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**Sucrose Metabolism in Developing Fruits of
Cultivated and Wild *Lycopersicon* Species**

A.B. Bennett, A.A. Schaffer

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Sucrose Metabolism in Developing Fruits of Cultivated and Wild
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Investigators' Names
(Principal listed first)

Alan B. Bennett

Arthur A. Schaffer

Investigators' Institutions

University of California, Davis

A.R.O. - Volcani Center

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Alan B. Bennett
Signature
Principal Investigator

Signature
Institution's Authorizing Official

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ABSTRACT

Soluble solids of tomato fruit are primarily comprised of sugars, organic acids, and salts. The soluble solids concentration is a major determinant of fruit quality, especially for processing tomatoes and there are several wild relatives of tomato that accumulate extremely high levels of soluble solids. We have adopted an approach to enhance tomato fruit soluble solids by evaluating components of carbohydrate metabolism in developing fruit of wild tomato species characterized by high soluble solids content. It was reasoned that unique biochemical traits identified in these species may provide insights into mechanisms conferring high soluble solids content to tomato fruit and provide a basis for transferring the appropriate biochemical trait(s) to *L. esculentum*. Under BARD Grant US-1321-87 we characterized the genetic and biochemical basis of one such trait, that of sucrose accumulation, in two wild tomato species and transferred the trait to *L. esculentum*. Analysis of the progeny resulting from the transfer of the trait of sucrose accumulation to *L. esculentum*, which normally accumulates hexose, supported our hypothesis that sucrose accumulation contributes to high soluble solids content in tomato. In addition, the genetic and biochemical basis of the trait of sucrose accumulation was determined. In the case of *L. chmielewskii*, the trait was controlled by a single recessive gene that maps to chromosome three. It was furthermore determined that the sucrose-accumulating fruit were deficient in acid invertase. The tomato acid invertase cDNA was cloned to determine if this gene is responsible for controlling the trait of sucrose accumulation.

INTRODUCTION

Soluble solids of tomato fruit are primarily comprised of sugars, organic acids, and salts (3). Collectively, the soluble solids content of the fruit is a major determinant of fruit quality, especially for processing tomatoes. Standard tomato cultivars have soluble solids content in the range of 4-6%, whereas processed tomato paste is at least 24% soluble solids (7). Consequently, fruit with low soluble solids result in lower yields of paste per ton of fruit, and additional energy is expended in evaporating more water from low solids fruit. Thus, from the standpoint of total production capacity and processing efficiency, increasing the soluble solids content of tomato fruit is of major economic significance. In California alone an increase in soluble solids of 0.1% (i.e. from 5% to 5.1%) is estimated to result in a \$7 M savings to the tomato processing industry (13). Because of these considerations the improvement of tomato soluble solids continues to be a major goal of tomato breeders and processors.

Although soluble solids content represents a commercially valuable trait, progress in enhancing this trait by classical genetic approaches has been slow. Indeed, because of a strong negative correlation between yield and soluble solids content (25, 26), soluble solids levels have tended to decrease in association with the development of high yielding tomato varieties.

Several strategies to increase soluble solids levels have been explored, including the transfer of high soluble solids from a wild *Lycopersicon* species (9, 21). Recent efforts have attempted to use restriction fragment length polymorphism DNA markers to facilitate the introgression of high soluble solids-conferring genes from *L. chmielewskii* to *L. esculentum* with some limited success (17, 18, 27). However, in these efforts, selections for increased soluble solids were made in F₁ populations and so only dominant genes that contributed to high soluble solids content were selected for. Our results suggest that recessive genes may control specific biochemical traits important in soluble solids accumulation.

An alternative approach to the enhancement of tomato fruit soluble solids is to evaluate components of carbohydrate metabolism in developing fruit tissue in order to identify biochemical steps whose modification may lead to increased soluble carbohydrate content in the fruit. Once identified, these biochemical processes could then be modified by

classical genetic means, using direct biochemical selection, or by molecular genetic strategies. Several studies have described sink metabolism in *L. esculentum* (2, 5, 10, 14, 16, 28). We expanded upon this approach in the course of BARD grant #US-1321-87 by examining carbohydrate metabolism in two wild *Lycopersicon* species (*L. chmielewskii*, USA; *L. hirsutum*, Israel) that are characterized by high (>10%) soluble solids content. It was reasoned that unique biochemical traits identified in these species may provide insights into mechanisms conferring high soluble solids content to tomato fruit and provide a basis for transferring the appropriate biochemical trait(s) to *L. esculentum*.

Both *L. chmielewskii* and *L. hirsutum* were found to accumulate sucrose as the predominant soluble sugar (15, 30), unlike *L. esculentum* fruit which accumulate hexose (3). It was reasoned that sucrose, as opposed to hexose, accumulation may contribute to enhanced soluble solids levels in several ways. First, osmotic considerations suggest that sucrose-accumulating fruit can accumulate twice as much soluble carbohydrate as hexose-accumulating fruit while maintaining an equivalent osmotic potential. In this regard it is interesting to note that while *L. chmielewskii* fruit accumulated twice as much total soluble carbohydrate when expressed on a glucose equivalent basis, the osmotic concentration of soluble carbohydrate was approximately the same in *L. chmielewskii* as in *L. esculentum* (30). To the extent that soluble sugar uptake and accumulation is regulated by turgor (29) or by osmotic potential (5) the trait of sucrose accumulation might favor elevated soluble sugar accumulation. Secondly, sucrose is metabolically less active than hexose (19) and may be less accessible for loss through respiration, contributing to higher levels of accumulation. It is significant to note that storage organs accumulating very high levels of sugars do so by accumulating sucrose (6, 8, 12, 20). For instance, in sweet melon sugar accumulation occurs in two stages with only low levels of hexose accumulating early in development followed by high levels of sucrose accumulating in later stages of development (23, 24). These considerations suggest that sucrose accumulation may contribute to the ability of these two wild tomato species to accumulate high levels of total soluble solids. Introgression of the trait of sucrose accumulation into *L. esculentum* supports this contention. Under BARD grant #US 1321-87 we characterized the genetic and biochemical basis of the sucrose-

accumulating trait in the wild tomato species and transferred this trait through several backcross generations to *L. esculentum*.

OBJECTIVES OF BARD GRANT #US 1321-87

The goals of the project were to:

- 1) Characterize sucrose metabolism in cultivated *Lycopersicon esculentum*, which stores hexose rather than sucrose, and compare it to wild species of *Lycopersicon* which store significant amounts of sucrose and are characterized by a high soluble solids content.
- 2) Determine the inheritance of the unique metabolism of the wild species that allows for sucrose accumulation

RESULTS OF BARD GRANT #US 1321-87

Carbohydrate Metabolism in Wild Tomato Species.

L. chmielewskii. We previously showed that *L. chmielewskii* fruit accumulate sucrose, rather than hexose. These same fruit were also reported to be deficient in invertase and sucrose synthase and it was proposed that sucrose accumulation resulted from the lack of enzymic degradation of imported sucrose. This conclusion was supported by analysis of import of ([¹⁴C]-fructosyl)-sucrose (asymmetric label) which suggested that sucrose was taken up and stored without intervening metabolism (30).

L. hirsutum. Fruit of *L. hirsutum* were also found to accumulate sucrose as the predominant soluble sugar. As in *L. chmielewskii*, invertase levels were greatly reduced and declined even further during the period of greatest sucrose accumulation. In addition, sucrose phosphate synthase (SPS) activity sharply increased during this same period (Fig. 1) and it was proposed that enhanced capacity for sucrose synthesis (via SPS), in addition to low invertase levels, is required for sucrose accumulation in this species (15). The proposed pathway of sucrose accumulation implies that imported sucrose is degraded in the apoplast, taken up as hexose and resynthesized in the cytosol by SPS prior to storage as sucrose.

Figure 1. Activity of insoluble invertase and sucrose phosphate synthase (SPS) in *L. hirsutum* fruit throughout fruit development. The pattern of sucrose accumulation in the same fruit is also shown. (Redrawn from Miron & Schaffer, 1990.)

Genetic Analysis of Sucrose Accumulation.

L. chmielewskii. To analyze the genetic basis for sucrose accumulation, a cross between *L. esculentum* and *L. chmielewskii* was made and the progeny analyzed. No sucrose-accumulating progeny were identified in the F₁ hybrids, suggesting that recessive genes contribute to this trait. Among the segregating population of F₂ progeny, several sucrose-accumulating individuals were identified and one was self-pollinated and crossed to *L. esculentum* (cv UC204C) to produce F₃ and BC₁F₁ seed, respectively. All plants analyzed in the F₃ population accumulated sucrose rather than hexose, and the total sugar content was 57% higher than in *L. esculentum* (Table I). The fact that true-breeding sucrose-accumulating lines could be obtained indicated that the trait of sucrose accumulation could be stably transferred from *L. chmielewskii* to *L. esculentum*.

As expected from analysis of the F₁ population, all fruit of the BC₁F₁ population accumulated hexose. The BC₁F₁ plants were self-pollinated to produce BC₁F₂ seed. Of the 62 BC₁F₂ plants that were planted, only 56 were analyzed since six of them were sterile and did not set fruit. Of the 56 plants analyzed, 46 were hexose accumulators and 10 were

sucrose accumulators (Table II). Thus the ratio of sucrose- to hexose-accumulating plants was approximately 1:6 in the BC₁F₂ population. In mature fruit (40 d after anthesis) 32% more sugar was present in sucrose-accumulating than in hexose-accumulating fruit of the same BC₁F₂ population (Table II). The increased level of soluble sugar in mature fruit resulted in a comparable (30%) increase in total soluble solids of sucrose-accumulating fruit relative to hexose-accumulating fruit in the same BC₁F₂ population. This result is consistent our expectation that the trait of sucrose accumulation may enhance total soluble solids levels in tomato fruit. This effect of sugar composition is now (1990) being further assessed by comparing soluble solids levels of BC₂F₃ families of sucrose and hexose accumulators derived from the cross between *L. chmielewskii* and *L. esculentum*.

In cooperation with Dr. J. DeVerna (Campbell's Institute for Research & Technology) the BC₁F₂ population was further analyzed by restriction fragment length polymorphism and the trait of sucrose accumulation was found to be associated with a single region on chromosome 3 (Fig. 2). This chromosomal region has been shown to carry a recessive allele associated with sterility in crosses of *L. chmielewskii* with *L. esculentum* (A. Paterson, personal communication). As mentioned above, 6 plants in the BC₁F₂ population failed to set fruit. Interestingly, all 6 of these fruit were homozygous for the RFLP marker linked to the trait of sucrose accumulation suggesting that, if fruit had been present on these plants, they would have been sucrose accumulators. If this presumption is correct the true ratio of sucrose to hexose accumulators in the BC₁F₂ population was approximately 1:4 (16:62). Based on analysis of segregation ratios in other populations we have concludes that the trait of sucrose accumulation derived from *L. chmielewskii* is most likely controlled by a single recessive gene (Table IV).

***L. hirsutum*.** Genetic analysis of the trait of sucrose accumulation derived from *L. hirsutum* was carried out by crossing *L. hirsutum* to *L. esculentum* and backcrossing the F₁ progeny to both parents. In the BC₁F₁ to *L. hirsutum* all progeny accumulated sucrose and in the BC₁F₁ to *L. esculentum* 8 of 40 progeny accumulated sucrose. All of the sucrose accumulators had high total soluble solids levels (~12%) suggesting, that as in progeny from *L. chmielewskii*, sucrose accumulation confers enhanced soluble solids levels. The genetic analysis of the *L. hirsutum* progeny is not sufficiently advanced to draw firm

conclusions regarding the basis of its inheritance but the initial results suggest that the trait of sucrose accumulation is incompletely dominant. This suggests that the genetic mechanism(s) determining sucrose accumulation in *L. hirsutum* and its progeny differ significantly from those that determine the same trait in *L. chmielewskii* and its progeny.

Table I. Level of soluble carbohydrates in ripe fruits of *Lycopersicon esculentum*, *Lycopersicon chmielewskii*, and F₃ population. The F₃ population is derived from the highest sucrose accumulator of the F₂ population.

Population	Hexose ($\mu\text{mol glc/g fr wt}$)	Sucrose ($\mu\text{mol glc/g fr wt}$)	Sucrose/ Hexose ratio	Total Sugar ($\mu\text{mol glc/g fr wt}$)
<i>L. esculentum</i>	128.82	8.94	0.07	137.76
<i>L. chmielewskii</i>	27.4	235.20	8.58	262.60
F ₃ population	23.09	194.36	8.42	217.45

Table II. Level of hexose and sucrose in tomato fruits of the BC₁F₂ population.

Population	Age (day)	Hexose ($\mu\text{mol glc/g fr wt}$)	Sucrose ($\mu\text{mol glc/g fr wt}$)	Sucrose Hexose ratio	Total Sugar ($\mu\text{mol glc/g fr wt}$)
Hexose Accumulator (46/56)	20	70.9 $\pm$ 3.2	7.2 $\pm$ 1.4	0.11 $\pm$ 0.02	78.1 $\pm$ 3.9
	40	176.3 $\pm$ 6.8	5.1 $\pm$ 1.8	0.05 $\pm$ 0.01	181.4 $\pm$ 6.5*
Sucrose Accumulator (10/56)	20	36.6 $\pm$ 5.6	48.9 $\pm$ 4.51	1.34 $\pm$ 0.23	85.5 $\pm$ 7.36
	40	71.9 $\pm$ 12.6	165.0 $\pm$ 18.9	4.22 $\pm$ 1.26	236.5 $\pm$ 18.4*

* Values for total sugar are different at the 99.9% confidence level.

Table III. Activity of invertase and sucrose synthase in tomato fruit of the BC₁F₂ population.

Population	Days after anthesis (days)	Invertase activity (nmol glc/g fr wt min)	Sucrose Synthase activity (nmol glc/g fr wt min)
Hexose Accumulator (46/56)	20	3216 $\pm$ 271	397 $\pm$ 52
	40	12839 $\pm$ 572	N.D.*
Sucrose Accumulator (10/56)	20	42 $\pm$ 20	346 $\pm$ 57
	40	32 $\pm$ 29	N.D.

* Sucrose synthase levels not determined (N.D.) at 40 days after anthesis since levels are very low in both *L. esculentum* and *L. chmielewskii* at this developmental stage.

Table IV. Segregation ratios of the sucrose-accumulating trait in several populations derived from the an initial cross between *Lycopersicon esculentum* and *Lycopersicon chmielewskii*.

**SUCROSE-ACCUMULATING TRAIT (*suc*) SEGREGATES
AS A SINGLE RECESSIVE GENE**

Generation	PHENOTYPE		RATIOS	
	Hexose	Sucrose	Observed	Expected
F ₂	85	6	14:1	3:1 ***
BC ₁ F ₂	46	10	4.6:1	3:1
RBC ₁	133	117	1:1	1:1

Biochemical Analysis of Sucrose Accumulation.

L. chmielewskii. In order to assess the role of specific metabolic processes contributing to the *L. chmielewskii*-derived trait of sucrose accumulation, invertase and sucrose synthase activity were assayed in fruit of the BC₁F₂ population (Table III). Fruit of the 46 hexose accumulators showed a high level of both invertase and sucrose synthase, while low invertase but high sucrose synthase activities were measured in fruit of the 10 sucrose accumulators. These results indicate that low invertase levels are closely associated with sucrose-accumulation in this population. Invertase was purified from *L. esculentum* (Fig. 3) and found to resolve into three highly similar isoforms when analyzed by chromatofocusing (Fig. 4A). Invertase purified from fruit of *L. chmielewskii* or of a sucrose-accumulating BC₁F₂ plant was far less abundant but was comprised of identical isoforms (Fig. 4B). An antibody raised to carrot invertase (courtesy of Dr. M. Chrispeels, UC San Diego) was used to probe immunoblots of protein from *L. esculentum*, *L. chmielewskii* and sucrose- and hexose-accumulators of the BC₁F₂ population (Fig. 5). In all cases low invertase activity was associated with a low level of immunologically detectable invertase protein, indicating that the low invertase in sucrose-accumulating fruit is caused by lack of enzyme rather than by the presence of an invertase inhibitor, an activity proposed to be present in senescent tomato fruit (15, 16). We have not determined the subcellular localization of the invertase that is deficient in sucrose-accumulating plants and abundant in hexose-accumulating plants, but speculate that it is vacuolar. Experiments to determine its subcellular localization are in progress and we anticipate will be completed prior completion of the current grant period.

Cloning tomato fruit invertase. To determine whether the invertase gene or perhaps a gene encoding a factor regulating invertase expression in fruit controls the trait of sucrose accumulation, we cloned the invertase cDNA. It was reasoned that once the invertase cDNA was isolated, the chromosomal location of the invertase gene could be determined by restriction fragment length polymorphism (RFLP) analysis and compared to the chromosomal location of the trait of sucrose accumulation (see Fig. 2). To accomplish this, we determined the amino acid sequence of CnBr-fragmented tomato fruit invertase and

used corresponding oligonucleotide probes to isolate the cDNA. Based on comparison to a previously published carrot acid invertase cDNA, the nucleotide sequence of the isolated tomato fruit invertase cDNA confirmed its identity as encoding invertase (Fig. 7).

L. hirsutum. As described above, *L. hirsutum* fruit accumulated sucrose and the timing of sucrose accumulation paralleled a decrease in invertase and an increase in SPS activity (15). In segregating populations of sucrose- and hexose-accumulating individuals, genotypes with high invertase failed to accumulate sucrose. However, individuals with low invertase did not all accumulate sucrose, suggesting that biochemical factors in addition to low invertase (such as elevated SPS) are required for sucrose accumulation in *L. hirsutum* and its progeny. A similar role of SPS in sucrose accumulation in muskmelon has been proposed. In preliminary experiments with *L. chmielewskii*, such an increase in SPS was not observed, suggesting that the biochemical basis of sucrose accumulation may differ in the two wild tomato species that we have examined. If elevated SPS levels are required for sucrose accumulation in *L. hirsutum*, this would imply that the pathway of sucrose storage involves hydrolysis of imported sucrose in the apoplast followed by resynthesis in the cytoplasm. The absence of vacuolar invertase would also be required to prevent hydrolysis following resynthesis. It is interesting to note that in all sucrose accumulators, even though invertase levels are greatly reduced, a low level of invertase is present which may reflect apoplastic invertase.

The physiological benefit of the proposed pathway of sucrose hydrolysis and resynthesis in *L. hirsutum* may be in the maintenance of two separate sugar concentration gradients:

- 1) A sucrose gradient from the source leaf (or phloem) to the site of apoplastic unloading and
- 2) A hexose gradient from the apoplast to the cytoplasm where sucrose resynthesis would occur.

Such a model is consistent with previous studies indicating that sugar uptake from the apoplast in tomato pericarp is not an energy dependent process and that the concentration gradient for sugars is the most likely driving force for sugar uptake (2, 11).

Figure 2. Chromosomal location of the sucrose accumulating trait as determined by RFLP mapping of BC₁F₂ progeny of a cross between *L. chmielewskii* and *L. esculentum*. All of the indicated RFLP markers were analyzed for linkage with the trait of sucrose accumulation. The boxed region of chromosome 3 corresponds to the chromosomal location of the trait.

Figure 3. SDS polyacrylamide gel electrophoresis of tomato fruit invertase at various stages of purification. The amount of protein in each sample lane was 30 μ g, crude extract; 15 μ g, Concanavalin; 5 μ g, anion exchange; 3 μ g, gel filtration; and 2 μ g each, Inv I, Inv II and Inv III. The gel was stained with Coomassie blue. Inv I, Inv II and Inv III refer to invertase isoforms separated by chromatofocusing (see Fig. 4).

Figure 4. Chromatofocusing of tomato fruit invertase on Mono P. Approximately 2.5 mg of protein was applied to a Mono P column and eluted with polybuffer 75. The flow rate was 0.3 ml/min and 0.3 ml fractions were collected and assayed for invertase activity ($\square$) and the pH ($\circ$) of each fraction was determined. Peaks of invertase activity are designated isozymes I, II and III in order of their elution from the Mono P column. Inset: A native gel of total invertase following purification on Mono Q and of peak fractions of invertase isozymes II and III following purification on Mono P. Approximately 5 μg of each fraction was separated by non-denaturing polyacrylamide gel electrophoresis and stained for invertase activity as described in Materials and Methods.

Figure 5. Chromatofocusing of invertase purified from A) *L. esculentum* or a hexose-accumulating fruit of a *BC₁F₂* plant or from B) *L. chmielewskii* or a sucrose-accumulating fruit of a *BC₁F₂* plant. Total invertase purified from 50 g of pericarp tissue of each genotype was applied to the chromatofocusing column. Note the different scales of the ordinate in panels A and B, indicating approximately 100-fold higher levels of invertase in *L. esculentum* and hexose-accumulating *BC₁F₂* fruit (panel A) as compared to *L. chmielewskii* and sucrose-accumulating *BC₁F₂* fruit (panel B). Chromatofocusing was carried out on a Mono P column and FPLC (Pharmacia).

Figure 6. Immunological detection of invertase in *L. esculentum* or *L. chmielewskii* and in hexose- or sucrose-accumulating BC₁F₂ fruit. Protein from hexose-accumulating fruit (*L. esculentum* or BC₁F₂) or from sucrose-accumulating fruit (*L. chmielewskii* or BC₁F₂) at either 20 or 40 d after anthesis. 100 μ g of protein was loaded per lane and silver stained (upper panel) or blotted to PVDF membrane and invertase detected with antibodies to carrot invertase (lower panel).

GATTCCTCTATCTTCTATTATGGCCACTCAGTGTTATGACCCCGAAAACCTCCGCCTCTCGTTACACATTACTCCCGGATCAACCCGATTCCGGCCACCGG	100
M A T Q C Y D P E N S A S R Y T L L P D O P D S G H R	27
AAGTCCCTTAAATCATCTCCGGCATTTTCTCTCCGTTTCTTTTCTGTTCTGTAGCCTTCTTTCGGATCCTCAACAACCAAGTCACCGGACTTGCAAA	200
K S L K I I S G I F L S V F L L L S V A F F P I L N N Q S P D L Q	60
TCGACTCCCGTTCCGCCGGCGCCCGCTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAAAACCTTTTCGAGATGTAGCCGGTGCTAGTCACGTTTCTTATGC	300
I D S R S P A P P S R G V S Q G V S D K T F R D V A G A S H V S Y A	94
GTGGTCCAATGCTATGCTTAGCTGGCAAAGAACGGCTTACCATTTTCAACCTCAAAAAAATGGATGAACGATCCTAATGGACCATTTGTATCACAAGGGA	400
W S N A M L S W Q R T A Y H F Q P Q K N W M N D P N G P L Y H K G	127
TGGTACCACCTTTTTTATCAATACAATCCAGATTAGCTATTTGGGGAATATCACATGGGGCCATGCTGTATCCAAGGACTTGATCCACTGGCTCTACT	500
W Y H L F Y Q Y N P D S A I W G N I T W G H A V S K D L I H W L Y	160
TGCCTTTTGCCATGGTTCTGATCAATGGTATGATATTAACGGTGTCTGGACAGGGTCCGCTACCATCCTACCCGATGGTCAGATCATGATGCTTTATAC	600
L P F A M V P D Q W Y D I N G V W T G S A T I L P D G Q I M M L Y T	194
CGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGCCTACCCCGCAACTTATCTGATCCTCTCCTTCTAGACTGGGTCAAGTTCAAAGGCAACCCG	700
G D T D D Y V Q V Q N L A Y P A N L S D P L L L D W V K F K G N P	227
GTTCTGGTTCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACTACTGCTTGGACCGGACCACAAAATGGGCAATGGCTGTTAAACAATCGGGT	800
V L V P P P G I G V K D F R D P T T A W T G P Q N G Q W L L T I G	260
CTAAGATTGGTAAACGGGTGTTGCACCTTGTTTATGAACTTCCAACCTTCAAGCTTTAAGCTATTGGATGGAGTGTGCATGCGGTTCCGGGTACGGG	900
S K I G K T G V A L V Y E T S N F T S F K L L D G V L H A V P G T G	294
TATGTGGGAGTGTGTGGACTTTTACCCGGTATCTACTAAAAAACAACCGGTTGGACACATCATATAACGGGCCGGGTGTAAGCATGTGTTAAAGCA	1000
M W E C V D F Y P V S T K K T N G L D T S Y N G P G V K H V L K A	327
AGTTTAGATGACAATAAGCAAGATCATTATGCTATTGGTACGTATGACTTGGGAAAGAACAAATGGACACCCGATAACCCGGAATTGGATTGTGGAATTG	1100
S L D D N K Q D H Y A I G T Y D L G K N K W T P D N P E L D C G I	360
GGTTGAGACTAGACTATGGGAAATATTATGCATCAAAGACTTTTATGACCCGAAGAAAGAACGAAGAGTACTGTGGGGATGGATTGGGAAACTGACAG	1200
G L R L D Y G K Y Y A S K T F Y D P K K E R R V L W G W I G E T D S	394
TGAATCTGCTGACCTGCAGAAGGGATGGGCATCTGTACAGAGTATTCGAAGGACAGTGCTTTACGACAAGAAGACAGGGACACATCTACTTCAGTGGCCA	1300
E S A D L Q K G W A S V Q S I P R T V L Y D K K T G T H L L Q W P	427
GTGGAGAAATGAAAGCTTAAGAGTGGGTGATCCTACTGTTAAGCAAGTCGATCTTCAACCAGGCTCAATTGAGTACTCCGTGTTGACTCAGCTGCAG	1400
V E E I E S L R V G D P T V K Q V D L O P G S I E L L R V D S A A	460
AGTTGGATATAGAAGCCTCATTGAAGTGGACAAAGTCGCGCTTCAGGGAATAATTGAAGCAGATCATGTAGGTTTCAGTTGCTCTACTAGTGGAGGTGC	1500
E L D I E A S F E V D K V A L Q G I I E A D H V G F S C S T S G G A	494
TGCTAGCAGAGGCATTTTGGGACCATTTGGTGTATAGTAATTGCTGATCAAACGCTATCTGAGCTAACGCCAGTTTACTTTTACATTTCTAAAGGAGCT	1600
A S R G I L G P F G V I V I A D Q T L S E L T P V Y F Y I S K G A	527
GATGGTCGTGCAGAGACTCACTTCTGTGCTGATCAAAGTAGATCCTCTGAGGCTCCGGGAGTTGGTAAACAAGTTTATGGTAGTTTCACTACCTGTGTTGG	1700
D G R A E T H F C A D Q T R S S E A P G V G K Q V Y G S S V P V L	560
ACCGTGAAAAACATTCAATGAGATTATTGGTGGATCACTCAATTGTGGAGAGCTTTGCTCAAGGAGGAAGAACAGTCATAACATCGCGAATTTACCCAAC	1800
D G E K H S M R L L V D H S I V E S F A Q G G R T V I T S R I Y P T	594
AAAGGCAGTAAATGGAGCAGCAGACTCTTTGTTTTCAACAATGCCACAGGGCTAGCGTTACTGCCTCCGTCAAGATTGGTCACTTGAGTCAGCTAAT	1900
K A V N G A A R L F V F N N A T G A S V T A S V K I W S L E S A N	627
ATTCAATCCTTCCCTTTGCAAGACTTGAATCTTCTTTATTTTCGTTTTTTTTCTTTTTCATTTGAAGGTTATTTACCGGACGTCCTCAAGAAAGGG	2000
I Q S F P L Q D L 636	
AAGAGGGAGATCAATATATGTAGTGTATTTCGCCCTACCTTAGGATTAGATGTATCTAGCAATGTCAAACTAGTAGAGTATACAATGTATGGGTTCTT	2100
GGAAACCGAGTAGAGCTTACCTGGATTCTATGTAACCTAAGAAAGCTCAGCAATATATGCACAAATAATTTACAGAAAAAAAAAAAAAAAAAAAAA	2200
AAAAAAAAAAAAAAAAAAAAA 2223	

Figure 7. Nucleotide and amino acid sequence of the tomato fruit acid invertase cDNA. Underlined regions correspond to sequences confirmed by sequencing fragments of the protein. Predicted signal sequence cleavage site is indicated by an arrow, and the N-terminal tyrosine residue of the mature protein indicated by *.

PUBLICATIONS FROM BARD GRANT #US 1321-87.

- Yelle S., Chetelat R.T., DeVerna J.W., Bennett A.B. 1991. Sink metabolism in tomato fruit. IV. Genetic and biochemical analysis of sucrose accumulation. *Plant Physiol.* 95:1026-1035.
- Miron D., Schaffer A.A. 1991. SPS, SS and invertase in developing fruit of *Lycopersicon esculentum* Mill. and the sucrose-accumulating *L. hirsutum* Humb. and Bonpl. *Plant Physiol.* 95:623-627.
- Bennett A.B., Klann E.M., Lashbrook C.C., Yelle S., Chetelat R.T., DeVerna J.W., Fischer R.L. 1992. Genetic and molecular genetic regulation of soluble and insoluble carbohydrate composition of tomato fruit. In: Bills, D. (ed), *Biotechnology and Nutrition*. Butterworths, (in press).

RESEARCH ACHIEVEMENTS WITH RESPECT TO ORIGINAL OBJECTIVES

We have accomplished most of the original objectives of BARD Grant #US 1321-87.

Specifically:

1. We have characterized carbohydrate metabolism in *L. chmielewskii* and *L. hirsutum* in relation to sucrose accumulation in these wild tomato relatives. These studies have indicated that the lack of acid invertase (which we presume to be vacuolar) may be primarily responsible for sucrose accumulation in *L. chmielewskii* and progeny derived from *L. chmielewskii*.
2. Similar studies in *L. hirsutum* suggest that, in addition to greatly reduced levels of acid invertase, increased levels of SPS are required for sucrose accumulation.
3. The trait of sucrose accumulation from both *L. chmielewskii* and *L. hirsutum* was transferred to *L. esculentum* by classical genetic means. In both cases transfer of this trait was associated with increased levels of soluble solids, suggesting that this biochemical trait contributes to enhanced soluble solids levels.
4. In crosses with *L. chmielewskii*, the sucrose accumulating trait appeared to be inherited as a single recessive gene, the location of which was mapped using RFLP markers to chromosome #3.
5. Interestingly, in crosses with *L. hirsutum* the trait of sucrose accumulation appeared to be incompletely dominant, suggesting that the genetic determinants of sucrose accumulation in these two wild relatives of tomato differ.

Overall our progress in the first period of the grant has established evidence supporting the role of the sucrose accumulating trait as a determinant of enhanced soluble solids levels, characterized the biochemical basis of the trait of sucrose accumulation in *L. chmielewskii* and *L. hirsutum* and in progeny derived from crosses of both wild species with

L. esculentum and demonstrated that the trait of sucrose accumulation can be stably transferred to *L. esculentum*. This information is critical to the development of tomato lines that incorporate the trait of sucrose accumulation as a means to enhance total fruit soluble solids.

COOPERATION

The USA lab has focused on the sucrose-accumulating trait derived from *L. chmielewskii* and the Israel lab on the same trait derived from *L. hirsutum*. In 1990, Dr. Schaffer visited the USA lab (UC Davis) where detailed experimental information was exchanged. The two labs have also exchanged procedures and reagents to facilitate the research of each lab. We have planned to exchange material as a means to test for potential allelic relationship between the genes controlling sucrose accumulation in *L. hirsutum* and *L. chmielewskii*.

LITERATURE CITED

1. Bennett A.B., Damon S., Osteryoung K., Hewitt J. 1986. Mechanism of retrieval and metabolism following phloem unloading. In: J. Cronshaw, W. Lucas, R. Giaquinta, Alan R. Liss, Inc. Eds. Phloem Transport pp. 307-316.
2. Damon S., Hewitt J., Neider M., Bennett, A.B. 1988. Sink metabolism in tomato fruit. II. Phloem unloading and sugar uptake. *Plant Physiol* 87:731-736.
3. Davies J.N., Hobson G. (1981) The constituents of tomato fruit-the influence of environment, nutrition and genotype. *CRC Crit. Rev. Food Sci. Nutr.* 15:205-280.
4. DellaPenna D., Alexander D., Bennett, A.B. 1986. Molecular cloning of tomato fruit polygalacturonase: Analysis of polygalacturonase mRNA levels during ripening. *Proc. Natl. Acad. Sci. USA* 83:6420-6424.
5. Ehret D, Ho L.C. (1986) Effects of salinity on dry matter partitioning and fruit growth in tomatoes grown in nutrient film culture. *J Am Soc Hort Sci* 61:361-367
6. Giaquinta RT (1979) Sucrose translocation and storage in the sugar beet. *Plant Physiol* 63:828-832
7. Hankin L. (1986) Quality of tomato paste, sauce, puree and catsup. Bulletin 828. Conn. Agr. Exp. Sta. 4 pp.
8. Hatch M.D., Sacher J.A., Glasziou K.T. (1963) Sugar accumulation cycle in sugar cane. II. Relationship of invertase activity to sugar content and growth rate in storage tissues of plant grown under controlled environments. *Plant Physiol* 38:344-348
9. Hewitt J.D., Garvey, T.C. 1987. Wild sources of high soluble solids in tomato. In: *Tomato Biotechnology*, D.T. Nevins and R.A. Jones, eds. pp. 45-54. New York.

10. Ho L.C. (1988) Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. *Ann. Rev. Plant Physiol. & Plant Mol Biol.* 39:355-378.
11. Johnson C., Hall J.L., Ho L.C. 1988. Pathways of uptake and accumulation of sugars in tomato fruit. *Ann. Bot.* 61:593-603.
12. Kato T., Kubota M. (1978) Properties of invertase in sugar storage tissues of citrus fruit and changes in their activities during maturation. *Plant Physiol* 42:67-72
13. Kunimoto L. (1986) Commercial opportunities in plant biotechnology for the food industry. *Food Tech.* Oct. 58-60.
14. Manning K., Maw G.A. (1975) Distribution of acid invertase in the tomato plant. *Phytochemistry* 14:1965-1969
15. Miron D., Schaffer A.A. (1990) SPS, SS and invertase activities in developing fruit of *Lycopersicon esculentum* Mill. and the sucrose-accumulating *L. hirsutum* Humb. and Bonpl. *Plant Physiol.* (submitted).
16. Nakagawa H., Kawasaki Y., Ogura N., Takehana H. 1971. Purification and some properties of two types of fructofuranosidase from tomato fruit. *Agric. Biol. Chem.* 36:18-26.
17. Osborn T.C., Alexander D.C., Fobes J.S. 1987. Identification of restriction fragment length polymorphism linked to genes controlling soluble solids content in tomato fruit. *Theor. Appl. Genet.* 73:350-356.
18. Paterson A.H., Lander E.S., Hewitt J.D., Peterson S., Lincoln S., Tanksley S.D. (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* 355:721-726.
19. Pontis H.G. (1978) On the scent of the riddle of sucrose. *Trends Biochem Sci* 3:137-139
20. Ricardo C.P.P., Ap Rees T. (1970) Invertase activity during development of carrot roots. *Phytochem.* 9:239-247
21. Rick C.M. (1974) High soluble-solids content in large fruited tomato lines derived from a wild-green fruited species. *Hilgardia* 42:493-510
22. Schaffer A.A. 1986. Invertases in immature and mature *Citrus* leaves. *Phytochemistry* 25:2275-2277.
23. Schaffer A.A., Aloni B.. 1987. Sucrose accumulation in mature sweet melon fruits. *Plant Physiol.* 82:S-105.
24. Schaffer A.A., Aloni B., Fogelman E.. 1987. Sucrose metabolism and accumulation in developing fruit of sweet and non-sweet genotypes of *Cucumis*. *Phytochemistry* 26:1883-1887.
25. Stevens M.A. 1986. Inheritance of tomato fruit quality components. *Plant Breeding Reviews* 4:274-310.

26. Stevens M.A., Rudich J. 1978. Genetic potential for overcoming physiological limitations on adaptability, yield and quality in the tomato. *HortScience* 13:673-678.
27. Tanksley S.D., Hewitt J. (1988) Use of molecular markers in breeding for soluble solids content in tomato - a re-examination. *Theor. Appl. Genet.* 75:811-823.
28. Walker A.J., Ho L.C. (1977) Carbon translocation in the tomato: Carbon import and growth. *Ann Bot* 43:813-823
29. Wyse R.E., Zamskie E., Tomos A.D. (1986) Turgor regulation of sucrose transport in sugar beet taproot tissue. *Plant Physiol* 81:478-481
30. Yelle S., Hewitt J.D., Robinson N.L., Damon S., Bennett A.B. (1988) Sink metabolism in tomato fruit. III. Analysis of carbohydrate assimilation in a wild species. *Plant Physiol* 87:737-740
31. Yelle S., Chetelat R.T., DeVerna J.W., Bennett A.B. 1991. Sink metabolism in tomato fruit. IV. Genetic and biochemical analysis of sucrose accumulation. *Plant Physiol.* 95:1026-1035.