Muskmelon Fruit Soluble Acid Invertase and Sucrose Phosphate Synthase Activity and Polypeptide Profiles during Growth and Maturation

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ABSTRACT. Muskmelon [*Cucumis melo* L. (Reticulatus Group)] fruit sugar content is the single most important consumer preference attribute. During fruit ripening, sucrose accumulates when soluble acid invertase (AI) activity is less than sucrose phosphate synthase (SPS) activity. To genetically heighten fruit sugar content, knowledge of sugar accumulation during fruit development in conjunction with AI and SPS enzyme activities and their peptide immunodetection profiles, is needed. Two netted muskmelon cultivars, Valley Gold a high sugar accumulator, and North Star a low sugar accumulator, with identical maturity indices were assayed for fruit sugars, AI and SPS activity, and immunodetection of AI and SPS polypeptides 2, 5, 10, 15, 20, 25, 30, 35, or 40 (abscission) days after anthesis (DAA). Both cultivars, grown in the Fall, 1998 and Spring, 1999, showed similar total sugar accumulation profiles. Total sugars increased 1.5 fold, from 2 through 5 DAA, then remained unchanged until 30 DAA. From 30 DAA until abscission, total sugar content increased, with 'Valley Gold' accumulating significantly more than 'North Star'. During both seasons, sucrose was detected at 2 DAA, which coincided with SPS activity higher than AI activity, at 5 through 25 DAA, no sucrose was detected which coincided with SPS activity less than AI activity. At 30 DAA when SPS activity was greater than AI activity, increased sucrose accumulation occurred. 'Valley Gold' at abscission had higher total sugar content and SPS activity, and lower AI activity than 'North Star'. 'North Star' had AI isoforms at 75, 52, 38, and 25 kDa (ku) that generally decreased with maturation, although the isoform at 52 ku remained detectable up to anthesis (40 DAA). 'Valley Gold' had the same four AI isoforms, all decreased with maturation and became undetectable by 20 DAA. Both 'Valley Gold' and 'North Star' had one SPS band at 58 ku that increased with DAA, and coincided with SPS activity. 'Valley Gold' had a more intense SPS polypeptide band at abscission than 'North Star'. Thus, netted muskmelon fruit sugar accumulation may be increased, either by genetic manipulation or by selecting for cultivars with a specific number of down-regulated AI isoforms, and higher SPS activity during fruit ripening.

The component dictating muskmelon fruit eating quality is sugar concentration or sweetness (Lester and Turley, 1990; Yamaguchi et al., 1977). During muskmelon [*Cucumis melo* (Reticulatus Group)] fruit maturation and ripening, sucrose accumulation occurs in sweet genotypes as a result of a decrease in soluble acid invertase (AI) and a concomitant increase in sucrose phosphate synthase (SPS) activities (Hubbard et al., 1989; McCollum et al., 1988; Pharr and Hubbard, 1994; Shaffer et al., 1987). Sucrose in muskmelon fruit at harvest is controlled by two factors, the rate of sucrose accumulation, and the duration of accumulation until abscission (Stepansky et al., 1999). Muskmelon fruit do not accumulate starch, therefore, the total amount of sugars at harvest represents the total sugar content postharvest

(Lester and Dunlap, 1985). The capacity for sugar accumulation in muskmelon fruit has been described as a mathematical difference between sucrose biosynthesis (SPS activity) and sucrose degradation (AI activity) (Hubbard et al., 1989). Thus, when SPS activity is greater than AI activity, sucrose accumulates. The implication of this mathematical difference, according to Pharr and Hubbard (1994), is sugar accumulation is genetically controlled and that molecular techniques may be used to enhance melon sugar accumulation. To proceed with molecular technologies altering the decline in AI and/or the intensity of SPS activities, knowledge of fruit sugar profiles during developmental changes in conjunction with AI and SPS enzyme polypeptide profiles is necessary.

In the present study, we examined sugar accumulation, SPS activity, AI activity and their polypeptide profiles using two commercial muskmelon cultivars with identical abscission dates but divergent sucrose accumulation profiles. Prior studies (Schaffer et al., 1987; Stepansky et al., 1999) have compared sucrose accumulation, and SPS and AI activities among divergent *Cucumis melo* groups and cultivars with dissimilar maturity indices, but no studies have copresented immunodetection of AI and SPS polypeptide profiles. The objective of this study was to demonstrate the potential genetic regulation of AI and SPS activities via polypeptide profile differences in conjunction with their enzyme activities and resultant sucrose accumulation at abscission during spring and fall growing periods.

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Materials and Methods

PLANT MATERIAL. Seeds of 'North Star' and 'Valley Gold' netted muskmelons were germinated in Sunshine #3 mix (Sun Gro Horticulture, Bellevue, Wash). Single plants at the one to two true leaf stage were transplanted into 16-L pots filled with Sunshine #4 mix containing 26 to 27 g 14N-4.2P-11.6K Osmocote (The Scotts Co., Marysville, Ohio). Pots were irrigated as needed and fertilized twice a week with Peters 20N-8.8P-16.6K water soluble fertilizer during the plant and fruit growth stages or 9N-19.8P-12.5K water soluble fertilizer (The Scotts Co.) during the flowering stage. Plants were maintained in a greenhouse. During cloudy days, supplemental lighting of $300 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ was provided by high-pressure sodium-vapor lamps. Average day/night temperatures were $30 \pm 8/23 \pm 4^\circ \text{C}$ and average humidities were $72 \pm 10/98 \pm 2\%$. Flowers were hand pollinated, and one fruit per plant was allowed to develop. Fruit were harvested at 2, 5, 10, 15, 20, 25, 30, or 35 d after anthesis (DAA) and abscission. Fruit designated as 40 DAA, abscised at 38 to 41 DAA. All fruit were harvested at 0800 h.

TISSUE ISOLATION. Fruit were weighed immediately after harvest and the rind and hypodermal tissues were removed with a vegetable peeler. Middle mesocarp sections for moisture, soluble free sugar, enzyme, and polypeptide assays were removed from the equatorial region of the fruit, frozen in liquid N_2 , sealed under N_2 , then stored at -80°C until analyses.

Fig. 1. (A) Total sugar (T.SUG) and sucrose (SUC) content profiles of 'North Star' (NS) and 'Valley Gold' (VG) muskmelon fruit produced in the spring, and (B) T.SUG and SUC content profiles of 'North Star' (NS) and 'Valley Gold' (VG) muskmelon fruit produced in the fall. Each mean is based on $n = 3$ and the vertical bars = SE.

DETERMINATIONS OF MOISTURE, SOLUBLE SOLIDS CONCENTRATION, AND SUGARS. Moisture was determined on 1 to 10 g of lyophilized mesocarp tissue. Soluble solids concentration, in juice from fresh mesocarp tissue expressed through a hand held garlic press, was determined using a temperature corrected, digital refractometer (Reichert Scientific Instruments, Buffalo, N.Y.). Fruit sugars were extracted from 0.3 g lyophilized mesocarp tissue by stirring with 5 mL of 90°C , 80% ethanol for 2 min. The solution was filtered through filter paper (No. 1; Whatman, Maidstone, United Kingdom) and the residue washed with additional 5 mL hot 80% ethanol before determination of fructose, glucose, and sucrose using the high-performance liquid chromatography procedure described previously (Lester and Dunlap, 1985).

AI AND SPS ACTIVITIES. Enzyme extraction and assays for soluble AI (EC 3.2.1.26) and SPS (EC 2.3.1.14) were conducted as described by Hubbard et al., (1989).

ELECTROPHORESIS, BLOTTING, AND IMMUNODETECTION OF AI AND SPS PROTEINS. Total proteins from melon middle-mesocarp tissue were extracted according to Kausch and Handa (1997) and $50 \mu\text{g}$ were resuspended in 30 to $50 \mu\text{L}$ of SDS sample buffer [2% (w/v) SDS, 2% (w/v) 2-mercaptoethanol, 10% (v/v) glycerol, and 41.7 mM Tris HCl (pH 6.8)]. Samples were boiled for 1 min before addition of the tracking dye, 0.08% bromophenol blue. Electrophoresis was carried out with the buffer system of Laemmli (1970) using a 5% (w/v) stacking and a 12% (w/v) polyacrylamide resolving gel. The gels were stained with Coomassie blue. Molecular weight protein standards (Bio-Rad Lab., Hercules, Calif.) were used.

Fig. 2. (A) Sucrose phosphate synthase (SPS), left Y-axis, and soluble acid invertase (AI), right Y-axis, activity profiles of 'North Star' (NS) and 'Valley Gold' (VG) muskmelon fruit produced in the spring, and (B) AI and SPS activity profiles of 'North Star' (NS) and 'Valley Gold' (VG) muskmelon fruit produced in the fall. $n = 3$, vertical bars = SE.

to estimate molecular weight. Proteins were blotted to Hybond-C nitrocellulose membranes (Amersham Pharmacia Biotech, Inc., Piscataway, N.J.) following the protocol of Blake et al. (1984) using a 1:1000 antibody dilution. The blots were washed with PBS/Tween [PBS and 0.3% (w/v) Tween 20] and stored dry. Antibodies against AI from carrot (*Daucus carota* L.) were kindly provided by A. Strum (Friedrich Miescher Institute, Basel, Switzerland) and antibodies against SPS from banana (*Musa acuminata*), were kindly provided by F. Lajolo (Sao Paulo, Brazil).

Results and Discussion

SUGAR ACCUMULATION PROFILES. Comparisons of spring and fall grown 'North Star' and 'Valley Gold' fruit showed similar total sugar and sucrose accumulation profiles (Fig. 1). Total sugar content was similar for both cultivars in both seasons at 2 DAA, it increased 1.5 fold by 5 DAA, then remained unchanged until 30 DAA. Between 30 DAA and abscission (40 DAA) total sugar content increased in both cultivars during both growing seasons, however, 'Valley Gold' accumulated more total sugars than 'North Star'. There were seasonally related differences in total sugar accumulation of 'Valley Gold' versus 'North Star' fruit. In the fall, 'Valley Gold' accumulated significantly more sucrose at 30, 35 and 40 DAA than 'North Star', whereas in the spring, sucrose content was significantly higher in 'Valley Gold' versus 'North Star' at 35 and 40 DAA.

SUGAR ENZYME PROFILES. Regulation of total sugar and sucrose profiles in the two melon cultivars, although influenced by spring versus fall production, was highly regulated by AI and SPS activities (Fig. 2). In both cultivars, grown in the spring and fall, sucrose was detected at 2 or 5 DAA and coincidentally SPS activity was greater than AI activity at this time. Between 5 and 25 DAA, AI activity was always much greater than SPS activity, regardless of cultivar or growing season, which coincided with little or no sucrose detection. In both cultivars, grown in the spring, SPS activity was lowest at 10 DAA then increased steadily until abscission. In the fall, a significant increase in SPS didn't occur until 30 DAA. In both cultivars, during both seasons SPS activity was greater than AI activity 30 DAA. This change in dominance of SPS activity versus AI activity coincided with a rise in sucrose accumulation in both cultivars. During spring and fall production, 'North Star' fruit showed a

significant decrease in SPS activity from 35 DAA to abscission. During this period, the decline in AI activity for 'North Star' which began at 25 DAA, stopped and remained constant. 'Valley Gold', in contrast, did not experience a significant decrease in SPS activity and continued to have a decline in AI activity throughout fruit maturation. Schaffer et al. (1987), and Pharr and Hubbard (1994) have demonstrated that sucrose accumulation in melon fruit is strongly influenced by developmentally regulated changes in the activity of sucrose metabolizing enzymes, (i.e., AI and SPS), within the edible mesocarp. Pharr and Hubbard (1994) have further stated that a negative enzyme balance, whereby SPS activity is less than AI, would result in little or no sucrose accumulation and a positive balance would result in sucrose accumulation. Our data concur with these prior findings in that sucrose accumulation in melon fruit is developmentally controlled by changes in SPS and AI activities. Additionally, our data demonstrated using two commercial genotypes with identical maturity indices but dissimilar patterns of sucrose accumulation, that sucrose accumulation, although affected by seasonal variations, is highly likely genetically regulated.

IMMUNOCHEMICAL DETECTION OF AI AND SPS. To garner insight into the polypeptide influence on melon fruit sucrose accumulation, AI and SPS immunochemical detection was conducted on 'North Star' and 'Valley Gold' fruit grown in the fall. Fall versus spring produced fruit were chosen for having the greatest differences in AI and SPS activities during their developmental and ripening phases.

Total polypeptides from both cultivars, 2 DAA through maturation, were extracted, blotted and probed with antibodies: anti-AI and anti-SPS. Both cultivars had four AI isoforms (Figs. 3 and 4). 'North Star' presented one major band at 75 ku that was strongly evident through 20 DAA, that became faint and eventually nonevident by 35 DAA; a second band at 52 and a third band at 38 ku were evident up to abscission and 30 DAA respectively; and a fourth band at 25 ku, which increased with DAA, upto 25 DAA, then disappeared (Fig. 3). 'Valley Gold' exhibited the same four AI isoforms, but all four declined sharply with DAA and no bands were detectable after 20 DAA (Fig. 4). Since sucrose accumulates when AI activity is less than SPS activity, it is safe to speculate, that the higher sugar accumulation in 'Valley Gold' was due to the early reduced AI activity as a result of all four AI isoforms eventually declining to nondetection after 20 DAA. However, in 'North Star', the higher AI activity at abscission, probably resulting from AI polypeptide

Fig. 3. Immunoblot illustrating detection of 'North Star' muskmelon mesocarp proteins at different days after anthesis (DAA) cross reacting with soluble acid invertase (AI) antibodies. Proteins from each DAA sample (50 µg per lane) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with AI antibodies. The numbers to the left indicate molecular weight markers in unified atomic mass units (u). The arrows point to the 75, 52, 38, and 25 ku bands corresponding to the four isoforms of AI.

Fig. 4. Immunoblot illustrating detection of 'Valley Gold' muskmelon mesocarp proteins at different days after anthesis (DAA) cross reacting with soluble acid invertase (AI) antibodies. Proteins from each DAA sample (50 µg per lane) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with AI antibodies. The numbers to the right indicate unified atomic mass units (u). The arrows point to the 75, 52, 38, and 25 ku bands corresponding to the four isoforms of AI.

Fig. 5. Immunoblot illustrating detection of 'North Star' muskmelon mesocarp proteins at different days after anthesis (DAA) cross reacting with sucrose phosphate synthase (SPS) antibodies. Proteins from each DAA sample (50 µg per lane) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with SPS antibodies. The numbers to the right indicate unified atomic mass units (u). The arrow points to the 58 ku band corresponding to SPS.

Fig. 6. Immunoblot illustrating detection of 'Valley Gold' muskmelon mesocarp proteins at different days after anthesis (DAA) cross reacting with sucrose phosphate synthase (SPS) antibodies. Proteins from each DAA sample (50 µg per lane) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with SPS antibodies. The numbers to the right indicate unified atomic mass units (u). The arrow points to the 58 ku band corresponding to SPS.

detection up to abscission, likely regulated the lower sucrose accumulation.

Given the wide diversity of invertase isoforms found in higher plants (Sturm, 1999), it is likely that each isoform, may fulfill a different function as has been demonstrated in other fruit (Godt and Roitsch, 1997). Interestingly, the different pH optima and substrate specificities of extracellular and vacuolar plant invertases are determined by the substitution of a single amino acid (Goetz and Roitsch, 1999). It is unclear if one or all AI isoforms correspond with detected enzyme activity since the mere presence of a protein in a gel does not necessarily mean that it is active.

'North Star' (Fig. 5) and 'Valley Gold' (Fig. 6) each had a single SPS band at 58 ku that was first detected, with great intensity, at 10 DAA and appeared to increase in intensity with maturation. At abscission, 'Valley Gold' had a more intense SPS polypeptide band than 'North Star'. The increased polypeptide detection with DAA accompanied an increase in SPS activity in both cultivars. Increased SPS enzyme protein content and activity have been detected in other ripening fruit (do Nascimento et al., 1997; Komatsu et al., 1996; Langenkamper et al., 1997), which

indicates the important role this enzyme plays in fruit sugar accumulation.

Both AI and SPS have been genetically manipulated in other plants. Antisense AI plants exhibit altered development and sucrose partitioning (Tang et al., 1999) as well as abnormal sink metabolism (Weber et al., 1999), sugar composition, and size (Klann et al., 1996). On the other hand, antisense SPS plants exhibit altered leaf carbohydrate partitioning (Worrel et al., 1991) or an inhibition of the water stress-induced synthesis of sucrose (Geigenberger et al., 1999).

We propose that an increase in sugar accumulation in muskmelon fruit may be achieved via genetic manipulation and or by selection of cultivars with a specific number of AI isoforms, down-regulated long before abscission, and with concomitant high SPS activity throughout fruit maturation.

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