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Changes in carbohydrate content and related enzyme activity during *Cyphomandra betacea* (Cav.) Sendtn. fruit maturation

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Abstract

Changes in sugar content and activities of invertase (EC 3.2.1.26) and sucrose synthase (EC 2.4.1.13) were measured throughout fruit development in tree tomato (*Cyphomandra betacea* (Cav.) Sendtn.). Fruit of *C. betacea* accumulated predominantly reducing sugars similar to *Lycopersicon esculentum* and in contrast to sucrose accumulation, which is characteristic of *L. peruvianum*. Soluble acid invertase, fructose and glucose were localized principally in the vacuole. The soluble acid invertase activity was highest in ripe fruit (80 days past anthesis), while sucrose synthase activity was highest in the young fruit and declined with development. Otherwise, the activity of cell wall-bound acid invertase was less and may be greatly decreased by interaction with an invertase inhibitor protein, located in the cell walls of *C. betacea* fruit. The highest soluble acid invertase activity was associated with fruit ripening and during early stage of development. Sucrose synthase is the dominant enzyme in metabolizing imported sucrose.

Keywords: *C. betacea* (Cav.) Sendtn.; Solanaceae; Soluble acid invertase; β-D-Fructofuranoside fructohydrolase; Sucrose metabolism; Organic acids; Invertase inhibitor

1. Introduction

Cyphomandra betacea (Cav.), renamed by Sendtner to *Solanum betaceum* (Cav.), popular name tamarillo or tree tomato is a fruit-bearing subtropical tree that belongs to the Solanaceae family (Bohs, 1995). Tree tomato fruit are egg shaped and have attractive glossy, purplish-red or golden skins. Inside, they look somewhat like a tomato (*Lycopersicon esculentum*), but their succulent flesh has piquancy. Like tomatoes, they can be eaten as a vegetable and used in sauces, soups or other ways. Tamarillos are excellent sources of provitamin A (carotene 150 International Units per 100 g), Vitamin B_6, Vitamin C (25 mg per 100 g), Vitamin E and iron. They are low in carbohydrates; an average fruit contains less than 40 cal (Lost Crop of the Incas, 1989; Cantwell, 2002).

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Sugars are an important component of fruit quality and there are species of tomato that accumulate sucrose or reducing sugars. Slightly higher amounts of fructose, in comparison to glucose, are typical in ripe fruit of *L. esculentum*. Manning and Maw (1975) reported that an increase in soluble invertase activity during fruit development in *L. esculentum* and in the wild red-fruited species, *L. pimpinellifolium*, was directly correlated with the hexose content of the mature fruit. Otherwise, sucrose accumulation is associated with decreasing invertase activity during development in two green-fruited species, *L. chmielewskii* and *L. hirsutum* (Miron and Schaeffer, 1991; Yelle et al., 1988).

In a previous work, we have reported the presence of soluble acid invertase and an invertase inhibitory protein in *C. betacea* mature fruit (Ordóñez et al., 2000). The purpose of the present work was to determine the changes in carbohydrate content, the participation of invertase and sucrose synthase in the tree tomato fruit sink strength and their relationship with fruit growth. Then, to compare these results with other related species like *Lycopersicon*.

2. Materials and methods

2.1. Plant material

Cyphomandra betacea (Cav.) Sendtn. fruit were used. The tree tomato is unknown in the wild state. It is perhaps native to southern Bolivia (Department of Tarija) and northwest Argentina (Jujuy and Tucumán provinces). *C. betacea* plants were grown in a greenhouse in San Miguel de Tucumán. Flowers were tagged at the onset of anthesis.

2.2. Tissue sampling

Fruit age was determined as days after anthesis (DAA). Ten fruit were harvested at 7 day intervals for 120 days. At the time of harvest, a representative portion about 10 g of whole fruit was frozen in liquid nitrogen and stored at −20 °C for analysis of sugar content and enzyme activities.

2.3. Sugar determinations

Aliquots of frozen tree tomato powder samples from different growth stages (2–10 g) were boiled in 5 ml 80% (v/v) ethanol for 20 min and then centrifuged at 10 000 × *g* for 10 min at 4 °C. The resulting pellets were further extracted three times in 80% (v/v) ethanol at 100 °C for 20 min and the combined supernatants taken to dryness using a vacuum desiccator and freeze drier. The resulting residue was redissolved in distilled water.

Glucose was determined by the method of glucose-oxidase (Jorgensen and Andersen, 1973) and fructose by enzymatic assay of D-fructose dehydrogenase (Ameyama, 1982). Sucrose was estimated by the resorcinol method (Cardini et al., 1955). Total neutral sugars were determined by phenol–sulfuric acid reaction (Dubois et al., 1956). Starch was determined in the ethanol-insoluble fraction by incubating the pellets with 35% $HClO_4$ at 4 °C overnight. The liberated glucose was analysed with the anthrone–H_2SO_4 reagent as described by Fairbairn (1953).

2.4. Organic acids determination

Aliquots (2 g) of frozen tree tomato powder (20–120 DAA) were diluted in distilled water. After centrifugation at 10 000 × *g* for 10 min, the supernatant was collected and 100 μl of each sample was used for the assay. L-Malate and citrate content were analysed using standard enzymatic analytical kits (Boehringer–Mannheim) for the detection of these compounds in foods. The assay was carried out following the kit instructions and the formation of products derived enzymatically from the acids was measured spectrophotometrically. Duplicate measurements of each analysis were performed.

2.5. Enzyme extraction

Aliquots (2 g) of frozen tomato powder (20–120 DAA) were extracted three times in 3 ml of 10 mM sodium acetate buffer (pH 4.5), containing 50 mM NaCl and 1 mM of 2-mercaptoethanol (Buffer A). Extracts were microfuged at 10 000 × *g* for 10 min at 4 °C and the clarified supernatants were then desalting and concentrated by lyophilization. The concentrate was diluted with distilled water to 500 μl and dialyzated against Buffer A for 2 h. The preparation was used to determine the total soluble acid invertase (vacuolar and apoplasmic forms) and sucrose synthase activities. The pellet was resuspended in 500 μl of 50 mM sodium

acetate buffer (pH 6.5), containing 50 mM NaCl and 1 mM of 2-mercaptoethanol (Buffer B) and used to determine the cell wall bound invertase activity.

2.6. Cellular localization of invertase and invertase inhibitor from C. betacea fruits

2.6.1. Efflux kinetic method

The compartmental analysis of sugars was carried out using efflux kinetic method (Damon et al., 1988). The assay was performed in triplicate on 5 g of fruit tissue (tissue disk was removed from the equator of the fruit using a cork borer) from different growth steps (20–120 DAA). The cuticles were removed and the discs were incubated for 1 h in an aerated ^{3}H-inulin solution (Amershan Pharmacia, 0.55 μCi/nmol) at 4 °C. The discs were stirred for 15 s in 1 l of distilled water to remove surface radioactivity and then were consecutively washed in 2 ml volumes of water over a 6 h period at 4 °C. The amount of radioactivity in each 2 ml wash was determined by liquid scintillation spectrometry (Beckman Coulter, LS 6500). From the efflux kinetics of the ^{3}H-inulin, an apoplastic, cytoplasmic and vacuolar efflux time was determined.

Pericarp discs (5 g) from fruit at each developmental stage were stirred for 15 s in 1 l of cold distilled water to remove the sugar from the cut cell. Then they were placed in 2 ml of aerated cold water during 8, 50 and 270 min (efflux times). The fractions (apoplastic, cytoplasmic and vacuolar) were concentrated by lyophilization and then were used to determine soluble sugars.

2.6.2. Protoplast preparation

Pericarp tissue (5 g) from fruit of different growth step (20–120 DAA) was sliced into small pieces. The pieces were washed with water and macerated in 15 ml of a digestion solution composed of 25 mM of MES–NaOH buffer (pH 6), 0.8 M manitol, 0.6% KCl, 1% $MgCl_2$, 2% macerozyme R-10, 0.8 UE pectinase, 3% cellulase, 0.2 mM EDTA and 0.02% amilase (Buffer C). The digested mixture was incubated in a shaker for 1 h at room temperature. Digested fruit tissue was filtered through two layers of gauze and then centrifuged for 5 min at $300 \times g$. The pellet was suspended in 5 ml of Buffer C without digestion enzymes and centrifuged. The operation was repeated twice to wash the preparation. A Neubauer chamber was used for counting the suspended protoplast. Half of the suspension was used as the protoplast preparation and the other half was kept for vacuole preparation.

The protoplast preparation was centrifuged for 5 min at $300 \times g$ and the pellet was suspended in 5 ml of Buffer A. This preparation was centrifuged at $10\,000 \times g$ for 10 min at 4 °C and the clarified supernatants were then concentrated by lyophilization.

2.6.3. Vacuole preparation

Half of protoplast preparation was centrifuged at $300 \times g$ for 5 min. The pellet was suspended in 5 ml of 25 mM MES–NaOH buffer (pH 6), 0.4 M manitol, 0.6% KCl, 1% $MgCl_2$ (Buffer D) and left to stand for 30 min. The suspension was centrifuged for 5 min at $300 \times g$ and the pellet was suspended in 5 ml of the same buffer and centrifuged. The operation was repeated twice to wash the preparation. Then, the number of vacuoles was counted in a Neubauer chamber. The vacuolar suspension was centrifuged for 5 min at $300 \times g$ and the pellet was resuspended in 5 ml of Buffer A. This preparation was centrifuged at $10\,000 \times g$ for 10 min at 4 °C and the clarified supernatants were then concentrated by lyophilization.

2.6.4. Solubilization of cell wall associated invertase and invertase inhibitor activities

2.6.4.1. Procedure I. All procedures were carried out at 4 °C and all centrifugations at $10\,000 \times g$ for 15 min. *C. betacea* mature fruit (280 g) were homogenized in 150 ml of 0.1 M sodium phosphate buffer (pH 7) and maintained at 4 °C for 1 h. The crude homogenate was filtered through gauze. The solid material was washed several times with water and resuspended in 100 ml of 10 mM sodium acetate buffer (pH 4.5) with 1 M NaCl and 1 mM mercaptoetanol (Buffer E) during 1 h at 4 °C. The liquid fractions obtained with Buffer E were pooled and centrifuged to eliminate debris. The supernatant was named S1. The pellet was washed several times with 50 ml of Buffer B and was then resuspended with 30 ml of 50 mM sodium acetate buffer (pH 6.5), containing 5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 0,4% PEG-6000, 0.1 mM phenylmethylsulfonylfluoride (PMSF) and 2% cellulase. The preparation was incubated with shaking at 30 °C for 1 h. Then, the preparation was centrifuged; the pellet was washed several times with 30 ml of Buffer B. All soluble fractions ob-

tained were pooled and solid $(NH_4)_2SO_4$ was added to saturation. Solids were gathered by centrifugation, resuspended and dialyzed against Buffer B. The solubilized cell wall material was named S2.

2.6.4.2. Procedure II. The proteinaceous inhibitor from *C. betacea* fruit was extracted of cell wall preparations at pH 1.7 according to Ordóñez et al. (2000). Briefly, ripe fruit (1 kg) were homogenized in cold water at pH 3 and centrifuged at 15 000 × *g* for 15 min. The insoluble fraction (cell wall) was suspended in 200 ml of 0.25 M NaCl. Then, the pH was adjusted to 1.7 and centrifuged at 15 000 × *g* for 15 min. The supernatant was adjusted to pH 4.75 and concentrated by tangential ultrafiltration.

2.7. Enzymatic assays

2.7.1. Invertase

The reaction mixture consisted of 20 μl of different preparations of soluble invertase or 40 μl of cell wall invertase, 40 μl of 0.2 M sodium acetate buffer (pH 4.5), 15 μl 0.6 M sucrose and distilled water in a final volume of 100 μl. Incubations were performed at 37 °C during 15 min and the reactions were stopped by the Cu alkaline reagent (Somogyi, 1945). Reducing power was measured by the method of Nelson (1944).

One unit of invertase activity was defined as the enzyme amount that catalysed the hydrolysis of 1 μmol of sucrose per minute at 37 °C and pH 4.5.

2.7.2. Sucrose synthase (synthesis direction)

Sample solution (25 μl) was incubated 15 min at 30 °C with 25 mM of fructose and 25 mM of UDP-glucose in 50 mM Hepes–KOH buffer (pH 7.5) containing 15 mM $MgCl_2$. The reaction was stopped by transferring to a boiling water bath and by adding 70 μl of 30% KOH. The concentration of sucrose was determined (Cardini et al., 1955).

2.7.3. Sucrose synthase (cleavage direction)

Sucrose synthase cleavage activity was measured in the mixture of 20 μl of 0.05 M Hepes–NaOH buffer (pH 7.0), containing 60 mM sucrose and 20 mM UDP, 25 μl of sample solution and distilled water until a final volume of 100 μl. Reaction was incubated during 30 min at 25 °C and stopped by transferring to a water bath (80 °C). The fructose was measured by enzymatic (Ameyama, 1982) and colorimetric (Somogyi, 1945 and Nelson, 1944) methods.

2.7.4. α-Glucosidase

α-Glucosidase was measured using 10 μl of 0.2 M sodium acetate buffer (pH 4.5), 30 μl of 20 mM α-metil-glucoside, 30 μl of different preparations (protoplast, vacuole and the fractions obtained by efflux kinetic method) and distilled water until a final volume of 100 μl. The reaction mixture was incubated during 30 min at 37 °C. Glucose liberation was measured by Jorgensen and Andersen (1973) method.

2.7.5. β-Galactosidase

β-Galactosidase was measured using 20 μl of 0.2 M sodium acetate buffer (pH 4.5), 25 μl of 0.2 M *p*-nitro phenyl β-D-galactoside (PNF), 25 μl of different preparations (protoplast, vacuole and the fractions obtained by efflux kinetic method) and distilled water until a final volume of 100 μl. The reaction mixture was incubated 30 min at 37 °C and the enzymatic activity was measured according to Etcheberrigaray et al. (1981).

2.7.6. Peroxidase

Peroxidase was measured using 2.84 ml of 20 mM sodium acetate buffer (pH 4.5), 0.05 ml of 20 mM guayacol and 0.1 ml of all preparations. Then, the reaction was started with 0.01 ml of H_2O_2 and incubated 30 min at 37 °C. The absorbance at 470 nm was measured (Rathmell and Sequeira, 1974).

2.8. Invertase proteinaceous inhibitor activity determination

C. betacea soluble acid invertase obtained according to Ordóñez et al. (2000) (10 μl) was incubated with 100 μl of all preparations in 80 mM sodium acetate buffer (pH 5.0) and 60 mM of sucrose at 37 °C during 15 min. The remaining invertase activity was measured as described above. Mixtures that contain no inhibitor were used as control.

2.9. Protein determination

Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.10. Determination of fructose, glucose and sucrose

Soluble sugars (glucose, fructose and sucrose) were measured in vacuole, protoplast and fractions obtained by efflux kinetic method, as previously described.

2.11. Immunodetection of invertase and invertase inhibitor

Protoplast and vacuole preparations, fractions obtained by efflux kinetic method and eluted fractions from cell wall preparations were seeded on 1 cm × 1 cm pieces of nitrocellulose (0.45 μm, Schleicher and Schuel, Germany). Each membrane was analysed as immunoblots by probing with antibodies raised against purified potato soluble acid invertase or potato invertase inhibitor (Isla et al., 1998, 1999). Cross-reacting bands were identified using anti-rabbit immunoglobulin conjugate labeled with alkaline phosphatase (Sigma Immunochemical) and staining with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT).

2.12. Tissue printing

Intercellular compartmentation of acid invertase within fruit in different stages of growth was studied using a technique described by Sayago et al. (2002). Nitrocellulose filters were soaked in 0.2 M $CaCl_2$ for 30 min and air-dried. Different fruit samples were cut transversely, placed on the filter and pressed gently by hand. The filter was air-dried. Total proteins in the tissue prints were stained with amido black 1% (w/v) in 7% acetic acid for 15 min and then the membranes were destained in 7% acetic acid until a clear image was detectable. The enzymes were visualized using a specific antiserum. The filters were first incubated for 1 h with 10% (w/v) bovine serum albumin in Tris-buffered saline (TBS) (20 mM Tris-(hydroximethyl) aminomethane HCl (pH 7.4) with 0.137 M NaCl) at room temperature with shaking. They were then incubated with antibodies raised against acid soluble invertase (1:1000 diluted invertase antiserum in TBS containing 5% (w/v) BSA) from *S. tuberosum* tubers followed by washing and incubation (30 °C, 2 h) with anti-rabbit inmunoglobulin conjugate with alkaline phosphatase (Sigma Inmunochemicals). After washing, phosphatase activity was visualized by incubating in 100 mM Tris–HCl (pH 9.5), 50 mg/l BCIP, 340 mg/l NBT, 100 mM NaCl and 0.1% of 50 mM $MgCl_2$ (30 °C, 15 min). Specificity of the labeling pattern obtained with the invertase antibodies was assessed by a control test conducted using the filters with preimmune serum incubation, instead with anti-invertase antibodies.

3. Results and discussion

Fig. 1A shows the *C. betacea* fruit growth and ripening pattern. Tree tomato fruit reached about 3.8 cm diameter when fully ripe. Growth curve was sigmoidal

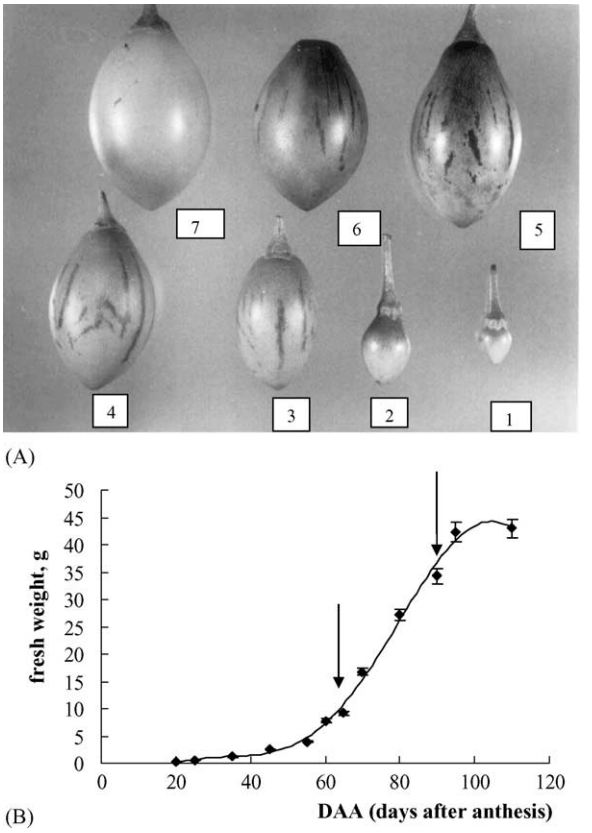

Fig. 1. (A) *C. betacea* fruit in different maturation stages (1) 20 DAA, (2) 30 DAA, (3) 40 DAA, (4) 55 DAA, (5) 65 DAA, (6) 75 DAA, (7) 95 DAA. (B) Fresh weight of *C. betacea* fruit during different maturation stages (20–95 DAA). The arrows show when the fruit changed colour (70 DAA) and when the fruit were commercially mature (90 DAA).

(Fig. 1B), firstly, there is a slow-growing period from approximately 20–45 DAA (until 5 g of fresh weight). This is followed by a phase when the fruit accumulates most of its dry matter from 45–95 DAA until the fruit had attained the senescence. *C. betacea* fruit had high soluble solids content at the ripe stage (at 90 DAA). Because sugars are the major component of fruit soluble solids, we examined the accumulation of carbohydrate and levels of carbohydrate metabolizing enzymes in tree tomato fruit throughout the development.

3.1. Starch accumulation

Starch accumulates in *C. betacea* young fruit reaching a peak approximately 55 DDA (15.1 mmol kg^{-1}) and then decreasing to 2.7 mmol kg^{-1} at fruit maturity (95 DAA) (Fig. 2A). Davies and Cocking (1965) observed a similar pattern of transient starch accumulation in different cultivars of tomato fruit. Between 14 and 49 DAA, *L. esculentum* had a significantly higher level of starch than *C. betacea*. A positive correlation has been observed between the rate of starch accumulation and the rate of fruit growth in *L. esculentum* (Yelle et al., 1988), but in *C. betacea* fruit starch accumulation does not seem to be associated with fruit growth and the high soluble solids content.

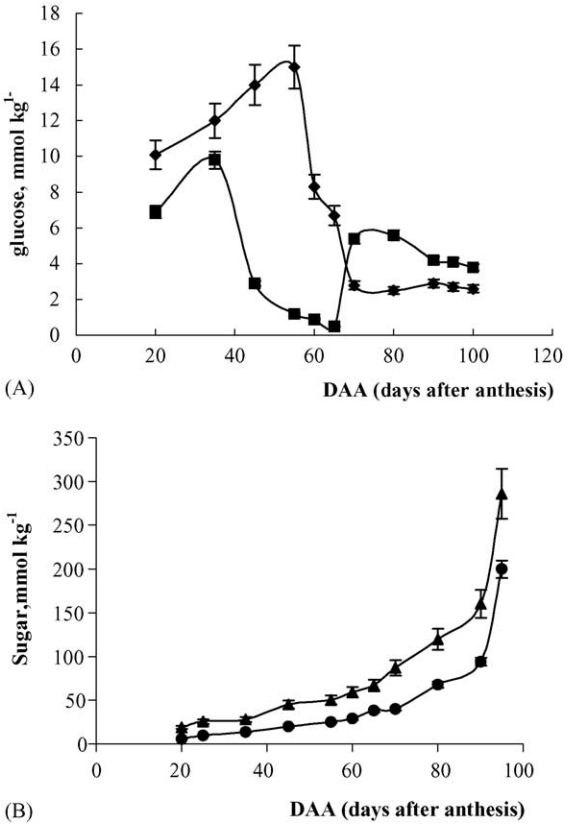

Fig. 2. Carbohydrate levels in *C. betacea* whole fruit throughout all development stages (20–95 DAA). (A) ■, Sucrose; ♦, starch; (B) ▲, hexoses; ●, fructose.

3.2. Sugar content and relationship between pH and sugar composition

Data from numerous measurements of the development-related changes in soluble sugars in different tomato species and cultivars are available at present (Davies and Kempton, 1975; Wang et al., 1993, 1994; Stommel, 1992; Klann et al., 1993). Analysis of soluble sugars in *C. betacea* whole fruit throughout the development stages is shown in Fig. 2A and B. The glucose and fructose content of tree tomato fruit increased with fruit development from 70 DAA until fruit maturation. The highest level of reducing sugars was 286 mmol kg^{-1} at 90 DAA which corresponded to the red-ripe stage. Hexoses represented 94% of the total soluble sugar. Slightly higher amount of fructose in comparison with glucose was typical in *C. betacea* ripe fruit. In contrast, *C. betacea* fruit have very low level of sucrose throughout fruit development. The maximal concentration was the 10.5 mmol kg^{-1} at 35 DAA (Fig. 2A).

Soluble sugar levels in the apoplast were determined throughout fruit development in tree tomato. Glucose and fructose concentrations ranged from 1.3 to 6.1 mmol kg^{-1}. Sucrose concentration in the apoplast was much lower in red fruit than hexoses concentration (Table 1). This low sucrose concentration may reflect the hydrolysis of the disaccharide by an extracellular invertase.

Otherwise, protoplasts from tree tomato fruit were obtained by tissue digestion and vacuoles by gentle osmotic shock of the protoplast. Microscopic examination of the vacuolar preparation and determination of vacuolar (α-glucosidase and β-galactosidase) and extravacuolar (peroxidase) markers showed a homogeneous protoplast-free suspension. Fructose was the main sugar stored in the vacuole of ripe fruit

Table 1
Levels of hexose, fructose and sucrose in different subcellular compartment throughout fruit development

	Reducing sugars	Fructose	Sucrose
Green fruit (immature)			
Protoplast	16.35 ± 1.50	5.2 ± 0.39	6.4 ± 0.55
Vacuole	12.08 ± 1.20	4.1 ± 0.23	4.8 ± 0.41
Apoplast	2.4 ± 0.46	1.3 ± 0.10	3.5 ± 0.29
Red fruit (mature)			
Protoplast	290 ± 26	176 ± 12	2.5 ± 0.34
Vacuole	198 ± 15	114.6 ± 10	1.7 ± 0.09
Apoplast	12.2 ± 1.40	6.1 ± 0.57	2.7 ± 0.10

Values are expressed as mmol kg^{-1} of sugar on a fresh weight basis. Mean ± S.E. values were obtained from five determinations.

(114.6 mmol kg^{-1}). Vacuolar sucrose concentration in ripe fruit was 1.7 mmol kg^{-1} (Table 1).

Stepansky et al. (1999) studied genotypes of *Cucumis melo* and found different behaviour with respect to fruit sugar accumulation. They observed a relationship between fruit pH and sucrose level. The high sucrose genotypes had high pH values and acidic genotypes had low sucrose concentrations. Tree tomato fruit had low pH value in whole fruit, with 3.0 for green fruit to 3.8 for red fruit. The citric acid content was found to be at a very low level throughout development with a maximum value of 4.8 mmol kg^{-1}. However, malic acid accumulated in young fruit reaching a peak approximately at 60 DAA (62.75 mmol kg^{-1}) and then decreased slowly (Fig. 3). In *L. esculentum* fruit, one of the major organic acids present is citric acid. This acid accumulates to concentrations of 6–30 mmol kg^{-1} in the whole fruit and 10–34 mmol kg^{-1} in the pericarp tissue (Oleski et al., 1987). It is likely that this high level of citric acid was largely concentrated in the vacuole and therefore might be transported across the tonoplast. The accumulation of organic acids in the fruit cell vacuoles could represent the main feature of the fruit acidity. Grape berries were characterized by very low vacuolar pH values increasing from 2.5 during the green stage to 3.5 in ripe fruit, which arises as a consequence of the accumulation of malic and tartaric acids in large excess (Terrier et al., 1998).

3.3. Invertase and sucrose synthase activities

Two enzymes involved in sucrose breakdown were studied, sucrose synthase and invertase. The temporal patterns of sucrose synthase and invertase activities were different. The developmental profiles of soluble invertase activity found in *C. betacea* fruit (Fig. 4) were similar to those found for *L. esculentum* (Stommel, 1992; Godth and Roitsch, 1997; Husain et al., 2001a,b). An increase in invertase activity was observed between 40 and 90 DDA. The rise of soluble invertase activity for *C. betacea* fruits is associated with an increase of hexose concentration in the tissue (Fig. 2B). Green fruit had much less soluble invertase activity than orange or red fruit (Table 2). The correlation between soluble invertase activity and hexose accumulation in the vacuole

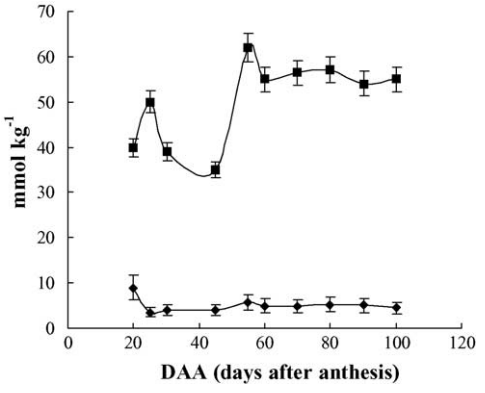

Fig. 3. Changes in organic acids content through *C. betacea* fruit development. (♦) citric acid and (■) malic acid were expressed in mmol kg^{-1}. Data points represent the means of five determinations ± S.E.

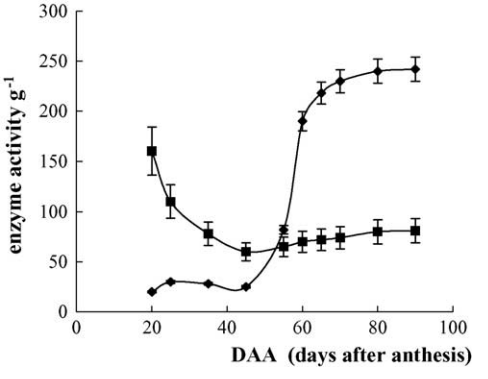

Fig. 4. Levels of (♦) soluble acid invertase and (■) sucrose synthase in fruit of *C. betacea*. Enzyme activities are expressed as mmol of reducing sugar produced per kg of fresh weight per hour. Data points represent the means of five determinations ± S.E.

(Table 1) suggest that acid invertase is mostly located in the vacuole. This hypothesis has been supported by the subcellular localization studies.

Sucrose synthase is an alternative enzyme capable of degrading sucrose to UDP-glucose and fructose. Sucrose synthase activity was higher in the first week after anthesis, reaching a peak early in development (around 20 days after anthesis, when the fruit is about 1 cm in diameter) and subsequently declining (Fig. 4). Sink strength in the first phase of fruit development could be determined by the peak of SS activity. Lower level of sucrose synthase activity in later stages of *C. betacea* fruits development indicates an essential role for invertase in generating hexose for maintenance of tissue metabolism and cell growth in *C. betacea*.

Similar behaviour for sucrose synthase activity was found in *L. esculentum* (which is highest when the fruit is very young; Wang et al., 1993; Chengappa et al., 1999).

3.4. Subcellular localization of invertase activities during tree tomato fruit development

No acid invertase was detected in the cytoplasmic fraction. The activity of soluble acid invertase was found in isolate vacuoles and was lower in green fruit than in red fruit. The contribution of cell wall bound invertase to the total fruit invertase activity was less than 2% in red fruit and 26% in green fruit (Table 2). Perhaps, the activity of invertase in cell wall preparation of red fruits may be greatly decreased by interaction with an invertase inhibitor protein located in cell wall of *C. betacea* fruit (Ordóñez et al., 2000). No invertase inhibitor was detected in isolate vacuoles or protoplast preparation.

Table 2
Acid invertase activity[a] in different fruit development stages (green and red)

	Soluble invertase activity (EA[b])	Cell wall bound invertase activity (EA[b])
Green fruit (inmature)	22 ± 2.3	5.8 ± 0.35
Red fruit (mature)	250 ± 30	2.3 ± 0.44

[a] Mean ± S.E. values were obtained from five determinations.
[b] EA: enzyme activity was defined as micromoles of reducing sugar produced per gram of fresh weight per hour.

Immunoblots have showed that invertase protein is present in all fractions eluted from cell wall and vacuole preparations of the tree tomato fruits in all mature stages. We suggest that the measured differences in soluble acid invertase activity throughout development are due to changes in the absolute amount of protein rather than changes in the activation state of the enzyme. We are investigating the developmental changes in the production of the tree tomato invertase inhibitor to determine whether the inhibitor could act as a cell wall invertase regulator in vivo (Greiner et al., 2000).

3.5. The intercellular location of acid invertase

The intercellular distribution of invertase protein was investigated by using tissue blotting of immature and mature fruit samples. The invertase protein was present in all development stages and was uniformly distributed across the different tissues of fruit (epidermis, pericarp and gelatinous central core). The pericarp tissue of the mature fruit was less defined due to the high liquid content. Nevertheless, this stage contains qualitatively more invertase protein per unit surface area than green fruit and this is according to the invertase activity determination.

4. Conclusions

Ripe fruit had the highest reducing sugar contents and soluble invertase activity. In all cases, the activity of invertase was much greater than that needed to hydrolyse the translocated sucrose. Sucrose is not accumulated in tree tomato fruit. The fruit storage efficiency of hexoses is greater than that of sucrose. A major function of high acid invertase activity would be maintaining high cellular hexose concentrations and the hydrolysis of sucrose in the vacuole. Vacuolar sugar storage may be important in fruit cell growth when less sucrose is available for the sink organs. Otherwise, sugars provide an additional store of carbon skeletons, increasing the soluble solids content, which is an important factor to the fruit industrial processing.

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