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Aspects of Sugar Metabolism in Melon Fruit as Determinants of Fruit Quality

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TABLE OF CONTENTS

ABSTRACT	2
OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL	3
RELEVANT DATA, METHODOLOGY, RESULTS AND DISCUSSION	4
A. STACHYOSE TO SUCROSE METABOLISM	4
1. GALACTOSIDASES	4
2. GALACTOKINASE	15
3. PYROPHOSPHORYLASES	16
4. METABOLIC PATHWAY DURING YOUNG FRUIT DEVELOPMENT	25
5. METABOLIC PATHWAY IN MATURE FRUIT: LOCALIZATION AND COMPARISON OF SUCROSE ACCUMULATORS AND NON-ACCUMULATORS	35
6. EFFECT OF FRUIT TEMPERATURE AND LEAF REMOVAL	43
EVALUATION OF THE RESEARCH ACHIEVEMENTS	47
DESCRIPTION OF THE COOPERATION	47
CONCLUSIONS	48
LIST OF PUBLICATIONS	49
REPORT ON PATENTS	50
APPENDIX: STEPANSKY ET AL., 1999	51

ABSTRACT

The cucurbit family, including melon, translocates the galactosyl-sucrose oligosaccharides, raffinose and stachyose, in addition to sucrose, from the source leaves to the fruit sink. The metabolism of these photoassimilates in the fruit sink controls fruit growth and development, including the horticulturally important phenomenon of sucrose accumulation, which determines melon fruit sweetness.

During this research project we have characterized the complete pathway of galactosyl-sucrose metabolism in developing fruit, from before anthesis until maturity. We have also compared the metabolic pathway in sucrose accumulating genotypes, as compared to non-accumulating genotypes. Furthermore, we studied the pathway in different fruit tissues, in response to pollination, and also analyzed the response of the individual steps of the pathway to perturbations such as low temperature and leaf removal.

The results of our studies have led to the conclusion that generally galactosyl-sucrose metabolism functions as a coordinately controlled pathway. In one case, as an immediate response to the absence of pollination, the activity of a single enzyme, UDPglu pyrophosphorylase, was drastically reduced. However, during young fruit development, sucrose accumulation, and in response to perturbations of the system, groups of enzymes, rather than single enzymes, respond in a concerted manner.

Our research has characterized in detail the initial enzymes of galactosyl-sucrose metabolism, including the galactosidases, galactokinase and the UDPgal- and UDPglu pyrophosphorylases. We have discovered a novel alkaline α -galactosidase which hydrolyzes both stachyose and raffinose and thereby may have solved the dilemma of cytosolic galactosyl-sucrose metabolism, since prior to this research there was no known alkaline α -galactosidase capable of hydrolyzing raffinose.

OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

The overall objective of the research proposal was to broaden our understanding of carbohydrate metabolism in melon fruit, especially as it pertains to sugar accumulation. As we stated in the original proposal, quality of melon fruits is determined primarily by its sweetness and sugar content which has been shown to be due to the accumulation of the disaccharide, sucrose. The melon plant, as all the cucurbits, translocate as its photosynthetic product, not only sucrose but also the galactosyl-sucrose saccharides, raffinose and stachyose. Accordingly, the metabolism of these saccharides in the fruit sink may play a controlling role in the partitioning and accumulation of sugar to the fruit.

The metabolism of stachyose and raffinose to their sugar derivatives is outlined in Fig. 1. The major objective of this proposal was to characterize this metabolic pathway in developing melon fruits. Our attention focused on the enzyme activities during fruit development, the comparison of the pathway in fruit of sweet and non-sweet genotypes, the purification and characterization of some of the key enzymes that have not previously been studied, and the effect of perturbations such as the increasing or decreasing fruit temperature and leaf removal on the metabolism leading to sucrose accumulation.

Fig.1. The metabolism of galactosyl-sucrose in melon fruit.
(from Feusi et al., 1999)

Proposed metabolism of raffinose family oligosaccharides (RFOs) by a pathway involving form III PPase from melon meso-carp tissue. Enzymes: 1, α -galactosidase; 2, galactokinase; 3, UDP-Gal PPase; 4, UDP-Glc 4-epimerase; 5, UDP-Glc PPase; 6, phosphoglucomutase; 7, phosphoglucose isomerase; 8, sucrose phosphate synthase; 9, sucrose-6-P phosphatase; 10, invertase; 11, sucrose synthase. Note that reactions 3 and 5 can be catalyzed by a single enzyme, form III PPase.

RELEVANT DATA, METHODOLOGY, RESULTS AND DISCUSSION

A. GALACTOSIDASES

Galactosidase is the first enzyme of the metabolic pathway catabolizing the galactosyl-sucrose saccharides, stachyose and raffinose. We discovered that in the melon fruit there exist multiple forms of the enzyme. One of the forms is capable of hydrolyzing both raffinose and stachyose at acidic pH. Another form is capable of hydrolyzing only stachyose at neutral-alkaline pH. During the course of this research we discovered a third form, which may be the physiologically most important one. This enzyme is capable of hydrolyzing both raffinose and stachyose at neutral-alkaline pH. The significance of this enzyme is that until its discovery, raffinose metabolism in cucurbits was considered to be a dilemma, since there was no known enzyme that could carry out the crucial step at the likely physiological pH, based on earlier studies.

The detailed report of this study is presented in the following publication (Plant Physiology 119:979-988). Some of the highlights of the study are as follows:

- 1- The enzyme is extremely sensitive to ammonium sulfate so that previous studies of galactosidases in cucurbits did not uncover its presence since the enzyme activity was lost during the classical purification schemes.
- 2- We assayed activity with the natural substrates, raffinose and stachyose, rather than the synthetic substrate p-nitrophenyl galactose, which allowed us to distinguish between the enzyme forms.
- 3- The novel form has a number of distinctive characteristics, including a relatively broad range of substrates and a lack of product inhibition.
- 4- In the mature fruit the novel form is the major galactosidase present.
- 5- This novel form can be found in other galactosyl-sucrose translocating plants, including other cucurbits as well as *Coleus blumei* of the Lamiaceae.

Furthermore, we purified the novel enzyme to apparent homogeneity and the protein was partially sequenced (Technion Institute Protein Sequencing Unit). The N-terminal sequence and two additional peptide sequences were translated into degenerate primers and are presently being used in the attempt to clone the gene for the alkaline alpha-galactosidase.

Considering the potential use of this enzyme in biotechnological processes, particularly in the food industry, a patent application was submitted (Israel Patent Applic. 125423).

4. METABOLISM IN YOUNG FRUIT

In addition to the detailed study of the initial enzymes in galactosyl-sucrose metabolism we developed assay methods for the measurement of activity of all the enzymes in the stachyose to sucrose metabolic pathway in melon fruit tissue. These included acid and alkaline invertase, sucrose synthase, UDPglu-4'-epimerase, UDPglu pyrophosphorylase, PGM, PGI, in addition to the three alpha-galactosidases, galactokinase and UDPgal pyrophosphorylase. These assay methods allowed us to study the whole pathway during development, in order to discern unique developmental patterns of individual enzymes that may hint at physiological significance. Our strategy is based on the supposition that the study of a whole pathway prevents the drawing of incorrect conclusions based on a developmental pattern of only one or a few enzymes in a pathway. In addition, it can discern coordinated patterns of pathway control.

Furthermore, the comparison of activities of all the enzymes in a pathway also allowed us to carefully study the effect of single exogenous factors (in this study, pollination) and to discern over a short time period the first enzyme in the pathway which is responsive to the external factor.

The details of this study are presented in the following paper, "Carbohydrate metabolism during early fruit development of sweet melon" (*Physiologia Plantarum*, 1999). The key points of the study were as follows:

- 1- During early fruit development, prior to the onset of sucrose accumulation, practically all of the enzymes in the pathway show a developmental loss of activity. Only two enzymes show a distinct developmental pattern of maintenance or increase in activity. These two enzymes, alkaline alpha-galactosidase form I (the novel enzyme reported earlier) and acid invertase are capable alone of hydrolyzing the imported photosynthate (stachyose, raffinose and sucrose) to its component hexoses for further metabolism.

2- The initial enzymes in the pathway, the alpha-galactosidases and galactokinase, show the lowest extractable in vitro activity of the enzymes in the pathway. Nevertheless, the activity observed was adequate to account for metabolic flux during this period.

3- The enzyme alkaline invertase, whose function is not clear, was the primary invertase during the pre-anthesis stage of ovary development, during the period that acid invertase activity was rising.

4- We studied the effect of pollination on the growth and metabolic activity of the ovary during the initial two days after anthesis. We found that in the absence of pollination the ovary ceases to grow already during the day of anthesis so that by the following day there is a striking difference in ovary size between the pollinated and non-pollinated ovaries. In the comparison of the metabolic pathway enzyme activities we found that by the second day most of the pathway has become less active. However, only one enzyme, UDPglu pyrophosphorylase, showed a significant decrease in activity already by the first day. Our results showed that pyrophosphorylase activity using UDPglu as substrate was decreased by over 50% while activity with UDPgal remained unchanged. This further increases the significance of the observation since, as we noted in the previous study, the UDPgal pyrophosphorylase shows equal activity with UDPglu. Accordingly, it is possible that the 50 % activity we observed with UDPglu was in fact, due to the UDPgal pyrophosphorylase enzyme. This would lead to the striking conclusion that in the absence of pollination the activity of the specific UDPglu pyrophosphorylase is lost almost completely.

This enzyme likely plays a role in cell wall synthesis, for the production of the UDPglu for the cellulose synthase reaction. As such, its loss of activity would mark the cessation of futile ovary growth in the absence of pollination, even before the initial metabolism of imported photosynthate at the galactosidase or invertase steps. We are continuing this line of research, focussing our attention on the detailed characterization of the UDPglu specific pyrophosphorylase.

5. METABOLISM IN MATURE FRUIT: LOCALIZATION AND COMPARISON OF SUCROSE ACCUMULATORS AND NON-ACCUMULATORS

One of the major goals of the project was to compare the metabolic pathway in fruit of genotypes which differ in their capacity to accumulate sucrose. The results of our initial research project showed that the genetic trait of developmental sucrose accumulation was correlated with a developmental loss of soluble acid invertase activity, as well as a developmental increase in sucrose-phosphate synthase (SPS) activity.

In the present research project we compared the whole pathway between high sucrose accumulators (cv. C-8 and cv. Eshkolit) and non-accumulators (*C. melo* var. 'faqus' and *C. sativus* cv. 'Delilah'). The strategy of this approach was that by comparing the whole pathway of at least two representatives of each genetic type we could focus in on those enzymes that are significantly different in, and perhaps characteristic of, the sucrose accumulators. We could then determine if the differences we observed in invertase and SPS activities are limited to these two enzymes or perhaps are characteristic of a modified pathway as a whole.

In addition, in the previous project we observed that the vascular enriched tissues of the melon fruit show especially high activities of the enzymes sucrose synthase and SPS and this led us to hypothesize that at least part of the metabolic pathway is associated with the unloading phenomenon. Therefore, in this study we also compared the enzyme activities and sugar contents of the vascular tissue and the inner flesh.

In the comparison of sugar content of the tissues of the 4 genotypes (Fig. 3, Table 1) two conclusions can be made. One, that the phenomenon of sucrose accumulation is limited to the fruit flesh (tissue "C") and is not present in either the vascular tissue or in the rind tissue between the main vascular bundles. Second, that in addition to sucrose accumulation in the two sweet genotypes, there is also a higher glucose and fructose content in these genotypes. However, additional studies on our part have shown that the two,

Table 1:

Sugar composition and concentration in different parts of melon

Genotypes	Location	Sucrose	Glucose	Fructose	Stachyose	Raffinose
		(mg/g FW)				
C-8	A	1.5	11.4	15.3	1.2	0.6
	B	1.2	21.3	23.1	0.4	0.3
	C	58.0	30.0	27.7	1.4	1.7
ESH	A	4.3	7.2	12.3	2.8	1.3
	B	4.7	21.5	25.6	0.7	0.8
	C	39.0	24.0	25.1	1.0	1.2
Cucumber	A	2.0	5.6	7.1	1.6	2.0
	B	1.6	12.6	13.7	1.3	1.8
	C	0.9	11.2	15.4	1.3	2.4
Faqus	A	1.1	7.4	10.7	3.5	0.4
	B	0.6	12.1	13.5	3.5	0.3
	C	4.2	12.2	13.2	3.6	0.3

sucrose and hexose levels, are not genetically linked when comparing a broader spectrum of genotypes (Stepansky et al., 1999, see attached appendix).

The comparison of the activities of the 13 enzymes between the 4 genotypes and 3 tissues (Fig. 4) led to the following observations:

1- The vascular tissue is frequently enriched in enzyme activity. This enrichment is observed, in comparison with the parallel tissue "B", for the following enzymes: alkaline alpha galactosidase I and II, galactokinase, UDPgal pyrophosphorylase, UDPglc epimerase, UDPglu pyrophosphorylase, PGI, PGM, SPS, sucrose synthase, and the invertases. For some of these enzymes the relative enrichment is less than for others but, with the exception of the acid alpha galactosidase the activities of all the enzymes in the pathway are enriched in the vascular tissue.

2- The following enzyme activities are higher in the two sucrose accumulators, compared to the two non-accumulators: alkaline alpha galactosidase I, galactokinase, PGI, PGM and SPS. For two of these enzymes (alkaline galactosidase I and SPS) activity is higher in both tissue "A" and "C", while in the others the difference is clear only for the flesh tissue ("C", PGI, PGM, galactokinase). For alkaline galactosidase II, activity is slightly higher in both sucrose accumulators only in the vascular tissue. With regard to acid invertase, the loss of activity is observed only in the sucrose accumulating flesh tissue while activity remains high in the vascular tissue. In the flesh tissue the activity of the 'faqus' genotype was also low, since in very ripe fruit its activity also decreases.

We conclude from this study the following:

1- The metabolism associated with the vasculature is significant and also associated with sucrose accumulation in the flesh, even though the vascular tissue itself does not accumulate sucrose. This is most strikingly evident for the enzyme presumably responsible for sucrose synthesis, SPS. The cause for the lack of sucrose accumulation in this tissue is likely the high invertase activity which prevents sucrose accumulation. Accordingly, it would appear that there is a

futile cycle in the vascular tissue, which may be related to the energy requirements of this tissue.

2- The genetic differences in activities of acid invertase and SPS we previously observed were corroborated, while an additional 5 enzymes were also found to be associated with sucrose accumulation. The association of three of these, the alkaline galactosidases I and II and galactokinase is not surprising since these initial enzymes of the galactosyl-sucrose metabolic pathway may be responsible for the increased flux of photoassimilate into the maturing, sucrose accumulating fruit. More surprising, though, is the strikingly higher activities of the two hexose-P enzymes, PGI and PGM in the sucrose accumulating flesh tissue. These two enzymes catalyze the conversions between glu-1-P, glu-6-P and fru-6-P. One possibility is that the glu-1-P is used for the synthesis of UDPglu, which together with the fru-6-P, serves as the substrate for sucrose synthesis by SPS. Further study of this system, including measurements of the metabolites, nucleotides and nucleotide sugars may shed light on the significance of these reactions to sucrose accumulation.

3- Of the 13 enzymes in the pathway, 7 are differentially active in correlation with sucrose accumulation while 6 (acid galactosidase, UDPgal- and UDPglu pyrophosphorylases, epimerase, sucrose synthase and alkaline invertase) are therefore unlikely to be causal to the genetic trait of sucrose accumulation. The large number of enzyme activities related to sucrose accumulation would imply a coordinated control of the metabolic pathway rather than multigenetic control of individual enzymes.

The activities of the enzymes in the pathway were also assayed during fruit development of the sucrose accumulating cv. C-8, particularly prior to the onset of sucrose accumulation (Fig. 5, 30 DAA) and at maturity (44 DAA). We hypothesized that those enzymes whose activities were higher (or lower in the case of invertase) in the flesh of the sucrose accumulating genotypes would also show a developmental increase in activity beginning from the onset of sucrose accumulation.

Fig. 5. Changes in sugar content of fruit flesh ("C") of cv. C-8 during development.

The following table (Table 2) indeed shows that all the enzymes with higher activity in the flesh of the sucrose accumulating genotypes also showed a significant increase (or decrease for invertase) in activity between 30 and 45 DAA. None of the other enzymes, with the exception of epimerase, showed an increase in activity during this period.

Table 2: Comparison of enzyme activities in flesh of the sucrose accumulating cv. C-8. Activity of each enzyme was measured on a minimum of 3 fruit. 30 DAA is prior to the onset of sucrose accumulation. * indicates significant difference in activity between the two dates.

Enzyme	Activity(nmol/gfw/min)	
	30 DAA	45 DAA
Alk a-gal I	108	147 *
Alk a-gal II	33	39
Acid a-gal	57	65
galactokinase	123	214 *
UDPgal PPase	1293	766
epimerase	447	594 *
UDPglu PPase	2296	2372
PGI	1166	2127 *
PGM	2765	3504 *
Acid invertase	186	80*
Alk invertase	78	57
SPS	185	295*
Sucrose synthase	103	99

In conclusion, the results of these two studies point to a coordinated control of metabolism associated with sucrose accumulation. The control is manifested by the developmental loss of invertase activity in the flesh, together with developmental increases in activity in the flesh of the enzymes alkaline α -galactosidase I, galactokinase, PGI, PGM and SPS. In addition, higher activity of both alkaline α -galactosidases and SPS in the vascular tissue of the sucrose accumulating fruit may also be involved in maintaining carbohydrate import into the sucrose accumulating fruit.

6. EFFECT OF FRUIT TEMPERATURE AND LEAF REMOVAL

In our previous research project we observed that an environmental modification, such as lowering the greenhouse night temperature from 18 to 12 degrees, can produce melon fruit with an increased sucrose content, presumably due to the effect of the lower night temperature on the developmental pattern of sucrose accumulation. We studied this phenomenon in more detail during the present project to determine whether the effect of the low temperature in delaying the loss of invertase activity was via the effect of the temperature on the fruit itself.

Melon plants were grown under greenhouse conditions of 15 degrees minimum night temperature and the plants were trained to 1 fruit per plant, until fruit were 30 DAA (T_0), just prior to the onset of sucrose accumulation. Temperature-controlled fruit chambers were developed so that the individual fruit could be placed in the chamber and the temperature of that fruit could be controlled by the flow of water from circulating heated or cooled water baths, without affecting plant leaf temperature. Three temperature treatments were applied for a period of 7 days: the control (ambient air temperature, minimum 15 degrees), fruit warming (30 degrees night temperature) and fruit cooling (10 degrees night temperature).

The comparison of sugar accumulation (fig. 6) shows that during the 7 day treatment fruit cooling to 10 degrees led to a decrease in sucrose accumulation, compared to the ambient control and the warmed fruit. Other sugar levels were not affected.

Fig. 6. Effect of fruit temperature on sugar content.

The comparison of acid invertase and SPS activities of these fruit (Table 3) showed that the cooled fruit had both a higher invertase activity and a lower SPS activity than either the ambient fruit or the warmed fruit. It appears that SPS activity did not increase during the 7 day sucrose accumulation period and the developmental loss of invertase activity was partially inhibited. These results corroborated the hypothesis that sucrose accumulation is correlated with the developmental changes in these enzymes. Regretfully, at the time we performed this experiment we had not yet discovered the important alpha-galactosidase I enzyme and did not compare its activity. Of the other enzymes in the pathway only acid alpha galactosidase and sucrose synthase showed indications of decrease in activity in response to fruit cooling.

Table 3: Effect of fruit temperature on enzyme activities before and after one week of fruit cooling or warming.

Enzyme	T ₀	7 days		
		control	30	10
		Activity (nmol/gfw/min)		
SPS	173	269	231	185
acid invertase	141	50	40	90
acid a-gal	30	28	29	21
alk a-gal II	18	24	31	29
galactokinase	146	147	155	148
UDPgal PPase	367	238	241	277
epimerase	180	272	144	310
UDPglu PPase	2768	2529	1769	2277
PGM	1993	2850	2627	2629
PGI	1306	996	1008	1112
sucrose synthase	76	78	80	65
alk invertase	118	81	70	91

Our previous research had clearly shown that sucrose accumulation in the melon fruit is determined by the carbohydrate metabolism in the fruit tissue. Nevertheless, we observed that there was some form of signalling between the supply of photoassimilate and the metabolism in the fruit during development (Hubbard and Pharr, 1990, "Sucrose metabolism in ripening muskmelon fruit as affected by leaf area" J. Am. Soc. Hort. Sci. 115:798-802). This was shown by leaf removal experiments in which the removal of the photoassimilate supply led to an inhibition of the developmental loss of invertase activity and to a decrease in the rise of SPS activity.

We repeated this experiment and expanded it to include the whole metabolic pathway in order to determine whether the observed effects were limited to these two enzymes or whether, in fact, there was a coordinated effect on the pathway.

The effect of leaf removal on the sugar content of the fruit is seen in Fig. 7. As in the previous experiment on fruit cooling, the experiment began at the onset of sucrose accumulation in the fruit, at 30 DAA. Removal of half the photoassimilate supply resulted in a decrease in sucrose content but not in hexose content. Removal of all the leaves led to reduced sucrose and hexose levels. Nevertheless, there was an increase in sucrose content during the 7 days after leaf removal and the source for this is perhaps the carbohydrate reserves in the plant stem or perhaps the synthesis of sucrose from some of the hexose, particularly glucose which decreased during the 7 day period. The effect on sugar content is seen also in the effect on fruit TSS (Table 4).

Fig. 7. Effect of leaf removal on sugar content of melon fruit.

The survey of enzyme activities showed that the effect of leaf removal was clearly not limited to SPS and invertase (Table 4). We observed that SPS indeed did not increase in activity in the absence of its photoassimilate supply. Acid invertase however, did decrease even in the absence of leaves. Significantly, though, is the result that practically the whole pathway studied showed decreases in activity in response to complete leaf removal. The only exceptions are the epimerase and UDPgal pyrophosphorylase reactions whose activities were not affected by leaf removal. Again, regretfully, we did not assay the activity of the alkaline α -galactosidase I in this study.

Table 4: Effect of leaf removal on enzyme activities before and after one week following treatment.

	To	7 days		
		control	half	none
Fresh weight (g)	839	902	1006	881
TSS (Brix)	5.1	8.1	7.1	4.5
<u>Enzyme</u>		<u>Activity (nmol/gfw/min)</u>		
SPS	173	284	277	156
acid invertase	141	20	22	25
acid α -gal	30.1	28.1	27.9	25.7
alk α -gal II	17.9	23.3	22.6	17.9
galactokinase	146	177	155	106
UDPgal PPase	367	353	336	367
epimerase	180	170	165	154
UDPglu PPase	2768	3419	3593	2320
PGM	1993	2841	2684	1628
PGI	1306	1898	1574	1084
sucrose synthase	76	110	111	70
alk invertase	117	35	30	33

EVALUATION OF THE RESEARCH ACHIEVEMENTS

We have dealt with and accomplished, to varying degrees, practically all of the original objectives of the BARD proposal. Throughout our studies we have utilized the strategy of studying the complete metabolic pathway of stachyose metabolism which has proved to be important for determining conclusions related to metabolic control of the pathway. Specifically, we have:

- developed assay methods for 13 enzymes in the pathway,
- characterized in detail the initial key enzymes in the pathway,
- discovered a novel enzyme which may solve the dilemma of raffinose metabolism in galactosyl-sucrose translocating species including non-cucurbits such as *Coleus*,
- implicated a coordinated control of the metabolic pathway in determining metabolic control in response to the genetically controlled phenomenon of sucrose accumulation, and the effect of temperature and photoassimilate supply,
- shed light on the tissue localization of the pathway. We had hoped to carry out localization studies at the cellular level but, regrettably, our attempts to produce specific antibodies to the alkaline α -galactosidases and to the pyrophosphorylases were not successful.

DESCRIPTION OF THE COOPERATION

The Israeli and US labs maintained contact and cooperation throughout the period of the grant. AAS and DMP met for detailed discussions and analysis of results at the 94th Annual International Conference of the American Society of Horticultural Science (Salt Lake City, Utah, July 23-26, 1997) where each was also invited to participate in a colloquium on "The carbohydrate economy of horticultural crops". Between the two groups the major enzymes of galactosyl-sucrose metabolism were characterized. AAS and DMP authored together a major review of carbohydrate metabolism in cucurbits and a publication from each group will appear together in the same issue of *Physiologia Plantarum*.

CONCLUSIONS

The work resulting from this project leads to a number of conclusions and opens up further areas for future research. The major general conclusion is that galactosyl-sucrose metabolism in melon fruits, especially as it relates to the processes of fruit growth and sucrose accumulation, is a coordinated process. During fruit set and early development most of the pathway enzymes show a similar developmental pattern while two of the enzymes show distinctly similar patterns. A coordinated pattern of development is seen also in relation to the genetically controlled phenomenon of sucrose accumulation, as well as the response to some perturbations such as fruit temperature and leaf removal. The response to pollination is unique in that the pollination signal seems to affect one enzyme in particular, and this surprising and potentially significant observation is the subject of our continuing research.

Another major conclusion from this project is that we now have a complete picture of galactosyl-sucrose metabolism in the melon fruit. The discovery of the alkaline alpha-galactosidase I allows for the complete cytosolic hydrolysis of imported stachyose and raffinose. The detailed characterization of the pyrophosphorylases, together with the repeated inability to observe activity of the Leloir pathway uridyltransferase enzyme, indicates that the metabolism of the galactokinase-produced gal-1-P is via the three enzyme system of UDPgal pyrophosphorylase, epimerase and UDPglu pyrophosphorylase.

LIST OF PUBLICATIONS

Feusi MES, Burton JD, Williamson JD and Pharr DM (1999) Galactosyl-sucrose metabolism and uridinephosphogalactose pyrophosphorylase from *Cucumis melo* L. fruit. *Physiologia Plantarum* (in press)

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Stepansky A, Kovalski I, Schaffer AA and Perl-Treves R (1998) Variation in sugar levels and invertase activity in mature fruit representing A broad spectrum of *Cucumis melo* genotypes. *Genetic Resources and Crop Evolution* (in press)

REPORT ON PATENTS

A patent covering the novel enzyme alkaline α -galactosidase I was applied for as Israel Patent Applic. No. 125423