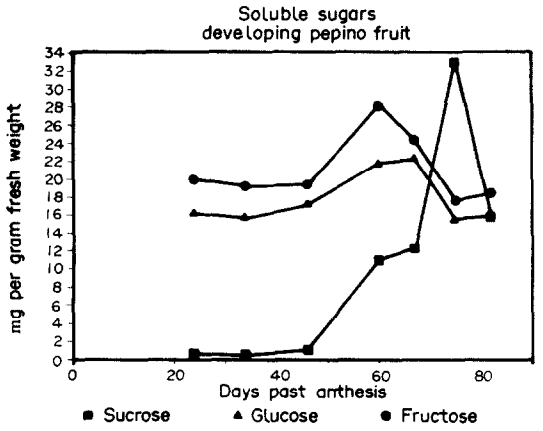

Fig. 2. Sugar content of developing pepino fruit. Each point is the mean value from three fruits. ■, sucrose; ▲, glucose; ●, fructose.

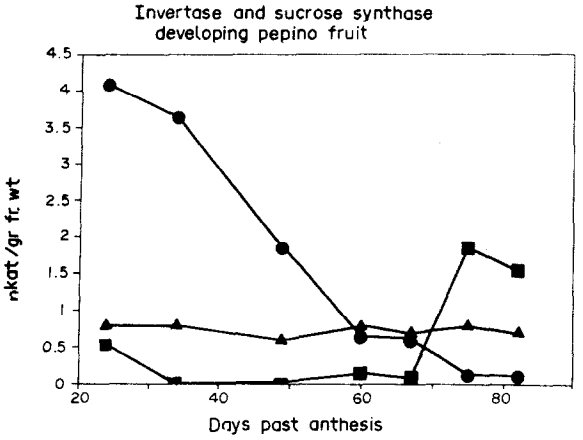

Fig. 3. Soluble acid invertase and sucrose synthase (cleavage) activities in developing pepino fruit. Each point is the mean value from three fruits. ●, Soluble acid invertase; ■ sucrose synthase; ▲ insoluble acid invertase.

fairly constant throughout development (Fig. 3). Accordingly, at the final stages of development the insoluble form of invertase made up the major fraction of total invertase activity.

Our results show that the pepino fruit can be classified as a sucrose accumulator and that the onset of sucrose accumulation is accompanied by a decrease in soluble acid invertase activity. In this respect, the pepino fruit resembles other sucrose accumulators such as sweet melon [7, 8], sugar beet [2], carrot root [6], citrus fruits [4, 5] and sugar cane stem [3], among others. The dependency of sucrose accumulation on the loss of soluble acid invertase activity is presumably due to the proposed compartmentation of both in the vacuole [9]. Accordingly, such a dependency should characterize all storage organs in which sucrose undergoes vacuolar compartmentalization. The cause of this loss of activity is not known. Invertase inhibitors have been reported in a number of tissue (see ref. [10] for review). Mixing experiments of crude extracts of young and mature fruit did not indicate the presence of a soluble invertase inhibitor and we are continuing studies to determine the cause of the apparent loss of activity.

The role of sucrose synthase in sucrose storing tissues is less clear. It is often assumed that its *in vivo* role is in the cleavage direction. However, this assumption is at least partially based on studies with starch-storing tissues, such as potato tuber and corn, rice, wheat and barley grain (see ref. [10] for review) where there is frequently a correlation between starch synthesis and sucrose synthase activity. Although the level of activity we observed in pepino at the earliest stage studied (24 dpa) may be related to starch synthesis, the increase in activity at maturity cannot. Alternatively, its role may be to supply nucleotide sugars for cell well synthesis [11] but such a role is unlikely in ripe, fully grown fruits.

A possible synthetic role for sucrose synthase cannot be ruled out [7, 12]. Accordingly, sucrose storage may occur via two mechanisms [7, 13]: (i) The intact vacuolar compartmentation of imported sucrose and (ii) the synthesis of sucrose via sucrose synthase and subsequent transport into the vacuole. A dual mechanism of sucrose accumulation has been proposed [14] for red beet where the second mechanism utilizes a UDP glucose-dependent-translocator for synthesis of sucrose.

The alternative hypothesis, that the rise in enzyme activity is related to sucrose breakdown, might suggest a degradative role associated with fruit senescence and over-ripening. Such a role could, in fact, explain the decrease in sucrose content of pepino between 75 and 82 dpa. Alternatively, the remaining invertase activity associated with the insoluble fraction may also account for the loss of sucrose.

Avigad [10] has pointed out that in light of the compartmental separation of sucrose and sucrose synthase, the relatively poor affinity of sucrose suggests that cleavage reaction rates are normally far below their potential V_{max}. In the case of pepino fruit, sucrose concentrations may be expected to reach 150 mM. Assuming efficient compartmentation, the cytoplasmic concentration might be significantly less and a K_m of 49 mM sucrose which we observed could indicate that cleavage may be substrate limited. However, in the case of a senescing, overripe tissue, if accompanied by loss of organellar and tonoplast membrane integrity, the compartmentalized sucrose may be released into the cytoplasm, allowing sucrose synthase to efficiently cleave it.

In conclusion, sucrose synthase potentially may fulfil either a synthetic or a degradative role in the sucrose accumulating, non-starch storing, pepino fruit. Its *in vivo* role may be determined by the state of sucrose compartmentation and organellar integrity of the tissue, in addition to UDP and UDP-glucose levels, as suggested by Avigad [10]. These may in turn be controlled by the activities of UDPase [15] and UDP-glucose pyrophosphorylase and PPi levels [11]. Whether the *in vivo* role of sucrose synthase is degradative or synthetic, the increase in sucrose synthase activity in the ripe sucrose storing fruit is likely to be of physiological significance.

EXPERIMENTAL

Plant material. Plants of *S. muricatum* Ait (breeding line no. 3) were grown in a screenhouse in Bet Dagan, Israel, and flowers

were tagged on the day of anthesis, during April–May 1986. Fruit growth was estimated by measuring the diam of selected fruits with a caliper throughout the expt. Fruits were harvested and the central portion of the fruit flesh was frozen, lyophilized, powdered and stored at −20°. Ten fruit of each developmental stage were measured for fruit size while three fruit of each developmental stage studied were individually sampled for carbohydrate and enzyme activity measurements.

Carbohydrate extraction and analysis. Lyophilized plant material (200 mg) was extracted × 3 in hot 80% EtOH and starch content measured on the insol. fraction after treatment with amyloglucosidase as described in ref. [7]. Sol. sugars were measured by HPLC as follows. Sugars were passed through a column of 1 ml insol. PVP and 1 ml Dowex 50 and eluted with double dist. H_2O as described in ref. [16]. Samples were sepd on an Alltech 700 CH Carbohydrate column heated to 90° and LDC refractometer at sensitivity to detect μg sugar. Solvent was double dist. H_2O delivered at 0.5 ml/min. Eluted sugars were identified by their R_t and quantified by ref. to stds. Maltose was not observed in ripe fruits when sugars were sepd on an amino column (Micropak NH_2-10) under conditions separating sucrose from maltose (MeCN–H_2O, 4:1).

Enzyme extraction and assay. Sol. acid invertase activity and sucrose synthase activity in the cleavage direction were determined as described in ref. [7]. Lyophilized tissue (200 mg) was homogenized in 10 ml buffer containing 50 mM HEPES–NaOH (pH 7.5), 0.5 mM EDTA, 0.5 mM $MgCl_2$, 3 mM DTT, 3 mM diethyldithiocarbamate (DIECA) and 2% Polyclar AT in a Kinematica homogenizer. After centrifugation (20 000 *g*, 15 min) the supernatant was dialysed overnight against the same buffer without Polyclar AT and used as crude enzyme extract. The insol. fraction was homogenized 2 × in extraction buffer without DTT, DIECA and Polyclar, resuspended in 5 ml of the same buffer and was then used as crude insol. enzyme.

Invertase activity was assayed in 0.6 ml 0.1 M K_2HPO_4–0.1 M citrate buffer (pH 5), 0.2 ml, 1.0 M sucrose and 0.2 ml enzyme extract (*ca* 100 μg protein for sol. fraction). Reducing power was measured with dinitrosalicylic acid reagent [17]. Enzyme activity over a pH range of 4–8 showed peak activity at pH 5 in young fruits with no discernible second peak in the neutral–alkaline range in ripe fruit.

Sucrose synthase activity was assayed in the cleavage direction according to a modification of the method of ref. [18]. The additional fructose produced at pH 7 in the presence of 5 mM UDP, as compared to reaction mixts without UDP, was attributed to sucrose synthase activity. All sucrose synthase activity in 200 mg of mature (75 dpa) fruit was recovered when extd together with an equal amount of young (34 dpa) fruit tissue.

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