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Length polymorphism and homologies of microsatellites in several Cucurbitaceae species

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Abstract The objectives of this research were to assess (1) the degree of Simple Sequence Repeats (SSR) DNA length polymorphism in melon (*Cucumis melo* L.) and other species within the Cucurbitaceae family and (2) the possibility of utilizing SSRs flanking primers from single species to other genera or species of Cucurbitaceae. Five melon $(CT/GA)_n$ SSRs were isolated from a genomic library. Two cucumber (*Cucumis sativus* L.) SSRs were detected through a search of DNA sequence databases, one contained a $(CT)_8$ repeat, the other a $(AT)_{13}$ repeat. The seven SSRs were used to test a diverse sample of Cucurbitaceae, including 8 melon, 11 cucumber, 5 squash, 1 pumpkin, and 3 watermelon genotypes. Five of the seven SSRs detected length polymorphism among the 8 melon genotypes. PCR amplification revealed between three and five length variants (alleles) for each SSR locus, with gene diversity values ranging from 0.53 to 0.75. Codominant segregation of the alleles among F_2 progeny was demonstrated for each of the five SSR loci. Four of the seven SSRs detected polymorphism among the 11 cucumber genotypes, with gene diversity values ranging between 0.18 and 0.64. Primers specific to SSRs of *C. melo* and *C. sativus* also amplified DNA extracted from genotypes belonging to other genera of the Cucurbitaceae family.

Key words Simple Sequence Repeats (SSR) · Microsatellites · DNA markers · *Cucumis melo* L. · Cucurbitaceae · Melon

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Introduction

Analysis of restriction fragment length polymorphism (RFLP) in several species has shown that certain species such as maize, *Brassica napus*, and *Arabidopsis thaliana* demonstrate a high rate of polymorphism among individuals, while in others, such as tomato and melon, the level of polymorphism is considerably lower (Helentjaris et al. 1985; Bernatzky and Tanksley 1986; Figdore et al. 1988; Shattuck-Eidens et al. 1990). DNA polymorphism in several species of the Cucurbitaceae has been assessed by several investigators. Neuhausen (1992) evaluated restriction fragment length polymorphism in *Cucumis melo* and found that only 33% of the probes distinguished between accessions within *C. melo*, while 80% detected a polymorphism between *C. melo* and *C. sativus*. Kennard et al. (1994) studied RFLPs and random amplified polymorphic DNA (RAPD) in *Cucumis sativus* and found relatively few DNA polymorphisms, a result agreeing with previous studies that demonstrated a narrow genetic base for cucumber (Perl-Treves et al. 1985; Knerr et al. 1989; Knerr and Staub 1992). Our study on length polymorphism of Simple Sequence Repeats (SSR) within the Cucurbitaceae family was prompted by the accumulating information on the low level of polymorphism in the *Cucumis* genus, with the idea that this highly polymorphic class of markers might be efficient even in a narrow genetic background.

SSR, also termed microsatellites, consist of a variable number of tandem repeats of one to five nucleotides that form highly informative, locus-specific genetic markers. These markers have been described as "second-generation" markers (Davies 1993) and were recommended as the standard markers to be used in the preparation of highly saturated genetic maps of any eukaryote genome (Beckmann and Soller 1990). They are abundant and evenly dispersed throughout the human and other mammalian genomes (Hamada et al. 1982). SSR markers can be efficiently analyzed 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. The polymorphism is detected by sequencing gel separation that allows accurate sizing to within 1 base pair (bp). Several previous investigations of plant SSR demonstrated that the application of SSR markers may be as important in plant genetics as in mammalian genetics. Condit and Hubbell (1991) reported that SSR occur abundantly in plant species. Akkaya et al. (1992) assessed the presence and the degree of SSR length polymorphism in the soybean [*Glycine max* (L.) Merr.] proving, for the first time, the usefulness of this marker in higher plant species and suggesting its application as a complement to the RFLP and RAPD markers. Rongwen et al. (1995) used seven SSR markers to characterize a group of 96 soybean genotypes and demonstrated gene diversity values that are much higher than the values obtained with RFLP markers. These gene diversity values are similar to the average values obtained for human SSR markers. Akkaya et al. (1995) demonstrated that SSR are evenly dispersed throughout plant genomes by mapping 40 SSR markers in soybean. The usefulness of microsatellites has since been proven in other species including rice (Wu and Tanksley 1993; Zhao and Kochert 1993), maize (Senior and Heun 1993), *Brassica napus* (Lagercrantz et al. 1993), grape (Thomas and Scott 1993), barley (Saghai-Maroof et al. 1994), sunflower (Brunel 1994), avocado (Lavi et al. 1994), *Arabidopsis thaliana* (Bell and Ecker 1994) and wheat (Devos et al. 1995).

We present here an evaluation of length polymorphism of SSR in *C. melo* and *C. sativus*, as well as other genera of the Cucurbitaceae family (*Cucurbita* and *Citrulus*). Moreover, the potential of using SSR markers from a single species (*C. melo* or *C. sativus*) to detect polymorphism in other genera or species of the Cucurbitaceae family is discussed.

Materials and methods

Plant material

Eight *Cucumis melo* L. genotypes were selected for investigation of the polymorphism detected by SSR. The genotypes belong to the following groups, as classified by Whitaker and Davis (1962) (1) *Cucumis melo* var *reticulatus* Naud. (muskmelon): 'Noy Yizre'el', 'Krymka', 'Eshkolit Ha'amaqim' (all bred at Newe Ya'ar), 'Ducle' (Bonanza); (2) *C. melo* var inodorus Naud. (casaba): 'Q36' (bred at Newe Ya'ar); (3) *C. melo* var acidulus Naud.: 'PI414723' (courtesy of M. Pitrat, INRA, Monfavet, France); (4) *C. melo* var *conomon* Mak.: 'Freeman Cucumber' (courtesy of H. M. Munger, Cornell University USA) and pickling melon ('P15-S_5' breeding line: *C. melo* var '*conomon*' Mak. × *C. melo* var *reticulatus* Naud.). F_1 plants of the crosses 'Noy Yizre'el' × 'Krymka'; 'Eshkolit Ha'amaqim × 'Krymka', and 'PI414723–S_5' × 'Dulce' (bred at Newe Ya'ar) and 112 F_2 plants of the cross 'PI414723–S_5' × 'Dulce' (Danin-Poleg et al. 1996) were also tested for SSR segregation.

The *Cucumis sativus* L. genotypes tested for SSR polymorphism included *C. sativus* L. var *sativus*: 'Bet Alfa' and 'Shimshon' (Hazera'), 'Ashley' (Hollar), 'Pickmore' (Harris), 'Dasher' F_1, 'Calypso' (Petoseed), and 'Colet' (Sluis Royal). GY14, G421, H19, and *C. sativus* L. var *Hardwickii* ('PI183967') were obtained from Dr. J. Staub, (USDA-ARS, Wisconsin U., Madison, USA).

Cucurbita genotypes chosen for the polymorphism assessment were: *Cucurbita pepo*. L.: 'Sihi Lavan' (Hazera'), 'Vegetable Spaghetti' (Sakata), 'True French' (Thompson & Morgan), 'Beirut' (Hazera'); *C. moschata* Poir.: 'Butternut' (Agway); *C. maxima* Duch.: 'Big Moon' (Petoseed).

Citrullus lanatus (Thunb.) Matsum & Nakai genotypes chosen for the polymorphism assessment were 'Sugar Baby' (inbreds selected at Newe Ya'ar, the original from Lofts), 'Tri-x-313' (Sluis & Groot), and 'Malali' (Hazera').

DNA was isolated from the bulked leaf tissue of the plants of each genotype or from single plants of the F_2 population using a modification of the mini preparation procedure described by Dellaporta et al. (1983).

DNA library construction and screening

Melon DNA ('Noy Yizre'el') fragments of 200–400 or 400–600 bp obtained by *Sau*3AI (New England Biolabs, USA) were cloned into the *Bam*HI (New England Biolabs, USA) site of pBluescript II KS$^+$ (Stratagene, La Jolla, Calif.). The recombinant plasmids were used to transform the XL-1 Blue *E. coli* strain, and this was followed by blue/white color selection. Selected colonies were isolated onto microtiter plates containing LB medium with ampicillin (50 µg/ml). Procedures outlined by Sambrook et al. (1989) were followed throughout. Filters were hybridized to a γ [^{32}P]-ATP labeled $(CT)_{15}$-GGG oligonucleotide probe using T4 polynucleotide kinase (New England Biolabs, USA). The hybridization was conducted in an hybridization incubator (model 1000, Robbins Scientific) overnight at 37 °C. Membranes were washed three times for 5 min at 37 °C in 6 × SSC and exposed to an X-ray film (Kodak or Fuji). Positive clones were sequenced and checked for the presence of SSRs as described by Rongwen et al. (1995).

Table 1 Five melon microsatellite loci identified from a DNA library of cv 'Noy Yizre'el' and two cucumber loci from EBML database, including locus designation, core motif, number of repeat unit (*n*), and the sequence of primers used in the PCR amplification

Locus	Core motif and number of repeats	Sense primer 5′ to 3′	Antisense primer 5′ to 3′
From library			
CMTC13	$(TC)_{12}(CG)_5(AG)_3$	TGGATGGATAAGGTGGTAAG	TTCCCCTAGTCGCTCTCT
CMCT58	$(CT)_2CC(CT)_5C(CT)T(CT)$	CTTCAGCCTTAGCCCCTACT	TTCATTCTCCCACGTAGCT
CMAG59	$(GA)_2A(AG)_8$	TTGGGTGGCAATGAGGAA	ATATGATCTTCCATTTCCA
CMGA127	$(GA)_{13}A(GA)_2$	GAACTAAGACTCTCCAATTAA	ATGTCCCTAACTGCCAAACATA
CMGA128	$(GA)_{10}AA(GA)_2$	ATGAAGAAGGGATATTCAAAG	ACTCCATTGTTGCTAACCTTT
From database			
CSLHCPA	$(GA)_8$	TTCTCCATGTTTGGATTCTTT	ACCACAAATAATAATTCAACA
CSHPRAG	$(AT)_7(N)_{37}(AT)_{13}$	GTTTAACTCAATCCAACTCAA	CGAAACATTCATAACTCTACT

Search in sequence databases

Using the program FASTA (Genetics Computer Group, Madison), we found two SSRs by searching the EMBL and GenBank databases. Both were from the 3′-end of the non-coding regions of two *Cucumis sativus* genes: EMBL accession numbers M16057 and X58542 (*CSLHCPA* and *CSHPRAG*, respectively, Table 1).

PCR primers selection and synthesis

To select primers to regions flanking SSRs, we analyzed sequence data with the Primer Detective, Primer Design Software Program (Clontech Laboratories, Palo Alto, Calif.). Primers with expected PCR product lengths of 100–250 bp were selected and synthesized on an Applied Biosystems International 391 DNA synthesizer.

PCR amplification of microsatellite loci

Reaction mixtures for the PCR amplification of microsatellite loci contained 60 ng of plant genomic DNA, 1 m*M* of Mg^{2+}, 8–10 pmole of 3′ and 5′ primers, 166 μ*M* dATP, dTTP, dGTP, 2 μ*M* dCTP, 0.1 μl 3000 Ci/mmol α-[^{32}P] dCTP, 1 × *Taq* Buffer (Advanced Biotechnologies, UK), 1 unit of *Taq* DNA polymerase (Advanced Biotechnologies, UK), in a total volume of 15 μl. The amplification program was as follows: 30 s denaturation at 94 °C, 30 s annealing at 51 °C, and 60 s extension at 72 °C for 34 cycles on a thermocycler (PTC-100 MJ Research). 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. The sequencing reactions of the four nucleotides of M13 ssDNA were used as molecular-weight standards to determine the exact nucleotide length of the denatured PCR products. After drying, the gels were exposed to a Kodak XAR-5 film (Eastman Kodak).

Calculation of pairwise polymorphism

The amount of polymorphism detected by an SSR in a group of homozygous individuals, from a self-pollinating species, was calculated as:
Gene diversity $= 1 - \Sigma P_{ij}^2$ (Rongwen et al. 1995). Where P_{ij} is the frequency of the *j*th allele for the *i*th SSR locus, it is summed across alleles. In the case of the *CMGA127* locus in melon, allele frequency was calculated as 2 out of 16 gametes (for the 8 melon genotypes), except for the frequencies of each of the 2 alleles produced by 'P15' (Table 2 and see below), which was calculated as 1 out of 16.

Fig. 1 Radiolabeled PCR products amplified from the DNA of 8 melon genotypes and 2 F_1 progeny using primers of the *CMAG59* locus. Fragments were separated on a standard DNA-sequencing gel. The sequencing ladder is M13mp18 ssDNA. Lanes are as follows: *1* 'Noy Yizre'el', *2*, *6* 'Krymka', *3* 'Noy Yizre'el' × 'Krymka', *4* 'Eshkolit Ha'amaqim' × 'Krymka', *5* 'Eshkolit Ha'amaqim', *7* 'Freeman Cucumber', *8* 'Q36', *9* 'P15', *10* 'PI414723', *11* 'Dulce'

Results

SSR selection and characterization

Two approaches were applied for SSR selection: (1) screening of a genomic library, which resulted in the isolation of five melon SSR, and (2) search of DNA sequence databases, which provided two cucumber SSR. A genomic library of melon ('Noy Yizre'el') was constructed, and 7300 colonies were screened with CT-dinucleotide probes. Forty (0.55%) positive clones were identified, of which 26 were sequenced. Three clones did not contain any SSR, 17 clones contained a $(CT)_n$ with $n < 10$, and six clones contained repeats with $n > 10$ (*n* equal the number of repeats). Unique primers for flanking regions of five repeats (presented in Table 1) were synthesized and used to amplify DNA extracted from several different Cucurbitaceae species. Primers were also designed to amplify the two cucumber SSRs that were found through a search of DNA sequence databases (Table 1). Of these seven SSRs that were tested throughout the study, six contained CT/GA repeats and one was an AT/TA repeat. The two cucumber SSRs from the EMBL database had an uninterrupted core motif, while the five melon SSRs selected from a genomic library had interrupted core motifs. In these interrupted repeats, *n* was counted in the largest expanse of uninterrupted repeats. The value of *n* ranged from five to 13. One SSR (*CMTC13*) had a CG/GC repeat flanked by TC/AG repeats. The seven SSR markers described above were used to assess length polymorphism in melon, cucumber, squash, pumpkin, and watermelon.

Length polymorphism of SSRs in melon (*Cucumis melo* L.)

Of the seven SSRs tested in eight melon varieties, five (71%) (*CMTC13*, *CMAG59*, *CMGA127*, *CMGA128*, and *CSLHCPA*) were polymorphic, and two (*CMCT58* and *CSHPRAG*) were not polymorphic. Polymorphism in melon was detected by four melon SSRs and one cucumber SSR with a (CT/GA) core motif. The smallest SSR which detected polymorphism in melon had eight repeats of the core motif. The only tested SSR with a

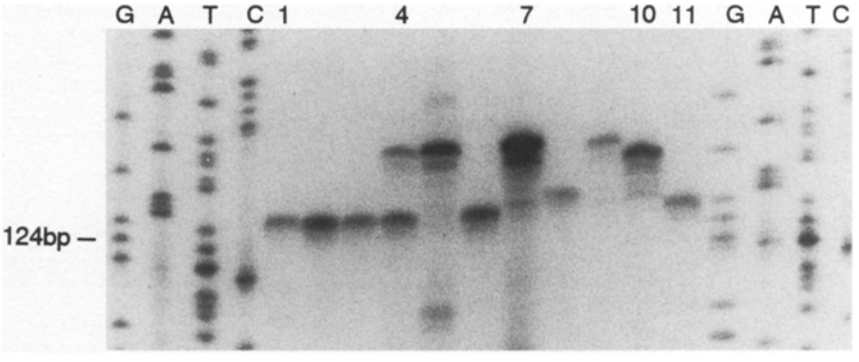

Table 2 Distribution of SSR alleles, described by their length in base pairs, in the different Cucurbits species tested (*ns* no signal, *ncs* no clear signal, *nt* not tested)

	Genotype	Alleles (bp)						
		CMTC13	*CMCT58*	*CMAG59*	*CMGA127*	*CMGA128*	*CSLHCPA*	*CSHPRAG*
Melon								
Cucumis melo var								
reticulatus	Noy Yizre'el	92[a]	144[a]	124[a]	138[a]	119[a]	205	106
reticulatus	Krymka	92	144	124	138	125	209	106
reticulatus	Eshkolit Ha'amaqim	100	144	132	138	119	205	106
reticulatus	Dulce	100	144	125	138	119	205	106
inodorus	Q36	100	144	126	138	119	205	106
conomon	P15	91	144	132	138, 142	122	205	106
conomon	Freeman Cucumber	91	144	132	140	122	211	106
acidulus	PI414723	91	144	131	146	125	211	106
F_1 hybrid	Noy Yizre'el × Krymka	92	144	124	138	119, 125	205, 209	106
	Eshkolit Ha'amaqim × Krymka	92, 100	144	124, 132	138	119, 125	205, 209	106
	PI414723 × Dulce	91, 100	nt	125, 131	138, 146	119, 125	205, 211	nt
Cucumber								
Cucumis sativus var								
sativus	Bet Alfa	90	144	123	ns	ns	211[a]	154
sativus	Ashley	90	144	123	ns	ns	211	154
sativus	Pickmore	90	144	133	ns	ns	211	144[a]
sativus	Calypso	90	144	ncs	ns	ns	211	144
sativus	Colet	90	144	ncs	ns	ns	211	0
sativus	Shimshon	90	144	ncs	ns	ns	211	154
sativus	GY14	90	nt	133	nt	nt	211	144
sativus	G421	90	nt	133	nt	nt	211	144
sativus	H19	90	nt	133	nt	nt	211	144
hardwickii	PI183967	0	nt	0	nt	nt	212	142
F_1 hybrid	Dasher	90	144	123, 133	ns	ns	211	144, 158
Squash, Pumpkin								
Cucurbita pepo	Sihi Lavan	78	144	106	ns	ns	178	ns
	Vegetable Spaghetti	78	144	106	ns	ns	178	ns
	True French	78	144	106	ns	ns	178	ns
	Beirut	78	144	106	ns	ns	178	ns
Cucurbita moschata	Butternut	ns	144	121	ns	ns	178	ns
Cucurbita maxima	Big Moon	83	144	106	ns	ns	176	ns
Water melon								
Citrullus lanatus	Sugar Baby	82	144	122	ns	ns	197	ns
	Tri-x-313	82	144	122	ns	ns	197	ns
	Malali	82	144	122	ns	ns	197	ns

[a] Expected size of the amplification products obtained with the 'Noy Yizre'el' variety from which the library was constructed, or expected size according to database sequence

core motif smaller than eight repeats was *CMCT58*, having a $(CT)_5$, which was not polymorphic.

Figure 1 depicts PCR amplification patterns of melon varieties obtained with *CMAG59*, which demonstrated polymorphism both between and within melon groups. The number and size of the alleles found within these varieties for each of the tested SSRs is presented in Table 2 and summarized in Table 3. The size of the alleles, expressed in base pairs, was estimated on the basis of an M13 sequencing ladder. All five amplification products obtained with the 'Noy Yizre'el' variety, from which the library was constructed, were of exactly the expected size (Table 2).

Of the eight melon varieties tested, seven were homozygous and expressed a single allele for each of the five polymorphic SSR primers. One genotype ('P15'), a breeding line that was not homozygous, demonstrated the presence of two alleles with one of the five SSRs (*CMGA127*). The number of alleles detected by each of the markers and the corresponding gene diversity values are presented in Table 3. The most polymorphic marker was *CMCT59* with five length-variant alleles in the sample of eight varieties, corresponding to a gene diversity value of 0.75. Four of the five SSRs detected two alleles among varieties within muskmelon. Two of the SSRs, *CMTC13* and *CMCT59*, enable a distinction to be made between two close F_1 hybrids sharing a single common parent (Fig. 1 lane 4; Table 2).

Mendelian inheritance of SSR alleles

Each of the parental lines of the cross 'PI4114723-S_5' × 'Dulce' produced a single allele with each of the five polymorphic SSR markers. These alleles differed in size between the parental lines for all five SSR loci. The

Table 3 Gene diversity values and number of alleles detected in 8 varieties of melon and 11 varieties of cucumber, using five melon SSRs and two cucumber SSRs (*ns* no signal)

SSR locus	Melon		Cucumber	
	Number of Alleles	Gene diversity	Number of Allelles	Gene diversity[a]
Melon				
CMTC13	3	0.66	2	0.18
CMCT58	1	–	1	–
CMCT59	5	0.75	3	0.57
CMGA127	4	0.49	ns	–
CMGA128	3	0.63	ns	–
Cucumber				
CSLHCPA	3	0.53	2	0.18
CSHPRAG	1	–	5	0.64

[a] Calculated per the 10 homozygous varieties

F_1 hybrids were heterozygous and contained, as expected, the two parental alleles (Table 2).

Figure 2 depicts the segregation of the two parental alleles amplified with *CMTC13* in F_2 plants of the cross 'PI414723-S_5' × 'Dulce'. Table 4 summarizes the segregation of the alleles obtained with each of the polymorphic SSRs and tested in 112 F_2 plants of the same cross. The segregation of these alleles in the five SSR loci was tested for deviation from the expected codominant segregation ratio of 1:2:1. Segregation at four SSR loci fitted the 1:2:1 expectation with a probability value greater than 0.05 (P values ranging between 0.51 and 0.90). At the fifth locus, *CSLHCPA*, a chi-squared value of 6.86 ($P = 0.03$) was obtained. However, deviation from the expected was not significant at the 1% level of probability.

Length polymorphism of SSRs in cucumber (*Cucumis sativus* L.)

Five of the seven SSRs described above produced clear amplification products with DNA isolated from 11 cucumber cultivars. Of these five SSRs, four (80%) were polymorphic between melon and cucumber (Table 2). The fifth SSR, *CMCT58*, did not detect any polymorphism, and this indeed was the smallest SSR tested. The size of the amplification products obtained with the two cucumber SSRs in several cucumber varieties fitted the published sequence size (Table 2). Polymorphism within cucumber was detected by the two cucumber SSRs and by two melon SSRs (*CMTC13* and *CMAG59*). Figure 3 depicts the amplification pattern obtained with the DNA of cucumber varieties using the *CSHPRAG*

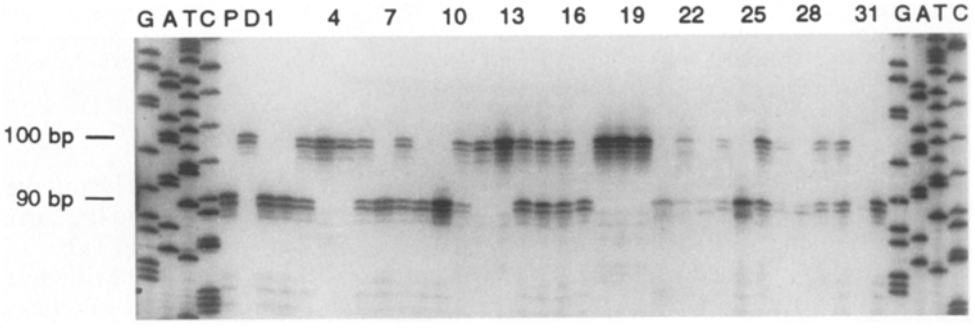

Fig. 2 Radiolabeled PCR products amplified from the parental lines and 31 F_2 plants of the cross 'PI414723' × 'Dulce', using primers flanking the melon *CMTC13* locus. Fragments were separated on a standard DNA-sequencing gel. The sequencing ladder is M13mp18 ssDNA

Table 4 Segregation of SSR alleles in F_2 progeny of the cross between 'PI414723-S_5' (P_1) and 'Dulce' (P_2)

Locus	Number of F_2 plants	Number of F_2 plants in genotypic classes[a]			χ^2	
		P_1	Heterozygote	P_2	1:2:1	P
CMTC13	112	33	51	28	1.34	0.51
CMAG59	112	26	57	29	0.20	0.91
CMGA127	112	23	59	30	1.20	0.55
CMGA128	112	30	54	28	0.21	0.90
CSLHCPA	112	32	64	16	6.86	0.03

[a] Progeny were classified as: homozygous for the allele from the maternal parent (P_1), heterozygous, or homozygous for the allele from the male parent (P_2), with an expected codominant segregation ratio of 1:2:1

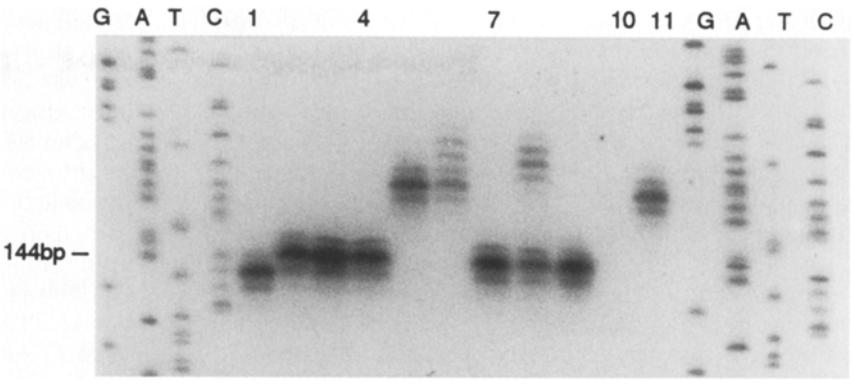

Fig. 3 Radiolabeled PCR products amplified from the DNA of 11 cucumber (*Cucumis sativus*) genotypes using primers flanking the cucumber *CSHPRAG* locus. Fragments were separated on a standard DNA-sequencing gel. The sequencing ladder is M13mp18 ssDNA. Lanes are as follows: *1* 'PI18396', *2* 'GY14', *3* 'G421', *4* 'H19', *5* 'Bet Alfa', *6* 'Ashley', *7* 'Pickmore', *8* 'Dasher' F_1, *9* 'Calypso', *10* 'Colet', *11* 'Shimshon'

primers. Three different size alleles and one null allele were detected in the 10 homozygous cucumber cultivars tested, corresponding to a gene diversity value of 0.64. An additional size allele was detected in the 'Dasher'-F_1 hybrid (Table 2). The polymorphism detected with the *CSLHCPA* and *CMTC13* primers was much lower (Table 3). A null allele was determined only after several repetitions of the assay to ensure that it was not a failure of the reaction. The same template DNA produced a clear product at other loci.

CMAG59 primers detected a high level of polymorphism (gene diversity value of 0.57), yet the amplification pattern obtained with this marker was not as clear as with the other four SSRs. This may be due to a small mismatch between the melon primers and the cucumber DNA.

Of the 11 cucumber varieties tested, 10 expressed only a single allele following amplification with each of the four polymorphic SSR primers, as is expected from homozygous varieties. We also tested one hybrid ('Dasher'-F_1) which demonstrated two alleles with two of the polymorphic SSRs (*CSHPRAG* and *CMAG59*).

Cross homology of microsatellites between different species of the Cucurbitaceae family

In addition to the cross reaction of SSRs within the genus *Cucumis*, it was found that primers of melon and cucumber SSRs amplified DNA extracted from other genera belonging to the Cucurbitaceae family. Figure 4 depicts amplification patterns of DNA obtained from *Cucurbita*, *Citrullus*, and *Cucumis* cultivars using the melon *CMTC13* primers. Specific SSR signals were produced also with *CMAG59* and *CSLHCPA* primers (Table 2). However, the intensity of the signals within the genus *Cucumis* was usually stronger than that produced with DNA of *Citrullus* or *Cucurbita* cultivars. In some cases, a stronger signal was obtained with DNA of *Citrullus* varieties and in some cases with DNA from the different *Cucurbita* cultivars, suggesting mismatches of primer sequences. The SSR results were not always in accordance with the results obtained for other groups of repetitive DNA. For example, ribosomal DNA spacer has been shown to exhibit strong cross reaction within the *Cucurbita* genus while showing no hybridization

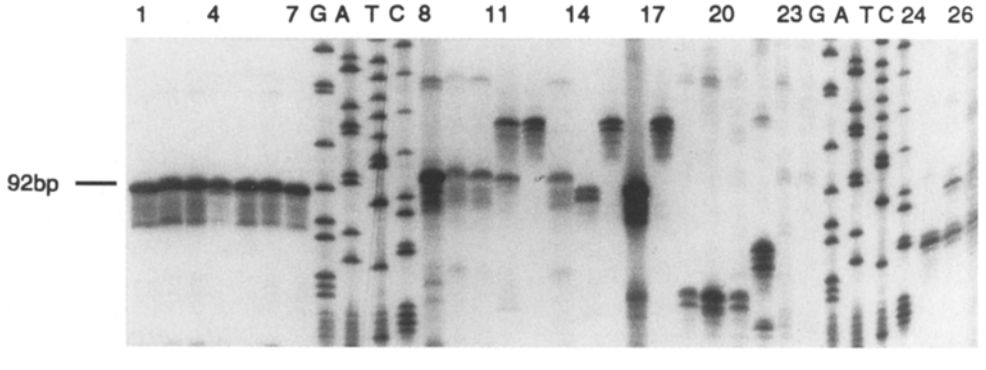

Fig. 4 Radiolabeled PCR products amplified from the DNA of Cucurbits genotypes using primers flanking the melon *CMTC13* locus. Fragments were separated on a standard DNA-sequencing gel. The sequencing ladder is M13mp18 ssDNA. Lanes are as follows: *Cucumis sativus*: *1* 'Bet Alfa', *2* 'Ashley', *3* 'Pickmore', *4* 'Dasher' F_1, *5* 'Calypso', *6* 'Colet', *7* 'Shimshon'; *Cucumis melo*: *8* 'Noy Yizre'el', *9,13* 'Krymka', *10* 'Noy Yizre'el' × 'Krymka', *11* 'Eshkolit Ha'amaqim' × 'Krymka', *12* 'Eshkolit Ha'amaqim', *14* 'Freeman Cucumber', *15* 'Q36', *16* 'PI414723' *17* 'Dulce'; *Cucurbita pepo*: *18* 'Sihi Lavan', *19* 'Vegetable Spagetti', *20* 'True French'; *Cucurbita maxima*: *21* 'Big moon'; *Cucurbita moschata*: *22, 23* 'Butternut'; *Citrullus lanatus*: *24* 'Sugar baby', *25* 'Tri-x-313', *26* 'Malali'

with the DNA of *Cucumis* species (Ramon et al. 1991; Zentgraf et al. 1992).

Polymorphism within the genus *Cucurbita* was detected with the three SSR markers mentioned above. *CMCT13* primers detected polymorphism between *Cucurbita pepo* and *C. maxima*, whereas no specific signal was produced from *C. moschata* (Fig. 4). *CMAG59* primers distinguished *C. moschata* from *C. pepo* and *C. maxima*. *CSLHCPA* primers detected polymorphism only between *C. maxima* and the other two species. No polymorphism was detected by any of the primers among the four varieties tested of *C. pepo*. (Table 2).

A specific signal was also detected in *Citrullus lanatus* with the same three SSRs (Fig. 4), but no polymorphism was observed between the Israeli 'Malali' variety and the two American varieties tested (Table 2). The low polymorphism found within *Citrullus lanatus* was consistent with previous findings of high homology within cultivated watermelon (Navot and Zamir 1987).

Discussion

Informativeness of microsatellite markers

The high level of polymorphism associated with microsatellites was demonstrated in the present study in melon and in related species. Five of the seven SSRs tested (71%) detected polymorphism in a sample of eight melon varieties. For comparison, only 84 out of 220 RAPD primers (38%) detected polymorphism in the same sample of varieties (data not shown). Of the five polymorphic SSRs, two distinguished the closely related muskmelon varieties, 'Noy Yizre'el' and 'Eshkolit', while only one of 28 RAPD primers distinguished between them. Gene diversity values obtained with SSRs in melon were high (0.49–0.75), with three to five alleles for each SSR in a sample of eight varieties. The high gene diversity values obtained by using microsatellites in melon are similar to those obtained for microsatellites within other plant species (e.g., Senior and Heun 1993; Rongwen et al. 1995).

The informativeness of microsatellites is even more significant when these results are compared with the low level of variation detected by other methods. Shattuck-Eidens et al. (1990) found a relatively low number of base substitutions by sequencing different regions of the melon genome (two bp differed out of 1572 that were sequenced). Neuhausen (1992) studied RFLPs and found that only 33% (resembling the RAPD results) of the tested probes were useful in differentiating at least one of the seven melon accessions tested. Furthermore, the majority of the informative RFLPs detected only two hybridization patterns in a sample of 44 melon lines. Except for SSRs, the only markers able to detect a high polymorphism between melon varieties are telomeric arrays probes (Broun et al. 1992). However, due to their location at chromosome ends, telomeric arrays are of limited use in genome mapping and marker-assisted selection.

A high level of polymorphism was detected in the present study between *C. sativus* var *sativus* and *C. sativus* var *hardwickii*: two of the four polymorphic SSRs produced length polymorphism between the two sub-species. Moreover, when the two null alleles are also taken into consideration, all four polymorphic SSRs distinguished between the two sub-species. For comparison, using 29 isozymes Perl-Treves et al. (1985) did not find any polymorphism between the two. Kennard et al. (1994) conducted a large RFLP survey and found that only 25% of the clones detected polymorphism between *C. sativus* var *sativus* and *C. sativus* var *hardwickii*. Gene diversity for the polymorphic SSRs in cucumber ranged between 0.18 and 0.64. In view of the narrow genetic background of *C. sativus*, that slowed progress in the development of a saturated genetic map (Kennard et al. 1994), SSR markers provide a useful source by which to complement the RFLP and RAPD markers used so far.

SSR structure

The informativeness of SSR increases with the number of the repeats (Weber 1990). The core motifs of the informative SSRs described above are short in comparison to those of other mammalian and plant SSRs (Akkaya et al. 1992, 1995; Beckmann and Weber 1992; Wang et al. 1994). Among the SSRs tested, eight repeats were sufficient to detect polymorphism. Polymorphic SSRs with eight repeats in their core motif have also been reported in other plants, including maize (Senior and Heun 1993), sunflower (Brunel 1994), and *Brassica* (Lagercrantz 1993). SSR length is not necessarily an indicator of polymorphism. For example, in melon only the smaller of the two cucumber SSRs (eight repeats compared with 13) was polymorphic. In addition, an SSR which was polymorphic in one species was not necessarily polymorphic in the other (Table 3, *CSHPRAG* versus *CSLHCPA*). The two cucumber SSRs had uninterrupted core sequences, while the melon SSRs had cores with interrupted sequences.

Microsatellite homology between species

Three of the five melon SSRs tested produced distinct amplification products in cucumber, and the two cucumber SSRs produced distinct amplification products in melon. In one case, an amplification product of melon DNA obtained with cucumber primers (*CSLHCPA*) was excised from a gel and sequenced. The sequence was nearly identical to that of cucumber with an $(AG)_9$ repeat (to be described in details elsewhere). This homology between *C. melo* and *C. sativus* was in agreement with results from previous studies. Using RFLP analysis, Neuhausen (1992) found that 94% of the melon probes tested hybridized to cucumber DNA. It is expected that a cross-homology detected by SSR would be

lower than by RFLP, as the use of SSR primers requires a near-complete matching of DNA sequences, while that of RFLP requires only partial homology within relatively long DNA sequences. Based on RFLP results, Neuhausen (1992) suggested that the basis of polymorphism within *C. melo* were point mutations, whereas between *C. melo* and *C. sativus* structural changes were involved. However, the results here demonstrate that the length polymorphism between *Cucumis* species, as detected by three SSR markers (*CMTC13*, *CMAG59*, and *CSLHCPA*), was within the range of length polymorphism detected witin species (Table 2). This implies that the polymorphism discussed above resulted from mechanisms typical of those introducing mutations into microsatellites rather than from major rearrangements. Most of the mechanisms proposed in the literature suggest strand slippage during replication, repair, or recombination (Litt and Luty 1989; Weber 1990). Two SSR markers (*CMGA127* and *CMGA128*) did not cross-react between the species, which may be explained in terms of either base substitution or rearrangement. The last polymorphic SSR (*CSHPRAG*) produced a clear signal in melon that differed in size from the cucumber alleles. To test whether the melon product does indeed contain the AT repeat of CSHPRAG we need to carry out further studies.

The possibility of using the same microsatellite flanking primers in more than one species is encouraging in view of the efforts and expenses involved in designing each of them. The conservation of microsatellites has been previously demonstrated in mammalian genomes (Moore et al. 1991) and in plants (in *Brassica* species, Lagercrantz et al. 1993; Kresovich et al. 1995; and in *Vitis* species, Thomas and Scott 1993). On the other hand, there are reports indicating limited conservation among related species (wheat, barley, and rye, Röder et al. 1995) as well as within wheat (Devos et al. 1995). It was suggested that wheat microsatellites often occur in fractions of the genome that diverged between species (Röder et al. 1995). While further study is required in order to evaluate the generality of the conservation phenomenon, our results suggest that SSR markers may be a useful tool for evolutionary studies.

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