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Full Length Research Paper

Effects of grafting on carbohydrate accumulation and sugar-metabolic enzyme activities in muskmelon

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Carbohydrates are the most important biochemical compounds in determining the quality of muskmelon fruit, and their accumulation in the muskmelon (*Cucumis melo* L.) fruits as substrate tub plants in solar greenhouse with different scion/rootstock graft combinations were studied. The results indicated that Zhongmi1/Shengzhen1 graft combination (GS) reduced all kinds of main sugars contents except galactinol and stachyose throughout the whole fruit development. However, the effects of Zhongmi1/RibenStrong graft combination (GR) on carbohydrates accumulation was less compared to GS, in which sucrose content was lower than that in self-rooted muskmelon (CK) only on 32 DAA, decreased raffinose content merely on 48 DAA, increased stachyose content only on 16 DAA and reduced total sugar accumulation dramatically. Grafting (GR and GS) enhanced starch content in the later fruit development. On the other hand, grafting GS significantly enhanced acid invertase or neutral invertase activity during the whole development, sucrose synthase activity only on 8 DAA and reduced sucrose phosphate synthase activity dramatically compared to CK during 16 to 48 DAA. Grafting GR increased acid invertase activity only on 24 and 40 DAA and sucrose phosphate synthase activity dramatically on 48 DAA. Grafting significantly increased alkaline α-galactosidase activity during 8 to 24 DAA; there were no significant differences between 32 to 48 DAA. Overall, considering the carbohydrate accumulation, Zhongmi1/RibenStrong graft combination was a superior graft combination, followed by Zhongmi1/Shengzhen1 graft combination.

Key words: Raffinose family oligosaccharides, scion/rootstock graft combination, galactose metabolism, alkaline α-galactosidase, raffinose, galactinol, stachyose, starch.

INTRODUCTION

Melons are cultivated widely, as one of the global top ten economic cultivated fruits, for their delicious sweetness and high nutrient quality with flavor (Cheng et al., 2007; Hui et al., 2009). However, due to limited availability of arable land and high market demand for off-season cucurbits (plants in the family Cucurbitaceae), melons (including muskmelon, oriental melon among others) are continuously cultivated under unfavorable conditions worldwide including environments that are too cold, wet and dry, or are cool low-light winter greenhouses. Successive cropping can increase salinity, the incidence of cucurbit pests, and serious devastating soil-borne diseases such as melon wilt (*Fusarium oxysporum* f. milonis) and root-knot nematode disease (*Meloidogyne* spp.) (Pavlou et al., 2002; Lee and Oda, 2003). These could cause various physiological and pathological disorders leading to severe crop loss. To complicate issues, chemical control is costly, not always effective and could harm the environment.

Grafting cultivation is widely being applied to overcome these problems, especially as countermeasures to the terrible muskmelon diseases and continuous cultivation barrier (Lee, 1994; Zhang and Ge, 2002; Angela et al., 2008). However, substantial studies confirmed that grafting altered the muskmelon fruit quality to varying degree (Jiao et al., 2000; Lee and Oda, 2003; Li et al., 2009). For example, total sugar contents in grafted melon and watermelon were lower than that in self-rooted ones (Liu et al.,

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2006; Xu et al., 2006). Therefore, the study about grafting effects on carbohydrate content in muskmelon is of profound significance for high yield and superior quality cultivation.

Carbohydrates are the most important biochemical compounds in determining the quality of muskmelon fruit, of which the soluble sugars mainly consist of sucrose, glucose, fructose and so on (Gross and Pharr, 1982; Lalonde et al., 2003). The kind and amount of various carbohydrates directly influence fruit flavor components such as sweetness. Carbohydrate accumulation is closely related to stachyose metabolism (Taji et al., 2002), photosynthates translocation capacity in phloem (Li, 2000; Brian et al., 2003), galactose metabolism (Dai et al., 2003) and sucrose metabolism in fruit (Sarah and James, 1987). The terminal sucrose metabolism is more important for its influence in the final carbohydrates accumulation (Zhang et al., 2003). In addition, cucurbitaceae are model plants in the study of the raffinose family oligosaccharides (RFOs) metabolism and translocation, in which photoassimilates will coexist usually in the forms of stachyose, partial raffinose and sucrose, of which stachyose and raffinose will be under galactose metabolism upon being unloaded to the fruits, or they will be under hydrolysis by α-galactosidase prior to sucrose metabolism. Previous researches though have indicated that grafting lessen sugar content in fruits, these are only restricted to the grafting effects on total soluble sugar or sucrose metabolism (Xu et al., 2005; QI et al., 2006; Xu et al., 2006). Therefore, study about the grafting effects on various carbohydrates accumulation, RFOs hydrolytic metabolism and sucrose metabolism in muskmelon fruit with different rootstocks may not only serve a basis in selecting superior graft combinations and in breeding special rootstock cultivars for muskmelons, but also pave a way for the further research in depth about mechanism of grafting effects on muskmelon carbohydrate metabolism.

MATERIALS AND METHODS

Plant materials and sampling

Scion cultivar (*Cucumis melo* L. cv. Zhongmi1), a popular muskmelon (commercial maturity on 40 days after anthesis [DAA] roughly) and rootstocks (*Cucurbita maxima×moschata* cv. RibenStrong and Shengzhen1) were grown in a sand/soil/peat (1:1:1 by volume) mixture. For grafting, rootstock seeds were sown for 7 days to produce seedlings with approximately the same size of hypocotyls as that of muskmelon. Tongue approach grafting was carried out at the two-leaf stage of growth. Ungrafted plants (self-rooted control), Zhongmi1/RibenStrong and Zhongmi1/Shengzhen1 graft combinations were named as CK, GR and GS for short abbreviation, respectively. Approximately 20 days after grafting, the seedlings were transplanted into plastic pots (with upper diameter, base diameter and height of 30, 24 and 25.5 cm, respectively) with NPK (2:1:2) fertilizer in Liaoshen-2 solar greenhouse (Patent right possessed by High-efficiency Industrialized Agricultural Engineering Technology Research Center of Liaoning Province). All plants were managed as in normal production after planted. To identify fruit of known age, freshly opened female flowers were tagged on the day of hand-pollination and one fruit per plant was allowed to develop at 12th node. Fresh weight per fruit was determined immediately on 8, 16, 24, 32, 40 (maturity) and 48 DAA (delayed harvest period). Mesocarp was also collected on 8, 16, 24, 32, 40, 48 DAA, between 8:00 am and 9:00 am, frozen in liquid nitrogen and stored at -80℃ for carbohydrates and enzymatic activity assessment. All experiments were repeated at least three times with three replicates in each case.

Soluble carbohydrate analysis

Soluble carbohydrates in mesocarp were extracted with 80% (v/v) ethanol (15 ml per 6 g sample FW) at 80℃ for 1 h. Ethanol extracts were collected and the pellets re-extracted twice using the same method. The extracts were subsequently cleaned via a Waters Sep-Pak column (C_{18}, Accell Plus QMA and Accell Plus CM), combined and dried in a centrifugal evaporator (MAXI dry Lyo). The dry extracts were dissolved in 500 ul ultra-pure water, filtered through an acetate filter (0.22 um pore size, Nalgene) and 20 ul samples were analyzed for sugar content by high performance liquid chromatography (HPLC) using a Waters 600 controller fitted with a Luna 5U NH_2 100R column (Phenomenex Separation Products, USA), Waters 2410 Refractive Index Detector, Waters In-Line Degasser AF and Waters 600 pump as previously described (Madore et al., 1990). The separations were performed at 35℃ and eluted with 75/25 (v/v) acetonitrile/H_2O at a flow rate of 1 ml/min. Fructose (Product No. 31140), glucose (Product No. G5400), sucrose (Product No. 84099), raffinose (Product No. R0250), galactinol (Product No. 79544) and stachyose (Product No. S4001) were determined by co-elution with standards (SIGMA-ALDRICH Co., 3050 Spruce Street, St. Louis, MO63103 USA). According to retention time to distinguish different kinds of sugars, fructose, glucose, sucrose, raffinose, galactinol and stachyose were detected at 4.858, 5.315, 6.710, 11.664, 12.885 and 21.923 min in Auto-Scaled Chromatogram of HPLC, respectively (Figure 1). Waters Millennium software was used for controlling and processing of data.

Enzyme extractions and activity assays

Mesocarp samples were finely grounded in liquid nitrogen using a mortar and pestle. Approximately 1 g of the ground tissue was suspended in 1 ml of ice-cold extraction buffer containing 50 mM HEPES-NaOH (pH 7.5), 10 mM ascorbic acid, 2.5 mM dithiothreitol (DTT), 10 mM $MgCl_2$, 10% (v/v) ethylene glycol (pH 7.5) and 1 mM EDTA. The extracts were filtered through cheesecloth and centrifuged at 26,000 g (unit of speed) for 20 min at 4℃. The supernatant was desalted by dialyzing for more than 20 h at 4℃.

Acid invertase (AI) and neutral invertase (NI) (EC 3.2.1.26) activities were assayed in a final volume of 25 ml, containing 0.2 ml of dialyzed enzymatic extract, 0.8 ml of reaction solution contained 100 mM Na_2HPO_4, 100 mM sodium citrate, 100 mM sucrose and pH 4.8 or 7.2 for acid invertase and neutral invertase, respectively. The activities were measured by the quantity of reducing sugars released in the assay media with dinitrosalicylic acid. The reducing sugars were revealed by incubation at 100℃ for 5 min and read at 520 nm in a Cary 100UV:VIS spectrophotometer (GBC Scientific Equipment Pty Ltd., Heareus, Germany) (Pinheiro et al., 2005).

Sucrose synthase (SS) (EC 2.4.1.13) activity was measured by using 0.4 ml reaction mixture containing 50 mM fructose, 0.82% UDPG, 100 mM Tris, 10 mM $MgCl_2$, adding 0.2 ml enzyme at 37℃ for 30 min and bathing for 1 min at 100℃ and a volume of 1 ml reaction products adding 0.1 ml 2 M NaOH was placed in boiling water bath for 10 min, cooled in water and then 3.5 ml 30% HCl and 1 ml 0.1% resorcinol was added. Blank controls were obtained by adding distilled water to the reaction medium containing resorcinol. The reducing sugars were revealed by incubation at 80℃ for 10 min

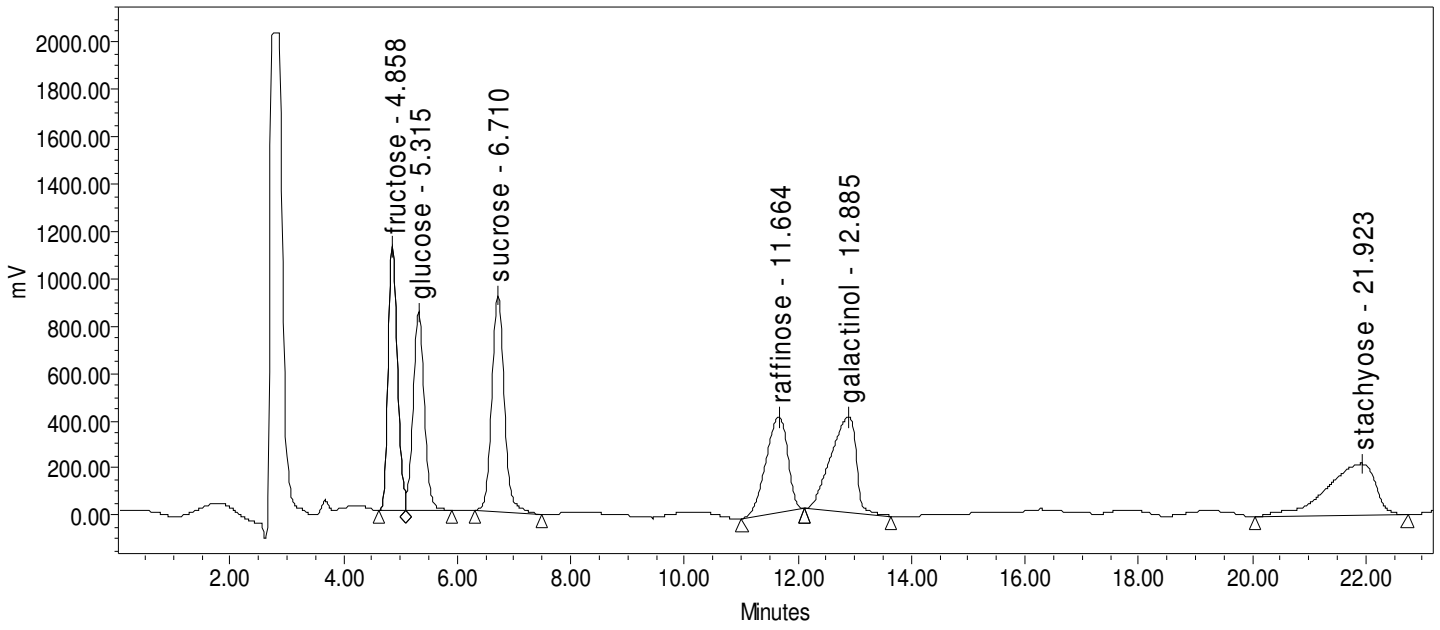

Figure 1. Auto-scaled chromatogram of high performance liquid chromatography on fructose, glucose, sucrose, raffinose, galactinol and stachyose.

and read at 480 nm in a Cary 100UVVIS spectrophotometer (GBC Scientific Equipment Pty Ltd., Heareus, Germany). Sucrose phosphate synthase (SPS) (EC 2.4.1.14) was assayed by measurement of sucrose produced from fructose 6-phosphate plus UDP-glucose (Vassey and Sharkey, 1989; Wardlaw and Willenbrink, 1994).

The alkaline α-galactosidase (AGA) (EC 3.2.1.22) was assayed using p-nitrophenyl-α-galactosidase as substrate. The reaction mixture contained 5 mM p-nitrophenyl-α-galactosidase and 100 mM HEPES buffer (pH 7.5) for alkaline form activity. Reaction was started by adding 30 ul enzyme extract and terminated after 20 min by adding 1 ml 5% (w/v) Na_2CO_3. The enzyme activity was expressed as micromoles of nitrophenol formation per minute by reading at 410 nm in a Cary 100UV:VIS spectrophotometer (GBC Scientific Equipment Pty Ltd., Heareus, Germany) (Monika et al., 1999).

Data Analysis

SPSS 13.0 and Excel 2007 were used for data analysis and graphing.

RESULTS

Grafting effects on various sugars contents in muskmelon fruit

Fructose and glucose contents in muskmelon mesocarp were quite high as 22.27 and 18.18 mg/g or so, respectively, followed by sucrose, while the contents of galactinol, raffinose and stachyose were extremely low in all treatments (Figures 1A, 1B, 1C, 1D, 1E and 1F).

It is suggestive that upon arrival at the sink tissue, the galactosel moieties of the translocated stachyose, raffinose and sucrose were initially hydrolytically removed into galactose metabolism efficiently which may take place in the fruit pedicel or be associated with the fruit vasculature. Therefore, fructose and glucose were accumulated heavily during the early fruit development (Figures 2A and 2B). Their contents increased progressively prior to considerable accumulation of sucrose (Figure 2C) in mesocarp; however, these decreased slightly after the onset of massive sucrose accumulation. Sucrose accumulation was very low during 8 to 24 DAA and sucrose was piled up rapidly during 32 to 48 DAA. Throughout the whole fruit development, raffinose and stachyose contents showed a decreasing trend gradually (Figures 2D and 2F). It is noteworthy that stachyose level dropped dramatically during the 8 to 24 DAA, then remained as low as 0.06 mg/g. On the other hand, galactinol content rose a little during the 8 to 16 DAA and declined slowly during the 16 to 24 DAA, then rapidly lowered during the 24 to 48 DAA (Figure 2E). On the whole, carbohydrate content in muskmelon had varying degrees response to different grafting stocks. Grafting GS reduced all kinds of main sugars contents ($P < 0.01$) except galactinol and stachyose throughout the whole fruit development. In addition, on 16 DAA grafting, GS increased stachyose content dramatically ($P < 0.01$). By contrast, the effects of GR on these sugars accumulations were less than that of GS. Therefore, sucrose content in GR was significantly lower than that in CK on 32 DAA ($P < 0.01$) and had no significant difference during the other periods. Grafting GR decreased raffinose content only on 48 DAA ($P < 0.05$) and increased stachyose content on 16 DAA ($P < 0.05$); however, it failed to meet the significant level during other

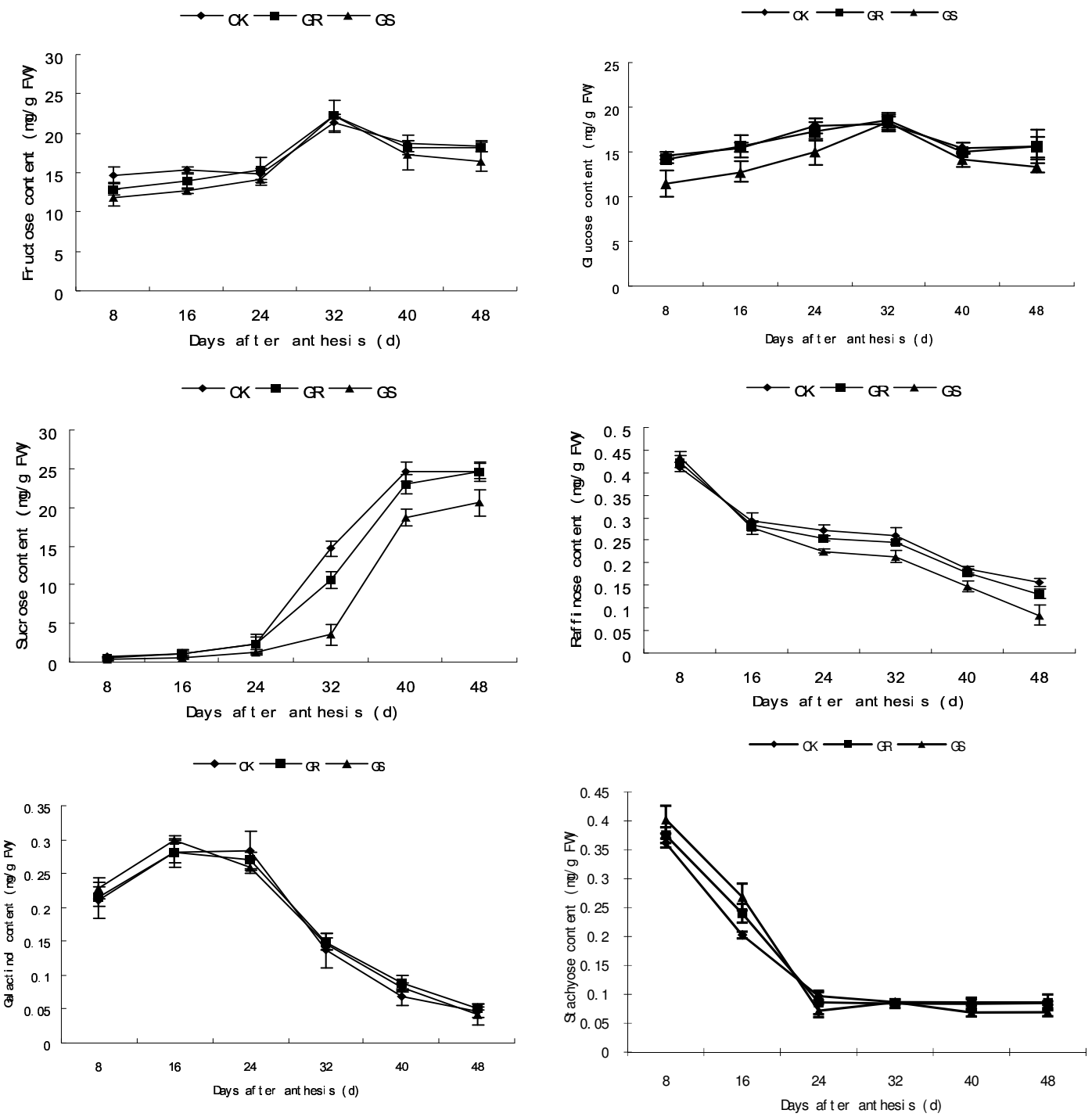

Figure 2. The effects of different rootstocks on fructose, glucose, sucrose, raffinose, galactinol and stachyose contents in muskmelon fruit.

periods prior to considerable accumulation.

Grafting effects on total sugar and starch content in muskmelon fruit

During muskmelon fruit development, total sugar content increased gradually (Figure 3A). The accumulation rate of total sugar was greater during 24 to 40 DAA and the development trend was not changed by grafting. Total sugar accumulation in GS was significantly lower than that in CK ($P < 0.01$). In comparison, the effect of GR on total sugar content was not noticeable.

Basically, the starch content experienced the same

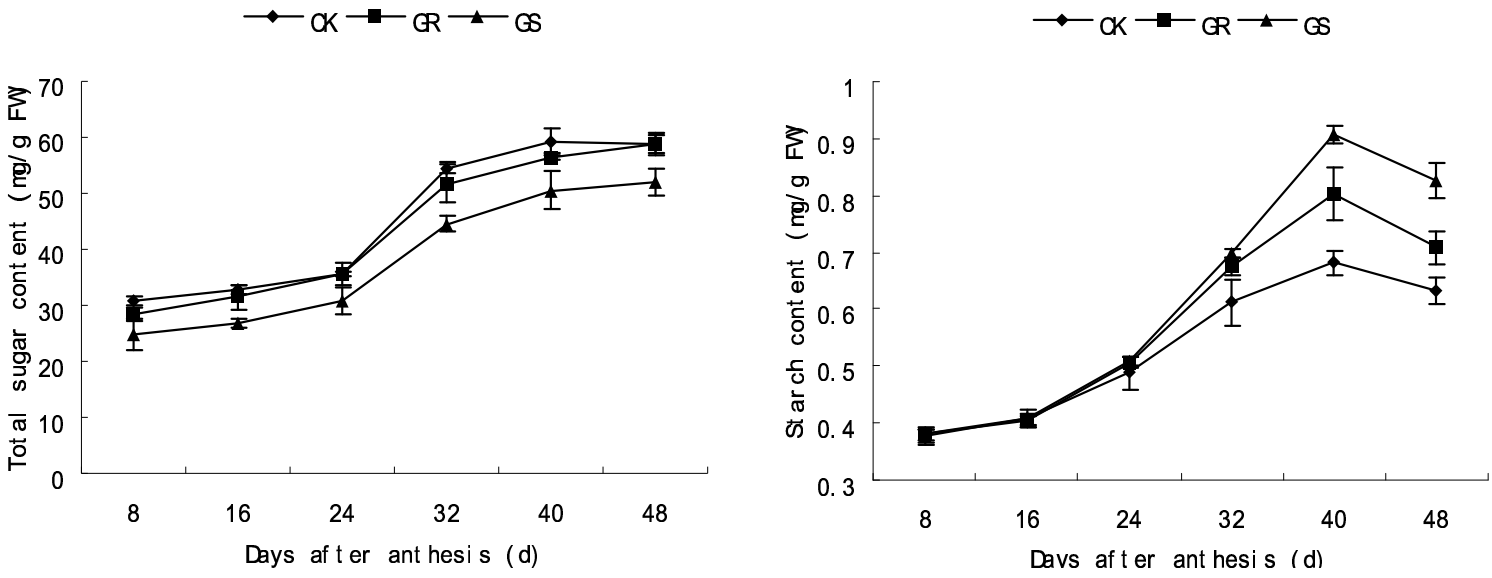

Figure 3. The effects of different rootstocks on total sugar and starch contents in muskmelon fruit.

tendency in different graft combinations and CK, which increased steadily during 8 to 40 DAA, peaking at 40 DAA thereafter decreased (Figure 3B). Starch contents in GS and GR were significantly higher than that in CK during the 32 to 48 DAA ($P < 0.01$), while increment of starch content in GS was greater than that in GR when compared to CK ($P < 0.01$).

Grafting effect on sugar metabolic enzyme activities in muskmelon fruit

As shown in Figures 4A and 4B, basically, acid invertase (AI) and neutral invertase (NI) activities depicted the same decreasing trend in different graft combinations and CK. Therefore, acid invertase activity gradually decreased in different graft combinations and CK throughout fruit development. Grafting GS significantly increased acid invertase activity in all fruit development stages ($P < 0.01$), but grafting GR increased acid invertase activity only at 24 ($P < 0.01$) and 40 DAA ($P < 0.05$). In addition, neutral invertase activity had a gradual decreasing trend during the whole fruit development in which overall activity was lower than acid invertase during the same period after anthesis. Grafting GS increased neutral invertase activity during 16 to 48 DAA ($P < 0.01$), however, grafting GR did not enhanced neutral invertase activity significantly in the whole fruit development.

Grafting did not alter the development trend of sucrose synthase (Synthesis Direction of SS), sucrose phosphate synthase (SPS) and alkaline α-galactosidase activities (AGA) (Figures 5A, 5B and 5C). Grafting GS increased sucrose synthase activity on 8 DAA significantly in early fruit development ($P < 0.01$), but decreased it during 40 to 48 DAA ($P < 0.05$). In contrast, the effect of GR on sucrose synthase activity was not significant. Sucrose phosphate synthetase activity experienced a gradual increase tendency throughout the whole fruit development and faster growth during 24 to 40 DAA. Grafting GS reduced sucrose phosphate synthase activity dramatically compared to CK during the 16 to 48 DAA ($P < 0.05$). Grafting GR enhanced sucrose phosphate synthase activity significantly on 48 DAA ($P < 0.05$), but significant differences were not found in the rest periods (Figures 5A and 5B). Alkaline α-galactosidase activities in all treatments were at high level in early stage of fruit development and quickly dropped after 24 DAA (Figure 5C). Grafting significantly increased alkaline α-galactosidase activities during 8 to 24 DAA ($P < 0.01$); however there were no significant differences during 32 to 48 DAA. Therefore, alkaline α-galactosidase was induced by grafting in early fruit development in which activity was enhanced evidently.

DISCUSSION

Response of carbohydrate accumulation in muskmelon fruit to different grafting rootstocks

Cucurbitaceae are model plants to study on RFOs metabolism and translocation (Figure 6). Cucurbitaceae are distinguished from most of sucrose-translocation plants that generally carry out sucrose metabolism simply, because their photoassimilates will coexist usually in the forms of stachyose, partial raffinose and sucrose, of which stachyose and raffinose will be under galactose metabolism upon being unloaded to the fruits, or they will be under hydrolytic metabolism particularly by α-galactosidase prior to sucrose metabolism (Donald et al., 1992;

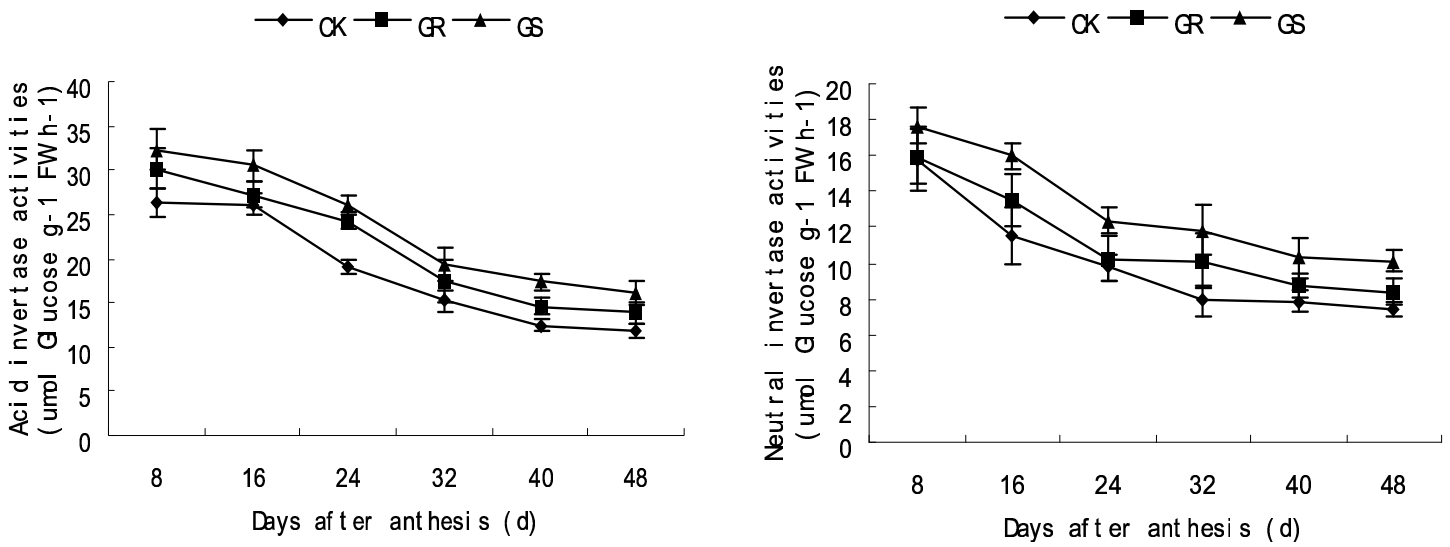

Figure 4. The effects of different rootstocks on acid invertase and neutral invertase activities in muskmelon fruit.

Monika et al., 1999; Brian et al., 2003; Nir et al., 2006). Galactosyl sucrose unloaded from phloem will be under hydrolysis by alkaline α-galactosidase, a fact resulting in the less content of raffinose and stachyose in muskmelon fruits, a typical sucrose-accumulation plant with fructose and glucose accumulation as the principle in early fruit development, while sucrose accumulation occur as a characteristic of its maturity (Gross and Pharr, 1982; Monika et al., 1999). Carbohydrates unloaded to the fruits after a series of energy consumption process and under catalysis with sucrose metabolic enzymes will be turned into a large amount of fructose and glucose mainly to provide carbon sources for tissue growth and the rest may serve as major source for reservation and energy (Nir et al., 2003; Yu et al., 2008).

As this experiment indicates, besides the large proportion of fructose, glucose and sucrose, there is a slight amount of raffinose, stachyose and galactinol in muskmelon mesocarp (Figure 2). In addition, fructose and glucose content experienced a gradual rise in early fruit development (during 8 to 32 DAA) and a gradual decline in the later development (during 32 to 48 DAA). Meanwhile a large amount of sucrose accumulation begins after 32 DAA (Figures 2A, 2B and 2C). During the whole development, raffinose and stachyose contents depict a trend of progressive decline; however, galactinol content showed a rising trend at first followed by a fast decline (Figures 2D, 2E and 2F). In other words, it is interpretative fact that a large portion of stachyose and raffinose among translocation stream are unloaded into the fruit, or it was not only sucrose metabolism, but galactose and stachyose metabolism as well in muskmelon fruits (Gao et al., 1999; Nir et al., 2006). Though some researches have indicated that grafting may lessen sugar content in the fruits, they are only restricted to the grafting effect on total soluble sugar (Xu et al., 2005; Qi et al., 2006; Xu et al., 2006; Angela et al., 2008). However, the experiment presented here is focused on the grafting effects on various carbohydrates contents in the muskmelons. And the results in the present paper demonstrated that grafting GS decreased remarkably all kinds of carbohydrates contents ($P < 0.01$) except for galactinol and stachyose during the whole development period; meanwhile grafting GS increased stachyose content markedly on 16 DAA ($P < 0.01$). However, by contrast, the effects of GR on these sugars accumulations were less than that of GS. On 32 DAA, the sucrose content in GR is evidently less than that in CK ($P < 0.01$) while the difference is not obvious in other periods. The raffinose content in GR is evidently less than that in CK only on 48 DAA ($P < 0.05$). Grafting GR significantly increased stachyose content only on 16 DAA ($P < 0.05$), though it did not affect it remarkably as expected in the other periods. As seen from the whole development period, total sugar accumulation in GS is significantly less than that in CK ($P < 0.01$) but GR graft combination (Figure 3A). On the other hand, the starch contents in the fruits on dissimilar grafting rootstocks were enhanced remarkably ($P < 0.01$) (Figure 3B). Starch, which composition depends on sucrose supply, though its accumulation depends on the rate of starch synthesis and decomposition, is the temporary sink for carbohydrates during the whole fruit development (Tjaden et al., 1998; Stark et al., 1991; Anna et al., 2007). The marked increase of the starch content in the grafted muskmelon mesocarp may result from the observation that grafting has accelerated the transformation from soluble sugars to starch, so that part of the soluble sugar may be reserved temporarily in the form of starch and it may viewed as another reason for the less total sugar content in the grafted ones compared to self-rooted muskmelons. Meanwhile, the development process of grafted muskmelon may be so delayed compared to CK that the transformation rate from starch to

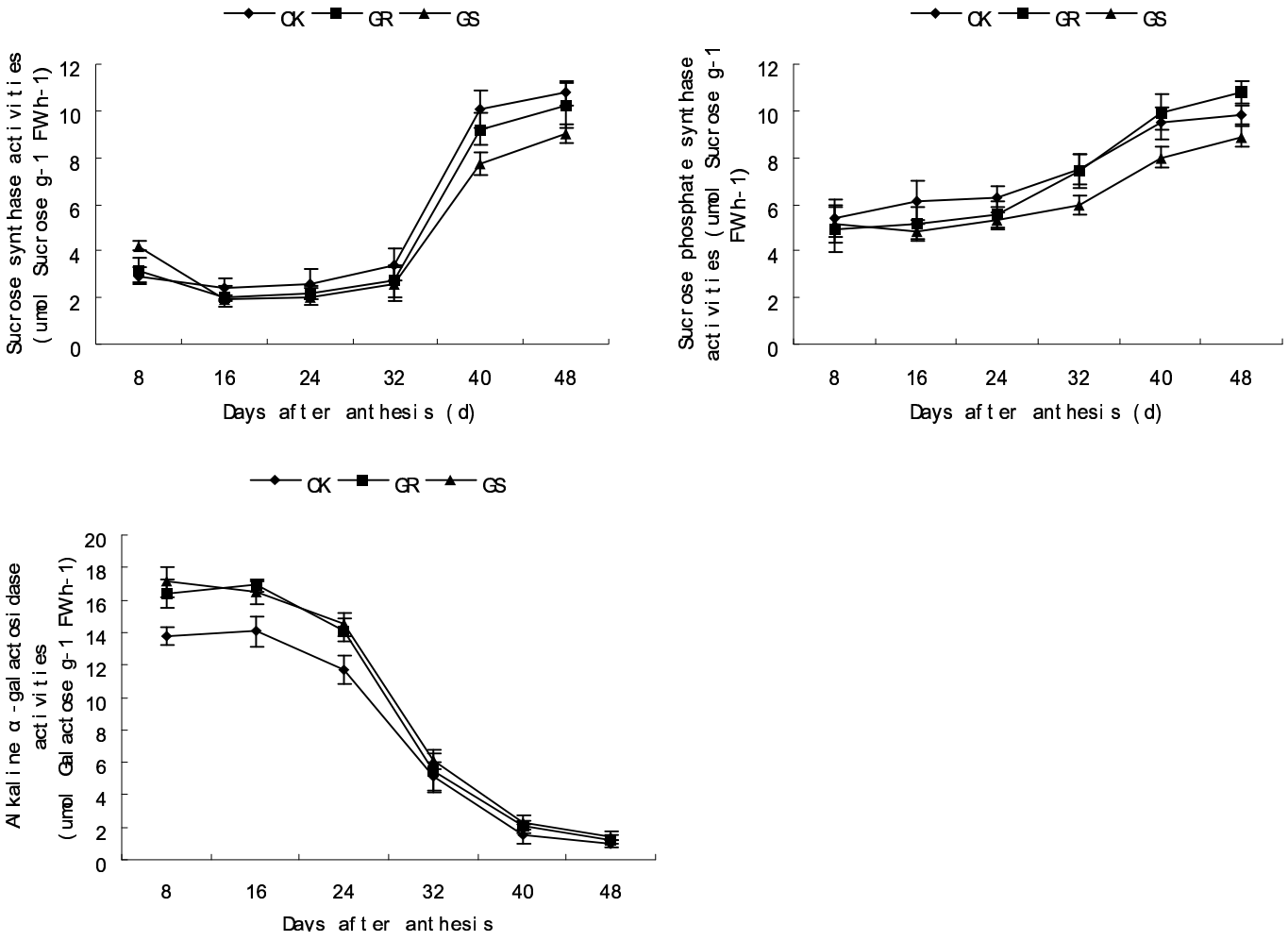

Figure 5. The effects of different rootstocks on sucrose synthase, sucrose phosphate synthase and alkaline α-galactosidase activities in muskmelon fruit.

soluble sugar could be slowed down and large amount of starch accumulates in grafted muskmelon fruits finally. Thus it is clear that the regulation on starch synthesis and decomposition will be one of the critical steps in changing carbohydrates contents in the grafted muskmelon fruits.

Response of carbohydrate metabolic enzymes in muskmelon fruit to different grafting rootstocks

It was essential to elucidate the response of enzymes involved in carbohydrate metabolism in muskmelon to different grafting rootstocks for further studying in depth about mechanism of grafting effects on muskmelon carbohydrate metabolism. Sucrose metabolism is an important part of carbohydrate accumulation (Figure 6B) and sucrose-metabolic enzymes play a key role in fruit sugar accumulation (Hubbard et al., 1989; Jiang and Li, 2005; Qi et al., 2005). In addition, as the initial enzymes in RFOs catabolism, alkaline α-galactosidase hydrolytically removes the terminal galactose moiety of RFOs unremittingly for carbohydrates unloading (Figure 6A). The alkaline α-galactosidase are also classified as acid and alkaline forms according to the optimal pH for their maximal activities and most of the eukaryotic α-galactosidase studied to date are acidic α-galactosidase, with broad pH optima in the acidic range (Nir et al., 2003). However, a large number of studies on acidic form α-galactosidase have shown at present that they play a major role just in seed development and germination. By contrast, the alkaline α-galactosidase studied in the present paper hydrolyze both raffinose and stachyose efficiently, accounting for the distribution of carbohydrates and the catabolism of translocated RFOs in cucurbits (Gao et al., 1999; Nir et al., 2003). It is also a key enzyme for fruit development and sucrose accumulation to provide a guarantee prior to the catabolism of UDP-galactose/glucose pyrophosphorylase for normal operation of sucrose metabolic pathway. This study showed alkaline α-galactosidase is related with the maintenance of fruit

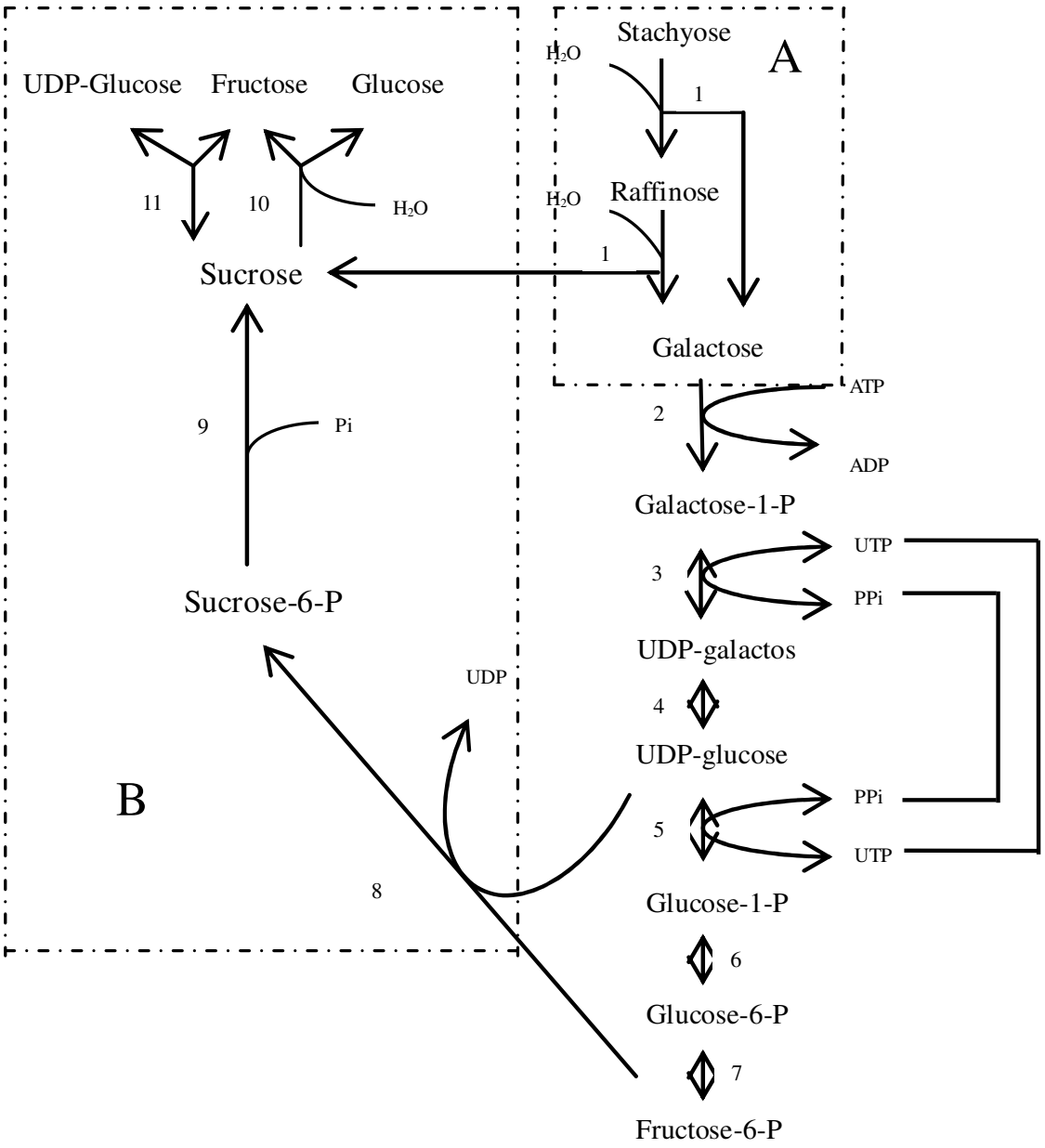

Figure 6. Proposed metabolism of raffinose family oligosaccharides (RFOs) in muskmelon mesocarp tissue (Hubbard et al., 1989; Monika et al., 1999; Nir et al., 2006; Anna et al., 2007). 1. α-galactosidase; 2. galactokinase; 3. UDP-Gal PPase and UDP-Galactose/Glucose Pyrophosphorylase; 4. UDP-Glc 4-epimerase; 5. UDP-Glc PPase; 6. Phosphoglucomutase; 7. Phosphoglucose isomerase; 8. Sucrose phosphate synthase; 9. Sucrose-6-phosphatase; 10. Invertase; 11. Sucrose synthase (Synthesis Direction and Decomposition Direction). Frame A and B in figure refer to RFOs Hydrolytic Metabolism by α-galactosidase and Sucrose Metabolism by sucrose phosphate synthase, invertase and sucrose synthase, respectively.

sink strength and enlargement closely. It is one of the intrinsic reasons for GS's increasing single fruit weight (data not shown) that grafting GS significantly increases acid and neutral (except 8 DAA) invertase activities during the whole fruit developmental stages ($P < 0.01$) and enhances alkaline α-galactosidase activities during the early fruit developmental stage ($P < 0.01$) (Figures 4A, 4B and 5C). However, it is not conducive for sucrose accumulation that grafting GS reduces sucrose synthase and sucrose phosphate synthase activities (Figures 5A and 5B). By contrast, grafting GR increased acid invertase activities dramatically on 24 ($P < 0.01$) and 40 DAA ($P < 0.05$) only, however, slightly reduce sucrose synthase and sucrose phosphate synthase activities during the whole development apart from 48 DAA. It had no significant effects on neutral invertase activity. In general, it is

suggestive that GR may be a more appropriate grafting combination for faintly influencing sucrose metabolic enzymes activities as a whole.

The relationship between carbohydrate accumulation and carbohydrate metabolic enzyme activities in grafted and self-rooted muskmelon

As the first step toward the further research in depth about mechanism of grafting effects on muskmelon carbohydrate metabolism, it is necessary to elucidate enzymes involved in carbohydrate metabolism and clarify the relationship between carbohydrate accumulation and carbohydrate metabolic enzyme activities in the grafted and self-rooted muskmelons. In this experiment, invertase activity was at a much high level during early fruit development stage. It declined rapidly as the fruit development proceeds and acid invertase activity was higher than neutral invertase activity in the corresponding stages (Figures 4A and 4B). Sucrose synthase activity was relatively higher in the early development, but declined to the lowest levels before it got a progressive rise again (Figure 5A). Sucrose phosphate synthase activity is rather lower in the early and middle developmental stages, while it is at a rapid rise in the later stages (Figure 5B). As what has been indicated, acid invertase with high activity and sucrose phosphate synthase with low activity in the early development stages can be considered as one of the prerequisites for the large amount of glucose and fructose accumulation (Lingle and Dunlap, 1987; Hubbard et al., 1989). Invertase with high activity when participating in metabolism may not only supply hexose as carbon source for the rapid growth of the sink tissues, but also play an important role in maintaining sink strength for ensuring a constant import of sucrose, which is also an element in respiratory substrate simultaneously in the fruitlet (Koch, 2004). Invertase in the muskmelon fruits will change sucrose into glucose and fructose under sucrose hydrolysis to retain osmotic pressure for the development of cells, since higher osmotic pressure in early fruit growth stage can ensure favorable moisture absorption. It is similar to the results gotten in this experiment which showed that tissues under rapid growth generally enjoy high acid invertase activity (Tian et al., 2009), with a lower neutral invertase activity in immature fruits. In addition, the large accumulation of sucrose commences when sucrose phosphate synthase activity increased rapidly and acid invertase activity remained at the relative low level during later fruit growth stage. This provided the conditions for sucrose accumulation. But as some research has indicated, acid invertase and neutral invertase activity during early development stage for specific melon breeds are not so high, while sucrose synthase (Decomposition Direction) activity is particularly high so as to make up for the deficiency of invertase activities to hydrolyze sucrose. Thus it is clear that sucrose metabolic enzymes in different kinds of fruits may play different roles in sugar accumulation (Zhang et al., 2003). The overall effect of sucrose metabolic enzymes should be taken into consideration in the study on sugar accumulation mechanism using the net activity of enzymes as an index for such a general effect (Zhao et al., 2001). Thus it could be seen from this experiment that the category and activity significance in sugar metabolic enzymes in the grafted muskmelon exerted an indirect influence on the sugar accumulation. In addition, many past researches have made it clear that there were some kinds of α-galactosidase as acid and alkaline forms in other Cucurbitaceae such as cucumbers and pumpkins. Alkaline α-galactosidase in the pumpkin leaves during early development period (pH = 7.5) is four times as much as that of acid α-galactosidase (pH = 5.4) (Gaudreault and Webb, 1983). Alkaline α-galactosidase activity can alter as plant development proceeds and regulate unloading in sinks and as it has been shown that alkaline α-galactosidase activity in the pumpkin leaves in the early development is much high, while it declined as the sink activity declines (Gaudreault and Webb, 1982). It is indicated that alkaline α-galactosidase is critical in grape fruit development (Han-Chul and Seon-Hwa, 2001), because alkaline α-galactosidase activity experiences a fast growth from 4 weeks to 8 weeks, though not so high 4 weeks prior to grape fruiting. In this experiment, the alkaline α-galactosidase activity depicts a high level in early development stage, but declined rapidly up to its extremity after 24 DAA (Figure 5C). The higher alkaline α-galactosidase activity in early growth stage guarantees the generation of a large amount of sucrose and galactose. This is favorable in providing plentiful glucose and fructose as carbon sources as the fruits enlarge rapidly. Though, it will be advantageous to the decomposition of stachyose and sucrose along with the accumulation of glucose and fructose that invertase and alkaline α-galactosidase activities increased while sucrose synthase and sucrose phosphate synthase activities decreased in grafted muskmelon fruits, the glucose and fructose contents in the grafted muskmelon fruits were still reduced and total sugar content also decreased as well by grafting rootstocks. There are several reasons for this. First, the translocated sugars in sink fruit were reduced by the competition from vigorously growing grafting rootstocks and the reduction extent was greater than sucrose decomposition capacity into glucose and fructose. In other words, due to relative reduction of sugars translocated to the fruit for the competition from root sink strength, hexose (fructose plus glucose) as carbon source was consumed by the rapid growth of the sink fruit. Secondly, grafting accelerates the transformation from soluble sugar to starch dramatically. In addition, grafting may influence the carrier-mediated membrane transport or long-distance transport governed by mechanisms of retention and reclamation along the

transport pathway (Brian et al., 2003). Thus, further studies in depth are still required on the grafting mechanism affecting carbohydrate accumulation in muskmelon fruits.

Conclusion

The category and activity significance in sucrose metabolic enzymes in grafted muskmelon fruits exert an in- direct influence on sugar accumulation. According to the carbohydrates accumulation in muskmelon fruits, Zhong mi1/RibenStrong graft combination is much superior for its effects on carbohydrate metabolic enzymes activities. This is followed by Zhongmi1/ Shengzhen1 graft combination. Thus, it is expected that the agriculturists and scientists in the world will decrease the number of negative quality issues arising from grafted muskmelons and breed improved scion/rootstock graft combinations as well as screen for superior ones in order to harvest higher carbohydrates accumulations level or quality muskmelon fruits. Predictably, the desired goal that grafting can enhance muskmelon fruits quality under better specific rootstocks and cultivation circumstances should be achieved

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REFERENCES

Angela R, Davis P, Perkins V, Yoshiteru S, Salvador LG, Jose VM, Sang-Gyu L, Yun-Chan H, Zhanyong S, Alfredo M, Stephen R, King RC, Jung-Myung L (2008). Cucurbit Grafting. Crit. Rev. Plant Sci. 27: 50-74.

Anna L, Uwe S, Alisdair RF (2007). The complex network of non-cellulosic carbohydrate metabolism. Curr. Opin. Plant Biol. 10: 227-235.

Brian GA, Felix K, Robert T (2003). Symplastic Continuity between Companion Cells and the Translocation Stream: Long-Distance Transport Is Controlled by Retention and Retrieval Mechanisms in the Phloem. Plant Physiol. 131: 1518-1528.

Cheng ZJ, Wang HS, Zhang ZB, Guo SR, He CX (2007). Genetic diversity of melon (*Cucumis melo* L.) germplasm based on AFLPs. Acta Bot. Boreali-Occidentalia Sinica. 27: 244-248.

Donald E, Michelle VG, Monica AM (1992). Patterns of Assimilate Production and Translocation in Muskmelon (*Cucumis melo* L.). Plant Physiol. 99: 959-965.

Gaudreault PR, Webb JA (1982). Alkaline alpha-galactosidase in leaves of Cucurbita pepo. Plant Sci. Lett. 24: 281-288.

Gaudreault PR, Webb JA (1983). Partial purification and properties of an alkaline α-galactosidase from mature leaves of *Cucurbita pepo*. Plant Physiol. 71: 662-668.

Gao Z, Marina P, Eli Z, Arthur AS (1999). Carbohydrate metabolism during early fruit development of sweet melon (*Cucumis melo*). Physiol. Plant. 106: 1-8.

Gross KC, Pharr DM (1982). A Potential Pathway for Galactose Metabolism in *Cucumis sativus* L., A Stachyose Transporting Species. Plant Physiol. 69: 117-121.

Han-Chul K, Seon-Hwa L (2001). Characteristics of an α-galactosidase associated with grape fresh. Phytochem. 58: 213-219.

Hubbard Natalie L, Steven CH, Mason P (1989). Sucrose Phosphate Synthase and Acid Invertase as Determinants of Sucrose Concentration in Developing Muskmelon (*Cucumis melo* L.) Fruits. Plant Physiol. 91: 1527-1534.

Hui F, Xiao-ming L, Zhi-yong L, Peng W, Rui-qin J (2009). A co-dominant molecular marker linked to the monoecious gene *CmACS-7* derived from gene sequence in *Cucumis melo* L. Afr. J. Biotechnol. 8: 3168-3174.

Jiang J, Li T (2005). Molecular biology research of sucrose metabolism key genes in tomato. Biotechnology, 15: 82-85.

Jiao ZG, Wang CQ, Dong YM, He QW, Sun CH (2000). Effects of grafting on development and quality of cucumber. Shandong Agric. Sci. 1: p. 26.

Koch K (2004). Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Curr. Opin. Plant Biol. 7: 235-246.

Lalonde S, Tegeder M, Throne-Holst M, Frommer WB, Patrick JW (2003). Phloem loading and unloading of sugars and amino acids. Plant Cell Environ. 26: 37-56.

Lee JM (1994). Cultivation of grafted vegetables I: Current status, grafting methods and benefits. Hort. Sci. 29: 235-239.

Lee JM, Oda M (2003). Grafting of herbaceous vegetable and ornamental crops. Hort. Rev. 28: 61-124.

Li TL (2000). Relation between the vascular system and photosynthate translocation pathways in tomato plants. J. Jpn. Soc. Hort. Sci. 69: 69-75.

Li T, Liu Y, Qi H, Bai C (2009). Grafting effects on root activity and amino acid contents in bleeding sap of muskmelon. Hort. Environ. Biotechnol. 50: 1-6.

Lingle SE, Dunlap JR (1987). Sucrose Metabolism in Netted Muskmelon Fruit during Development. Plant Physiol. 84: 386-389.

Liu HY, Zhu ZJ, Diao M, Guo ZP (2006). Characteristic of the sugar metablolism in leaves and fruits of grafted watermelon during fruit development. Plant Physiol. Commun. 42: 835-840.

Madore MA (1990). Carbohydrate metabolism in photosynthetic and nonphotosynthetic tissues of variegated leaves of *Coleus blumei Benth*. Plant Physiol. 93: 617-622.

Monika E, Studer F, James DB, John DW, Pharr DM (1999). Galactosyl-sucrose metabolism and UDP-galactose pyrophosphorylase from *Cucumis melo* L. fruit. Physiol. Plant 106: 9-16.

Nir C, Genfa Z, Marina P, Zhifang G, Yoram E, David G, Arthur AS (2003). Cloning and functional expression of alkaline α-galactosidase from melon fruit: similarity to plant SIP proteins uncovers a novel family of plant glycosyl hydrolases. Plant J. 33: 97-106.

Nir D, Marina P, Vitaly P, Nurit K, Pharr DM, Arthur AS (2006). Cloning and expression analysis of a UDP-galactose/glucose pyrophosphorylase from melon fruit provides evidence for the major metabolic pathway of galactose metabolism in raffinose oligosaccharide metabolizing plants. Plant Physiol. 142: 294-304.

Pavlou GC, Vakalonnakis DJ, Ligoxigakis EK (2002). Control of root and stem rot of cucumber, caused by F. *oxysporum* f. sp *radicis cucumerinum*, by grafting onto resistant rootstocks. Plant Dis. 86: 379-382.

Pinheiro C, Rodrigues AP, Chaves MM (2005). Sugar metabolism in developing lupin seeds is affected by a short-term water deficit. J. Exp. Bot. 56: 2705-2712.

Qi H, Li T, Liu H, Zhang J (2005). Studies on carbohydrate content and sucrose-metabolizing enzymes activities in different parts of tomato. Acta Hort. Sinica. 32: 239-243.

Qi H, Li T, Liu Y, Dan L (2006). Effects of Grafting on Photosynthesis Characteristics, Yield and Sugar Content in Melon. J. Shenyang. Agric. Univ. 37: 155-158.

Stark DM, Timmermann KP, Barry GF, Preiss J, Kishore GM (1991). Regulation of the amount of starch in plant tissues. Science, 258: 287-292.

Taji T, Ohsumi C, Iuchi S, Seki M, Kasuga M, Kobayashi M (2002). Important roles of drought- and cold-inducible genes for galactinol sythase in stress tolerance in *Arabidopsis thaliana*. Plant J. 29: 417-426.

Tian H, Qingguo K, Yanqing F, Yu X (2009). Cloning and characterization of a soluble acid invertase-encoding gene from muskmelon. Mol. Biol. Rep. 36: 611-617.

Tjaden J, Monhlmann T, Kampfenkel K, Henrichs G, Neuhaus HE (1998). Altered plastidic ATP/ADP-transporter activity influences potato (*Solanum tuberosum* L.) tuber morphology, yield and composition of tuber starch. Plant J. 16: 531-540.

Wardlaw IF, Willenbrink J (1994). Carbohydrate storage and mobilisation by the culm of wheat between heading and grain maturity: the relation to sucrose synthase and sucrose-phosphate synthase. Austra. J. Plant Physiol. 21: 255-271.

Xu SL, Chen QY, Li SH, Zhang LL, Gao JS, Wang HL (2005). Roles of sugar-metabolizing enzymes and GA3, ABA in sugars accumulation in grafted muskmelon fruit. J. Fruit Sci. 22: 514-518.

Xu CQ, Li TL, Qi HY (2006). Effects of grafting on development, carbohydrate content and sucrose metabolizing enzymes activities of muskmelon fruit. Acta Hort. Sinica. 33: 773-778.

Yu X, Wang X, Zhang W, Qian T, Tang G, Guo Y, Chengchao Z (2008). Antisense suppression of an acid invertase gene (*MAI1*) in muskmelon alters plant growth and fruit development. J. Exp. Bot. 59: 2969-2977.

Zhao ZZ, Zhang SL, Xu CJ, Chen KS, Liu ST (2001). Roles of Sucrose Metabolizing Enzymes in Accumulation of Sugars in Satsuma Mandarin Fruit. Acta Hort. Sinica. 28: 112-118.

Zhang JM, Ge ZD (2002). Effects of grafting application on muskmelon. Chin. Cucurbit. Veg. 1: 26-27.

Zhang MF, Li ZL, Chen KS, Qian QQ, Zhang SL (2003). The relationship between sugar accumulation and enzymes related to sucrose metabolism in developing fruits of muskmelon. J. Plant Physiol. Mol. Biol. 29: 455-462.