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**Study of Carbohydrate Metabolism and
Partitioning as Controlling Factors of Fruit
Quality in Sweet Melon**

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FRUIT QUALITY IN SWEET MELON**

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ABSTRACT

Sweetness is the most important factor determining quality in melon fruits and is controlled by a complex of source-sink relationships. These include: metabolism of assimilates and partitioning within the source leaf, subsequent translocation and partitioning among the various sinks and, finally, metabolism at the fruit sink which determines the fate of imported assimilates. The objective of BARD project IS 1063-86 was to study these factors in order to determine the controlling and limiting factors of sugar accumulation in melon fruit.

Throughout most of our studies we have utilized the strategy of genotypic comparisons to critically show differences of physiological significance. This was accomplished by studying a broad spectrum of pure breeding genotypes and also by studying segregating populations from crosses between these genotypes.

1) We have studied the potentially key steps in fruit sucrose accumulation, stachyose synthesis in the source leaves and primary photosynthate production, and found them not to play roles in determining genotypic differences in sucrose accumulation.

2) We have characterized the biochemical trait of sucrose accumulation and have identified three major factors as playing controlling roles: the loss of soluble acid invertase, SPS activity and the duration of the sucrose accumulation period. The development of a reliable assay method for SPS was a major achievement and pointed to the contribution of sucrose synthesis in the fruit to the phenomenon of sucrose accumulation. This also paved the way for additional studies of sucrose metabolism in fruit other than melons, both by our laboratories and others.

3) We have pointed to the potential contribution of stachyose to sucrose metabolism along the translocation pathway, including the peduncle and fruit vasculature and are continuing research in this area.

4) We have laid the groundwork for the ongoing study of the genetic control of the metabolically complex phenomenon of sucrose accumulation in melons. In particular, our studies emphasize the importance of timing of loss of invertase activity in the fruit and ongoing research is focussed on studying the inheritance of this trait by classical genetic means and also on learning the molecular control of this trait.

OBJECTIVES OF BARD GRANT IS 1063-86

The overall objective of the research proposal was to characterize the factors involved in determining sugar content in the melon crop in order to, in the future, develop appropriate agrotechniques for existing cultivars and to identify useful germplasm for the breeding of new varieties with high fruit quality.

The specific objectives of this proposal were to study source and sink carbohydrate metabolism throughout fruit development and, particularly, during the period of sugar accumulation. Specific studies were planned to:

- a) characterize the primary photosynthate production of the fruiting melon plant,
- b) determine the factors controlling partitioning between exportable and non-exportable assimilates in the source leaves,
- c) study the physiology of uptake and compartmentation of sugars in the fruit sink,
- d) assess the contribution of fruit sink metabolism to sugar accumulation in order to relate sink metabolism to the rate and fate of assimilate import into the fruit,
- e) compare source and sink metabolism in sweet melon to that of non-sweet genotypes of *Cucumis melo* in order to focus on the controlling factors that determine sugar accumulation in sweet melon and to reveal existing genetic variability in sugar metabolism in the species.

RESULTS OF BARD GRANT IS 1063-86

Our preliminary study (1) comparing sugar accumulation and invertase activity in a sweet melon cultivar (Galia) and the non-sweet cucumber showed that the key to sugar accumulation lies in the ability to accumulate the disaccharide sucrose. Sucrose accumulating melons were

developmentally characterized by a biphasic accumulation of sucrose in which sucrose accumulation began only when fruit growth was more than half complete. Sucrose accumulation was accompanied by a concomitant loss of the enzyme soluble acid invertase in the the sucrose-accumulating fruit flesh. This study also pointed to differences in the activity of the enzyme sucrose synthase between the two genotypes which it was hypothesized may play a role in the synthesis of sucrose in the fruit tissue.

A. THE ROLE OF ACID INVERTASE

The U.S. team initially expanded the above work and compared sucrose accumulation and metabolism in developing fruits of three genotypes of *Cucumis melo*, two high sugar genotypes (Galia and Noy Yizre'el) and one low sugar genotype (Bird's Nest) which also exhibits sucrose accumulation but to a more modest extent. They observed that the transition to the sucrose accumulation phase in all three genotypes was characterized by the loss of extractable acid invertase activity. The results of this study indicated that the decline in acid invertase activity appears to be a normal function of fruit maturation and is not the primary factor that controls sucrose accumulation. (2)

Further expansion of the research by the Israeli team introduced the variety 'Faqus' which was found to be incapable of sucrose accumulation, as compared to the previously studied genotypes, Bird's Nest (*ca.* 10 mg gfw⁻¹ sucrose) and Noy Yizre'el and Galia (*ca.* 40 mg gfw⁻¹). In Faqus, acid invertase activity remained high, even in the mature fruit. This is the first report of a *Cucumis melo* genotype with a sucrose accumulation pattern similar to *Cucumis sativus* (cucumber) and in which invertase activity remained relatively high throughout fruit development. This allowed us to expand our research to include genetic studies based on crosses between sucrose-accumulating and non-accumulating melon genotypes. In addition, it permitted us to test biochemical-physiological hypotheses using segregating genetic populations, as will be described.

In a developmental study of eight genotypes (seven *C. melo* and one *C. sativus*), exhibiting a wide range of sucrose accumulation from none to high, the contribution of acid invertase was further confirmed. Sucrose accumulation began developmentally in the six accumulating genotypes

only when acid invertase activity declined to below an activity of 10 $\mu\text{moles reducing sugars gfw}^{-1} \text{ hr}^{-1}$. In the two genotypes that did not accumulate sucrose ('Faqus' and 'Delila' cucumber) invertase activity remained above the 10 μmole threshold throughout development. Fig. 1 shows the relationship between acid invertase activity and sucrose content of individual fruit from various developmental stages of the eight genotypes studied. Only in fruit in which the threshold is passed is sucrose accumulated.

FIG. 1: Activity of acid invertase of developing fruit of eight genotypes of *Cucumis* in relationship to sucrose concentration in the fruit flesh (Burger and Schaffer, unpublished)

Crosses between a high sucrose accumulator (Noy Yizre'el) and the non-accumulator 'Faqus', as well as between Noy Yizre'el and the moderate sucrose accumulator Bird's Nest were performed and F_1 , F_2 and backcross ($F_1 \times$ each parent) populations were grown and mature fruit studied. From both groups of crosses (high $\times$ non and high $\times$ low) the results clearly showed that of the three major fruit sugars (sucrose, glucose and fructose) only genetic variation in sucrose content contributed to differences in total sugar content (Fig. 2). Fructose and glucose levels did not vary among the wide gamut of total sugar content observed among the segregating individuals.

FIG. 2: Relationship between individual sugar content and total sugar content in fruit of a segregating population of a high sucrose (Noy Yizre'el) x low sucrose (Bird's Nest) cross. Data from the Noy Yizre'el x Faqus (non-sucrose) is not presented. (Burger and Schaffer, unpublished)

These populations allowed us to test the hypothesis that low acid invertase activity is a requisite for sucrose accumulation. From the results of the high x low (Noy Yizre'el x Bird's Nest) populations there was no segregation for mature fruit invertase activity since both parents are characterized by the developmental loss of invertase activity. This is evidence, though, that the genetic control of developmental loss of invertase activity in these two genotypes is allelic and not controlled by independent genetic factors.

The results of the high x non populations (Noy Yizre'el x Faqus) showed a hyperbolic relationship between invertase activity in the mature fruit and sucrose accumulation. Sucrose accumulation occurs only when invertase activity is below the same threshold of 10 μ mole observed in the genotype developmental studies. Of further significance is the observation that the relationship is not linear, implying that other factors

than final invertase activity are responsible for determining final sucrose content.

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The correlative evidence presented by segregating genetic populations is a more powerful corroboration of the hypothesis set forward than the correlation presented based on individual genotypes. We conclude from the studies described that the loss of invertase is a requisite for sucrose accumulation in melon fruits. However, it is not the causal factor that determines final sucrose content and invertase activity may decline to below threshold levels and yet sucrose concentration will be low.

B. THE ROLE OF SUCROSE PHOSPHATE SYNTHASE

As already mentioned, the analysis of the role of acid invertase in the previous studies points to additional factors that determine sucrose accumulation. The American team developed a sensitive assay for the labile enzyme, sucrose phosphate synthase (SPS), whose role in sucrose synthesis had previously been defined in sucrose synthesizing photosynthetic tissue. Using this assay method, they were able to detect significant activity of SPS in sucrose accumulating fruit tissue for the first time. (2)

In the comparative study between the two high and one low sucrose genotypes (Galia, Noy Yizre'el and Bird's Nest) it was found that the two high sucrose genotypes showed higher SPS activities during their respective sucrose accumulating stages (Fig. 3). In addition, SPS activity rose significantly, concomitant with sucrose accumulation. These results suggested that both acid invertase and SPS are determinants of sucrose accumulation in melon fruit and that the capacity for sucrose synthesis, reflected in the activity of SPS, appears to determine sucrose accumulation.

FIG. 3: SPS activities in three melon genotypes (O: Galia; ○: Noy Yizre'el; □: Bird's Nest. (Hubbard, et al. 1989)

The low sucrose BN showed a significant, albeit low, rise in SPS activity during fruit development. When SPS activity in developing fruit of the non-accumulating 'Faqus' was measured it was found to be consistently low, although measurable with the developed assay, throughout development. The Israeli team then compared SPS activity throughout development in the eight genotypes which ranged from non to high sucrose accumulation. For each of the eight genotypes there was a good correlation between the rise in SPS activity during development and the onset of sucrose accumulation. However, there was not a good correlation between SPS activity and sucrose content in the mature fruit flesh of the eight genotypes.

SPS activity in the eight genotypes throughout development showed an important relationship with sucrose content, as shown in Fig 4.

FIG. 4: Relationship between SPS activity and sucrose concentration in developing fruit of eight *Cucumis* genotypes (Burger and Schaffer, unpublished)

Two conclusions may be drawn from this relationship. The first is that sucrose accumulation to any significant extent (over 10 mg/gfw) occurs only in the presence of a threshold value of SPS activity. The corollary conclusion is that there is no correlation between SPS activity above this threshold value and sucrose content in the fruit flesh.

Additional supporting evidence for these conclusions was obtained from the study of SPS activities in mature fruit of the segregating populations of the NY x Faqus cross. While correlatory studies of segregating populations provide evidence not provided by correlatory studies of discrete genotypes, an inherent disadvantage of such populations is that they cannot be studied developmentally due to the limited number of fruit per plant. The results of this study (fig. 5) showed a poor correlation between SPS activity and sucrose content in mature ripe fruit, providing further evidence that factors other than SPS activity *per se* determine sucrose accumulation in melon fruit.

FIG. 5: Relationship between SPS activity and sucrose content in mature fruit of a segregating population of Noy Yizre'el x Faqus. (Burger and Schaffer, unpublished)

To be sure of these negative results the correlation between SPS activity and sucrose content in an additional NY x Faqus backcross population (F1 x NY) was studied. In this population there is an expected segregation of sucrose accumulators. However, this additional study confirmed the lack of strong correlation between final sucrose content and SPS activity in the mature fruit.

C. ROLES OF SUCROSE SYNTHASE, ALKALINE INVERTASE

In the initial study (1) a rise in sucrose synthase activity was observed in developing Galia melons and it was hypothesized that this enzyme may play a synthetic role in developing sucrose accumulating fruit. However, in the study comparing Galia, Noy Yizre'el and Bird's Nest (2), sucrose synthase activity was low and there were no apparent changes in activity that were associated with sucrose accumulation. A similar conclusion was reached with regard to alkaline invertase, whose activity is sometimes positively correlated with sucrose accumulation in plant tissues (2).

In the comparison of the eight genotypes there was similarly no correlation between either of these two enzyme activities and sucrose content. However, in those genotypes in which acid invertase activity declines alkaline invertase activity is clearly observable and it becomes the major invertase present.

TABLE 1: ALKALINE INVERTASE IN SUCROSE ACCUMULATING MELON FRUIT. A HIGH RATIO OF ACTIVITY, pH 7 TO pH 5 IS INDICATIVE OF ALKALINE INVERTASE ACTIVITY. (Burger and Schaffer, unpublished)

<u>GENOTYPE</u>	<u>RATIO OF ACTIVITY, pH 7 TO pH 5</u>	
	<u>IMMATURE FRUIT</u>	<u>MATURE FRUIT</u>
FAQUS	0.4	0.4
NOY YIZRE'EL	0.4	2.1

Although there does not seem to be, based on the above studies, good indication that either sucrose synthase or alkaline invertase play roles in determining sucrose accumulation in melon fruit tissue, the evidence from the segregating populations of NY x Faqus indicate otherwise. SS activity in Faqus was low throughout development as compared to NY. The segregating F₂ population indicated a linear correlation between SS activity and sucrose content in the ripe fruit. With regard to alkaline invertase, data from the segregating populations did not contradict the lack of correlation observed in the previously mentioned studies.

D. PHYSIOLOGICAL-BIOCHEMICAL MECHANISMS OF SUCROSE ACCUMULATION

Based primarily on the results of the study comparing the three genotypes, Galia, Noy Yizre'el and Bird's Nest, a simple arithmetic scheme which calculated the difference in activity of sucrose synthesizing enzyme (SPS) and the potential sucrose degrading enzymes (sucrose synthase, acid invertase and alkaline invertase) was found to correlate well with sucrose content of the fruit tissue (Fig 6). Sucrose accumulation

occurred only after the enzymatic capacity to synthesize sucrose exceeded the enzymatic capacity to degrade sucrose. 12

Fig. 6: Relationship between sucrose concentration and the difference between activities of SPS and the potential sucrose degrading enzymes. (from Hubbard et al., 1989)

Further analysis of the results of the enzyme activities from the expanded study of the eight genotypes pointed to time as a controlling factor in sucrose accumulation. For example, one of the eight genotypes studied developmentally, P221, with a low sucrose content (10 mg gfw) exhibited the characteristic loss of invertase activity and rise in SPS activity to levels similar to some of the high sucrose genotypes (Galia and Krymka). The striking difference between the development of these genotypes was the number of days from the period that the invertase activity declined to below threshold levels until fruit abscission. Since sucrose accumulation is a developmental, additive process, the longer the fruit remains attached to the plant from the onset of potential sucrose accumulation, the more sucrose it will accumulate.

A simple mathematic calculation which takes into consideration SPS activity during the maturing phase of fruit development, multiplied by the

number of days from the loss of invertase activity until abscission, was found to account for sucrose content of the mature fruit in seven of the eight genotypes studied (Fig. 7).

Fig. 7: Correlation between sucrose content and SPS activity during the maturing phase of fruit development, multiplied by the number of days from the loss of invertase activity until abscission. (Burger, Petrovka and Schaffer, unpublished)

Accordingly, we view the start of potential sucrose accumulation as from the time of loss of invertase activity. However, as mentioned previously, this alone is not sufficient to insure sucrose accumulation; rather, it merely permits the process. Two of the major factors of fruit sugar metabolism that will determine final sucrose content are the sucrose synthetic capability of the fruit tissue (SPS) and the time period that sucrose accumulation takes place.

This scheme points to the significance of the timing of the loss of invertase activity in determining sucrose accumulation. The eight genotypes studied exhibit the range of genetic variability for developmental loss of invertase activity and show the potential for manipulating the timing of loss of activity by classical breeding methods. Further studies are presently being carried out in this direction, as well in determining the molecular control of the loss of activity.

E. ENVIRONMENTAL CONDITIONS DETERMINING PERIOD OF FRUIT DEVELOPMENT AND SUCROSE ACCUMULATION

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The significance of the factor of fruit development time period on sucrose accumulation was tested in two studies. In the first, the effect of night temperature (12° and 18° minimum) on fruit development from the period of anthesis until abscission was correlated with final sucrose content. Low night temperatures (12°) in a greenhouse grown crop in the winter extended the period of fruit development to 75 days from anthesis to abscission, as compared to 65 days for melons grown under high night temperatures. The low night temperature melons had a higher total sugar content which was solely due to an increased sucrose content.

TABLE 2: EFFECT OF NIGHT TEMPERATURE ON MELON FRUIT DEVELOPMENT

PARAMETER	NIGHT TEMPERATURE	
	12°	18°
DAYS FROM ANTHESIS TO MATURITY	74	64
SUCROSE (MG/GFW)	36	24
GLUCOSE (MG/GFW)	15	16
FRUCTOSE (MG/GFW)	17	16

Although invertase activity was not measured developmentally we explain these results as at least partially due to the additional days that the fruit were able to accumulate sucrose. Further studies will follow the developmental changes in melon fruit sucrose metabolism in response to night temperatures in order to ascertain whether, in fact, the increase in sucrose content is due to a lengthened sucrose accumulation period.

In the second study we studied the effect of growing seasons (summer and winter, greenhouse grown) on the development of three genotypes

representing high and low sucrose accumulators (Noy Yizre'el and Bird's Nest, respectively). Summer grown melon fruit develop more quickly than winter grown fruit, presumably due largely to higher day, as well as night, temperatures. We hypothesized that sucrose content would be modified for each genotype depending on the effect of growing season on the timing of loss of invertase for that genotype and the subsequent remaining period from loss of invertase activity to abscission.

The results of this study showed that the longer growing season, from anthesis to abscission, was due to a lengthening specifically of the fruit development period prior to the onset of sucrose accumulation. The period from the loss of invertase activity until abscission remained the same for both genotypes studied (*ca* 12 days). In this study sucrose concentration (mg/gfw) was also similar whether fruit were grown in summer or winter. However, considering that the summer grown fruit were larger (approx. 1300 gram vs. 900 gram for NY and 1200 gram vs. 400 gram for Bird's Nest), the total photosynthate imported into the fruit during the sucrose accumulation period was higher in the summer grown, as compared to the winter grown, fruit. This is presumably due the higher primary photosynthate production during the summer because of higher irradiance and longer days.

F. SUCROSE METABOLISM IN THE FRUIT PEDUNCLES

Although the cucurbits translocate primarily the tetrasaccharide stachyose we began our research with the working hypothesis that the sugar that fruit mesophyll cells are exposed to is sucrose. This was based on the observations that stachyose and raffinose are present in only low concentrations in fruit mesophyll tissue (Fig. 8), which was substantiated in our studies with the varieties mentioned (3). In addition, previous work with cucumbers (4,5) had suggested that the stachyose to sucrose conversion may occur in the fruit peduncle. In that work sucrose synthase was implicated as the enzyme catalyzing the final step of sucrose synthesis since SPS activity was not observed.

FIG. 8: The ratio of sucrose to galactosyl-sucrose (stachyose and raffinose) in different portions of the source to sink pathway of sucrose accumulating melon plants (Schaffer et al., unpublished)

In the present study we observed, for the first time, high SPS activities, in addition to sucrose synthase activity, in peduncle tissue of the sucrose accumulating genotype *Galia* (6). This was presumably made possible by using the improved assay developed (2). A developmental study showed that both enzymes increased in activity, concomitant with the rise in sucrose content in the fruit flesh (6). This led us to hypothesize that sucrose synthesizing capability in the peduncle may play a controlling role in determining fruit sucrose accumulation.

The SPS and sucrose synthase activities in the peduncles of the eight genotypes during development were measured (6). The results of these studies showed a good correlation between the activities in the peduncles of mature fruit and the sucrose content of the fruit flesh. (Fig. 9). In addition, the phenomenon of increasing SPS and sucrose synthase activity concomitant with sucrose accumulation was observed in some, though not all, of the sucrose accumulating genotypes.

FIG. 9: Correlation between sucrose synthesizing enzyme activities (SPS and sucrose synthase) in the mature peduncle and total soluble solids content in the fruit tissue of eight *Cucumis* genotypes differing in sucrose accumulation (Burger and Schaffer, 1990).

The activities of these enzymes were measured in portions of dissected peduncle tissue in order to determine the localization of these enzymes. The peduncle vasculature system (which included portions of adjacent tissue) was found to be particularly enriched in SPS and sucrose synthase activity. In addition, the vasculature tissue was also enriched in alkaline invertase activity, while the tissue exterior to the vasculature tissue was enriched in acid invertase activity.

Based on these results we suggested that these enzyme activities may play a role in the control of photosynthate import into the fruit, perhaps by contributing to the maintenance of a strong stachyose gradient from the source leaf to the fruit sink. The initial enzyme of stachyose catabolism, alpha galactosidase, which hydrolyzes stachyose to sucrose plus two galactose moieties, was also found to be active in peduncle tissue. Accordingly, sucrose synthesis would utilize and salvage these galactose moieties in a resynthesis scheme (Fig. 10) which consists of hexokinase, UDPgal pyrophosphorylase, UDPgal-4'-epimerase and subsequently either SPS or sucrose synthase.

FIG. 10: Metabolic scheme for synthesis of sucrose from stachyose in melon fruit (from Hubbard, et al., 1989)

In order to test that stachyose to sucrose transformation actually occurs in the peduncle tissue and that sucrose is the sugar that enters the fruit we collected the translocation sap from the sepal and petal vasculature of the fruit tissue and analyzed the sugars by HPLC. The distal three-quarters of sucrose accumulating fruit were cut and removed, leaving the proximal quarter of the fruit still attached to the plant. Droplets exuded from both the internal and external phloem of the sepal and petal vasculature tissue (near the outer perimeter of the fruit) and could be collected by micropipette. After precipitation of the protein in 80% ethanol the sugars were analyzed and were found to contain almost exclusively stachyose, indicating that in spite of the high sucrose synthesizing enzyme activities in the peduncle vasculature, the sugar continuing in the vascular pathway into the fruit is still stachyose.

We then measured SPS and sucrose synthase activities in different portions of the fruit tissue in order to determine if the fruit sepal and

petal vascular tissue is particularly enriched in these activities. Initially we observed that outer tissue contained lower activities of SPS than inner tissues, in correlation with the sucrose gradient found in the fruit flesh. (2) However, we found that the tissue along the fruit circumference which contained the sepal and petal vasculature was indeed enriched in SPS and sucrose synthase activities, as compared to the inner fruit flesh and particularly the outer fruit flesh without the major vasculature bundles.

TABLE 3: SPS AND SUCROSE SYNTHASE ACTIVITIES IN DIFFERENT PORTIONS OF SUCROSE ACCUMULATING MELON FRUIT

TISSUE	SPS	SS
	(umol suc / gfw / hr)	
enriched vascular bundles (v.b.)	28.6	10.1
external mesocarp tissue without v.b.	8.9	3.0
middle mesocarp tissue	15.4	5.7
inner mesocarp tissue	18.2	5.7

Although stachyose was the primary translocated sugar in the vasculature there was little observed in the fruit tissue itself, suggesting that stachyose is rapidly metabolized in the fruit. The observation that the sucrose synthesizing enzyme activities are enriched in the vasculature tissue, both in the peduncle and in the fruit, suggests the possibility that the stachyose to sucrose metabolic pathway may be part of the stachyose unloading system from the vascular path and would explain the absence of significant amounts of stachyose in the fruit tissue.

Accordingly, based on these accumulated results we hypothesize that translocated stachyose is metabolized to sucrose along the vasculature pathway, beginning from the peduncle and continuing into the fruit. This metabolism may be part of the unloading process. Such a scheme would indicate that the sugar that the accumulating mesophyll cells are exposed

is sucrose, consistent with our observations that tissue not enriched in major vasculature bundles contains insignificant amounts of stachyose.

Further studies will attempt to more definitively localize stachyose to sucrose metabolism in the fruit tissue by immunolocalization of the key enzymes potentially involved in the pathway (alpha-galactosidase, SPS and sucrose synthase). In this respect, it has recently been reported (6) that sucrose synthase showed companion cell specificity in the unloading zone of citrus fruit.

G. SUCROSE UPTAKE AND COMPARTMENTATION

The results of our research has clearly indicated that the loss of soluble acid invertase activity in developing *Cucumis melo* fruit is a prerequisite for sucrose accumulation. Our working hypothesis, at the time of the study was that sucrose translocated into the fruit, undergoes phloem unloading and subsequently uptake into the fruit mesocarp cells. In the absence of soluble acid invertase activity in the vacuole the sucrose does not undergo further metabolism and is stored intact.

We were therefor interested in characterizing sucrose uptake in melon fruit mesocarp tissue. As already described, although the Cucurbitaceae translocate primarily galactosyl-sucrose (stachyose) the sugar that the sucrose accumulating mesocarp cells are exposed to is most likely sucrose. Even considering that stachyose is the major sugar in fruit vascular bundles it is found in only small amounts in the mesocarp tissue. Therefore, it is important to understand the uptake and compartmentation of sucrose in melon fruit tissue.

One cm diameter discs approximately 3 mm thick were prepared from melon mesocarp tissue from fruit of various stages of development. Discs were preincubated in isoosmotic buffer (MES pH5.5) using mannitol as osmoticum, for one hour. Afterward discs were placed in isoosmotic solutions with various concentrations of either non-radioactive or ^{14}C -sucrose. After two hours of uptake discs were washed for two seconds to remove cut-end sugars and tissue sugars were extracted in 80% ethanol and separated by HPLC.

There is a slight saturable component at low concentrations (below 10 mM) but at higher concentrations sucrose uptake is linear with concentration. (FIG. 11). Uptake was not significantly inhibited by either PCMBS or CCCP.

FIG. 11: Sucrose uptake in sucrose accumulating melon fruit mesocarp discs. (Schaffer, et al., unpublished)

The linear uptake pattern was observed to concentrations of 500 mM sucrose. By using the technique of efflux kinetics for compartmental analysis of sugars we observed that in isoosmotic solution generally over 80% of the tissue sucrose is compartmentalized in a non-available compartment which we assume to be the vacuole. Lowering the osmoticum concentration or placing the tissue in 20% DMSO caused rapid efflux of this compartmentalized sucrose. Based on the efflux kinetics we utilized a two hour efflux of sugars to distinguish between

compartmentalized (vacuolar) and non-compartmentalized (presumably apoplast and cytoplasm) sugar.

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Just as we observed that sucrose uptake into melon mesocarp discs was linear up to 500 mM, so too was the amount of compartmentalized sucrose taken up. When we calculate the percent of taken up sucrose which was subsequently compartmentalized we find that independent of sucrose concentration, approximately 80% of the incorporated sucrose was compartmentalized in the concentration range of 125-500 mM external sucrose concentration (Fig. 12).

FIG. 12: Effect of external sucrose concentration on percent of compartmentalized sucrose of total sucrose incorporated into melon mesocarp discs. Total sucrose incorporated was linear with sucrose concentration. (Schaffer, et al., unpublished)

We calculated approximate compartmental concentrations of sucrose, taking into account the content of sucrose from the compartmentational analysis - efflux kinetics and assuming compartment volumes of apoplast, cytoplasm and vacuole of 10,10 and 80%, respectively. Calculation of concentrations, based on these volumes, do not indicate any significant uphill gradient of sucrose from the apoplast and cytoplasm to the vacuole.

Sugars of the sucrose accumulating Noy Yizre'el at various stages of development, from the pre-sucrose accumulation stage until the mature, ripe stage, were studied by compartmental analysis. In all cases sucrose was compartmentalized in approximately equal amounts from 70-90%, depending on the experiment, and no trend was observed. Furthermore, increases in tissue sucrose content with development were due to higher concentrations in all three compartments and no significant development of an uphill gradient was evident.

These results indicate that at physiological concentrations of sucrose uptake is linear and does not appear to be energy dependant. A significant uphill gradient of sucrose is not evident and as the total concentration increases so do the concentrations of the individual compartments. On the other hand, sugars which are compartmentalized in the vacuole diffuse out slowly, as compared to the speed of uptake and compartmentation, which indicates a passive, facilitated diffusion pathway of sucrose uptake. the conclusion from these studies is that sucrose uptake in melon fruit tissue will be primarily determined by sucrose supply to the apoplast.

In addition to the uptake and compartmentation of sucrose we tested the possibility that mesocarp tissue can synthesize sucrose *in vivo*. As we described, sucrose synthesis metabolizing enzymology (SPS and sucrose synthase) is present in sucrose accumulating tissue. We incubated tissue discs for two hours with ^{14}C -fructose and assumed that it could serve as substrate for sucrose synthase or SPS, after phosphorylation via fructokinase/hexokinase. After two hours incubation we observed that in addition to the incorporated ^{14}C -fructose there was both ^{14}C -sucrose and ^{14}C -glucose. After efflux kinetics of the tissue discs we found that in mature sucrose accumulating fruit in which soluble acid invertase activity had declined, all the synthesized sucrose was compartmentalized in the vacuole, in addition to some of the radioactive glucose and fructose. In immature fruit in which soluble acid invertase activity is high there was little sucrose remaining in the vacuole while vacuolar fructose and glucose levels were higher.

These results indicate that sucrose may be synthesized *in vivo*, either via sucrose synthase or SPS, and is subsequently, or concomittantly, compartmentalized in the vacuole. Even in immature fruit sucrose synthesis may occur but the sucrose synthesized will be hydrolyzed by vacuolar invertase. In

mature fruit the sucrose will be stored in the absence of vacuolar invertase. ²⁴ The source of the radioactive glucose may be from the isomerization via phosphoglucose isomerase from fructose-6-phosphate and subsequent phosphatase action. Alternatively, it may derive from synthesized sucrose, after isomerization of the hexose-6-phosphate and subsequent metabolism of glucose-6-phosphate to UDP-glucose, followed by sucrose synthesis with the incorporation of a radioactive glucose moiety.

H. SCHEME OF SUCROSE METABOLISM IN MELON FRUITS

The results of our studies to date, are consistent with the following scheme of sucrose accumulation in mesocarp cells of developing melon fruit, beginning from the point of photoassimilate arrival at the sink.

Although it is clear that stachyose is the major sugar translocated from the source leaves, it was not clear where the stachyose to sucrose transition occurs along the translocation pathway. Initially, based on the high activities of SPS in the mesocarp tissue we felt that the transition most likely occurs in the fruit mesocarp tissue itself (2). However, the lack of significant amounts of stachyose in this tissue, although possibly reflecting rapid metabolism, point to metabolism at an earlier point in transport. The recent results showing relatively high SPS and sucrose synthase activities in the vascular tissue of the fruit and peduncle, which have high stachyose and low sucrose concentrations, strongly suggests that the initial stachyose to sucrose metabolic transition occurs in that tissue.

These metabolic steps may occur at the point of unloading and, if so, suggest a metabolic controlling point for translocate unloading into the fruit tissue by the stachyose to sucrose synthetic pathway. Evidence suggestive to this is the strong correlation between SPS and sucrose synthase activities in the peduncle with sucrose content in the flesh. Although we have not yet studied this we hypothesize that a similar correlation will exist between these enzyme activities in the fruit vasculature and fruit sucrose content.

The significant conclusion from this work is that the sugar that bathes the sucrose accumulating mesocarp cells is most likely sucrose. Our study of sucrose uptake kinetics into melon fruit tissue discs shows that there

may be a small saturable component of uptake at low concentrations but at physiological concentrations (above 10 mM) uptake is linear, unaffected by metabolic inhibitors of uptake and is likely via facilitated diffusion. Therefore, sucrose accumulation can be expected to be dependent on sucrose concentration reaching the mesocarp cells.

The metabolic fate of the imported sucrose reaching the mesocarp cells is perhaps the most important factor determining sucrose accumulation and is determined by events occurring in the sink tissue. From our combined studies we conclude that the key factor that determines whether sucrose will be accumulated or not is the developmental loss of soluble acid invertase activity. However, the loss of activity is not causal, but rather permissive, to sucrose accumulation.

The results of our studies also show a role for SPS activity in sucrose accumulation. From some of our studies (for example, the study correlating SPS and SS activities in peduncles of the developing eight genotypes with flesh sucrose content), there is indication that absolute SPS activity is correlatory with final sucrose content. However, from the bulk of the results there does not appear to be a simple correlatory relationship between the two. Rather, we conclude that SPS activity, at least above a certain threshold value (10 μ moles sucrose/gfw/hr) is requisite for, but, again, does not assure sucrose accumulation.

If SPS is indeed requisite for sucrose accumulation, and if sucrose is indeed the sugar that bathes the accumulating mesocarp cells, this would imply a physiological-metabolic pathway that would presumably include hydrolysis and subsequent resynthesis of imported sucrose. We have reconfirmed our earlier report (1) that in sucrose accumulating melon tissue insoluble (presumably cell wall bound) acid invertase activity remains active even after the soluble activity is developmentally lost. This constant activity of insoluble invertase could account for apoplastic hydrolysis. Alternatively, the cytoplasmic enzymes alkaline invertase and sucrose synthase could account for sucrose degradation and the positive correlation between the activities of these enzymes and sucrose content in the segregating NY x Faqus population (section C) may be explained by such a role. Accordingly, the role of SPS resynthesis could be to increase the rate of sucrose accumulation by maintaining a hexose gradient and as such, SPS may play a role in determining sink activity. An alternative

role for SPS resynthesis of sucrose is as part of a salvage mechanism for the degradation products of sucrose diffusion out of the vacuole to the cytoplasm or apoplast and subsequent breakdown by the invertases or sucrose synthase.

The metabolic cost for the resynthesis scheme will depend on whether the invertases or sucrose synthase carries out the hydrolysis/cleavage of sucrose. In the case of the latter only one ATP per molecule sucrose resynthesized is required for the fructokinase/hexokinase phosphorylation of fructose. In the case that the invertases are responsible for hydrolysis the energy cost is significantly higher, requiring $2 \text{ ATP} + 1 \text{ UTP} \rightarrow 2 \text{ ADP} + 1 \text{ UDP} + \text{PPi}$ for the two kinase reactions and the UDPglucose pyrophosphorylase reaction to produce UDP glucose for the synthesis of sucrose *via* SPS, and one less kinase reaction if synthesis is *via* sucrose synthase.

Whether sucrose is accumulated intact or undergoes hydrolysis-resynthesis, accumulation can proceed only in the absence of soluble invertase activity. Even in the absence of a strong uphill gradient and exclusive compartmentation of sucrose in the vacuole, the bulk of the sucrose is stored in the vacuole merely by virtue of the relative volume which the vacuole accounts for. Therefore, vacuolar sucrose accumulation is irreconcilable with vacuolar invertase activity.

The metabolic and physiological processes described account for the phenomenon of sucrose accumulation. However, the phenomenon *per se* does not account for the amount of sucrose accumulated, which is an additive process determined by the rate of supply of photoassimilate from the source leaves and by the period of time that the accumulation phenomenon occurs. The contribution of the time factor has been discussed earlier. The role of photoassimilate supply in determining sucrose accumulation was also studied.

I. THE ROLE OF PHOTOASSIMILATE SUPPLY AND ENZYMOLOGY OF PARTITIONING

Since melon fruit lack a stored starch reserve they therefore depend on translocated photoassimilate from the leaf canopy for sugar accumulation during ripening. Therefore we have addressed the question of the

potential role of the strength of the photosynthetic canopy as a determining factor in the accumulation of sucrose within the fruit (8).

The low sucrose accumulating genotype 'Bird's Nest' was observed to have a lower photosynthetic rate ($9 \text{ mg CO}_2 / \text{dm/hr}$) than the high sucrose accumulating 'Noy Yizre'el ($24 \text{ mg CO}_2 / \text{dm/hr}$). On a per plant basis this translated to a primary photoassimilate production of 835 and 1485 mg CO_2 per hour for BN and NY, respectively. Because of these large differences in canopy photosynthesis during the period of fruit sweetening an experiment was designed to determine if limited primary photoassimilate production is the controlling factor in sucrose accumulation. To do this the photosynthetic canopy of NY was reduced during the period of sucrose accumulation so that its primary photoassimilate production approximated that of BN and we tested whether the limited photosynthetic canopy of NY would result in fruit with the sucrose content similar to the low sucrose BN.

The NY plants were grown in a greenhouse and after 24 days after anthesis three treatments were initiated: control, 50% defoliation (every other leaf removed) and 100% defoliation. Fruits were harvested at four day intervals until 52 days after anthesis. Fruits from control plants accumulated sucrose to a concentration of 58 mg/gfw . Removal of 50% foliage resulted in only a small decrease in sucrose content, to 46 mg/gfw , while totally defoliated plants expectedly had only 13 mg/gfw . The reduction in sucrose due to defoliation was associated with reduction of SPS activity in the fruit flesh, while the developmental loss of acid invertase activity was unaffected (Fig. 13).

Fig.13: Effect of leaf removal on sugar and sucrose metabolizing enzyme activities in developing Noy Yizre'el fruit (Hubbard et al., 1990)

There was no affect of 50% defoliation on leaf photosynthetic rates and therefore the 50% defoliation treatment was effective in reducing the canopy photosynthesis of NY to levels typical of BN. However, this reduction in primary photosynthate production did not result in the production of fruit with lower sucrose content characteristic of BN. The conclusion from this study is that insufficient assimilate supply is is not a factor responsible for genotypic differences in sucrose accumulation. However, it is obviously a factor controlling sucrose accumulation within a genotype and factors which will significantly impact on primary photosynthate production, such as significant defoliation, will lower sucrose accumulation in melon fruit.

As described, the galactosyl derivatives of sucrose, such as stachyose, are the primary transport metabolites of the cucurbits. As such the synthesis of stachyose is potentially an important control point of photosynthate partitioning between storage and transport forms in source leaves. Stachyose synthesis involves the transfer of the galactosyl moiety of galactinol to the acceptor raffinose and is catalyzed by the enzyme stachyose synthase, a galactosyltransferase. Its formation is a component of the raffinose saccharide biosynthetic pathway.

Stachyose synthase was isolated from mature leaves of young non-fruitle plants of NY, BN and also cucumber (9). The objective was to characterize the partially purified source leaf enzyme with respect to kinetic properties and potential metabolite effectors in order to identify

metabolic differences which might correlate with the genotypic differences in sink tissue. The conclusion of this study, described in detail in (9) is that there is no evidence for differential regulatory properties for source leaf stachyose synthase among the genotypes studied. The extractable enzyme from sweet and non-sweet melon genotypes during vegetative growth exhibited functionally similar kinetic properties even though the genotypes differ significantly in sink metabolism.

TABLE 3: Km values of stachyose synthase from different *Cucumis* genotypes

ENZYME	Km RAFFINOSE	Km GALACTINOL
CUCUMBER	15.3 mM	4.6 mM
BIRD'S NEST	15.0 mM	2.4 mM
NOY YIZRE'EL	15.0 mM	3.0 mM

It may be that metabolic differences in the source tissue between the genotypes becomes evident only under fruiting conditions, analogous to the developmental differences in fruit invertase activity. However, these results, together with the previously described study of primary photosynthate production, do not point to the source leaf as a controlling factor of genotypic differences in fruit sucrose accumulation.

M. THE EFFECT OF ROOT FLOODING ON SUCROSE ACCUMULATION IN MELON FRUITS

As mentioned, factors affecting photosynthate supply to the fruit strongly influence fruit sweetening. Studies were carried out to evaluate the whole plant response to root flooding in order to determine whether excess soil moisture can lead to intra-plant competition and alterations in the partitioning of assimilates to fruit (10).

Flooding the roots of NY plants for four days reduced sucrose accumulation by 36% in the inner mesocarp tissue from an apparent failure to accumulate sucrose. There were no alterations in activities of

the sucrose metabolizing enzymes, indicating that reduced synthesis or excess degradation was not responsible for the decreased sucrose content. There were also no differences in the apparent kinetic or regulatory properties of fruit SPS as a result of the flooding treatment.

Increased glucose consumption by anaerobic roots could represent a competing sink for translocated sugars. This study showed a doubling in glucose consumption by the flooded roots due to increased glycolytic activity (Pasteur effect). The conclusion of this study is that the increased demand for carbohydrates by anaerobic roots may lead to intra-plant competition and subsequently to a reduction in translocated carbohydrates available for sucrose accumulation by the fruit.

In a follow up study (11) it was also shown that there exists genotypic variation in the response to root flooding. Young plants of four genotypes (NY , BN and two additional sucrose accumulating genotypes, Ambrosia and Tam Uvalde) were subjected to five days flooding. In NY and BN primary photosynthate production was significantly decreased. Studies of leaf carbohydrate metabolism and translocation showed that indeed there is genotypic variation in the response to flooding. It may be assumed that in mature fruiting plants similar genotypic variation will exist for the extent that fruit sucrose accumulation will be affected by flooding.

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DESCRIPTION OF COOPERATION

The USA and Israeli labs have focussed on some similar lines of investigation which has at times resulted in similar but not identical results. The two labs have exchanged procedures and research was coordinated so that similar genotypes were studied in both countries. Research results were communicated frequently and such communication formed the basis for the research plans of both groups.

Cooperation between the two groups was strengthened by Dr. Schaffer's visit to North Carolina in 1988 and by his meeting with the USA research group during the American Society of Plant Physiologists conference in 1989. During both meetings data from each laboratory was analyzed, techniques were shared and research goals were defined.

RESEARCH ACHIEVEMENTS WITH RESPECT TO ORIGINAL OBJECTIVES

We have dealt with and accomplished, to varying degrees, all of the original objectives of the BARD proposal.

Throughout most of our studies we have utilized the strategy of genotypic comparisons to critically show differences of physiological significance. Specifically, we have

- 1- Studied the potentially key steps in fruit sucrose accumulation, stachyose synthesis in the source leaves and primary photosynthate production, and found them not to play roles in determining genotypic differences in sucrose accumulation.
- 2) We have characterized the biochemical trait of sucrose accumulation and have identified three major factors as playing controlling roles: the loss of soluble acid invertase, SPS activity and the duration of the sucrose accumulation period. The development of a reliable assay method for SPS was a major achievement and pointed to the contribution of sucrose synthesis in the fruit to the phenomenon of sucrose accumulation. This also paved the way for additional studies of sucrose metabolism in fruit other than melons, both by our laboratories and others.
- 3) We have pointed to the potential contribution of stachyose to sucrose metabolism along the translocation pathway and are continuing research in this area.

4) We have laid the groundwork for the study of the genetic control of the metabolically complex phenomenon of sucrose accumulation in melons. In particular, our studies emphasize the importance of timing of loss of invertase activity in the fruit and ongoing research is focussed on studying the inheritance of this trait by classical genetic means and also on learning the molecular control of this trait. 33

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