Carbohydrate Metabolism of Cucurbits

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3.1 INTRODUCTION

Carbohydrate metabolism and its regulation are of central importance to the growth and development of plants. Cucurbits have long been considered ideal materials for carbohydrates metabolism studies for a number of reasons. First, the cucurbits synthesize and translocate assimilates mainly in the form of raffinose family oligosaccharides (RFOs). As a result, the loading, translocation, and unloading mechanisms in cucurbits are largely different from those in sucrose-translocating plants. Second, cucurbit fruits are relatively fast growing. Some types of pumpkins show maximum dry weight gain of 1.71 g h⁻¹ during growth (Crafts and Lorenz, 1944). Recently (October 2014), a Swiss farmer claimed a new world record with a pumpkin weighing 953.5 kg. These evidences indicate that the mass transfer rate in cucurbit phloem is quite fast. Third, the long petioles and stem internodes and relatively abundant phloem sap exudation from the incised stem facilitate radiolabeling studies and sample collection and analysis.

In cucurbits, RFOs are not only primary translocated assimilates but also abundant soluble sugars throughout all plant parts, except immature seeds and fleshy fruits (Schaffer et al., 1996). The pathway of synthesis and catabolism of these galactosyl-sucrose (mainly stachyose and raffinose) is well established (Gross and Pharr, 1982; Handley et al., 1983a; Schaffer et al., 1987; Keller and Pharr, 1996; Dai et al., 2006), as shown in Figures 3.1 and 3.2.
Cucurbits accumulate lipids, proteins, starch, and soluble sugars in mature seeds as sources of nutrition for germination. Lipids are the primary component in cucurbit seeds; in mature cucumber (Cucumis sativus) seeds, about 38% of dry weight is that of lipids (Pharr and Motomura, 1989). The lipid level could reach to approximately 50% of dry weight in the mature seeds of watermelon (Citrullus vulgaris) and pumpkin (Cucurbita pepo) (El-Adawy and Taha, 2001). Mature cucurbit seeds are also rich in protein, and the protein level in mature seeds of watermelon and pumpkin is about 35% (EI-Adawy and Taha, 2001). There is a small amount of starch in cucurbit seeds, the content of which is less than 1 mg g⁻¹ in cucumber seeds (Handley et al., 1983b). Furthermore, cucurbit seeds also contain a certain amount of glucose, sucrose, raffinose, stachyose, and verbascose (Handley et al., 1983a,b; Botha and Small, 1985; EI-Adawy and Taha, 2001).

Stachyose is the primary soluble sugar in mature cucumber seeds. Stachyose and raffinose are rapidly consumed during the first 36 h after imbibition, accompanied by an increase in the concentration of their enzymatic degradation product sucrose (Handley et al., 1983b). α-Galactosidases catalyze the first step of RFO hydrolysis (Figure 3.1). There are six α-galactosidase genes in the cucumber genome, three of them are acid and the other three are alkaline, based on their activity response to pH (Yang, 2012). The author found that mRNA expression of all six genes gradually
increased during the 5 days after seed imbibition. Thomas and Webb (1978) also found that the activities of two acid α-galactosidases rose and RFO levels declined rapidly within 48 h of pumpkin seed germination. Blöchl et al. (2008) reported that the RFOs were broken down by both acid α-galactosidase and alkaline α-galactosidase together in pea (Pisum sativum) seeds. An acid α-galactosidase is predominately expressed during seed maturation and hydrolyzed RFOs at the early stage of germination, while alkaline α-galactosidase is only expressed in the latter stage of seed germination. It is not clear whether RFO catabolism in cucurbit seeds is similar to that in pea seeds.

After 36 h imbibition, lipids began to break down into cucumber seeds (Pharr and Motomura, 1989). Lipid degradation in watermelon seeds was processed even later than that in cucumber seeds (Botha and Small, 1985), indicating the importance of soluble sugars as an initial energy substrate during the early germination stage. Bharadwaj et al. (2012) reported that there were significant activities of protease and β-amylase after 12 h of germination in cucumber seeds, suggesting that the decomposition of proteins and starch may be earlier than lipids. Pharr and Motomura (1989) observed that there was starch synthesis in the cucumber seeds after 36 h of germination.

Galactinol synthase is a key enzyme in the RFO biosynthesis pathway (Figure 3.1). Its activity was undetectable in cucumber seeds during the first 36 h after imbibition, which then increased rapidly between 36 to 60 h. RFO levels maintained at a stable low level during the latter stage, indicating that RFOs may be resynthesized during the catabolism of lipids and other large biological molecules (Handley et al., 1983b; Pharr and Motomura, 1989). At this stage, vascular bundles in cotyledons to the roots and growing seedling axis gradually formed, and the resynthesized RFOs may be transported in the phloem from the cotyledons (source) to the roots or seedling axis (sink) (Schaffer et al., 1996; Savage et al., 2013).

Enzymes in lipid metabolism pathway are more sensitive to low oxygen stress than those in starch metabolism pathway. As a result, seeds storing lipids as the major food reserve (such as cucurbit seeds) were more sensitive to anaerobic stress than those containing starch predominantly (Al-Ani et al., 1985). Thus, utilization of starch is important for seed germination under low oxygen conditions. The inhibition of lipid degradation and improvement of the utilization of readily metabolizable carbohydrates under anaerobic stress have been reported by several research groups (Pharr and Motomura, 1989). Todaka et al. (2000) also found that β-amylase activity and sucrose level in cucumber cotyledons increased after water stress. The increased soluble sugar level may act as both energy sources and protective agents. It is suggested that selecting genotypes with a high seed starch reserve may be a good idea for breeding new cultivars with high germination rate under abiotic-stressed conditions (Schaffer et al., 1996).

### 3.3 CARBOHYDRATE METABOLISM IN LEAVES

#### 3.3.1 Sink-to-Source Transition

Young leaves of cucurbits are heterotrophic organs whose growth is maintained by imported soluble carbohydrates from mature leaves. Turgeon and Webb (1975) indicated that the leaf of C. pepo was first capable of net CO₂ fixation when 8% expanded, began to export excess photosynthate when the phase of rapid decrease in relative growth rate was almost complete at about 45% expansion, and reached the maximum net photosynthesis rate at 70% expansion. Similar to other dicotyledonous plants, the maturation (transition from sink to source) of the cucurbit leaf develops in a basipetal direction (Figure 3.3) (Turgeon and Webb, 1973; Savage et al., 2013). Assimilates import into the lamina tip of C. pepo leaves stops when the blade is 10% expanded, while the leaf base still needs assimilate supply at this time. As a result, sugars exported from the leaf tip are redistributed to the less-matured leaf base during this period, delaying export from the lamina until the blade is 35% expanded (Turgeon and Webb, 1973). During the sink-to-source transition, there may be a short period in which assimilates travel toward the leaf in the adaxial
phloem and away from the leaf in the abaxial phloem simultaneously (Peterson and Currier, 1969). In addition, the loss of import capacity of the petiole is also basipetal and dorsoventral (Turgeon and Webb, 1973).

Transition of cucurbit leaves from sink to source involves complex anatomical changes. First, intercellular air spaces increase as the leaf expands, which greatly enhances the CO₂ diffusion and fixation. Second, as the cucurbit leaf grows, maturation of veins develops progressively from the largest toward the smallest elements, and commencement of sugar export is coincident with maturation of the abaxial phloem of the minor veins (Turgeon and Webb, 1976).

Changes in soluble carbohydrate levels were observed during leaf development. In cucumber, sucrose and raffinose concentrations decrease, while galactinol and stachyose concentrations increase during leaf expansion (Pharr and Sox, 1984). In mature cucumber leaves, stachyose is the most abundant soluble carbohydrate (Pharr and Sox, 1984). Sucrose is synthesized at all development stages, whereas the first detectable synthesis of raffinose and stachyose coincides with the beginning of assimilate export from leaf tip (Turgeon and Webb, 1975). The raffinose pool in sink leaves is from the degradation of imported stachyose, rather than from in situ synthesis (Turgeon and Webb, 1975).

Activity changes of enzymes involved in stachyose degradation and synthesis play an important role during leaf sink-to-source transition. The function of acid α-galactosidase during leaf development is not clear. Thomas and Webb (1978) and Smart and Pharr (1980) indicated that there was no significant correlation between the acid α-galactosidase activity and the leaf development of cucumbers and pumpkins. However, Pharr and Sox (1984) found that acid α-galactosidase activity declined during leaf development. More evidences support that alkaline α-galactosidase is involved in the catabolism of RFOs imported from the phloem in sink leaves (Gaudreault and Webb, 1982, 1986; Pharr and Sox, 1984). They found that the activity of alkaline α-galactosidase is high in young leaves and declines as leaves expand. Yang (2012) reported that the mRNA level of cucumber alkaline α-galactosidase 1 (AGA1, Genbank accession number DQ157703) was positively correlated with the activity of alkaline α-galactosidase during leaf development, indicating AGA1 may play a role in RFO unloading in young leaves of cucumber. On the other hand, galactinol synthase activity increases during leaf growth, coinciding with the increasing accumulation of stachyose in maturing pumpkin leaves (Pharr and Sox, 1984).

### 3.3.2 Phloem Loading

The vascular anatomy of the cucurbit leaf affects assimilate loading profoundly, and the following description is based primarily on the studies of Turgeon et al. (1975) and Schmitz et al. (1987) (Figure 3.4). In cucurbits, veins in mature leaves are always classified into seven orders, among which veins of orders 1–3 are defined as major veins and veins of orders 4–7 as minor veins, according to whether they contain intermediary cells. Photosynthate loading of mature leaves occurs at the
The veins in cucurbit leaves are bicolateral, even including veins of order 7. In these smallest veins, the adaxial phloem and abaxial phloem are separated by one to two tracheid cells and a parenchyma cell. The whole minor vein is bounded by a single layer of bundle sheath cells. The areoles are delimited by small veins, yet the distance between any mesophyll cell and the next vein is no more than three to four cell diameters.

The adaxial phloem in the smallest vein is composed of one sieve element and one companion cell, and the adaxial sieve elements are approximately equal in diameter to their companion cells. These companion cells have typical characteristics of common companion cells, that is, a central vacuole surrounded by a dense layer of cytoplasm, which are termed as ordinary companion cells. In the adaxial phloem, plasmodesmata connection is observed between the companion cell and the sieve element but not between the companion cell or sieve element and the bundle sheath cells.

The adaxial phloem in the smallest veins consists of one to two sieve elements and two to four companion cells. The abaxial companion cell is specialized, has dense cytoplasm, and contains numerous mitochondria and small vesicles, which are termed as the intermediary cell. In the abaxial phloem, plasmodesmata connection is observed between the companion cell and the sieve element but not between the companion cell or sieve element and the bundle sheath cells.

The abaxial phloem in the smallest veins consists of one to two sieve elements and two to four companion cells. The abaxial companion cell is specialized, has dense cytoplasm, and contains numerous mitochondria and small vesicles, which are termed as the intermediary cell. The adjacent cell walls of intermediary and bundle sheath cells are traversed by numerous plasmodesmata, which occur in clusters in large pit fields. The plasmodesmata are highly branched, more so on the intermediary cell side than on the bundle sheath side. In the abaxial phloem, the intermediary cell is much larger than the sieve element, which is smaller than the sieve element in the adaxial element. There is also extensive symplastic contact between intermediary cells and sieve elements; these plasmodesmata are also branched on the intermediary cell side.
A considerable amount of evidence indicates that phloem loading of cucurbits occurs by a symplastic pathway at the abaxial phloem of minor veins. There are abundant plasmodesmata connecting intermediary cells to adjacent bundle sheath cells and intermediary cells to sieve elements. Dye-coupling studies were undertaken by Turgeon and Hepler (1989) to determine whether plasmodesmata between intermediary cells and bundle sheath cells in the minor veins of mature C. pepo leaves are open to passage of low-molecular-weight compounds, and the results indicated that the dye can spread from one intermediary cell to another and from intermediary cells to bundle sheath and mesophyll cells. Autoradiograms also indicated that the abaxial phloem of minor veins is responsible for carbon export from mature leaves of Cucumis melo (Schmitz et al., 1987). The authors also found that the release of $^{14}$C into the leaf apoplast was minor and PCMBS (a sucrose transporter inhibitor) only slightly reduced $^{14}$C export. Several studies have suggested that stachyose synthase is located in the intermediary cells and raffinose and stachyose for long-distance translocation are synthesized there (Holthaus and Schmitz, 1991a,b). In addition, concentrations of raffinose and stachyose in intermediary cells are much higher than that in mesophyll cells (Schmitz and Holthaus, 1986; Holthaus and Schmitz, 1991b; Beebe and Turgeon, 1992; Haritatos et al., 1996). Based on the results mentioned earlier and the research data from other RFO-transporting species, Turgeon (1991) suggested a phloem loading process known as polymer trapping. According to this model, sucrose from the mesophyll diffuses to the bundle sheath and then into the intermediary cells and raffinose and stachyose for long-distance translocation are accumulated there (Holthaus and Schmitz, 1991a,b). In addition, concentrations of raffinose and stachyose in intermediary cells are much higher than that in mesophyll cells (Schmitz and Holthaus, 1986; Holthaus and Schmitz, 1991b; Beebe and Turgeon, 1992; Haritatos et al., 1996). Based on the results mentioned earlier and the research data from other RFO-transporting species, Turgeon (1991) suggested a phloem loading process known as polymer trapping. According to this model, sucrose from the mesophyll diffuses to the bundle sheath and then into the intermediary cells and is converted to raffinose and stachyose. These RFOs are too large to diffuse back to the bundle sheath and mesophyll through the intermediary cell plasmodesmata. As a result, they accumulate to high concentrations in the intermediary cell. They further diffuse into the sieve element for long-distance translocation.

In lower-order (larger) veins, the number of SE–CC complex increases. Only those companion cells that have direct contact with the bundle sheath cells are specialized as intermediary cells; others are still ordinary companion cells. These veins (6–4) are considered to be responsible for both loading and transport, while veins of orders 1–3 may be involved mainly in long-distance transport (Schmitz et al., 1987).

Three pathways of phloem loading exist in the plant kingdom, symplastic and passive, symplastic followed by polymer trapping, and transporter driven via the apoplast (Rennie and Turgeon, 2009). Individual species always employ multiple loading strategies, as we have seen in cucurbits (Slewinski et al., 2013). If compartmentation is taken into account (cytosol made up only 5% of the mesophyll cell volume), sucrose level is higher in mesophyll cells than in intermediary cells (Haritatos et al., 1996). Thus, sucrose could be loaded through a symplastic and passive way. In addition, sucrose could be loaded at the adaxial phloem of minor veins through an apoplastic way. This may explain why the sucrose level in the phloem sap is higher than that in the sieve element–intermediary cell complex (Haritatos et al., 1996). Experimental data from CMV-infected C. melo support this view (Shalitin and Wolf, 2000; Gil et al., 2011, 2012). The authors found that CMV infection increases the sucrose–stachyose ratio in phloem sap of C. melo. Further research indicated that the enhancement expression and activity of a sucrose transporter was responsible for this increase. Furthermore, the phloem loading was inhibited by the sucrose transporter inhibitor PCMBS in this case. The physiological significance of CMV-induced quantitative shift from symplastic to apoplastic phloem loading is not clear. It is suggested that the plant partly closed the symplastic loading pathway to prevent CMV entering the phloem and improved the apoplastic loading pathway to ensure enough assimilate supply.

### 3.4 LONG-DISTANCE PHLOEM TRANSPORT

There are two distinct types of phloem in cucurbits, fascicular phloem (FP) and extrafascicular phloem (EFP). FP is bicolateral and presents in the vascular bundles on both sides of the xylem (so-called internal FP and external FP, respectively). EFP exists outside the bundles, which is composed of peripheral sieve tubes located at the margin of vascular bundles, entocyclic sieve tubes.
just inside the sclerenchyma ring, and laterally oriented commissural sieve tubes linking entocyclic sieve tubes, peripheral sieve tubes, and fascicular sieve tubes to each other. EFP also includes the longitudinal ectocyclic sieve tubes located outside the sclerenchyma ring (Figure 3.5) (Crafts, 1932; Zhang et al., 2012).

After loading at the minor vein, assimilates enter the phloem for long-distance translocation. Fruits of cucurbits usually grow fast (some giant pumpkins probably have the fastest-growing fruit in the plant kingdom), indicating the mass flow rate in the phloem is quite fast in these plants. Data from both fruit weight increase measurement and 14C labeling experiment suggest that assimilate translocation velocity is between 55 and 160 cm h⁻¹ in cucurbits (Crafts and Lorenz, 1944; Schaffer et al., 1996). Environmental factors, including light, temperature, and CO₂ concentration, affect assimilate translocation rate profoundly, either by altering the quantity of dry matter available for transport or by directly affecting the flow velocity in the phloem. Several reports have focused on the negative effect of low temperature on the assimilate translocation in cucurbit phloem (Murakami and Inayama, 1974; Toki et al., 1978; Kanahamam and Hori, 1980). In a typical Chinese energy-saving sunlight greenhouse, the temperature at daytime could reach 30°C or higher and would drop to 12°C or even lower at night. Thus, low translocation rate of photoassimilates from source to sink under low night temperature becomes one of the key limitations of cucumber production in these greenhouses in winter, and cultivars with relatively high assimilate translocation rate under low night temperature have been the target of Chinese cucumber breeders (Miao et al., 2009). On the other hand, Matsumoto et al. (2012) reported that heating bearing shoots near fruits promote sugar accumulation in melon fruit.

Stachyose, sucrose, and raffinose are major sugars translocated in the phloem of cucurbits (Weidner, 1964; Pharr et al., 1977; Richardson et al., 1982, 1984; Mitchell et al., 1992). It is difficult to sample “pure” phloem exudate from cucurbit plants and measure its exact sugar concentration since the fluid is always contaminated by adjacent parenchyma cells and diluted by the xylem sap (Zhang et al., 2012). Microdissecting FP tissues from freeze-dried stem is a more accurate method than stem cutting for phloem sap sampling. Using this technique, Zhang et al. (2010) found that the total sugar content is around 1 M in FP, which is consistent with other species and the measurements in cucurbit leaf cells. However, sugar concentration in EFP is much lower than that in FP, indicating...
that FP is largely responsible for assimilate transport (Zhang et al., 2010). If the exudate is collected from the incised stem or petiole, one should keep in mind that there are two types of cucurbits; the first group includes pumpkin (Cucurbita maxima) and Zucchini (C. pepo), whose exudate is primarily originated from the EFP, and the second group includes cucumber, watermelon, bitter apple (Citrullus colocynthis), luffa (Luffa acutangula), calabash (Lagenaria siceraria), and winter melon (Benincasa hispida), which exudate mainly from the FP (Slewinski et al., 2013). There is no significant difference of sugar content between external and internal FPs, suggesting that they may have similar capacity for sugar translocation (Zhang et al., 2010).

The composition of cucurbit phloem sap varies under different environmental conditions or during plant development. Mitchell and Madore (1992) found that 10°C chilling treatment for 72 h caused a general increase of stachyose, raffinose, and sucrose content in melon phloem sap. It has been reported that some abiotic (high temperature) or biotic (CMV infection) stresses can increase the sucrose to RFO ratio in the phloem sap of melon (Shalitin and Wolf, 2000; Gil et al., 2012). The diurnal rhythm of sugar content in the phloem sap was studied in several cucurbits. Mitchell et al. (1992) found that levels of stachyose, raffinose, and sucrose in melon phloem sap increased during the morning and early night. Jiang et al. (2006) reported that the concentration of stachyose achieved the maximal level at 4:00 p.m. while levels of other sugars showed no distinct diurnal patterns. In addition, according to Hu et al. (2009), total soluble sugar levels in cucumber phloem sap gradually increased during cucumber fruit development, and the level of raffinose increased smaller than those of stachyose and sucrose.

Two mechanisms may be involved in the composition change of cucurbit phloem sap. First, some environmental factors may alter the loading pathways, as mentioned in Section 3.2. A typical example is CMV infection caused by sucrose transporter activation in melon minor veins, which led to a quantitative shift from symplastic loading to apoplastic loading and an increase of sucrose to stachyose ratio in the phloem sap (Gil et al., 2011; Slewinski et al., 2013). Second, solutes are exchanged between the phloem and surrounding tissues, and the unbalanced exchange of some solutes may cause composition variation in the phloem sap during long-distance translocation. Richardson et al. (1982) suggested that monosaccharide concentration is higher in the phloem sap sampled near the sink tissue than that near the leaves. Ayre et al. (2003) proposed that in Coleus blumei (RFO-transporting species), sucrose leaks from the phloem symplast and is retrieved by transporters at similar rates. As a result, the concentration of sucrose remains unchanged during transport. Galactinol also leaks from the phloem but is not retrieved, and it is eventually depleted from the translocation stream. RFOs leak minimally and can be efficiently transported to sink tissues. The authors indicated that one advantage of transporting RFOs is it reduces solute leakage during long-distance transport and hence improves translocating efficiency.

### 3.5 ASSIMILATE PARTITIONING

In cucurbit plants, the older fruit gets higher priority in obtaining assimilates over the younger fruit, which is known as “first-fruit inhibition” (McCollum, 1934; Schaffer et al., 1996). This inhibitory effect may contribute to the mechanism of cucumber plants to obtain mature fruits and seeds in a shorter period when the total assimilate supply is limited. However, in cucurbit production, this limited simultaneous fruit set pattern greatly reduces the yield, especially in once-over harvest systems (Ramirez and Wehner, 1984).

Assimilate limitation is an obvious explanation for this inhibitory effect. In general, a fruit is a strong sink for assimilates. Several leaves act as principal assimilate suppliers for individual fruits. When assimilate supply is limited, fruit competition results in fruit drop, particularly among the later-set fruits (Ho, 1992). Ells (1983) observed that about 9–10 leaf nodes typically intervene between successfully growing fruits in picking cucumber plants, suggesting that assimilates from about 10 leaves may be required simultaneously for the growth of an individual fruit. During rapid growth period, about 3300–3400 mg assimilates are required to be transported to an individual fruit.
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...everyday (Schapendonk and Challa, 1980; Pharr et al., 1985), while the leaves can provide no more than 225 mg of assimilate dm$^{-2}$ during an 11 h photoperiod (Pharr et al., 1985). According to the average area of leaf blade, a balanced source–sink relationship between approximately 9–10 leaves and a single fruit can be calculated. In melon plants, assimilates from six leaves were needed for the growth of a single fruit (Hughes et al., 1983). In general, fruits tend to assimilate photosynthate from closer leaves (Hughes et al., 1983; Shishido et al., 1992). However, Zhang et al. (2015) found that in cucumber plants setting two fruits, assimilates from 14C-labeled leaves tended to move to the first fruit, although these leaves were closer to the second fruit. In watermelon plants, upper leaves of fruit set node were more important for fruit growth than lower leaves of fruit set node (Lee et al., 2000).

Another possible factor involved in the limited simultaneous fruit setting of cucumber plants is the level of plant hormones. It was reported that application of some plant growth regulators, such as chlorflurenol or 6-benzyl aminopurine (BA), could overcome the inhibition effect caused by the early fruit set (Cantliffe, 1976; Baniel et al., 2008; Zhang et al., 2015). Schapendonk and Brouwer (1984) also pointed out that limiting assimilate supply by defoliation caused reduced fruit growth rate rather than abortion, indicating other factors may be involved in the growth competition between fruits. Zhang et al. (2015) revealed that in cucumber plant with two fruits, sucrose and trehalose-6-phosphate (T6P) levels in the peduncle of the first fruit are higher than those of the second fruit, while sucrose nonfermenting 1–related kinase 1 [SnRK1] activity is lower in the peduncle of the first fruit than that of the second fruit from 0 to 8 days after anthesis. The growth rate and sink activity of the second fruit were enhanced by removing the first fruit or by treating it with BA, in comparison with an increase of sucrose and T6P levels and a decrease of SnRK1 activity in its peduncle. Furthermore, the mRNA level of CsAGA1 in cucumber calli was upregulated by exogenous trehalose treatment. These results suggested that T6P- and SnRK1-mediated signaling is involved in the regulation of cucumber first-fruit inhibition.

Growth competition exists not only between fruits on the same plant but also between fruits and vegetative tissues and between different vegetative tissues in plants without fruit. In fruiting plants, the fruit is the strongest sink. Compared with vegetative plants, the growth of young leaves, petioles, stems, and roots was significantly suppressed in fruiting plants (Pharr et al., 1985). The influence of fruit setting on the growth of root was greater than that of aboveground vegetative parts in cucumber (Marcelis, 1994). In vegetative plants, photoassimilates tended to translocate to root at first in melon (Schmitz et al., 1987), while in pumpkin, young leaves and seedling shoots were the regions most actively importing assimilates (Webb and Gorham, 1964). In cucumber plants with no fruit removed, the partitioning rates of 14CO2 in fruits, stems, roots, and leaves were 90%, 5%, 3%, and 2%, respectively; in vegetative cucumber plants, 14CO2 was mostly distributed into stems (Choi et al., 1997). The effects of restricted root growth on the leaf carbohydrate metabolism were studied by Robbins and Pharr (1988). The results showed that the reduced sink demand, induced by restricted root growth, may led to increased starch concentrations and a reduction in stachyose biosynthesis (indicated by a decrease of galactinol synthase activity) in cucumber source leaves.

Compared with the vegetative plants, the carbon exchange rate was higher in plants bearing fruits (Marcelis, 1991). Pharr et al. (1985) suggested that carbon exchange rate, carbon export rate, and starch accumulation rate in the day and starch degradation rate at night were higher in the plants with fruits than those without fruits. Higher carbon export rate is often accompanied by higher leaf stachyose synthase activity but has no relevance with sucrose phosphate synthase (SPS) and galactinol synthase (Pharr et al., 1985; Holthaus and Schmitz, 1991b; Miao et al., 2003), while the concentration of galactinol in the vegetative plants is significantly higher than fruiting plants (Pharr et al., 1985; Miao et al., 2003). Additional fruit loading did not increase the photosynthetic rate and assimilate partitioning rate to the fruits, which further confirmed that there is competition for assimilates among fruits (Marcelis, 1991). Girdling of the petiole had a similar effect on the leaf photosynthesis by removing fruits (Mayoral et al., 1985).
The diurnal fluctuation of the assimilate export rate of cucumber has been studied by several research groups. Some researchers suggested that the assimilate export rate is higher during daytime (Verkleij and Baan Hofman-Eijer, 1988; Verkleij and Challa, 1988). However, Toki et al. (1978) reported that photoassimilates were mainly exported from leaves during early night. According to these data, the authors proposed that higher temperature should be maintained during early night to facilitate carbon export, while relatively lower temperature during later night is beneficial to reduce respiration. Miao et al. (2009) found that under 28°C/22°C (day/night) condition, cucumber fruits grew fast during afternoon and early night and slowly during late night and morning, while under 28°C/12°C condition, the fruit growth during the night reduced significantly, indicating that carbon export from leaves was inhibited by low temperature. Verkleij and Baan Hofman-Eijer (1988) reported that under a short-day condition, the accumulation of dry matter and the volume expansion of cucumber fruit were out of phase during a diurnal cycle. Most assimilates were exported to the fruit during the 8 h light period, while about 70% fruit growth occurred during the 16 h dark period. This conclusion was confirmed in our study under a similar condition (Miao et al., 2009).

Branch removing, topping, and fruit thinning are common practices to manipulate the source–sink relationship during cucurbit cultivation. Fruit thinning is usually carried out in the production of cucurbits with large fruits, such as melon, watermelon, and pumpkin, since strong competition exists among fruits in these plants (Long et al., 2004). Besides fruits, the seedling shoot is another strong sink in the cucurbit plant. Thus, lower parts of topping plants always accumulate more assimilates than those of nontopping plants (Lee et al., 2000). Park et al. (2002) demonstrated that compared with one-stem plants, no more than four extra side shoots provided more assimilates to roots, stimulating the root activity and root growth of hydroponically grown cucumber plants. In addition, it has been reported that plants tend to accumulate more assimilates into roots under moderate nutrient deficiency conditions (Ciereszko et al., 1996; Hermans et al., 2006). Although little research is carried out on cucurbit plants in this area, we did observe similar phenomenon during cucurbits cultivation.

### 3.6 PHLOEM UNLOADING

An understanding of the vascular anatomy of the pedicel and fruit is beneficial to unveil the unloading mechanism in cucurbits. The vascular network of cucumber and melon were described by several research groups (Barber, 1909; Judson, 1929; Kanahama and Saito, 1987; Masuko et al., 1989). Basically, there are 10 main bundles in the pedicel. These bundles extend into the fruit, further branch and anastomose and extend inward to the placenta and outward to the epidermis. In addition to the main vascular bundles there is also an anastomosing minor vascular system in the fruit.

α-Galactosidase is the initial enzyme in the metabolic pathway of stachyose and raffinose (Figure 3.2). Plant α-galactosidase can be divided into two groups, acid and alkaline, based on their activity response to pH. There is a negative correlation between the activity of alkaline α-galactosidase and stachyose levels in cucumber pedicels, indicating that the alkaline activity was responsible for stachyose hydrolysis (Pharr and Hubbard, 1994) rather than the acid activity. Irving et al. (1997) also found that alkaline α-galactosidase activity was higher than acid activity in *Cucurbita maxima* fruit at anthesis. Two alkaline α-galactosidase genes were identified in the melon genome, namely, *CmAGA1* (AY114164) and *CmAGA2* (AY114165) (Carmi et al., 2003). *CmAGA2* was relatively specific for stachyose, whereas *CmAGA1* showed significant activity with both stachyose and raffinose. *CmAGA1* enzyme activity increased during the early stages of melon ovary development and fruit set, while *CmAGA2* declined in enzyme activity during this period. From these data, they supposed that *CmAGA1* may play a key role in melon assimilate unloading (Gao et al., 1999; Gao and Schaffer, 1999; Carmi et al., 2003). However, the data mentioned here cannot rule out the role of acid α-galactosidase in RFO catabolism during sink unloading in cucurbit plants.
There are two opinions about where RFOs are metabolized to sucrose when they reach the fruit sink. Some researchers thought that the catabolism of RFOs to sucrose takes place in the pedicel. There are several evidences supporting this view. (1) Little RFOs were detected in the fruits (Pharr et al., 1977; Gross and Pharr, 1982; Handley et al., 1983b; Hughes and Yamaguchi, 1983). (2) Significant activity of enzymes responsible for RFO catabolism, such as α-galactosidase, sucrose synthase (SS, synthetic activity), SPS, and UDP-galactose pyrophosphorylase, existed in the pedicel (Smart and Pharr, 1981; Gross and Pharr, 1982; Burger and Schaffer, 1991). (3) The ratio of RFO to sucrose in the pedicel was lower than that in the pediole and stem, indicating that RFOs were hydrolyzed to sucrose in the pedicel (Okawa et al., 2010).

However, other researchers suggested that RFOs are translocated into the fruit and rapidly metabolized to sucrose then. The evidences supporting this view are as follows: (1) RFOs indeed exist in the cucurbit fruit, although the level is quite low (Hubbard et al., 1989; Chrost and Schmitz, 1997; Irving et al., 1997; Kim et al., 2007; Hu et al., 2009). In addition, exudate from the fruit’s main bundles contained stachyose as the major sugar (Pharr and Hubbard, 1994; Schaffer et al., 1996). (2) Significant activities of alkaline α-galactosidase and other RFO metabolic enzymes were detected in cucurbit fruits (Pharr and Hubbard, 1994; Schaffer et al., 1996; Chrost and Schmitz, 1997; Irving et al., 1997; Gao and Schaffer, 1999; Carmi et al., 2003; Dai et al., 2006). (3) The sugar composition of phloem exudate collected from peduncles cut near the stem or fruit of the melon plant was identical, indicating RFOs are translocated into cucurbit fruits (Chrost and Schmitz, 1997).

Recently, we have studied vascular exudate from cucumber pedicels and fruits. We cut the pedicels and fruits from near the stem end to the fruit end and collected and analyzed exudate from each cut surface (from plant side). The results showed that RFO levels in the exudate gradually decreased from the stem end to the fruit end, indicating RFOs were hydrolyzed throughout the main vasculature along the pedicel and fruit (unpublished date). In addition, in melon pedicels, sucrose and hexoses were prevalent until day 18 after anthesis, while from the 25th day after anthesis, the RFO increased until fruit maturity (Chrost and Schmitz, 1997), suggesting the location of RFO catabolism may change from pedicel to fruit during fruit development.

Hu et al. (2011) reported that the sieve element-companion cell (SE–CC) complex of the main bundles in cucumber fruit is apparently symplasmically restricted and there are cell-wall invertase and sucrose transporter located at the SE–CC complex. The authors concluded that phloem unloading pathway in cucumber fruit is apoplasmic. In addition, the RFOs were absent in the tissue immediately adjacent to the main bundles, indicating the location of RFO metabolism is restricted in the main vasculature area (Schaffer et al., 1996). Cheng et al. (2015) cloned three cucumber hexose transporter genes, among which the protein of \( CsHT3 \) is located at the plasma membrane, and its expression level increased in peduncles and fruit tissues along with cucumber fruit enlargement, suggesting that \( CsHT3 \) probably plays an important role in apoplastic phloem unloading of cucumber fruit. If the apoplastic unloading pathway is true, acid cell-wall α-galactosidases should be responsible for the RFO hydrolysis when they are transferred to the free space around the SE–CC complex. However, there are abundant evidences indicating that alkaline α-galactosidase is important during RFO unloading (Pharr and Hubbard, 1994; Irving et al., 1997; Gao et al., 1999; Gao and Schaffer, 1999; Carmi et al., 2003). Another mystery is why RFOs cannot symplasmically diffuse from main vasculars to those anastomosing minor veins throughout the fruit. In summary, much remains to be studied to elucidate the full mechanism of assimilate unloading in cucurbits.

Little is known about how assimilates are unloaded in seedling shoots, young leaves, and roots of cucurbits. Activity of alkaline α-galactosidase is higher than that of acid α-galactosidase in immature cucumber leaves (Pharr and Sox, 1984). Furthermore, the activity of alkaline α-galactosidases is higher in immature leaves and roots than in mature leaves (Gaudreault and Webb, 1982, 1986). These results indicated that RFO may be translocated to immature leaves or roots symplasmically and then hydrolyzed by alkaline α-galactosidases.
3.7 CARBOHYDRATE METABOLISM IN FRUITS

Fruit size varies significantly among cucurbit plants. The final fruit size depends on how fast and how long it grows. However, Sinnott (1945) thought the size of cucurbit fruit is mainly dependent on the duration of growth; growth rate plays a minor role. The growth pattern of most cucurbit fruits generally follows an S-curve (Sinnott, 1945). Among those that belong in the Cucurbitaceae family, cucumber, melon, watermelon, and pumpkin are especially important for their economic value. As a result, most researches about fruit carbohydrate metabolism of cucurbits are focused on these four species.

3.7.1 Cucumber

Glucose and fructose are main soluble sugars in cucumber fruits (6–12 mg gFW⁻¹), and the level of sucrose is low (about 0.5 mg gFW⁻¹) (Handley et al., 1983b; Schaffer et al., 1987). Although a few RFOs were found in young fruits, little RFOs were detected in mature cucumber fruits (Pharr et al., 1977; Handley et al., 1983b). However, Hu et al. (2009) found that RFOs can be detected in cucumber fruits 20 days after anthesis, which make up 2.51% of total soluble sugars. Similar results were observed by Wang (2014). The different results may be due to the different cultivars they used or different fruit parts they sampled. Handley et al. (1983b) used a pickling cucumber variety, while Hu et al. (2009) and Wang (2014) used North China–type cucumber varieties. Furthermore, fruit tissue containing major veins may have relatively higher RFO levels, as mentioned in Section 3.6. In addition, starch grains in the cucumber mesocarp were observed by Patchareeeya et al. (2011). The content of starch of cucumber fruit ranged from 1 to 7 mg gFW⁻¹ (Schaffer et al., 1987; Hu et al., 2009).

The fluctuation patterns of carbohydrate levels during cucumber fruit development have been studied by several groups. Handley et al. (1983b) and Schaffer et al. (1987) suggested that the contents of glucose, fructose, sucrose, and starch in cucumber fruit almost remained unchanged during the development. However, Hu et al. (2009) reported that contents of glucose and fructose increased in the cucumber fruit from anthesis to 20 days after anthesis, while levels of sucrose, stachyose, raffinose, and starch decreased during this period. The different results may be due to the different varieties they used or different development stages they observed.

Little is known about the diurnal pattern of carbohydrates in cucumber fruits. Jiang (2006) reported that levels of glucose and fructose were low during 10:00–13:00 and had a peak value at 16:00, while levels of sucrose and starch remained stable during a photoperiod. The fluctuation of the hexose level should be caused by the diurnal changes of both assimilate translocation into the fruits and the carbon consumption by fruit respiration and growth and should be affected remarkably by the environment.

Significant acid invertase and SS (cleavage direction) were observed during cucumber fruit development, and the activities of sucrose degrading enzymes were always higher than that of sucrose-synthesizing enzymes in mesocarp tissues (Schaffer et al., 1987; Hu et al., 2009). These data may explain why levels of glucose and fructose are much higher than that of sucrose in cucumber fruits. Activities of raffinose synthase and stachyose synthase were also detected in cucumber fruits (Hu et al., 2009; Sui et al., 2012), indicating there may be basic RFO synthesis in the mesocarp tissues.

The contribution of green cucumber fruit photosynthesis to its own dry matter accumulation cannot be ignored. The quantity of fixed carbon by cucumber fruit was about equal to its respiration consumption (Todd et al., 1961). Marcelis and Baan Hofman-Eijer (1994) reported that photosynthetic contribution of cucumber fruits to their carbon accumulation was about 1%–5%. Obviously, chlorophyll level of the fruit epidermis, fruit shape, and fruit age are major factors impacting the importance of cucumber fruit photosynthesis.

3.7.2 Melon

Melon shows abundant genetic variation in fruit characteristics. Considerable variation in the sugar content and composition in mature fruits was observed (Stepansky et al., 1999). In general, the most
dominant soluble sugars in the fruit of sweet cultivars is sucrose, while glucose and fructose are primary sugars in nonsweet cultivars. One exception is the Soviet cultivar “Kuvsinka,” which belongs to the cantalupensis type, containing 82 mg gFW⁻¹ soluble sugars in the fruit, but the contents of sucrose, glucose, and fructose are 24, 19, and 28 mg gFW⁻¹, respectively (Stepansky et al., 1999). In most types, the content of glucose is always similar to that of fructose. There are also some exceptions. Stepansky et al. (1999) reported that an Indian cultivar that belongs to chito type contains 19.6 mg gFW⁻¹ glucose and 30.2 mg gFW⁻¹ fructose in the fruit. Hubbard et al. (1989) reported that about 0.15 ± 0.06 mg gFW⁻¹ starch was detected in melon fruit. The existence of a small amount of starch in the melon fruit was reported by a few other researches (Schaffer et al., 1987; Combrink et al., 2001). In addition, low concentrations of the RFO and galactose were also detected in melon fruits (Hubbard et al., 1989; Chrost and Schmitz, 1997; Kim et al., 2007). RFOs in the ovary will disappear after development, similar to what happens during cucumber fruit development (Hughes and Yamaguchi, 1983).

In general, soluble sugars were present in greater concentrations in the inner tissues than in the outer tissues in melon fruits (Lingle and Dunlap, 1987; Hubbard et al., 1989; Combrink et al., 2001). The difference in sugar concentration between inner and outer tissues is mainly caused by the concentration difference of sucros rather than those of hexose (Hubbard et al., 1989). The distribution pattern of RFO is similar to that of sucrose (Hubbard et al., 1989). In the other direction, sucrose and total sugar gradients were observed ascending from mesocarp adjacent to pedicle to mesocarp adjacent to umbilicus (Zhang and Li, 2005).

Generally, there are two types of melon: the high sucrose accumulation type and low sucrose accumulation type (Stepansky et al., 1999; Burger et al., 2009). For high sucrose accumulation type, in the early fruit development stage, the concentration of sucrose remains low, while glucose and fructose levels are relatively high. During the later stage, sucrose accumulates quickly, and its level achieves a similar level as hexose in some genotypes, or much higher than hexose levels in other varieties. For low sucrose accumulation type, the concentration of sucrose is maintained at a low level throughout the fruit development (Lingle and Dunlap, 1987; Schaffer et al., 1987; Hubbard et al., 1989; Gao et al., 1999; Zhang and Li, 2005). The gradient of sucrose concentration between the inner and outer fruit tissues is due to the faster rate or longer duration of sucrose accumulation in inner fruit tissues (Lingle and Dunlap, 1987; Hubbard et al., 1989; Wang et al., 2014). For high sucrose accumulation type, the exponential stage of fruit growth is always earlier than sucrose quick accumulation stage, and when the fruit growth slows down in the late stage of the S-curve, sucrose still accumulates rapidly; the time lag between the two periods varies with different varieties (Lingle and Dunlap, 1987; Gao et al., 1999; Villanueva et al., 2004). In some varieties, hexose and starch content remained little changed or slightly increased during fruit development (Lingle and Dunlap, 1987; Schaffer et al., 1987; McCollum et al., 1988, Hubbard et al., 1989; Zhang and Li, 2005). However, in other varieties, their levels gradually decreased with the accumulation of sucrose, suggesting the conversion from hexose and starch to sucrose during this period.

Acid invertase and SPS are two key enzymes regulating sucrose accumulation during melon fruit development (Lingle and Dunlap, 1987; Schaffer et al., 1987; Hubbard et al., 1989; Ranwala et al., 1991; Lee et al., 1997; Lester et al., 2001; Burger and Schaffer, 2007). Sucrose accumulation is always accompanied by a decrease in acid invertase activity and an increase in SPS activity. In addition, the inner tissue has higher SPS activity and low acid invertase activity than the outer tissue in melon fruit, further proving the important role of the two enzymes in determining the sucrose level (Lingle and Dunlap, 1987; Wang et al., 2014). The role of the SS and neutral invertase in sucrose accumulation may be different among varieties and varies according to the state of the plant growth. Hubbard et al. (1989) showed that the activities of SS and neutral invertase are low throughout the fruit growth, indicating minor roles of these two enzymes in sucrose accumulation. Wang et al. (2014) also discovered that there is no significant difference in the two enzyme activities between inner and outer fruit tissues. However, Ranwala et al. (1991) and Lee et al. (1997) found that sucrose accumulation was accompanied by the sharp decline of neutral invertase activity. Schaffer et al. (1987) suggested that SS activities in both synthesis and cleavage direction increased during sucrose accumulation and proposed...
that SS is related to sucrose accumulation. Lingle and Dunlap (1987) found SS activity (cleavage) was higher than that of SPS in the period of sucrose accumulation and the activity in the outer tissue was higher than in the inner tissue. They speculated that the SS may provide the substrate to glycolysis for cell growth. Burger and Schaffer (2007) studied sucrose accumulation in seven melon genotypes and found that SS and neutral invertase activities were positively correlated with sucrose accumulation. They proposed that final sucrose content was determined by the duration of “sucrose accumulation metabolism,” which was characterized by acid invertase activity less than threshold values, together with SPS, SS, and neutral invertase activities higher than threshold level. In addition, cell-wall acid invertase was also detected in the melon fruit (Schaffer et al., 1987; Ranwala et al., 1991). As mentioned in Section 3.6, there may be an apoplastic unloading pathway in the melon fruit. The cell-wall acid invertase may be responsible for the unloading of sucrose and the RFO.

Dai et al. (2011) analyzed the expression levels of 42 carbohydrate metabolism–related genes in melon fruits at different development stages by 454 pyrosequencing technology. They found that the expression levels of CmAIN2 (ICuGI contig ID: MU22596), CmSUS1 (MU21164), CmNIN3 (MU26813), CmHK1 (MU29719), CmFK3 (MU22877), CmAAG1 (MU20940), and CmAAG2 (MU39965) were high in the early stage of fruit development, while significant expressions of CmSPS1 (MU23155), CmSPP1 (MU23296), and CmSUS3 (MU31768) were observed in sucrose accumulation stage. The authors cannot correlate these expression patterns with the specific metabolic processes since most enzymes are encoded by more than one gene, so it is difficult to identify the contribution of each gene to the final activity. In addition, the functions of genes are also regulated at the posttranscription, translation, and posttranslation level, as well as in the presence of inhibitors. There may not be significant correlations between transcription levels and enzyme activities. Therefore, a lot of work needs to be done to clarify the function of these genes in fruit growth and carbohydrate metabolism in melon fruits.

3.7.3 Watermelon

Glucose, fructose, and sucrose are important sugars in watermelon fruits (Kano, 1991; Yativ et al., 2010; Zhang, 2010; Liu et al., 2013). Broad variations in total sugar concentration and the ratio among the three sugars were observed in different genotypes. The total sugar concentration was as high as 100 mg gFW\(^{-1}\) in sweet genotypes but was as low as 10 mg gFW\(^{-1}\) in nonsweet genotypes (Liu et al., 2013). Yativ et al. (2010) suggested that glucose and sucrose levels ranged from 20% to 40%, whereas the fructose levels changed within 30%–50% in the commercial watermelon varieties. Liu et al. (2013) showed that the levels of sucrose and fructose were similar at 35 days after anthesis in a high sugar variety, while Zhang (2010) suggested that sucrose content was higher than hexoses in ripening fruit of an inbred line. Generally, fructose level is always higher than glucose level in mature watermelon fruits (Kano, 1991; Yativ et al., 2010; Zhang, 2010; Liu et al., 2013). Unlike melon, there is no significant correlation between the total sugar concentration and the sucrose concentration in watermelon fruits among different genotypes (Yativ et al., 2010).

The change pattern of sugar levels in watermelon fruits during development was extensively studied in different genotypes. Soluble sugar levels are very low within the first 10 days after anthesis and then fructose and glucose begin to accumulate, while the sucrose level begins to increase rapidly at 18–35 days after anthesis, depending on the cultivars and planting conditions (Elmstrom and Davis, 1981; Kano, 1991; Yativ et al., 2010; Zhang; 2010; Liu et al., 2013). In the latter period of the sucrose accumulation, glucose and fructose levels slightly increase or remain at a stable level in some genotypes (Elmstrom and Davis, 1981; Brown and Summers, 1985; Zhang, 2010) or decrease with the increase of the sucrose level in other genotypes (Elmstrom and Davis, 1981; Kano, 1991; Yativ et al., 2010). For nonsweet genotypes, three sugars are maintained at very low levels during the entire growth period (Liu et al., 2013).

There is an upward gradient of sugar levels from outer tissue to inner tissue in watermelon fruits, both longitudinally and equatorially (Kano, 1991). In the early stage of fruit development (before the
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sucrose accumulation), the sugar gradient was mainly caused by the different distributions of glucose and fructose, while in mature fruit, it was primarily due to the higher sucrose concentration in the inner tissues. There were no concentration gradients of glucose and fructose between the inner and outer tissues at this time, indicating that more hexoses were converted into sucrose in the inner tissue than in the outer tissue. In addition, sugar content was high around the seeds (Kano, 1991).

Comparing with the low-sucrose-accumulation genotypes, lower soluble invertase activity and higher SPS and SS activities were observed in high-sucrose-accumulation genotypes, indicating the important roles of these enzymes in the sucrose accumulation of watermelon fruits (Yativ et al., 2010). The role of SPS in sucrose accumulation of watermelon fruit has also been demonstrated in other studies (Zhang, 2010; Liu et al., 2013). As in the melon, the functions of SS and neutral invertase in sugar accumulation may depend on the variety. In low sugar accumulation varieties, activities of all enzymes were maintained at low levels (Liu et al., 2013). The role of cell-wall invertase in the watermelon fruit development is controversial. Yativ et al. (2010) found that insoluble invertase activity was high and constant throughout fruit development in genotypes accumulating low levels of sucrose, while in genotypes accumulating high levels of sucrose, the activity declined sharply 4 weeks after pollination. However, Liu et al. (2013) showed that insoluble invertase was the only enzyme whose activity was positively correlated to sucrose accumulation during fruit development. Insoluble invertase may be responsive for both sucrose and RFO unloading (Section 3.6; Roitsch and Gonzalez, 2004) and sucrose hydrolysis in the fruit. Thus, the relationship between insoluble invertase and sucrose accumulation depends on how much hexoses from sucrose and RFO apoplastic unloading are transferred into the cell and used to resynthesize sucrose.

3.7.4 Pumpkin

Plants with edible fruits in genus *Cucurbita* are collectively called pumpkin or squash, mainly including *C. pepo*, *C. maxima*, *C. moschata*, and *C. argyrosperma*. Generally, there are two types of squash, summer squash and winter squash. Summer squash is harvested in the immature fruit stage, while winter squash is harvested in the mature fruit stage. Squash fruits contain glucose, fructose, sucrose, RFO, galactose, and starch; the content of these components changes among different types (Terazawa et al., 2011). Glucose and fructose are major soluble sugars in zucchini fruit, whereas galactose, RFO, and sucrose show very low concentrations. Irving et al. (1997) divided the developmental growth pattern of buttercup squash (*C. maxima*) into three phases: (1) early growth, from flowering up to 30 days after flowering; (2) maturation, from 30 days until 60 days after flowering (or harvest); and (3) ripening, from 60 days (or harvest) until about 100 days after flowering. During the early stage, dry matter and starch accumulation are largely completed. Fruits contain significant glucose, fructose, a little RFO, and very low sucrose. During the maturation stage, levels of dry matter and starch remain unchanged, while sucrose begins to accumulate. During ripening, starch is degraded and sucrose continues to accumulate. A similar pattern was observed during fruit development of other varieties of *C. maxima* and *C. moschata* (Tateishi et al., 2004; Sun et al., 2008). In addition, squash fruits contain a number of functional components such as polysaccharides and galactinol (Yang et al., 2008).

The content and property of starch largely determine the edible, storage, and processing quality of squash fruit. The fruit dry matter starch content ranges from 3% to 60% among different types (Stevenson et al., 2005), and the level of amylopectin is usually higher than that of amylose (Nakkanong et al., 2012). Structures and physicochemical properties of the starch from squash fruits were studied by Stevenson et al. (2005) and Zhou et al. (2013). They found that squash starch exhibited the B-type x-ray diffraction pattern with granules at the size of 1–15 μm. Scanning electron micrograph of pumpkin starch revealed that most of the granules of *Cucurbita moschata* starch were polygon-shaped, while those of *C. maxima* starch were oval-shaped (Yin, 2012). In addition, the gelatinization temperature and viscosity also vary among different types (Stevenson et al., 2005; Zhou et al., 2013).
As mentioned earlier, the sucrose concentration in the early development stage of squash fruit is very low. SS and acid invertase are two major enzymes responsible for the catabolism of sucrose translocated into the fruits. Irving et al. (1997) reported that SS activity was higher than that of acid invertase activity in young squash fruit, indicating that SS played a major role in sucrolysis during this period. However, data from Sun et al. (2008) showed that acid invertase was the major contributor toward sucrose decomposition at this stage. These results suggested that sucrose metabolism are different among various types; maybe in genotypes accumulating high concentration of starch, SS is more important, while in genotypes with abundant hexose, acid invertase plays a more significant role. On the other hand, high SPS activity in the late fruit development stage indicates that this enzyme is primarily responsible for sucrose accumulation during this period, although the import from mature leaf and the synthesis by SS in the synthesis direction cannot be ruled out (Irving et al., 1997; Tateishi et al., 2007). Nakkanonga et al. (2012) analyzed the expression levels of genes associated with starch biosynthesis of three genotypes and found that \( AGPase_L \) gene (encoding ADP-glucose pyrophosphorylase) expressed at early development stage and its transcript level was positively correlated with the starch content among different genotypes. A similar relationship was observed between transcript level of \( GBSSI \) (responsible for amylose biosynthesis) and the content of amylose. However, there was no significant correlation between \( SSII \), \( SBEII \), and \( ISAI \) (all involved in amylopectin biosynthesis) expression and the content of amylopectin. Data from Irving et al. (1999) suggested that starch breakdown during the fruit’s late development stage or storage was primarily catalyzed by \( \alpha \)-amylase in buttercup squash.

### 3.8 CARBOHYDRATE METABOLISM DURING SEED DEVELOPMENT

In the early stage of development, cucumber seeds contain relatively high levels of glucose, fructose, and sucrose, while the levels of RFO are quite low (Widders and Kwantes, 1995). As the cucumber seed developed, the contents of glucose and fructose decreased, and sucrose concentration first decreased and then increased at the late maturation stage. RFO concentrations increased throughout the fruit development (Handley et al., 1983b). Widders and Kwantes (1995) found that there was also a small amount of arabinose in cucumber seeds. In addition, the dry weight of seeds was positively related to fruit diameter in the cucumber (Widders and Kwantes, 1995), indicating a continuing assimilate input to cucumber seeds during fruit development. Differing from cucumber seeds, the main storage sugar in mature melon seeds is sucrose (Chrost and Schmitz, 1997).

Handley et al. (1983b) found that during the accumulation of RFO in cucumber seeds, galactinol synthase activity gradually increased. Funiculi from maturing fruit contained a high level of sucrose but little raffinose and stachyose. Holthaus and Schmitz (1991b) also reported that there are significant activities of raffinose synthase and stachyose synthase in the melon seeds. These results indicated that sucrose is transported to the seeds through funiculi and RFOs are biosynthesized in situ in the seeds. However, these evidences cannot exclude the possibility that RFOs are directly transported into the seeds through funiculi (Widders and Kwantes, 1995). In addition, cucumber seeds also contain a certain amount of starch (Handley et al., 1983b), but little is known about how starch is synthesized and accumulated during seed development.

### 3.9 CARBOHYDRATE METABOLISM IN ROOTS

The roots of Cucurbitaceae plants contain glucose, fructose, sucrose, and RFO (Du and Tachibana, 1994; Su et al., 1998). Du and Tachibana (1994) found that high temperature led to a significant increase of RFO in cucumber root. Further studies showed that this increase was not due to the inhibition of \( \alpha \)-galactosidase activity. Su et al. (1998) suggested that soluble sugar significantly increased in roots of \textit{Luffa cylindrica} and \textit{Momordica charantia} under high temperature and waterlogging stress. In addition, some cucurbits, such as \textit{Apodanthera biflora}, \textit{Cucurbita foetidissima}, and \textit{Cucurbita digitata}, store a large amount of starch in fresh roots (Berry et al., 1975; Bemis et al., 1978; Clark et al., 2012).
3.10 RFOs AND STRESS IN CUCURBITS

The roles of RFOs in plant response to abiotic stress have been well established. It is suggested that RFOs act as desiccation protectant with glassy state during seed maturation, osmoprotectant under dehydration stress, and antioxidant to scavenging reactive oxygen species (ElSayed et al., 2014). However, the relationship of RFOs and stress tolerance in RFO-transporting species, such as in cucurbits, has not been well characterized. Meng et al. (2008) reported that sucrose, glucose, fructose, galactose, raffinose, and stachyose were all upregulated by low-temperature treatment in cucumber seedlings. Stachyose synthase activity was higher in the cold-tolerant genotype than that in the cold-sensitive genotype and can be enhanced by abscisic acid (ABA) treatment. In addition, the expression of raffinose synthase gene could be induced by cold stress and ABA. As a result, raffinose synthase activity and raffinose content increased in the cucumber leaves under low temperature (Sui et al., 2012). Dong et al. (2011) also reported the levels of glucose, fructose, raffinose, and stachyose were increased in both leaves and roots by the treatment of salicylic acid, a stress-related phytohormone. These data indicated that RFO may also play a role in enhancing abiotic stress tolerance of cucurbits.

In fact, there was a controversy about the site of RFO synthesis during the 1970s and 1980s. Most researchers think that the minor vein in the leaf is the location of RFO synthesis for transport outside, as mentioned in Section 3.3.2. However, a few RFOs were found to be synthesized in mesophyll cells (Madore and Webb, 1982). Sprenger and Keller (2000) cloned two galactinol synthase genes from Ajuga reptans, a RFO-transporting species, and found that one gene is responsible for synthesis of transport RFO and another for storage RFO. There are four galactinol synthase genes in the cucumber genome; we have studied the expression of these genes under normal and different stress conditions recently, and a similar phenomenon was observed (unpublished). Based on these data, we proposed that in cucurbits, basic synthesis of RFO in mesophyll cells under normal growth conditions is less. The synthesis of RFO would be enhanced when plants experience stress conditions.

3.11 FUTURE PERSPECTIVES

Recent studies have revealed that sugars act as not only carbon sources but also critical signaling molecules affecting most processes in the plant life, including carbohydrate metabolism itself (Smeekens and Hellmann, 2014). Important sugar signaling systems identified recently are hexokinase (HXK) glucose sensor, T6P signal, target of rapamycin kinase system, SNF1-related protein kinase 1 (SnRK1), and C/S1 bZIP transcription factor network. Some key enzymes involved in sucrose and starch metabolism, such as SPS, SS, UDP-glucose pyrophosphorylase, and α-amylase, were proven to be regulated by sugar signals mentioned earlier in sucrose-transporting plants (Rolland et al., 2006; Lastdrager et al., 2014). However, till date little is known about the sugar signal systems in RFO-transporting species, including cucurbits. Further work needs to be done to elucidate if RFO metabolism-related enzymes are also regulated by these signal systems or if there are other molecular mechanisms to control carbohydrate partitioning that are specific to RFO-transporting species.

Compared with loading mechanism, phloem unloading and the role of vasculature within the fruit have attracted less attention. As mentioned in Section 3.6, the exact location of RFO catabolism still remains unclear. Hu et al. (2011) supposed that the RFO transporters should be located at the plasma membrane of companion cells in the cucumber fruit if RFOs are translocated into the fruit and unloaded through an apoplastic pathway. Greuter and Keller (1993) confirmed the existence of an active stachyose transporter on the tonoplast of artichoke (Stachys sieboldii) tuber vacuoles. Schneider and Keller (2009) suggested that raffinose was indeed transported across the chloroplast envelope by a raffinose transporter in the cold-treated common bugle (Ajuga reptans) leaf. However, no RFO transporters have been identified at the molecular level to date in any of the higher plants. Looking for these mysterious transporters is very important to fully clarify RFO metabolism and regulation in cucurbits.
Six putative α-galactosidase genes and four putative galactinol synthase genes were identified in the cucumber genome. It is hard to elucidate the exact function of each form of these enzymes since some forms may play important roles in multiple physiological processes. One example is a cell-wall acid α-galactosidase that may be responsible for both cell-wall polysaccharides hydrolysis and assimilate apoplasmic unloading. Thus, traditional gene knockout technology, such as constitutive RNAi, may cause serious deformity or even plant lethality. Microdissecting technology (Zhang et al., 2010) and chemical-induced RNAi (Guo et al., 2003) may enable us to enhance the research in this area.

We are lucky to live in a postgenomic era. The genome of cucumber, melon, and watermelon were sequenced in recent years (Huang et al., 2009; Garcia-Mas et al., 2012; Guo et al., 2013). The next-generation sequencing technology will accelerate the process of sequencing other cucurbit genomes and resequencing different types of cucumber, melon, and watermelon. The sharing of these massive data sets around the world enables us to develop high-throughput technologies such as transcriptome sequencing and microarray. These powerful genomic tools will provide a global view on the transcript dynamics of source–sink interaction and identify novel regulatory components and target genes and then have major impacts on the production and breeding of cucurbit crops.

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