Genetic Resources and Crop Evolution **46:** 53–62, 1999.

Variation in sugar levels and invertase activity in mature fruit representing a broad spectrum of *Cucumis melo* genotypes

Asya Stepansky[1], Irina Kovalski[1], Arthur A. Schaffer[2] & Rafael Perl-Treves[1*]
[1]*Department of Life Science, Bar-Ilan University, Ramat-Gan 52900 , Israel;* [2]*Department of Vegetable Crops – The Volcani Center, A.R.O., Bet-Dagan 50250, Israel (* Author for correspondence)*

Received 22 July 1998; accepted in revised form 28 July 1998

Key words: Cucumis melo, fructose, germplasm survey, glucose, invertase, sucrose

Abstract

Sugar accumulation is a very important physiological process that determines dessert-melon fruit quality. Considerable variation in the sugar content and composition in the mature flesh of *Cucumis melo* L. fruits was observed among 56 genotypes which represent the wide range of morphological and horticultural types found in this species. Sucrose accumulation was observed not only among 'dessert melons' of the *inodorus* and *cantalupensis* types, but also in representatives of other subgroups of *C. melo*, including some of the accessions from *agrestis* and *conomon* groups. Among the genotypes that accumulate sucrose, the levels of this sugar, and not of the hexoses, were correlated with the total sugar concentration. Hexose levels were correlated with total sugar levels only among low-sugar genotypes that did not accumulate sucrose. Activities of the sucrose hydrolyzing enzymes acid invertase (EC 3.2.1.26) and alkaline invertase (EC 3.2.1.27) were measured in the mature fruit flesh and the results of this survey support the hypothesis that low acid invertase activity is a prerequisite for sucrose accumulation. The sucrose accumulating, high sugar genotypes had less-acidic flesh pH values (pH$>$ 6) than the low-sugar genotypes. The latter were characterized by a broader range of pH values, including acidic (pH $<$ 5.5) fruit flesh.

Introduction

Sugar concentration is a primary determinant of dessert-melon fruit quality (Yamaguchi et al., 1977), and improvement of fruit sweetness is an important breeding goal. A better understanding of the physiology of sugar accumulation in the melon fruit, and its genetic control, is a requirement for designing rational breeding strategies, including modern biotechnological approaches.

Cucurbit fruits have been the object of a number of physiological studies on source-sink relationships and sugar metabolism (see Schaffer et al., 1996).In sweet melon varieties, the fruit undergoes a developmental metabolic transition to sucrose accumulation. During the early developmental phase when most of the fruit growth and expansion occurs, sucrose does not accumulate, as it is metabolized by invertase to the hexose sugars, fructose and glucose. The developmental transition to sucrose accumulation occurs in sweet genotypes of *Cucumis melo*, and is correlated with a marked decrease in soluble acid invertase activity in the pericarp tissue (Lingle and Dunlap, 1987; Schaffer et al., 1987; McCollum et al., 1988; Hubbard et al., 1989). In contrast, cucumber fruit, which does not accumulate sucrose upon maturation, retains relatively high acid invertase activity throughout development (Schaffer et al., 1987). A role for sucrose synthesis, via sucrose phosphate synthase (SPS, E.C. 2.4.1.14), has also been proposed by Hubbard et al. (1989) to explain sucrose accumulation in sweet melon genotypes. The final content of sucrose in the fruit pericarp of sweet melon is a function of two factors: the rate of sucrose accumulation, coupled with the duration of the accumulation period until abscission or harvest.

The species *Cucumis melo* shows extreme genetic variation in fruit characteristics, including morphology, aroma, pH and sugar composition and parameters of the maturation process (e.g., climacteric burst, abscission). The melon germplasm includes varieties

with high sucrose levels, as well as genotypes with practically no sucrose at all. These may be either inedible or consumed when immature as pickled or fresh vegetables. Some of the sweet varieties have climacteric maturation that terminates with development of aroma, early abscission or pigmentation changes, while others are long-keeping, non climacteric, non abscising fruits.

In the present study, we examined sugar composition and invertase activity in mature fruits representing the spectrum of varietal groups of *C. melo* described by Munger and Robinson (1991) and Hammer et al. (1986). This set of 56 *C. melo* cultivars, landraces and wild accessions spans much of the genetic variation found in this diverse species. Such a broad germplasm survey can describe important physiological correlations related to melon fruit composition, indicate the existence of genetic variation in different fruit taste components, and suggest new directions for the future exploitation of such diversity.

Materials and methods

Plant material

The set of 56 accessions assembled for this study included sweet 'dessert' cultivars from Europe, America and Japan, belonging to the *cantalupensis* and *inodorous* types; mildly sweet cultivars or landraces from Asia, Europe and Middle East of various types; non-sweet African and Asian wild accessions of the *agrestis* type; non-sweet landraces such as the *conomon*, *dudaim*, *momordica* and *chito* types, and 'snake melons' (*flexuosus* type) from various locations (Table 1). An effort was made to sample representatives of the main 'botanical varieties' (cultivar groups) and include many African, South-Asian, Central Asian and Far Eastern accessions, where primary and secondary centers of diversity of this crop occur (Pangalo, 1929). Seeds for this study came mainly from the Institut für Pflanzengenetik und Kulturpflanzenforschung at Gatersleben (IPK; Courtesy of Dr. Karl Hammer), and the Plant Introduction Station, Ames, Iowa (Courtesy of Dr. Kathy Reitsma), as well as cucurbit breeders in Israel, US and Spain (Table 1). Three plants of each accession were sown in a field plot near Rehovot during summer 1995, with spacing of 50 cm between plants, 150 cm between rows. Average day/night temperatures were approx. 30 °C/ 20 °C. Growth was duplicated in a net-house at Bar-Ilan to allow for additional samples when required. Drip-irrigation and fertilization were according to standard agronomic practice. At least three fruits from each accession were harvested according to the appropriate maturation parameters of each variety: *cantalupensis* types- abscission, development of full netting in the netted genotypes, softening, aroma and color change in some genotypes; *inodorus* types – color change (sometimes subtle) of fully-grown fruits; *flexuosus* – softening of fully-grown fruits; *conomon* – no change in fully grown fruits for approx. 10 days, color change in some genotypes; *chito* – abscission or color change; *dudaim* – abrupt color change and, in some genotypes, development of aroma; *momordica* – fruit splitting and/or abscission, color change; *agrestis* – abscission and/or softening, color change in some genotypes.

TSS and pH

Total soluble solids were examined with an Atago digital refractometer, using drops of juice pressed from a pericarp slice. Pericarp-juice pH was measured using pH-paper sticks (Acilit pH 0-6, Neutralit pH 5-10, Merck).

Soluble sugar analysis

Fruit portions (approx. 1 g fresh weight from the central region of the mesocarp) were extracted four times in 80% ethanol at 70 °C. Soluble sugars were separated by HPLC using a Bio-Rad Fast Carbohydrate column, according to manufacturer's directions, with refractometric detection, as described in Miron & Schaffer (1991). Sucrose, glucose and fructose were identified by their retention times and quantified according to standards.

Invertase activity

Enzyme extraction and assay of activity was carried out as in Miron & Schaffer (1991), with modifications. Mesocarp tissue (approx. 1 g fresh weight, frozen and stored at -70 °C) was homogenized in 5 ml extraction buffer (25 mm HEPES, 7 mm $mgCl_2$, 0.5 mm EDTA, 3 mm dithiothreitol (DTT), 2 mm diethyldithiocarbamic acid (DIECA), pH 7.5). After centrifugation, the supernatant was dialyzed against the same extraction buffer but without DTT and DIECA and used as a crude fruit extract. Invertase activity was measured in 0.6 ml of 0.1 M citrate/ phosphate buffer, (pH 5 or 7), 0.2 ml of crude extract and 0.2 ml of

Table 1. Melon accessions used in this study. Accessions are ordered alphabetically. Data on country of origin and a tentative assignment to describe melon types are presented. Seed source codes: 1 – Institut für Pflanzengenetik und Kultur-pflanzenforschung, Gatersleben, Germany; 2 – Plant Introduction Station, Ames, Iowa; 3 – Dr. A.P.M. den Nijs, Wageningen, The Netherlands; 4 – Mr. S. Niego and R. Herman, Zeraim Gedera Ltd., Israel; 5 – Dr. M. Gomez-Guillamon, C.S.I.C. La Majora, Spain; 6 – Prof. Y. Cohen, Bar-Ilan University, Israel; 7 – Dr. H. Munger, Cornell University, U.S.A.

Code	Origin	Name	Accession No.	Seed Donor	Melon Type (tentative)
ACK	Turkey	Acuk	PI 167057	2	flexuosus
AFG	Afghanistan		PI 125951	2	cantalupensis?
AGR	Africa			3	agrestis
AMA	Spain	Amarillo	C-610	5	inodorus, cassaba type
ARV	Israel	Arava		4	cantalupensis, Haogen type
BAK	South Balkan		CuM 53	1	
BNJ	USA Gem	Burpee's Netted		4	cantalupensis
CCA	Spain	cc26 (Amarillo orange flesh)	C-446	5	inodorus, Cassaba type
CHA	France	Charentais		6	cantalupensis, Charentais type
CHI	uncertain	Chito	PI 140471	7	chito/ dudaim
CHT	India	Chito	PI 164320	2	chito
COC	China		CuM 206	1	conomon
CON	Far East		line 85-893	7	conomon
COV	Vietnam	Kairyo Ogata Kogane Seumari	CuM 246	1	conomon
DHA	Israel	Dvash Haogen2		4	cantalupensis, Haogen type
DUA	Afghanistan		CuM 254	1	dudaim
DUD	uncertain	Dudaim	line 85-895	7	dudaim
DUG	Georgia		CuM 296	1	dudaim
END	Israel	Ein Dor		4	cantalupensis, Ananas type
FLI	India		CuM 227 (=VIR K2511)	1	flexuosus
FLN	India		CuM 225	1	flexuosus
FLR	Iraq		CuM 349	1	flexuosus
FLX	Lebanon	Facus		4	flexuosus
GIN	Japan	Ginsen Makuwa (Silver Spring)	PI 420176	2	conomon
GUO	China	Gou Gua	PI 532829	2	
HCR	Spain	Hilo Carrete	C-198	5	inodorus
HON	USA	Honeydew	line 89A-15	7	inodorus
IML	Kazakhstan	Imljskaja	PI 476342	2	cantalupensis
INA	India		PI 124111	2	
INB	India		PI 124112	2	
IRN	Iran		PI 140632	2	cantalupensis
ITA	Italy		CuM 298	1	cantalupensis
KAF	Afghanistan	Kamyaft	PI 125890	2	
KAK	India	Kakri	PI 164493	2	agrestis
KRK	Turkey	Kirkagac	PI 169305	2	inodorus
KRT	Greece		CuM 51	1	cantalupensis
KUV	URSS	Kuvsinka	PI 506460	2	cantalupensis
LYB	Libya		CuM 294	1	cantalupensis

Table 1. contd.

MEC	China		CuM 255	1	cantalupensis?
MOM	India		PI 414723	7	momordica
OGO	Japan	Ogon No.9	PI 266933	2	conomon
PDS	Spain	Pinonet Piel de Sapo	C-207	5	inodorus, Cassaba type
SAF	Afghanistan	Safed Sard	PI 116915	2	
SAL	Ukraine	Salgirskaja	PI 506459	2	cantalupensis
SEN	Senegal	G 22841	PI 436532	2	agrestis
SON	South Korea	Songwhan Charmi	PI 161375	2	conomon
TEM	Spain	Temprano Roget	C-209	5	inodorus, Cassaba type
TM	USA	Topmark		7	cantalupensis
USM	USA		PI 371795	2	momordica
VEL	India	Velleri	PI 164323	2	
WTP	Turkey	Winter Type	PI 169329	2	inodorus
ZA1	Zambia	ZM/A 5317	PI 505599	2	agrestis?
ZA2	Zambia	ZM/A 5384	PI 505602	2	agrestis?
ZM1	Zimbabwe	TGR 1843	PI 482429	2	agrestis?
ZM2	Zimbabwe	TGR 96	PI 482393	2	agrestis?
ZM3	Zimbabwe	TGR 228	PI 482399	2	agrestis?

0.1 M sucrose. After 1 h incubation at 37 °C, reducing sugars were measured using dinitrosalicylic acid reagent. Reactions with enzyme extract added after the incubation period were used as blank reactions. The sucrose hydrolysis activity measured at pH 5 consists of acid invertase activity (E.C. 3.2.1.26) together with substantial alkaline invertase activity (E.C. 3.2.1.27), since the latter is also active at pH 5 (estimated as 30% of the acitivity at pH 7). Similarly, the activity at pH 7 includes an acid invertase component, estimated as 30% of the acid invertase activity at pH 5 (Schaffer, 1986). In order to correct for the contaminating components, the residual contribution of the contaminating activity was subtracted from the activity measurement, as in Ricardo & ap Rees (1970), according to the equation:

Acid invertase activity, units = A - 0.3 B

Alkaline invertase activity, units = B - 0.3 A,

when A and B are the actual measurements at pH 5 and 7, respectively.

Results

Distribution of sugar patterns among melon varietal groups

We have assembled a set of 56 *Cucumis melo* genotypes from 23 countries to represent the range of genetic variability that can be found in the melon germplasm. A tentative assignment of the accessions to cultivar groups is presented in Table 1. It is based on classification by gene-bank curators, and on our own morphological observations. Intra-specific classification of melons is rather unclear (Munger & Robinson, 1991; Hammer et al., 1986; Bates & Robinson, 1995), and some of our accessions could not be assigned to described groups with any confidence. A fuller description of the morphological and molecular variation in this collection will be presented elsewhere (Stepansky, Kovalsky & Perl-Treves, manuscript in preparation).

The total soluble solids (TSS), sucrose, glucose and fructose concentrations and pH values of the 56 genotypes belonging to different varietal groups are shown in Table 2. Rapid estimates of fruit sugar content are usually obtained by refractometric measurement of the total soluble solids in juice expressed from flesh tissue. As expected, TSS values were cor-

Table 2. Varietal average and their standard deviation of fruit sucrose, glucose and fructose contents, pH and TSS in 56 melon varieties, ordered according to varietal groups.

Varietal Group	Var.	Sucrose ± SD	Glucose ± SD	Fructose ± SD	Total ± SD	TSS ± SD	pH ± SD
cantalupensis	AFG	50.9 ± 11.4	16.9 ±8.3	15.2 ± 5.6	83.0 ± 24.6	12.0 ± 0.4	6.2 ± 0.2
	ARV	43.5 ± 8.5	21.5 ±3.6	18.8 ± 3.2	83.7 ± 15.3	7.7 ± 0.7	6.0 ±0.0
	BNJ	55.5 ± 1.2	18.9 ±1.1	13.8 ± 1.1	88.2 ± 1.0	9.5 ± 1.1	6.8 ± 0.4
	CHA	59.0 ± 0.0	17.0 ±0.0	13.3 ±0.0	89.3 ± 0.0	12.0 ± 0.0	6.5 ±0.0
	DHA	41.8 ± 4.6	17.2 ±2.4	15.4 ±1.0	74.3 ± 7.9	10.0 ±0.0	6.0 ±0.0
	END	44.4 ± 0.0	13.5 ± 0.0	11.1 ± 0.0	69.0 ± 0.0	8.0 ± 0.0	6.5 ± 0.0
	IML	21.0 ± 7.3	20.6 ± 4.7	21.9 ± 6.5	63.5 ± 7.2	6.2 ± 0.4	6.8 ±0.4
	IRN	17.0 ± 7.8	13.5 ± 5.1	11.7 ± 1.1	42.1 ± 1.6	6.2 ±1.6	6.0 ±0.4
	ITA	63.1 ± 18.0	21.0 ± 2.2	17.9 ± 0.9	102.1 ± 19.4	10.4 ± 0.6	6.5 ± 0.0
	KRT	26.2 ± 2.1	16.3 ± 3.1	21.3 ± 2.4	63.8 ± 7.6	9.3 ±0.0	6.0 ±0.0
	KUV	24.5 ± 2.0	29.5 ±7.1	28.0 ± 6.1	82.0 ± 15.2	6.8 ± 0.6	5.7 ±0.2
	LYB	46.2 ± 7.8	19.6 ± 5.8	24.3 ±5.0	90.1 ± 14.0	8.8 ± 0.5	6.5 ± 0.0
	SAL	42.2 ± 0.9	20.0 ± 4.0	14.5 ± 5.6	76.7 ± 8.7	9.2 ± 1.2	6.0 ± 0.5
	TM	28.5 ± 1.1	14.4 ± 2.3	15.5 ± 0.5	58.4 ± 2.8	8.5 ± 0.5	6.8 ± 0.3
inodorus	AMA	35.1 ± 17.5	19.8 ± 1.3	19.4 ± 1.9	74.4 ± 18.5	8.1 ± 0.9	5.5 ± 0.0
	CCA	25.0 ± 8.5	14.9 ± 3.1	13.5 ± 1.1	53.3 ± 12.5	8.9 ± 1.2	6.5 ±0.0
	HCR	52.3 ± 8.5	11.4 ± 2.2	10.2 ± 2.4	73.9 ± 13.2	14.0 ± 1.0	6.0 ± 0.0
	HON	26.1 ± 0.5	22.9 ± 8.1	24.6 ± 9.9	73.6 ± 18.5	7.5 ± 1.5	6.0 ± 0.0
	KRK	6.3 ± 0.0	11.4 ± 0.0	12.7 ± 0.0	30.3 ± 0.0	4.0 ± 0.0	5.5 ± 0.0
	TEM	27.7 ± 19.1	21.4 ± 2.4	16.6 ± 4.6	65.6 ± 12.2	8.2 ± 0.8	6.0 ± 0.0
	PDS	48.1 ± 24.1	17.0 ± 4.5	13.4 ± 3.4	78.6 ± 32.0	11.0 ± 1.0	6.5 ± 0.0
	WNT	8.9 ± 1.3	13.7 ± 3.0	11.5 ± 2.0	34.0 ± 2.3	4.7 ± 0.2	6.3 ± 0.3
conomon	COC	56.1 ± 25.8	16.4 ± 9.6	13.6 ± 6.2	86.1 ± 20.3	9.5 ± 1.1	6.8 ± 0.4
	CON	1.4 ± 0.1	10.3 ± 0.5	10.6 ± 0.0	22.3 ± 0.6	6.2 ± 0.2	4.8 ± 0.2
	COV	9.4 ± 4.2	6.9 ± 2.2	10.7 ± 1.8	26.9 ± 8.1	4.3 ± 0.2	5.8 ± 0.2
	GIN	66.3 ± 9.3	16.1 ± 3.7	15.5 ± 2.7	97.8 ± 15.4	14.1 ± 1.4	5.6 ± 0.2
	OGO	26.5 ± 3.0	15.5 ± 1.1	13.5 ± 1.3	55.5 ± 0.7	8.3 ± 0.8	5.8 ± 0.3
	SON	34.4 ± 7.6	14.6 ± 2.9	12.9 ± 1.5	61.8 ± 6.7	7.9 ± 1.5	5.6 ± 0.2
chito, dudaim	CHI	3.0 ± 0.9	8.8 ± 1.9	11.1 ± 2.7	22.7 ± 4.0	6.2 ± 0.2	4.7 ± 0.4
	CHT	8.5 ± 0.7	19.6 ± 3.2	30.2 ± 1.5	58.4 ± 3.9	n.d.	4.8 ± 0.2
	DUA	7.2 ± 3.4	20.6 ± 9.2	20.4 ± 8.9	48.1 ± 21.6	6.2 ± 0.7	5.5 ± 0.0
	DUD	4.9 ± 0.9	7.7 ± 1.5	8.3 ± 1.3	20.9 ± 3.0	6.0 ± 0.2	5.0 ± 0.0
	DUG	33.6 ± 8.1	11.4 ±2.1	8.2 ± 0.9	53.2 ± 6.3	9.5 ± 0.7	5.7 ± 0.5
momordica	MOM	8.2 ± 1.0	15.6 ± 2.2	13.0 ± 1.2	36.8 ± 4.3	5.5 ± 1.0	5.0 ± 0.0
	USM	1.9 ± 0.4	13.2 ± 3.8	14.7 ± 4.8	29.7 ± 8.9	4.7 ± 0.2	5.0 ± 0.0
flexuosus	ACK	7.5 ± 8.1	11.9 ± 1.9	11.7 ± 1.7	31.0 ± 7.7	4.0 ± 1.2	5.0 ± 0.0
	FLI	0.8 ± 0.0	4.6 ± 0.0	5.7 ± 0.0	11.0 ± 0.0	2.4 ± 0.0	5.0 ± 0.0
	FLN	1.1 ± 0.3	8.1 ± 2.9	8.4 ± 1.5	17.5 ± 4.8	2.2 ± 0.2	5.0 ± 0.0
	FLR	1.6 ± 0.1	9.1 ± 0.5	9.8 ± 0.7	20.5 ± 0.1	3.5 ± 0.5	5.0 ± 0.0
	FLX	3.5 ± 0.5	5.2 ± 2.4	5.1 ± 1.3	13.7 ± 3.5	3.0 ± 0.0	5.0 ± 0.0
agrestis	AGR	1.2 ± 0.6	1.6 ± 0.4	1.1 ± 0.4	3.9 ± 0.7	3.7 ± 0.2	4.7 ± 0.3
	KAK	27.5 ± 10.0	7.1 ± 0.3	6.1 ± 0.7	40.7 ± 9.5	10.0 ± 0.0	5.0 ± 0.0
	SEN	24.7 ± 4.4	14.4 ± 3.8	18.4 ± 5.6	57.5 ± 11.7	9.0 ± 0.0	5.5 ± 0.0
	ZA1	2.4 ± 2.1	11.9 ± 4.5	12.3 ± 5.0	26.5 ± 11.4	3.7 ± 0.2	5.0 ± 0.0
	ZA2	1.4 ± 0.2	9.0 ± 1.2	9.1 ± 0.9	19.5 ± 2.0	4.2 ± 0.2	5.0 ± 0.0
	ZM1	1.0 ± 0.0	6.8 ± 0.0	6.8 ± 0.0	14.6 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
	ZM2	3.0 ± 0.0	15.2 ± 0.0	12.4 ± 0.0	30.5 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
	ZM3	1.4 ± 0.1	7.6 ± 0.2	8.5 ± 0.1	17.5 ± 0.2	4.5 ± 0.5	5.0 ± 0.0
var. non-defined	BAK	16.0 ± 1.5	12.0 ± 1.9	16.3 ± 2.1	44.2 ± 5.0	5.0 ± 0.0	6.8 ± 0.3
	INA	22.9 ± 9.2	21.6 ± 4.5	20.1 ± 3.3	64.6 ± 13.3	7.1 ± 1.6	5.4 ± 0.7
	INB	17.4 ± 8.8	10.9 ± 2.6	10.4 ±1.0	38.7 ± 11.7	7.0 ± 1.8	5.1 ± 0.2
	KAF	7.1 ± 0.7	17.7 ± 1.8	12.3 ± 4.8	37.0 ± 7.4	6.0 ± 0.0	5.5 ± 0.5
	MEC	6.2 ± 0.4	16.3 ± 2.9	15.0 ± 4.1	37.4 ± 6.6	5.7 ± 0.2	5.5 ± 0.0
	SAF	4.4 ± 0.6	20.3 ± 1.3	18.4 ± 1.4	43.1 ± 3.3	3.7 ± 0.7	5.5 ± 0.0
	VEL	1.8 ± 0.7	8.2 ± 1.4	8.3 ± 1.9	18.2 ± 3.9	3.5 ± 0.4	5.0 ± 0.0
	GUO	0.8 ± 0.1	3.7 ± 0.6	4.4 ± 0.4	9.0 ± 1.2	4.3 ± 0.2	5.0 ± 0.0

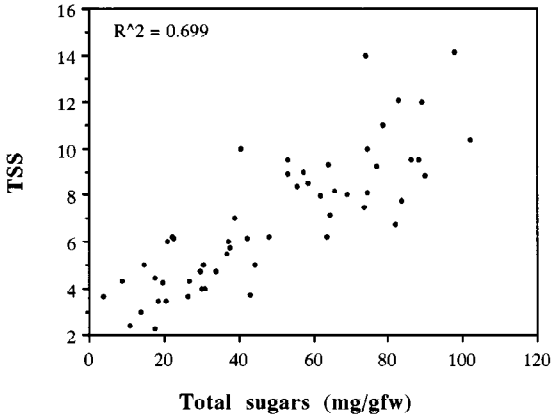

Figure 1. Correlation between total soluble sugars as measured by HPLC quantitation and total soluble solids (TSS) measured by refractometry in 56 *C. melo* accessions from a wide range of botanical varieties. Each point is the average of a minimum of three samples.

related with sum of the three soluble sugars (Figure 1).

Sucrose, glucose and fructose were the major sugars observed in all the genotypes studied. Of the three sugars, sucrose content showed the widest range among the genotypes. Sucrose content, but not the content of glucose or fructose, was positively correlated with the increase in sugar concentration above 40 mg/gfw total sugar (Figure 2), indicating that in the sweeter varieties sucrose is the most significant component that contributes to the variation in total sugars. Significantly, there was no correlation in this group between sucrose and hexose levels. In contrast, genotypes with low total sugars (<30 mg/gfw) do not accumulate sucrose, and total sugar content is correlated only with hexose levels. Interestingly, there were also genotypes with rather high total sugars from the *conomon* and *chito* groups, which contained primarily glucose and fructose, with low sucrose levels comprising only 10-15% of the total sugar (e.g., CHT, DUA). Figures 2B, 2C also indicate that varieties with lowest sugar often contain lower reducing sugars than sucrose accumulators.

Having divided our accessions into varietal groups (Tables 1, 2), we wished to determine whether we could correlate the pattern of sugar accumulation with the botanical/ horticultural classification of melons. The 14 genotypes classified as *cantalupensis* included aromatic, sweet-tasting, medium-large dessert melons from different countries, either netted or smooth-rinded; according to Munger & Robinson (1991), former var. *reticulatus* was incorporated into var.

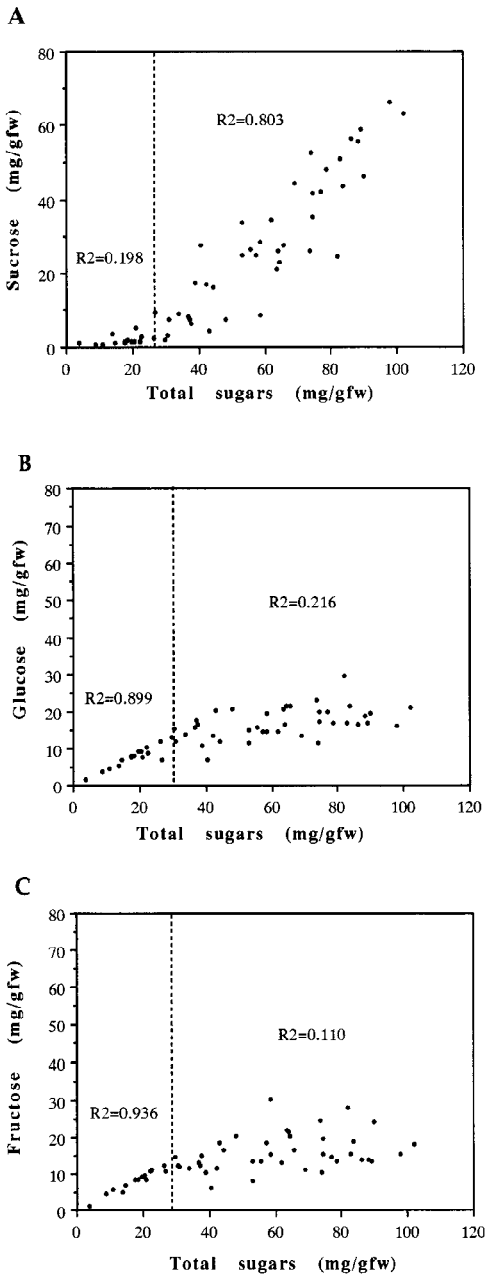

Figure 2. Scatter plots of sucrose, glucose and fructose contents versus total sugar content in 56 *C. melo* accessions. Sugar contents, averaged from 3 HPLC measurments are expressed in mg/gfw. Total sugars are the sum of the sucrose, glucose and fructose measurements. A. Sucrose versus total sugars. Sucrose is the major contributor to total sugars in the high sugar varieties (r^2= 0.8) and not in the low sugar ones (r^2=0.2). B. Glucose vs. total sugar. Glucose contributes about half of the sugar in the low-sucrose varieties, where correlation of glucose with total sugar content is high (r^2= 0.9), and a variable portion of the total sugar in the sucrose storers. C. Fructose versus total sugar. Fructose contributes about half of the sugar in the low-sucrose varieties (r^2= 0.9), and a variable portion of the total sugar in the sucrose storers.

cantalupensis. Their total sugars ranged between 40-100 mg/gfw, and sucrose levels were often above 40 mg/gfw. Sucrose generally consisted of 50-70% of the total sugars, although a few accessions with a lower percent of sucrose were recorded as well.

The *inodorus* group includes Winter Melons, Casaba and Honeydew cultivars with large, non-climacteric fruits. Among the eight accessions studied, both low sucrose- and high sucrose-accumulating genotypes were found. A few genotypes attained only ~30 mg/gfw total sugar, consisting mostly of hexoses (i.e. KRK, WNT); others had high sucrose levels (~50 mg/gfw), consisting of 60–70% of the total sugars.

Conomon melons from the Far-East include vigorous vines bearing smooth, apple-sized fruits with long-keeping, firm flesh. Most are non-sweet, pickled-vegetable types or mildly-sweet fruits, but sweet cultivars are present as well. Among the six *conomon* genotypes in this study, we observed genotypes with almost no sucrose (CON) as well as genotypes with intermediate and high sucrose levels and rather high total sugars (e.g., GIN, >60 mg/gfw).

The five genotypes belonging to the *chito* and *dudaim* groups have small, mildly sweet or non-sweet fruits. Four of them accumulated less than 10 mg/gfw sucrose, but one (DUG) had intermediate sucrose levels, and CHT had an unusual sugar pattern – it attained rather high levels of total sugar due to elevated hexose levels.

The varietal group *agrestis* consists of wild, weedy forms with small fruits that are often bitter and inedible. Most genotypes tested had extremely low levels of sugars. Rather surprisingly, two of the agrestis genotypes, KAK and SEN, had fruits that accumulated significant levels of sucrose (~25 mg/gfw) and rather high total sugars (41 and 58 mg/gfw, respectively).

The genotypes of two other varietal groups, *momordica* (mealy, insipid fruits of India) and *flexuosus* (elongated melons, eaten when immature as cucumbers) did not accumulate significant amounts of sucrose, and had rather low hexose levels as well.

Variation in invertase activity

Our hypothesis was that a developmental decrease in acid invertase activity is required for the accumulation of sucrose during fruit maturation. We therefore predicted that the high-sucrose varieties would have low acid invertase activity, while low-sucrose ones may maintain high activity. Figure 3A depicts the relationship between acid invertase activity and sucrose content. It clearly shows that all the accessions with significant sucrose accumulation (more than 15 mg/gfw) were characterized by low acid invertase activity (<1 unit/gfw), with the exception of KAK, an *agrestis*-like weedy accession that contained 2.3 units invertase and 27 mg sucrose /gfw.

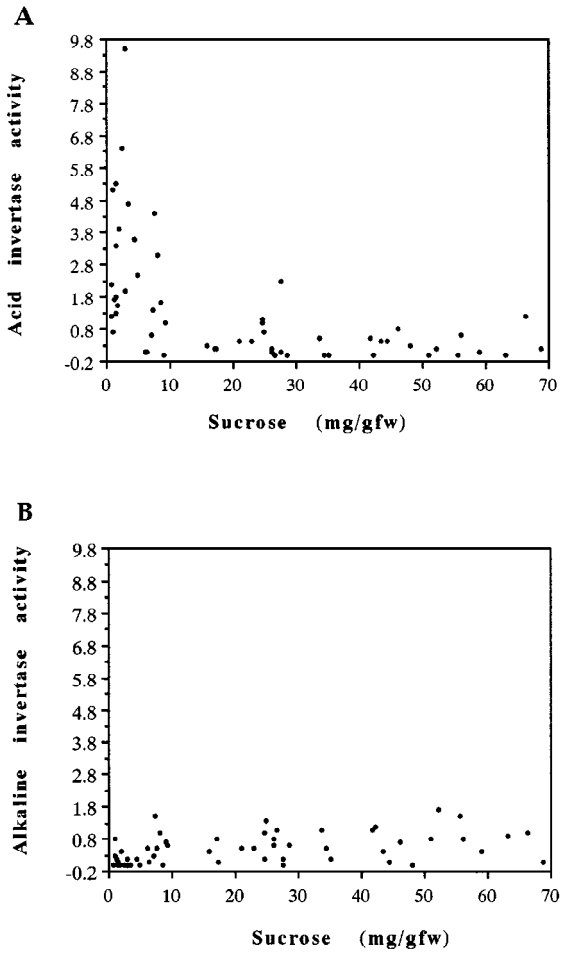

Figure 3. Scatter plot of invertase activity versus sucrose content in fruits of 56 *C. melo* accessions. Averages of invertase measurements from 2–4 fruits are presented, and are expressed in units/gfw (1 unit = 1 mg reducing sugar/h); sucrose measurements (mg/gfw) are averages from at least 3 fruits. A. Acid invertase. B. Alkaline invertase.

The low-sucrose region of the plot includes accessions with high acid invertase (e.g., ZM2, FLN, FLX), but also some with low invertase (ZM1, MEC, KRK), or with intermediate values (FLI, FLR, VEL). Similar heterogeneity is found, in fact, among individual fruits of some non-sweet accessions as well. For example, two CON samples had 5.75 and 0.92 invertase units, and two ACK samples had 7.53 and 0.82 units, respec-

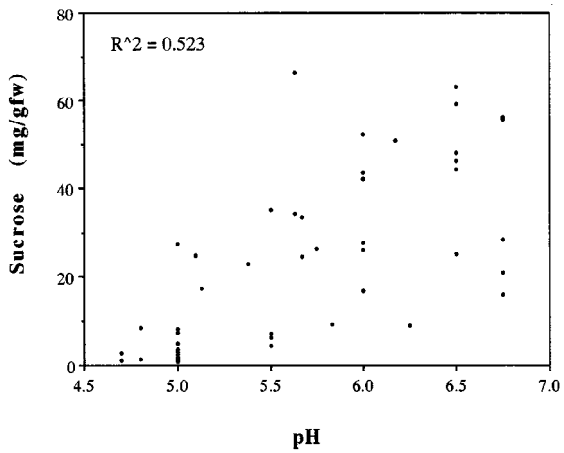

Figure 4. Scatter plot of fruit pH values vs. total sugars content in the fruits of 56 *C. melo* accessions. Average measurements from at least 3 fruits are shown, as detailed in Table 2.

tively. This may indicate that in the non sweet varieties invertase is high until late in the ripening process, but it eventually decreases as well. Since varieties such as *flexuosus* or *conomon* exhibit long and inconspicuous maturation stages, we may have picked a few samples that have undergone such a late decrease in invertase. In any case, this was not accompanied by sucrose accumulation. The activity of basic invertase varied over a narrow range (0 to 1.7 units/gfw), and did not exhibit significant correlation with the fruit sucrose content (Figure 3B).

In a study by Zrenner et al. (1996), potato cultivars were surveyed for hexose accumulation during cold storage. While hexose levels did not correlate with invertase activity, a strong correlation was observed between invertase activity and the hexose/sucrose ratio. We tested whether such correlation could be found in melon fruits, but it was rather loose (r^2=0.35): while most accessions with low hexose/sucrose ratio indeed had low invertase, those with higher ratios had variable invertase levels.

Variation in fruit pH

Fruit pH values are presented in Table 2 and the correlation between sucrose content and fruit pH is depicted in Figure 4. Almost all the sucrose-accumulating sweet varieties of the *cantalupensis* and *inodorus* types had near-to-neutral pH values (6-6.75). The low sucrose accumulating genotypes spanned the range of flesh pH values and included both acidic and neutral genotypes, but genotypes with low pH always had low sucrose levels. These included *flexuosus*, *chito*, *agrestis*, *momordica* and a few accessions of the *conomon* type.

Discussion

Changes in sucrose and hexose levels during melon fruit development and ripening have been studied at the physiological level in a few selected varieties of *Cucumis melo*, due to their commercial importance (Yamaguchi et al., 1977; Lester and Dunlap, 1985; Lingle and Dunlap, 1987; Schaffer et al., 1987; McCollum et al., 1988). Our broad survey of sugar profiles in ripe fruit of the *C. melo* species shows that soluble sugar content varies considerably, ranging from genotypes that accumulate very little sugar, to sweet genotypes which accumulate significant levels of sugar. Sucrose levels vary over a broader range than fructose and glucose levels. The genotypes with the lowest levels of soluble sugars (< 20 mg/ gfw) are characterized by the absence of sucrose accumulation as well as low glucose and fructose levels. In this subgroup total sugar levels are correlated with hexose levels alone. However, with the exception of this low sugar subgroup, total sugar levels in the broad spectrum of *C. melo* genotypes are correlated only with sucrose levels. Although higher hexose levels are found in the intermediate and high sucrose accumulators, as compared to the low sugar accumulators, there is no correlation between sucrose and hexose levels in those genotypes which accumulate sucrose. Accordingly, the conclusion from this observation is that variation in hexose levels does not explain the broad variation in total soluble sugar in *C. melo*, with the exception of low sugar genotypes that do not accumulate sucrose.

We thus observe that within *C. melo* all high sugar genotypes are based on sucrose accumulation, and there are no high sugar genotypes based on hexose accumulation. Although sucrose accumulation characterizes most species having sweet, fleshy fruits, hexose accumulation is found in a few species such as the grape berry which accumulates only hexoses (Coombe, 1960). The present study seems to indicate that a mechanism of high hexose accumulation, as a means to attain high-sugar fruits, has not evolved in *C. melo*, and it is unlikely that classical breeding strategies will produce such genotypes. Nevertheless, there exists variability in hexose levels in melon fruits, although the degree to which this variation is genetically determined or heritable cannot be determined

from the present study. Sucrose accumulation and hexose levels appear to be independently controlled. If the variability in hexose levels is heritable, this could have important implications for genetic advances in sugar levels in melons, since increased hexose levels could be selected for independently from increased sucrose levels.

An interesting, and surprising, conclusion of this study is that high sugar levels and sucrose accumulation are not restricted to the *cantalupensis* and *inodorus* 'dessert melon' types. Varieties that accumulate significant amounts of sugars are found among *conomon* (GIN, COC), *chito* (CHT), and even *agrestis* types (SEN, KAK). We do not know whether the diverse genetic backgrounds are indicative of distinct genetic loci controlling sugar accumulation patterns in each group. However, it does indicate that the evolution of sucrose accumulation in *C. melo* may be a complex story. Previously, Mallick & Masui (1986) suggested that the evolution of *C. melo* followed a pattern of domestication which consisted of the stepwise selection for sugar levels and low acidity. If so, identification of high-sugar varieties of other melon types would suggest parallel evolution of sweet genotypes. Our continuing study of the genetic relationships among the *C. melo* genotypes should shed light on the subject.

Significantly, sucrose accumulation was observed even in the *agrestis* group. These weedy-looking, wild-growing plants bear numerous small (<5 cm), often bitter, inedible fruits that lack distinct flesh. While accession AGR did not accumulate sucrose at all, and also had extremely low levels of reducing sugars, other accessions were heterogeneous, and some of their plants bore sweet fruits (KAK, SEN), whose sugar patterns clearly indicate the potential to accumulate sucrose. This was confirmed by growing additional accessions of var. *agrestis* from Afghanistan and Senegal, and these also included plants with sweet fruits (data not presented).

The presence of high sugar accumulation in the various botanical groups of *C. melo* is significant, since low yield, small-fruited wild accessions can contribute quality parameters in conventional plant breeding. This has been shown in tomato, where QTL alleles that increased yield and TSS could be identified in unadapted material (Paterson 1995; de Vicente & Tanksley, 1993; Eshed & Zamir, 1994). Moreover, the sucrose accumulation trait has been introgressed from *Lycopersicon chmielewskii* Rick (Chetelat et al. 1995) and *L. hirsutum* Humb. et Bompl. (Hadas et al. 1995) to *L. esculentum* Mill. Similar approaches may be used in melons, where breeders have traditionally restricted themselves to a narrow pool of sweet dessert cultivars of the *inodorus* and *cantalupensis* types. As much of the molecular and morphological variation in the germplasm resides outside such a narrow base (Staub et al. 1997), it may be possible to contribute genes for sugar accumulation from exotic accessions of poor horticultural quality, as indicated in our study.

The developmental loss of sucrose hydrolyzing activity (acid invertase) in the melon fruit has been proposed as the genetically controlled metabolic determinant of sucrose accumulation (Schaffer et al., 1987; Hubbard et al., 1989; Burger, Levin & Schaffer, unpublished). Our results support this, as many of the low sucrose accumulators had high invertase activities when ripe, while the the high sucrose accumulators had low acid invertase activities. However, not all genotypes with low invertase activity necessarily accumulated sucrose, indicating that the loss of invertase activity is one requisite for sucrose accumulation but other physiological components are also required in order for sucrose to accumulate. Hubbard et al. (1989) proposed that the sucrose phosphate synthase (SPS) pathway is also necessary for sucrose to accumulate. A more detailed study of fruit maturation and the developmental loss of invertase activity in these genotypes may shed further light on the relationship between invertase activity and sucrose accumulation. Since sucrose accumulation is a developmental, cumulative process, it is likely that in some genotypes the observed decrease in invertase activity occurs developmentally late, not leaving sufficient time for sucrose accumulation before harvest. Increasing the length of time that the melon fruit remains on the vine by agrotechnical means can increase the soluble sugar content (Welles & Buitelaar, 1988). Similarly, transgenic Charentais melons transformed with antisense ethylene forming enzyme (EFE) had a moderately increased soluble solids content, possibly due to an extended developmental period (Ayub et al., 1996).

We observed a relation between fruit pH and sucrose level, in which the high sucrose genotypes had high pH values, and acidic genotypes had low sucrose. The reason for such association is presently unknown, and two hypotheses may be advanced. The first is that one underlying physiological mechanism affects both pH and sugar level, e.g., that a common substrate (photoassimilate) is the precursor for both organic acid and sucrose accumulation, or that sucrose accumulation occurs physiologically only at higher pH. An alterna-

tive explanation would assume two separate traits that are either genetically linked or that evolved together under domestication.

Acknowledgements

The authors wish to thank Miriam Fogelman for her valuable assistance in the HPLC and invertase analyses. This research was supported by Grants No. IS-2129-92 and IS-2270-93 from BARD, the United States-Israel Binational Agricultural Research and Development Fund.

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