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Y. Danin-Poleg · N. Reis · G. Tzuri · N. Katzir

Development and characterization of microsatellite markers in *Cucumis*

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Abstract This study provides a set of useful SSR markers and describes their development, characterization and application for diversity studies. Sixty one *Cucumis* SSR markers were developed, most of them (46) from melon (*Cucumis melo* L.) genomic libraries. Forty of the markers (30 melon and 10 cucumber SSRs) were evaluated for length polymorphism in a sample of 13 melon genotypes and 11 cucumber (*Cucumis sativus* L.) genotypes. PCR-amplification revealed up to six size alleles among the melon genotypes and up to five alleles among the cucumber genotypes, with mean gene-diversity values of 0.52 and 0.28 for melon and cucumber, respectively. These differences are in accordance with the known narrower genetic background of the cucumber. SSR data were applied to phylogenetic analysis among the melon and cucumber genotypes. A clear distinction between the 'exotic' groups and the sweet cultivated groups was demonstrated in melon. In cucumber, separation between the two sub-species, *C.sativus* var. *sativus* and *C.sativus* var. *hardwickii*, was obtained. Conservation of SSR loci between melon and cucumber was proven by sequence comparisons.

Keywords Simple sequence repeats (SSR) · Melon · Cucumber · Diversity · Inter-species conservation

Introduction

Simple sequence repeats (SSRs, also termed microsatellites) consist of a variable number of tandem repeats, from two to five nucleotides, that form highly informative, locus-specific genetic markers. SSRs are abundant and evenly dispersed throughout Eukaryotic genomes. They can be analyzed efficiently by the polymerase chain reaction (PCR), using primers specific to their flanking regions. Variation in PCR product length is a function of the number of SSR units. SSRs have been recommended as standard markers to be used in the preparation of highly saturated genetic maps (Beckmann and Soller 1990), and have been studied in a large number of plant species including *Arabidopsis* (Bell and Ecker 1994), maize (Senior and Heun 1993), rice (Wu and Tanksley 1993) and soybean (Akkaya et al. 1992). The high level of polymorphism associated with SSRs in melon (*Cucumis melo* L.) and cucumber (*C. sativus* L.) and the cross homology of SSRs between the two species were demonstrated in a previous study (Katzir et al. 1996). This study was based on the only seven SSRs reported so far for the *Cucumis* genus. In addition, the application of SSR markers for mapping has been demonstrated (Danin-Poleg et al. 2000).

The genus *Cucumis* (Cucurbitaceae) includes two commercial vegetable crops, melon (*C.melo*) and cucumber (*C. sativus*). *C. melo* is considered to be the most morphologically diverse species in the genus *Cucumis* (Whitaker and Davis 1962; Kirkbride 1993). This morphological variation led to the intra-specific classification into eight groups by Naudin in 1859 (Whitaker and Davis 1962). Three groups include most of the edible cultivars (vars. *cantaloupensis*, *reticulatus* and *inodorus*) while most of the wild or exotic germplasm was classified into four groups (vars. *flexuosus*, *conomon*, *dudaim*, and *acidulus*). Several studies have applied various marker systems (including RFLP, RAPD, AFLP and ISSR) to assess genetic variation within *C. melo*. (Neuhausen 1992; Baudraco-Arnas et al. 1996; Katzir et al. 1996; Staub et al.1997, 2000; Wang et al. 1997; Danin-Poleg et al. 1998, 2000; Garcia et al. 1998;.Silberstein et al. 1999; Stepansky et al. 1999). Different estimates for the degree of genetic variation were obtained in these studies, reflecting differences in the selected sets of genotypes. A large-scale survey conducted by Stepansky et al. (1999) included 54 genotypes, representing most melon groups, which were analyzed using

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Y. Danin-Poleg · N. Reis · G. Tzuri · N. Katzir (✉)
Department of Vegetable Crops,
Agricultural Research Organization, Newe Ya'ar Research Center,
P.O.B. 1021, Ramat Yishay 30095, Israel
e-mail: geneweya@netvision.net.il

different (morphological and biochemical, RAPD and ISSR) marker systems. A major distinction between the 'exotic' melon groups and the cultivated groups was obtained in the above study based on all three categories of markers. In two other studies (Garcia et al. 1998; Staub et al. 2000) 32 and 46 genotypes were evaluated, most of which belong to the cultivated groups. Differentiation within the cultivated groups was less-clear in all these studies. It was suggested that this is partly due to the combining of genetic material by breeders and to the free hybridization between wild and cultivated germplasm (Stepansky et al. 1999).

Conservation of genomes between phylogenetically related species in the course of evolution has been reported in many plant families, including tomato and potato (Bonierbale et al. 1988), rice and maize (Ann and Tanksley 1993), and *Arabidopsis* and *Brassica* crops (Langercrantz 1998). Such conservation facilitates the identification of agronomic traits in one crop, based on mapping information of their homologues in a related crop. SSR markers have been examined and found to be informative across species in only a few cases, e.g. *Vitis* spp. (Thomas and Scott 1993), *Glycine* spp. (Peakall et al. 1998), and recently among *Lycopersicon* spp. (Areshchenkova and Ganal 1999). In other cases SSRs were not conserved across species, mainly those within the Gramineae (Devos and Gale 1997).

In the present paper, we describe the development, characterization and application of 40 useful and polymorphic *Cucumis* SSRs. The utility of these SSRs for diversity studies in both *C. melo* and *C. sativus* is presented. The conservation of SSRs between these two species is directly demonstrated by the DNA sequencing of SSR loci.

Materials and methods

Plant material

Thirteen *C. melo* L. accessions were selected to investigate SSR polymorphism (Table 1, Katzir et al. 1996). The accessions belonged to seven out of eight horticultural groups, classified by Whitaker and Davis (1962) after Naudin 1859. All accessions were bred and maintained by self-pollination and sib-pollination at Newe Ya'ar, except for two, Vedrantais and Songwhan Charmi (PI161375), which were obtained from M. Pitrat (INRA, Montfavet, France).

The following 11 *C. sativus* L. genotypes tested for SSR polymorphism have all been described previously (Katzir et al. 1996): *C. sativus* L. var. *sativus*: Bet Alfa, Shimshon, Ashley, Pickmore, Dasher F1, Calypso, Colet, GY14, G421 and H19; *C. sativus* L. var. *hardwickii*: PI 183967. The DNA of all genotypes was isolated from the bulked leaf tissue of ten plants of each genotype using the mini-preparation procedure described by Fulton et al. (1995).

Development of SSR markers

SSR markers were developed from: (1) a small-insert plasmid library constructed from a melon genomic DNA as described below; (2) a small-insert melon genomic phage library constructed as described below; (3) a cucumber cDNA library (obtained from R. Perl-Treves, Bar-Ilan University, Ramat Gan, Israel; Perl-Treves et al. 1998); and (4) database sequences.

The procedures for constructing the small-insert plasmid library and for the isolation of SSR-containing clones were as previously described (Katzir et al. 1996). Briefly, melon DNA (cv 'Noy Yizre'el') fragments of 200–400 or 400–600 bp, obtained by *Sau*3AI, were cloned into the *Bam*HI site of pBluescript II KS$^+$. The recombinant plasmids were used to transform the XL-1 Blue *Escherichia coli* strain, followed by blue/white color selection. Selected colonies were picked onto microtiter plates containing LB medium with ampicillin (50 mg/ml).

A second small-insert phage library of melon was constructed from melon (cv 'Noy Yizre'el') DNA fragments of 400–700 bp, obtained by *Acs*I (Boehringer Mannheim Biochemica, Germany). Fragments were ligated to the lambda ZAP II vector followed by packaging with the Gigapack III Gold packaging extract and transfection of the XL1-Blue cell (Stratagene).

Hybridization procedures, outlined by Sambrook et al. (1989), were followed throughout. Filters were hybridized to γ^{32}P-ATP end-labeled $(CT)_{10}$, $(TCC)_5$, $(CAC)_6$ and $(ATT)_6$ oligonucleotide probes at 37°C, overnight. Membranes were washed three times for 5-min at 37°C in 6×SSC and exposed to an X-ray film (Kodak). Positive purified phage clones were excised from lambda ZAP II according to the Stratagene protocol.

The cucumber cDNA library was screened with $(CT)_{10}$, $(TCC)_5$, $(CAC)_6$ and $(GTT)_6$ oligonucleotide probes as described for the phage library.

Positive clones were confirmed as follows: (1) a set of four plasmid clones derived from each positively hybridizing colony was screened by colony hybridization using the same probe; (2) Positive clones were re-screened by PCR to check the presence, nature and position of the microsatellites within the clones in three

Table 1 Description of melon genotypes studied

Melon group	Genotypes	Origin of genotype	Market class
C. melo var.:			
cantaloupensis	Vedrantais[a]	France	Charentais
reticulatus	Noy Yizre'el	Israel	Ha'Ogen
	Krymka	Crimea	Galia
	Eshkolit Ha'amaqim	Israel	Galia
	Dulce	U.S.A.	Americam Shipper
inodorus	Q36	Spain	Casaba-Rochet
	Baku[b]	Azerbaijan Republic	Casaba-Muskmelon
flexuosus	Faqus[b]	Mediterranean	Snake melon
conomon	P15	Mediterranean	Oriental pickling melon
	Freeman's Cucumber[b]	South East Asia	Oriental pickling melon
	PI161375[a]	Korea	
dudaim	Dudaim[b]	Turkey	Dudaim
acidulus	PI414723	India	Snap melon

[a] Baudracco-Arnas and Pitrat 1996 (grouping according to M.Pitrat, personal comunication)

[b] Originated from 'exotic' germplasm, self-pollinated and selected for uniformity for several generations (Dudaim – S1; Faqus – S3; Freeman's Cucumber – S4; Baku – S6)

Table 2 DNA sequences of primers for 34 newly developed SSR markers

SSR designation	SSR locus[a]	Sense primer 5′ to 3′	Antisense primer 5′ to 3′	Expected size (bp)[b]
From:				
Melon genomic library				
CMGA15		CGGCAAGACGATTGGCAGC	ATCACCGTAGCGAAGCACC	150
CMCT44		TCAACTGTCCATTTCTCGCTG	CCGTAAAGACGAAAACCCTTC	104
CMGA104		TTACTGGGTTTTGCCGATTT	AATTCCGTATTCAACTCTCC	125
CMACC146		CAACCACCGACTACTAAGTC	CGACCAAACCCATCCGATAA	152
CMCTT144		CAAAAGGTTTCGATTGGTGGG	AAATGGTGGGGGTTGAATAGG	192
CMTC47		GCATAAAAGAATTTGCAGAC	AGAATTGAGAAGAGATAGAG	168
CMAT141		AAGCACACCACCACCCGTAA	GTGAATGGTATGTTATCCTTG	176
CMCCA145		GAGGGAAGGCAGAAACCAAAG	GCTACTTTTGTGGTGGTGG	142
CMTC168		ATCATTGGATGTGGGATTCTC	ACAGATGGATGAAACCTTAGG	200
CMGA172		CAATCGCAGATACTTCCACG	TGCTTGTCCCAACGGTGTCAT	114
CMTC123		CGGATTGTACTTATTGCCAAG	CATGTGCATGTGTGCATGTAC	106
CMCT126		CTTTAGGTGTGAGATTGGTGG	GCACATCATTGGAGTAACTCG	192
CMTC158		CCCCCATATTCATCAAAACT	TCAGCTCACTTTTCCATTCA	174
CMGT108		CTCCTTCAAACATTGTGTGTG	GAGATAGGTATAGTATAGGGG	187
CMTC163		CTAAAACCTAACTCTTTTCC	TTTGTGGGACTCGTTGAATGA	86
CMTAA166		GGAACAGACACCTCTTCTGAG	TCCGTCTACAAGCGTGACTGT	167
CMTA170a		TTAAATCCCAAAGACATGGCG	AGACGAAGGACGGTTAGCTTT	125
CMCT170b		ATTGCCCAACTAAACTAAACC	CACAACACAATATCATCCTTG	160
CMGA165		CTTGTTTCGAGACTATGGTG	TTCAACTACAGCAAGGTCAGC	97
CMTA134a		ACGTGCTTCAGTAAACATG	CCGACATTGAAAACCAACTTC	159
CMCT134b		GCTCCTCCTTAACTCTATAC	GCATTATTACCCATGTACGAG	123
CMCT160a		GTCTCTCTCCCTTATCTTCCA	ACGGTGTTTGGTGTGAGAAG	88
CMTC160a+b		GTCTCTCTCCCTTATCTTCCA	GATGGTGCCTTAGTTGTTCCG	215
CMCT505		GACAGTAATCACCTCATCAAC	GGGAATGTAAATTGGATATG	219
Cucumber cDNA library				
CSCTTT15a		GTTTGATAATGGCGGATTGT	GTAGAAATGAAGGTATGGTGG	198
CSGTT15b		ACCTTGTTGATTCGGTTCTCC	AGTTCGGTTTAACTACCCACG	161
CSTCC813		GTTGTGCTCCCCAATAGTTG	CACCACTTCTTCCACCGAA	144
CSCT335		CCTTCACTTCCATCTTCATC	CGGTCCTTCATTTCATAGAC	120
Database melon				
CMAT35	CMEFEA	GTGGGTCATCATTATTGTTA	GCTTTTAGCCTATTAAGTTGC	110
CMTC51	CMACO1	ATTGGGGTTTCTTTGAGGTGA	CCATGTCTAAAAACTCATGTGG	160
Cucumber				
CSAT214	CSSEQ	TTGAGTACCATTGTCATAGAT	TTAGTTTAATTTCATCTCTGT	111
CSAT425	CSMASY	TAGGGCAGGTATTATTTCAG	ACGGACTGATTTAGTATAGGC	93
CSCCT571	CSG3PAT	CCTTTCTGCTGTTTCTTCTTC	GAAGGAAGGAGTGAGGGGAAG	209
CSTA050	CS-ACS1	GAATTATGCAGATGGGTCTT	CAAGAAGATCAAATGATAGC	163

[a] Data-base accession number

[b] Expected size of the amplification products obtained with the 'Noy Yizre'el' variety from which the library was constructed, or expected size according to the cucumber cDNA library, or the expected size according to data-base sequence

amplification reactions using T3 and T7 primers, and the microsatellite repeat sequence as primers together with the T3 or T7 primers. Confirmed positive clones were selected for DNA sequencing.

Search in sequence databases

The EMBL and GenBank databases were searched and two melon and six cucumber SSR-containing sequences were selected. Six of the SSRs were from the non-coding regions of two *C. melo* and four *C. sativus* genes. EMBL accessions CMEFEA, CMACO1, CSSEQ and CSMASY were used to generate SSR markers CMAT35, CMTC51, CSAT214 and CSAT425 respectively (Table 2). In addition, two SSR containing sequences, CSLHCPA and CSHPRAG (CSGA057 and CSAT542, see Table 4), had previously been described (Katzir et al. 1996). Two SSRs were from the coding region of *C. sativus* genes: EMBL accessions CSG3PAT and CS-ACS1 (CSCCT571 and CSTA050, Table 2).

Amplification of microsatellite loci

Microsatellite analysis, primer selection and primer synthesis were previously described by Katzir et al. (1996). Briefly, PCR-reaction mixtures contained 60 ng of plant genomic DNA, 1 mM of Mg^{2+}, 8–10 pmol of 3′ and 5′ primers, 166 µM of dATP, dTTP, dGTP, 2 µM of dCTP, 0.1 µl of 3000 Ci/mmol [α-^{32}P] or [α-^{33}P] dCTP, 1× *Taq* Buffer, 1 unit of *Taq* DNA polymerase (Advanced Biotechnologies, UK), in a total volume of 15 µl. The amplification program was as follows: 30 s at 94°C, 30 s at 51°C, and 60 s at 72°C for 34 cycles on a thermocycler (PTC-100 MJ Research Inc.). PCR products (3.0 µl/lane) were separated on a DNA sequencing gel, containing 6% polyacrylamide, 8 M urea and 1×TBE, at 60 W constant power for 1.5–2.5 h. An M13 DNA sequence was used as a size marker. After drying, the gels were exposed to a Kodak XAR-5 or MR film (Eastman Kodak) for ^{32}P- or ^{33}P-labeled products respectively.

Statistical analysis

Gene diversity was calculated as $1-\Sigma P^2_{ij}$, where P_{ij} is the frequency of the jth allele for the ith SSR locus; it was summed across alleles as described by Katzir et al. (1996). In most cases only one allele was detected per locus in each genotype. However, in a few instances two alleles per locus were detected; this resulted from a plant heterozygous for the locus in question. In these cases, allele frequency was calculated as 2 out of 26 gametes (for the 13 melon genotypes), except for the two alleles produced by a heterozygous genotype (see Table 4), whose frequencies were calculated as 1 out of 26. The same calculation was performed for the 11 cucumber genotypes.

Microsatellite data for all genotypes of a specific locus were scored as present (1), absent (0) or heterozygotus (0.5) for each of the two alleles presented. These data were used to calculate Nei's distance coefficients (Nei 1973, 1987) and to generate the corresponding matrix showing the genetic distance for each pair of 13 melon genotypes and 11 cucumber genotypes separately. These matrices of the genetic dissimilarity coefficients were used to determine the relationships among genotypes. The dendrogram was constructed by means of the UPGMA (the unweighed pair-group method with arithmetic average) with the Systat 7.0 computer software.

Results

SSR isolation and characterization

Three approaches were applied for the isolation of SSRs: (1) screening of melon genomic libraries, which resulted in the isolation of 46 SSRs; (2) screening of a cucumber cDNA library, which resulted in the isolation of six SSRs; and (3) a search of DNA sequence databases, which yielded two melon and seven cucumber SSRs.

The distribution of the 61 SSRs in melon genomic libraries provided an estimation of the frequency of their occurrence in the melon genome (Table 3). The CT repeat probe was the most effective for the isolation of SSRs from melon genomic libraries. Of the clones screened (covering approximately 12 Mbp), 0.2% contained a $(CT/GA)_n$ repeat with $n>7$, leading to an estimation of a CT/GA repeat appearing once every 250–500 kbp along the melon genome. Our results indicated that the minimum of seven repeats (*n*) was required for an SSR to be polymorphic in the case of a dinucleotide core motif, and a minimum of five repeats for a tri-nucleotide. Screening the cDNA library using the same CT repeat probe was less effective. Many of the 26 clones that were sequenced included a long repeat ($n>29$) at the 3′ end that was probably an artifact introduced during the construction of the library. Only one polymorphic CT/GA-containing SSR was finally developed from that search. Screening the cDNA library with triplet repeat-probes yielded one TCC/AGG SSR and one GTT/CAA SSR that had an additional repeat of CTTT/CAAA (Table 4).

Unique primers from the flanking regions of all 61 SSR loci were synthesized and used to amplify DNA extracted from melon (*C. melo*) and cucumber (*C. sativus*) genotypes. Fifty three SSRs amplified a clear product, from the DNA of at least one genotype, under the standard PCR-reaction conditions. Forty of the fifty three SSRs (30 from melon and 10 from cucumber) were further evaluated for length polymorphism in a sample of 13 melon genotypes and 11 cucumber genotypes (Table 4). The sequences of the primers used for the amplification of 34 SSR markers are presented in Table 2. The sequences of the remaining six SSRs were previously published by Katzir et al. (1996). SSR designation included: (1) the origin of the repeat sequence – CM for *C. melo* and CS for *C. sativus*; (2) the core motif of the repeat (the largest repeat in case of a compound one); and (3) an identification number. Primers were designed to amplify products in the range of 86–209 bp. In four cases two different pairs of primers were developed from the same DNA sequence (Table 2: CMTA170a and CMCT170b; CMTA134a and CMCT134b; CMCT160a and CMTC160a+b; CMAT35 and CMTC51); therefore, the 40 SSR markers represented 36 loci**.** A single product was amplified by each of 39 SSR markers. The 40th SSR marker (CSAT425) amplified one product from cucumber DNA and two products from melon DNA (Table 4). Of the 40 SSRs, only 14 contained a pure repeat with a single core motif (e.g. CMACC146, CMTC168). All others were either compound repeats, composed of more than one microsatellite repeat (e.g. CMTC13, CMTAA166), or interrupted repeats (e.g. CMCT 44, CSCT335), or both combined and interrupted repeats. The repeat number (*n*) ranged from 7 to 15 for the dinucleotide, and from 5 to 10 for the trinucleotide repeats.

SSR length polymorphism and genetic diversity in melon (*C. melo* L.)

The 40 SSRs were used to assess variation among the 13 *C. melo* L. accessions (Tables 1, 4). Thirty of the SSRs

Table 3 Estimated frequency of occurrence of SSR repeats on the melon genome, according to sequencing data obtained from SSR containing clones of melon genomic libraries

Repetitive element used as a probe	Size of library screened in Mbp.	No. of clones containing SSR[a]	Frequency in kbp
$(GA/CT)_n$	12.1	34	238–528[b]
$(CAC/GTG)_n$	1.92	3	640
$(TCC/AGG)_n$	2.11	1	–
$(ATT/TAA)_n$	1.31	5	262
Others	–	3	–

[a] Including clones with a $(GA)_n$ core motif where $n>7$ units, or triplets with $n>5$ units
[b] Data based on screening of bacterial (4.7 Mbp) and phage (7.4 Mbp) genomic libraries, respectively

Table 4 Gene-diversity values and number of alleles detected by 13 varieties of melon and 11 varieties of cucumber, using 30 melon SSRs and ten cucumber SSRs. * Calculated for 26 gametes (13 melon varieties), ** calculated for 22 gametes (11 cucumber varieties)

SSR designation	Core motif and number of repeats	Melon		Cucumber	
		Alleles[a]	Gene diversity[b]	Alleles	Gene diversity
From melon genomic libraries					
CMTC13*	$(TC)_{12}(CG)_5(AG)_3$	5	0.68	1	–
CMAG59	$(GA)_2A(AG)_8$	5	0.72	nss	
CMGA127*	$(GA)_{13}A(GA)_2$	4	0.39	ns	
CMGA128	$(GA)_{10}AA(GA)_2$	3	0.54	ns	
CMGA15	$(GA)_7$	2	0.43	1	–
CMCT44*	$(CT)_{10}TGTT(CT)_3$	4	0.33	2	0.17
CMGA104*	$(GA)_{14}AA(GA)_3$	6	0.72	ns	
CMACC146	$(ACC)_9$	3	0.46	1	–
CMCTT144*	$((CTT)_{10}CTAC(CTT)_4$	6	0.77	2	0.17
CMTC47*	$(TC)_9(CT)_6$	5	0.61	2	0.17
CMAT141	$(AT)_7(GT)_6$	4	0.49	nss	
CMCCA145**	$(CCA)_5$	2	0.36	2	0.24
CMTC168	$(TC)_{14}$	4	0.63	ns	–
CMGA172	$(GA)_9$	3	0.59	2	0.17
CMTC123*	$(TC)_9(TTTC)_2$	2	0.39	1	–
CMCT126	$(CT)_9$	1	–	ns	
CMTC158	$(TC)_{11}(ATT)_6$	1	–	ns	
CMGT108	$(GT)_9N65(CT)_7$	2	0.50	1	–
CMTC163	$(CTT)_2(TC)_8$	1	–	ns	
CMTAA166	$(TAA)_9N8(GA)_9(AT)_3$	2	0.14	2	0.17
CMTA170a	$(TA)_9T(TA)_3$	6	0.79	ns	
CMCT170b	$(CT)_8$	4	0.72	ns	
CMGA165**	$(GA)_{10}$	3	0.60	2	0.46
CMTA134a*	$(TA)_{12}$	5	0.76	ns	
CMCT134b*	$(TA)_2(CT)_8(AT)_7$	4	0.68	ns	
CMCT160a**	$(TC)_2(TCC)_2(CT)_8$	2	0.14	3	0.24
CMTC160a+b*,**	$(TC)_2(TCC)_2(CT)_8N122(TC)_8$	2	0.45	3	0.24
CMCT505*,**	$(CT)_{15}(AT)_{12}(AC)_{11}(AT)_4$	4	0.51	2	0.46
From cucumber cDNA library					
CSCTTT15a	$(CTTT)_6$	3	0.38	2	0.17
CSGTT15b	$(GTT)_6$	1	–	1	–
CSTCC813**	$(TCC)_7$	ns		2	0.24
CSCT335**	$(CT)_8N4(CT)_{11}$	ns		2	0.46
From database					
melon					
CMAT35	$(TA)_3AA(TA)_2C(AT)_7$	2	0.36	ns	
CMTC51	$(CT)_3GA(TC)_9(CT)_5$	1	–	2	0.17
cucumber					
CSGA057	$(GA)_8$	3	0.52	2	0.17
CSAT542**	$(AT)_7(N)_{37}(AT)_{13}$	1	–	5	0.66
CSAT214**	$(AT)_{13}$	ns	–	5	0.70
CSAT425a	$(AT)_{10}T(TA)_2$	4	0.60	2	0.17
CSAT425b	$(AT)_{10}T(TA)_2$	5	0.67	-	–
CSCCT571	$(CCT)_5CTT(CT)_2$	2	0.26	1	–
CSTA050	$(TA)_4(TTC)_3G(TA)_5TG(TA)_2$	1	–	2	0.17

[a] ns=no signal, nss=non specific signal
[b] Calculated for 13/11 homozygous varieties of melon/cucumber respectively

(75%) were polymorphic among 13 melon genotypes: 26 melon SSRs (86%) and four cucumber SSRs (40%) detected polymorphism (Table 4). For each polymorphic SSR locus two to six size alleles were detected, and gene-diversity values ranged from 0.26 to 0.79 (Table 4). The size variation among the alleles of a single locus tested in 13 accessions ranged between 1 and 44 base pairs.

Microsatellite data were employed for phylogenetic analysis among the 13 melon genotypes. One hundred and ten polymorphic bands (31 SSR loci) were used to generate a genetic-distance matrix (Table 5a). Genetic distances among the accessions ranged from 0.129 for the most-similar accessions (Noy Yizre'el and Eshkolit Ha'amakim) to 0.903 for the most-diverse ones (Noy Yizre'el and Freeman's Cucumber). The most-distant accession from all others was PI 414723 (average genetic distance of 0.755±0.0079). Cluster analysis was subsequently performed using the UPGMA method,

Table 5 Pairwise distance values (Nei's coefficient) between (a) 13 melon (*C. melo* L.) and (b) 11 cucumber (*C. sativus* L.) accessions based on SSR data

a)

Accession	Noy Yizre'el	Krymka	Eskolit Ha'amakim	Dulce	Q36	P15	FRC	PI414723	Faqus	Dudaim	Baku	Vedrantais	PI161375
Noy Yizre'el	0.000												
Krymka	0.355	0.000											
Eskolit Ha'amakim	0.129	0.452	0.000										
Dulce	0.323	0.419	0.290	0.000									
Q36	0.355	0.258	0.355	0.323	0.000								
P15	0.645	0.677	0.597	0.661	0.677	0.000							
Freeman's cucumber	0.903	0.871	0.839	0.774	0.871	0.500	0.000						
PI414723	0.774	0.774	0.806	0.710	0.774	0.726	0.742	0.000					
Faqus	0.403	0.548	0.371	0.435	0.452	0.677	0.790	0.758	0.000				
Dudaim	0.484	0.419	0.500	0.371	0.306	0.677	0.774	0.774	0.581	0.000			
Baku	0.274	0.371	0.306	0.306	0.371	0.597	0.806	0.742	0.387	0.452	0.000		
Vedrantais	0.419	0.452	0.355	0.226	0.419	0.694	0.677	0.742	0.435	0.419	0.339	0.000	
PI161375	0.839	0.806	0.774	0.774	0.839	0.419	0.194	0.742	0.726	0.790	0.742	0.613	0.000

b)

Accession	Bet Alfa	Ashely	Pickmore	Calypso	Colet	Shimshon	GY14	G421	H19	PI183967	Dasher
Bet Alfa	0.000										
Ashely	0.222	0.000									
Pickmore	0.306	0.222	0.000								
Calypso	0.278	0.083	0.167	0.000							
Colet	0.167	0.222	0.306	0.222	0.000						
Shimshon	0.000	0.222	0.306	0.278	0.167	0.000					
GY14	0.278	0.056	0.167	0.028	0.222	0.278	0.000				
G421	0.278	0.056	0.167	0.028	0.222	0.278	0.000	0.000			
H19	0.222	0.167	0.194	0.083	0.278	0.222	0.111	0.111	0.000		
PI183967	0.944	0.889	0.917	0.889	0.889	0.944	0.889	0.889	0.944	0.000	
Dasher	0.167	0.167	0.167	0.139	0.167	0.167	0.139	0.139	0.167	0.917	0.000

and the resulting dendrogram (Fig. 1a) showed six main branching nodes. Nodes 1 and 3 distinguished the exotic non-cultivated accessions belonging to three groups, *conomon*, *acidulus* and *flexuosus*, from the sweet cultivated melons belonging to three groups, *cantalupensis*, *reticulatis* and *inodorus* (node 4). Furthermore, the analysis distinguished between the three groups *conomon*, *acidulus* and *flexuosus* (nodes 2, 3). The sweet cultivated melons (*cantalupensis*, *reticulatis* and *inodorus*) were grouped together. The only exception was the accession Dudaim (var. *dudaim*), a non-sweet cultivated variety (node 6) that clustered closer to the sweet varieties. In addition, the orange-flesh cultivars were separated from the green/white-flesh cultivars (node 5).

SSR length-polymorphism and genetic diversity in cucumber (*C. sativus* L.)

The above 40 SSRs were also used to assess variation among the 11 cucumber accessions. All ten cucumber SSRs and 16 (53%) of the melon SSRs yielded a clear amplification product. Nineteen SSRs were polymorphic in cucumber: 80% of the cucumber-derived SSRs and 37% of the melon-derived SSRs (Table 4). Two to five alleles per locus were amplified, with corresponding gene-diversity values ranging from 0.17 to 0.70 (Table 4). Size variation among the alleles for a single SSR locus ranged from 1 to 15 base pairs.

Microsatellite data was used for the phylogenetic study among the 11 cucumber genotypes. Forty four polymorphic bands (18 SSR loci) were used to generate a genetic-distance matrix (Table 5b). Cluster analysis by the UPGMA method resulted in the construction of a dendrogram (Fig. 1b) with two main nodes. All genotypes belonging to the subspecies *sativus* were grouped together, while PI 183967, belonging to the subspecies *hardwickii*, was separated (Fig. 1b), with a genetic-distance range of 0.889 and 0.944 (Fig. 1b, Table 5b). Genetic distances among the accessions ranged from 0.0 for the most-similar to 0.944 for the most-diverged ones. Low genetic-distance values (0.0 to 0.306) were observed among the ten genotypes belonging to the subspecies *sativus*. The two most-similar pairs of accessions, with identical SSR profiles, were Bet Alfa and Shimshon, and GY14 and G421. The most-diverged varieties within var. *sativus* were Pickmore and each of the three cultivars Bet Alfa, Shimshon or Colet, with a genetic distance of 0.36 (Table 5b, Fig. 1b).

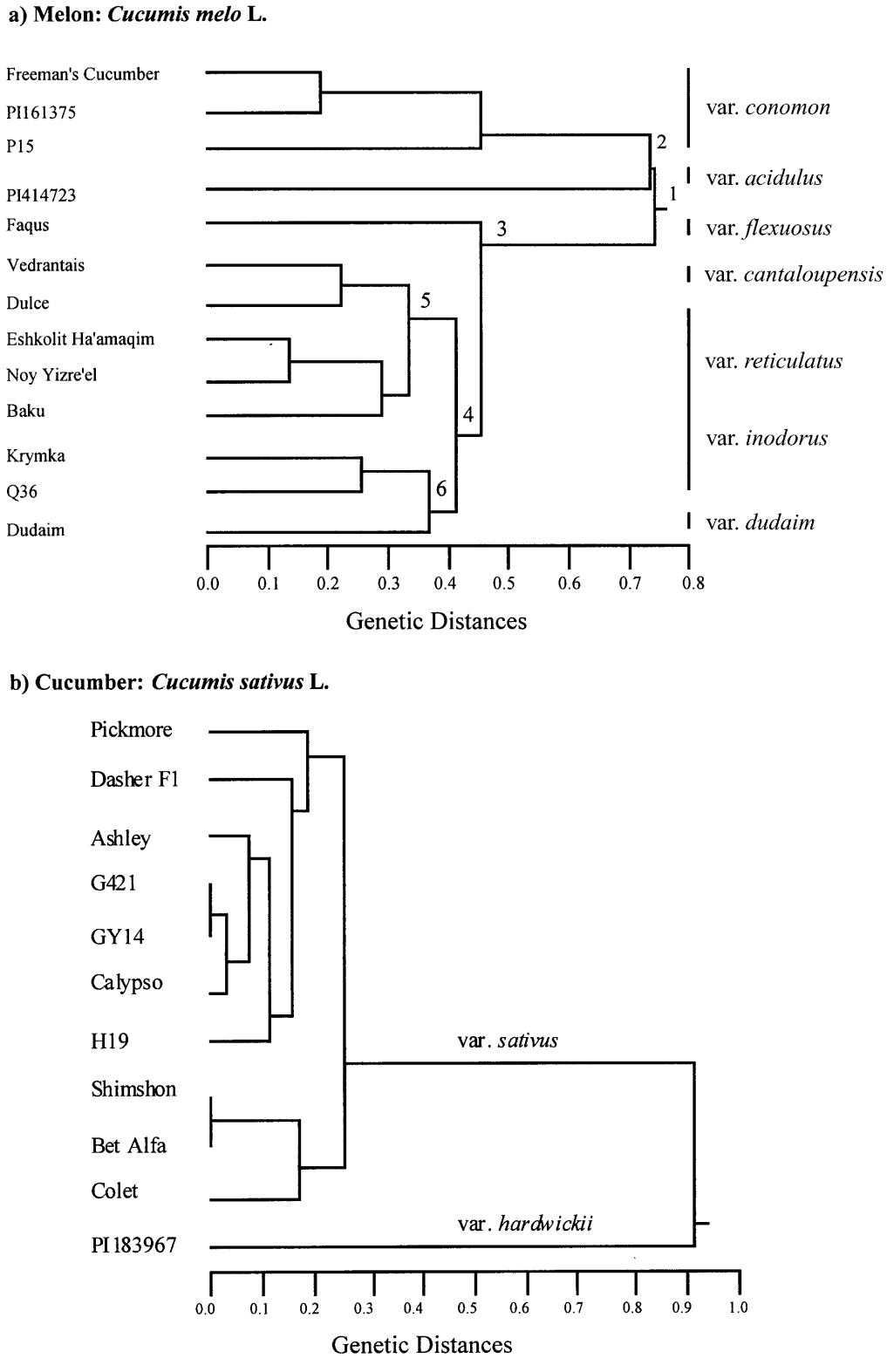

Fig. 1 Dendrograms presenting the association among (**a**) 13 melon genotypes and (**b**) 11 cucumber genotypes, based on UPGMA cluster analysis of 110 SSR amplification products of melon (31 SSR loci) and 44 SSR amplification products of cucumber (18 SSR loci), respectively

Conservation of SSR loci between the two *Cucumis* species, *C. melo* and *C. sativus*

More than 50% of the SSRs (22 out of 40) amplified a specific product in both melon and cucumber. The variation of size alleles between melon and cucumber were in the range of variation within each species (between 1 and 9 bp for 19 SSRs, and between 23 and 38 bp for the other three SSRs). Sequence comparisons between the two species were performed for a sample of five SSR loci. Melon products amplified by cucumber SSRs (CSCTTT15a, CSGA057, CSAT425), and cucumber

1. CMTC47 Melon sequence (168 bp) vs. cucumber sequence (160 bp)

```
C.melo      GCATAAAAGAATTTGCAGACACACACAACATTTATTCTATTTTATTTCCCTTTTT-------TTTTTCCCATTGGAAATATCAAAACAAAA
            |||||||||||||||||||||||||||| |||||||||||||||||||||||||||||||        ||||| ||||||||||||||||||||
C.sativus   GCATAAAAGAATTTGCAGACACACAC-ACATTTATTCTATTTTATTTCCCTTTTTTGTATTCTTTTTTCCATTGGAAATATCAAAACAAAC

C.melo      TTCATTTTTATGGATTATTTTCCCATTTCTAAATAATTTCTCTCTCTCTCTCTCTCCTCTCTCTCTATCTCTTCTCAATTCT
            |||||||||||||||||||| ||||||| |||||| |||||||||||||          |||||||||||||||||||
C.sativus   TTCATTTTTATGGATTATTTT-CCATTTCAAAATAATCTCTCTCTCTCTCT-------------CTCTATCTCTTCTCAATTCT
```

2. CMTC160a+b Melon sequence (215 bp) vs. cucumber sequence (217 bp)

```
C.melo      GATGGTGCCTTAGTTGTTCCGGCGACGTGGAACAAGAAAGCGGCGACCAACGCGAGGAGAGAGAGAGAGATCGAACCGGAAGGACGGTGGAGGAGGAGGA---TCGGCC
                          |||||| ||||||| |||||||||||||| ||||||    || ||||||||||||||| |||||||| ||||||||||||   | || |
C.sativus                  GCGACG-GGAACAACAAAGCGGCGACCAGCGCGAG----GAAAGAGAGAGATCGAACCCGAAGGACGGAGGAGGAGGAGGAGAATTGGGC

C.melo      GGAGGCCCGATGTGTGGTGACGGTGTTTGGTGTGAGAAGAAATTGAGCGAGTAGCTGTAAGAGAGAGAGAGAGAG----GGAGGAGAGACTGGAAGATAAGGGAGAGAGAC
            || || | |||||||||||||||| |||||||||||||||||| ||| || |||||||||||||||| ||||||    |||||||| ||||||||||||||||||||||
C.sativus   GGTGGTCTGATGTGTGGTGACGGCGTTTGGTGTGAGAAGAAATGGAGAAAGAAGCTGTAAGAGAGAGAAAGAGAGGGGGGGAGGAGAG-CTGGAAGATAAGGGAGAGAGAC
```

3. CSCTTT15a Melon sequence (184 bp) vs. cucumber sequence (198 bp).

```
C.melo                        TAATTAACGAAGAGGATTCTCTATTTCTCTTT--CTCTCTCTCTACTGTAATTCTCTCTTTTCTTTTTCATCACTTTCTTCCAATTTTCTT
                              |||||||||||||  ||||||||||||||||  |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
C.sativus   GTTTGATAATGGCGGATTGTTAATTAACGAAGATAATTCTCTATTTCTCTTTCTCTCTCTCTACTGTAATTCTCTCTTTTCTTTTTCATCATTTTCTTCCAATTTTCTT

C.melo      TGGATTTCTTCTT------------TTTTTTTTTTTTGAACTAATCAGAAGAACAAATCCATGG
            ||  ||  |||||             | ||||||||||||||||||||||||||||||||||
C.sativus   TGATTTCTTTCTTTCTTTCTTTCTTTTTTTTTTTGAACTAATCAGAAGAACAAATCCATGGTTCCACCATACCTTCATTTCTAC
```

4. CSGA057 Melon sequence (205 bp) vs. cucumber sequence (211 bp) (GeneBank accession CSLHCPA)

```
C.melo                          GTTCAGGCTATTGTTACTGGAAAGGGTCCATTGGAGAATTTGGCTGATCACCTTGCTGACCCAGTCAACAACAATGCTTGGGCC
                                |||||||||| |||| |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
C.sativus   TTCTCCATGTTTGGATTCTTTGTTCAGGCTATCGTTACCGGAAAGGGTCCATTGGAGAATTTGGCTGATCATCTTGCTGACCCAGTCAACAACAATGCTTGGGCC

C.melo      TACGCTACAAACTTTGTTCCTGGAAAGT--GAGAAATACGTA----GAAAGAGAGAGAGAGAGAGACTAAAGAGAGTTTCTATGGTGTTGAATTATTATTTGTGGT
            || |||||||||||||||||||||||||  |||||||| |||    |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
C.sativus   TATGCTACAAACTTTGTTCCTGGAAAGTGAGAGAAATATGTAGAAAGAAAGAGAGAGAGAGAGACTAAAGAGAGTTTCTATGCTGTTGAATTATTATTTGTGGT
```

5. CSAT425 Two melon amplification products 83 bp and 108 bp vs. cucumber sequence (93 bp) (GeneBank accession CSMASY).

```
a.
C.melo 83bp  TTAGGGCAGGTATTATTTCAGTTTTATATATATATATATATA--------CAAGTATGAAGT
             |||||||||||||||||||||||| ||||||||||||||||||        |||||||
C.sativus    TTAGGGCAGGTATTATTTCAGTTATATATATATATATATATTATTATTAAAAGCATGAAGTCCGCGCCATGGGCCTATACTAAATCAGTCCGT

b.
C.melo 108bp TAGGGCAGGTATTATTTCAGGTTTTATATATATATATATATATTATTATTAGGAGGATAAAGTGTGTGGGTG
             |||||||||||||||||||   |||||||||||||||||||||||||||||||||        |||       |  ||
C.sativus    TAGGGCAGGTATTATTTCAG---TTATATATATATATATATATTATTATTA-------AAA------GCATGAAGTCCGCGCCATGGGCCTATACTAAATCAGTCCGT
```

Fig. 2 Alignments of DNA sequences of *C. melo* and *C. sativus* for five SSR loci. Alignments span the complete amplified fragments. Two SSRs were developed from melon sequences (CMTC47, CMTC160a+b) and three were developed from cucumber sequences (CSCTTT5a, CSGA057, CSAT425). The DNA of the "non-source" amplification product was excised from the gel and directly sequenced

Table 6 Summary of the DNA sequence comparisons: repeat sequences, product length and percentage of similarity for five SSR loci

Source SSR[a]	Repeat		Product length in bp.		Percentage conservation
	Melon	Cucumber	Melon	Cucumber	
CMTC47	$(TC)_9(CT)_6$	$(TC)_9$	168	160	87
CMTC160a+b	$(GA)_8$, $(GGA)_4$, $(AG)_8$	$GAAA(GA)_4$, $(GGA)_5$, $(AG)_4AA(AG)_3$	215	217	87
CSCTTT15a	$(CT)_2TT(CT)_5$, $(CTT)_2T_{12}$	$(CT)_2TT(CT)_6$, $(CTTT)_6T_7$	184	198	86.5
CSGA057	$(GAAA)(GA)_8$	$(GAAA)_2(GA)_8$	205	211	94.8
CSAT425a	$T_3(TA)_9$	$T(TA)_{10}T(TA)_2$	83	93	77.5
CSAT425b	$T_3(TA)_{10}T(TA)_2$, $(GGA)_2$, $(GT)_3$	$T(TA)_{10}T(TA)_2$	108	93	76

[a] Markers designated CS were developed from *C. sativus* sequences, and markers designated CM were developed from *C. melo* sequences

products obtained with melon SSRs (CMTC47, CMTC160a+b), were excised from the gel and directly sequenced. Sequence similarities between the two species for all five loci were high (Fig. 2). The identity values for the complete sequences ranged from 76% to 94.8%, with a high conservation of the repeat. The repeat sequences of each of the five loci in both species are presented in Table 6. In one case (CSAT425), a product was

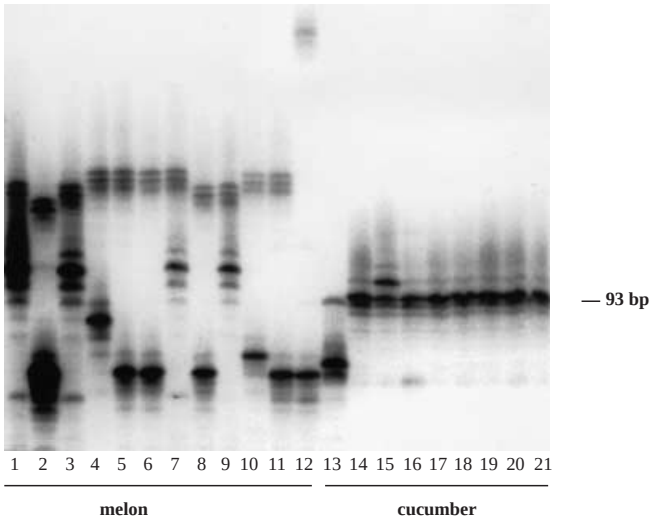

Fig. 3 PCR amplification products from the DNA of melon and cucumber lines using primers flanking the cucumber CSAT425 SSR. Separation was performed on a sequencing gel. *Lanes* were as follows: *C. melo*: *1*-Noy Yizre'el, *2*-Krymka, *3*-Eshkolit Ha'amaqim, *4*-PI 414723, *5*-Dulce, *6*-Freeman's Cucumber, *7*-Q36, *8*-P15, *9*-Faqus, *10*-Dudaim *11*-Védrantais, *12*-PI 161375; *C. sativus*: *13*-var. *hardwickii* PI 183967, var. *sativus*: 14-GY14, 15-Bet Alfa, 16-Ashley, 17-Pickmore, 18-Dasher F1, 19-Calypso, 20-Colet, 21-Shimshon

amplified from cucumber DNA while two products were obtained from melon DNA. These two melon products were mapped to two separate linkage groups (Danin-Poleg et al. 2000).

Discussion

SSR characterization

This study provides a set of SSR markers that have been proven to be beneficial for both genetic and breeding applications. The study of 61 SSRs allows us to draw several general conclusions, widening our previous report on the first seven *Cucumis* SSRs (Katzir et al. 1996). The majority of the SSRs developed from genomic libraries were $(CT/GA)_n$ repeats. This may be due to the high frequency of $(CT/GA)_n$ repeats and the higher efficiency of screening with $(CT/GA)_n$ probes, especially when compared with $(AT/TA)_n$ probes (Kresovich et al. 1995; Sun et al. 1998). Indeed, among the SSRs developed from database sequences $(AT/TA)_n$ repeats were the majority (five of eight). A high frequency of $(CT/GA)_n$ repeats has been reported for many plant genomes (Langencrantz et al. 1993; Bell and Ecker 1994; Wang et al. 1994; Plaschke et al. 1995; Jarret et al. 1997; Sun et al 1998). In the present study, the occurrence of $(CT/GA)_n$ repeats (n>7) along the melon genome was estimated to be once in every 250–500 kbp. This estimation was derived from the screening of genomic sequences that span 12 Mbp. This represented approximately 2.5% of the melon genome, which has been estimated to be 454–502 Mbp (Arumuganathan and Earle 1991). These values are within the range of frequency of $(CT/GA)_n$ repeats reported for other plant species including corn (every 168–710 kbp, Condit and Hubbel 1991), rice (every 225–330 kbp, Wu and Tanksley 1993), *Arabidopsis* (every 244 kbp, Bell and Ecker 1994), wheat (every 440 kbp, Rŝder et al. 1995), tomato (every 2 Mbp, Broun and Tanksley 1996) and alfalfa (every 210 kbp, Diwan et al. 1997). It should be noted that the different estimates were based on different numbers of repeats (n). The majority of the SSR motifs identified in the present study were compound or interrupted repeats, and many were isolated from repeat-rich sequences. Similar observations have been reported for other species of plants and animals (e.g. Blanquer-Maumont and Crouau-Roy 1995; van Treuren et al. 1997; Peakall et al. 1998; Areshchenkova and Ganal 1999). No correlation was found in the present study between the number of repeats and the informativeness of the SSRs as measured by gene-diversity values. Similar observations were reported for other plants including *Arabidopsis* (for repeat lengths up to 50 nucleotides, Bell and Ecker 1994); rice (Panaud et al. 1996); *Brassica* (Szewc-McFadden et al. 1996) and barley (Struss and Plieske 1998). Yet in other studies a positive correlation was found between the repeat length and the degree of polymorphism, e.g. for grapevine (Thomas and Scott 1993) and tomato (Smulders et al. 1997; Areshchenkova and Ganal 1999).

Polymorphisms detected by SSRs in both *Cucumis* species

Polymorphism detected by the *Cucumis* SSRs was high: 86% of the melon SSRs detected polymorphism among the melon genotypes and 80% of the cucumber SSRs detected polymorphism among the cucumber genotypes. The percentage of SSRs that detected polymorphism in the heterologous species was also high: 37% of the melon SSRs detected polymorphism in cucumber and 40% of the cucumber SSRs detected polymorphism in melon. The degree of polymorphism detected by the SSRs was measured by a gene-diversity value that incorporates both the number of alleles and the frequency of appearance of the alleles among the genotypes tested. Gene-diversity values obtained for the 30 melon SSRs among melon genotypes were high, with an average gene-diversity value of 0.522. By comparison, gene-diversity values obtained for other species were 0.43, 0.43, 0.59, 0.692 and 0.8 for *Arabidopsis*, tomato, corn, rice and soybean, respectively (Bell and Ecker 1994; Panaund et al. 1996; Diwan and Cregan 1997; Bresemijer et al. 1998; Senior et al.1998). The high informativeness of the microsatellites in melon was even more significant when compared with the level of variation detected by other methods. Various RFLP, RAPD and ISSR studies suggested different estimates for the polymorphism detected by these markers, most of them below or near the degree of polymorphism detected by the SSR markers

(Neuhausen 1992; Baudracco-Arnas et al. 1996; Katzir et al. 1996; Staub et al. 1997, 2000; Garcia et al. 1998; Silberstein et al. 1999; Stepansky et al. 1999). Additional advantages included the multiallelic nature of SSRs. The average number of alleles detected by SSRs in melon was 3.5, compared with only two size alleles detected by most RFLP probes. Rare alleles that were found, especially among the 'exotic' genotypes, were most valuable in diversity studies. Another advantage was the high reproducibility of SSR results, especially when compared with those of RAPD and ISSR. Finally, the simplicity of the procedure and the small amount of DNA required were general advantages of PCR markers, especially in comparison with RFLP.

A high level of polymorphism was detected by both cucumber and melon SSRs within *C. sativus.* Fourteen of the nineteen polymorphic SSRs (74%) distinguished between the two subspecies *sativus* and *hardwickii*, while nine of them (47%) were polymorphic within var. *sativus.* This is consistent with our previous study (Katzir et al. 1996). The average number of alleles detected by the 19 polymorphic SSRs was 2.4 and the average gene diversity value was 0.284. The relatively low number of alleles and gene-diversity values in cucumber, especially when compared with melon, reflect the known narrow genetic background of *C. sativus*. Studies based on other marker systems support this conclusion. For instance, Dijkhuizen et al. (1996) found that 33% of the 440 RFLP probes they used detected a unique pattern for at least one of the 16 accessions (including the *C. sativus* var. *hardwickii*) with an average of 2.2 alleles for each probe. In this study, the variation between the subspecies *sativus* and *hardwickii* (14 of 40 loci, 35%) was greater than that among var. *sativus* accessions (9 of 40 loci, 22.5%). The trend of these results is in agreement with the variation found with RFLPs, although lower variation was found in the latter case; 25% of the probes used by Kennard et al. (1994) distinguished between the two subspecies and 11% were polymorphic within the var. *sativus* genotypes. Similarly, 21% of the probes used by Dijkhuizen et al. (1996) were polymorphic between the subspecies and 12% were polymorphic within var. *sativus*.

Phylogenetic analysis of melons based on SSR data

The SSR data obtained in the present study were used to estimate the genetic distances among genotypes representing seven of the eight horticultural groups (Whitaker and Davis 1962). These SSRs had been previously proven to be well-distributed on the melon genome (Danin-Poleg et al. 2000). Here, the resulting dendrogram separated the 'exotic' non-sweet accessions belonging to three groups (*conomon, acidulus* and *flexuosus)* from the sweet cultivated melons belonging to four groups (*cantalupensis, reticulatis, inodorus* and *dudaim*). In general, this partitioning is in agreement with a previous survey by Stepansky et al. (1999) that included morphological, biochemical, RAPD and ISSR markers. The 54 genotypes that were studied by Stepansky et al. (1999) were classified according to Munger and Robinson (1991) into seven groups: *cantalupensis* (which included the *reticulatis* group in our study*), inodorus, conomon*, *dudaim*, *agrestis*, *momordica* (the equivalent of the *acidulus* in our study) and *flexuosus.* The major outcome of Stepansky et al. (1999) was the classification of the melon genotypes into two major groups: the first includes the *cantalupensis* and *inodorus* cultivars, and the second includes the more exotic varieties belonging to *conomon, chito, dudaim, agrestis, momordica* and *flexuosus.* Our dendrogram further separated the exotic accessions, vars. *conomon*, *acidulus* and *flexuosus*, into different nodes, in agreement with the classification of Whitaker and Davis (1962), after Naudin 1859. No clear distinction was observed within the sweet cultivated melons classified into the distinct horticultural groups vars. *cantalupensis*, *reticulatis* and *inodorus* (Whitaker and Davis 1962). This is due, most probably, to the extensive breeding programs that have combined the cultivated germplasm during the last century. Similar results were obtained in other studies of divergent genetic material (Staub et al. 1997; Silberstein et al. 1999; Stepansky et al. 1999). Separation among the cultivated melons was detected in two studies that included a larger number of accessions from these groups. Garcia et al. (1998) surveyed 32 breeding lines (of cultivated melons only) using RAPD and agronomical traits. They found a clear separation between the varieties with climacteric fruits, classified into *reticulatis* and *cantalupensis*, and those having non-climacteric fruits, classified as the *inodorus* group. The same general distinction between var. *cantalupensis* (that included var. *reticulatis*, according to Munger and Robinson 1991) as one group, and var. *inodorus* as a second group, was obtained using some of the SSR markers (54 bands, 17 loci) presented here (Staub et al. 2000). In the latter study, 46 genotypes were analyzed, most of them belonging to the var. *cantalupensis* and var. *inodorus* groups except for two 'exotic' accessions. In the same study (Staub et al. 2000), cluster analysis based on 135 RAPD bands resulted in the separation of the cultivated melon genotypes among four of the five melon market classes based on fruit characteristics. The distinction between the *inodorus* and the *cantalupensis* groups by the RAPD markers was less clear.

The ability of the SSR markers to detect rare allelles is advantageous, as demonstrated here by the example of the Near-East accession *flexuosus.* This group was clearly separated from the sweet groups both in the present study and in a previous study (Staub et al. 2000). The same group was found to be closer to the non-sweet acssesions, based on its unique phenotype, by Stepansky et al. (1999); yet other marker systems (RFLP, RAPD and ISSR) were not able to separate it from the sweet groups (Silberstein et al. 1999; Stepansky et al. 1999; Staub et al. 2000). The comparison of the different studies discussed above clearly supports the need for a large-

scale survey that will include both the original cultivars and the contemporary cultivars. In addition, the application of several marker systems in that survey is suggested, so that the unique evolutionary processes responsible for the polymorphism detected by each marker system can be taken into consideration (Lu et al. 1996).

Phylogenetic analysis of cucumbers based on SSR data

The cluster analysis presented for the 11 cucumber genotypes clearly separated var. *hardwickii* (PI 183967), which formed a distinct cluster, from the var. *sativus* accessions. These results are in agreement with previous studies based on other marker systems (isozymes, RAPD, RFLP) or morphological traits (Dijkuizen et al. 1996; Staub et al. 1997). Distinct clusters within var. *sativus* were not formed in our study (genetic distance 0.0–0.306). This is similar to the small genetic-distance values obtained in a survey of 40 RFLPs, ranging from 0.0 to 0.24 among glasshouse cultivars and from 0.09 to 0.55 among Mediterranean types (Dijkhuizen et al. 1996). The observation that GY14 and G421 were the most-similar accessions matches the findings of a previous study based on RAPD analysis (Staub et al. 1997). The number of SSRs available so far for cucumber is still low, yet their distribution on the cucumber genome (Danin-Poleg et al. 2000) indicates that they are adequate for diversity studies in *C. sativus*. The application of a larger number of SSRs in these studies might lead to separation within the var. *sativus* group.

Conservation of SSRs between *Cucumis* species

The results obtained here for cross-amplification of SSRs between the two *Cucumis* species, *melo* and *sativus*, are in agreement with our previous indications for conservation between the two species (Katzir et al. 1996). It has been claimed previously that sequencing data are required to minimize the risk of misinterpretation of PCR products and to maximize the genetic information (for a review see Peakall et al. 1998). The sequencing data in the present study, for five SSR loci, clearly demonstrate the high conservation of SSR loci between the two *Cucumis* species: there were mutations inserted within the five repeats, yet in all of them the repeats were well-conserved. In addition, mutations were also observed in the flanking regions of the repeats, as observed in previous studies of plants and animals (for a review see Peakall et al. 1998). In one case a duplication of a single cucumber locus occurred in melon (CSAT425, Fig. 2). Mapping of this duplication to two separate loci (Danin-Poleg et al. 2000) is of evolutionary interest. Future comparative studies, including synteny studies, will show the extent of duplication between these two genomes and the possible relationship with the different numbers of chromosomes in the two closely related species. The inter-specific conservation of SSRs reported in the present study suggests that these markers may prove to be a most useful tool in comparative studies.

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