

***Cucumis* SSR Markers Applied to the Study of Genetic Diversity in the *Cucurbita* Genus**

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The genus *Cucurbita* L. includes five cultivated species. *C. pepo* L., *C. maxima* Duchesne, and *C. moschata* Duchesne are the three most economically important. A high level of morphological and agronomical variability exists within these species. Large collections of *Cucurbita* landraces with a high potential value for breeding are maintained elsewhere. The COMAV's Genebank at the Polytechnic University of Valencia holds the largest collection of Spanish *Cucurbita* landraces. This collection also includes many accessions from the areas of origin and diversification of the different species (3).

Molecular tools, and particularly molecular markers, are very useful for the management of large germplasm collections. However, although new biotechnologies are increasingly being applied in *Cucurbita* breeding, it is still in the early stages when compared to other major Cucurbits. Only a few linkage maps with a low marker density have been reported. These maps are mainly based on dominant molecular markers, such as RAPDs, ISSRs or AFLPs. This type of markers has been used mostly to study the genetic variation in *Cucurbita* (3, 7).

SSRs are locus-specific markers assumed to be high quality (codominant, highly informative, and evenly dispersed throughout genomes). Identification of SSR markers can be attempted by screening genomic or cDNA libraries or by searching DNA sequence databases. None of these genomic tools are available for *Cucurbita* species. SSR markers have been largely reported in the *Cucumis* L. genus, also belonging to the Cucurbitaceae family. Katzir et al. (5) demonstrated that primers specific to melon and cucumber SSRs also amplified DNA from the *Cucurbita* genus. Seven (14%) of the 50 SSRs *Cucumis* primers tested (1) were found to be functional

and polymorphic among a collection of *C. pepo* accessions, but their utility in other species of *Cucurbita* was not tested (6, 7). Since these studies, the SSR density in the melon genetic map has been significantly increased (4), providing new SSRs which may be potentially useful in *Cucurbita*.

We have studied the cross homology of 60 *Cucumis* SSRs in the *Cucurbita* genus. These markers were selected from those mapped (from 3 to 10 per chromosome) in the last version of the Spanish genetic reference map (Piel de sapo x PI 161375) (4). These included both SSRs derived from genomic libraries (gSSRs) as well as from EST sequences (EST-SSR). Some of the *loci* are conserved across the two main species of the *Cucumis* genus. Accessions representative of the phenotypic diversity of each *Cucurbita* species (8, 12, and 15 for *C. maxima*, *C. moschata* and *C. pepo*, respectively) were used in the assays. This sample included Spanish landraces and some landraces from South and Central America. In the case of *C. pepo*, the eight edible cultivar groups were represented.

A high percentage (63.3%) of the SSRs assayed did not amplify in any species, even when the PCR conditions described for melon varied. Most of the amplified SSRs were monomorphic, but a small subset was polymorphic in one, two, or all three species. Of the 20 EST-SSRs assayed, 2 amplified in *C. pepo* (TJ2 and TJ31), 1 in *C. maxima* (TJ24), and 2 in the three species (CSWTA02 and CSTCC813), but only CSTCC813 was polymorphic in *C. moschata* and *C. pepo* (Table 1). Of the 40 gSSRs assayed, 28 were developed by Danin-Poleg, et al., (1) and the remaining 12 were among those recently described in Gonzalo, et al. (4). Of the first subset, 1 amplified in *C. pepo* (CSWCT01), 1

amplified in *C. moschata* and *C. maxima* (CMGA165), both of which being monomorphic, and 7 amplified in the 3 species. CMGA128 and CMGA15 were monomorphic, and the remaining five (CMTC51, CMTC123, CMAG59, CMGA172, CMTC168) were polymorphic within and between species (Table 1, Figure 1). The *loci* CSGA057, CMCT160a and CSTTT15a, previously reported as polymorphic in *C. pepo* (6), did not amplify in our assay. Of the second subset, 1 amplified in *C. pepo* and *C. maxima* (CMTCN56), being polymorphic between species, and 3 amplified in *C. pepo* and *C. moschata* (CMAGN75, monomorphic between and within species, and CMGAN21 and CMTAAN100, polymorphic between species). Three *loci* amplified in the 3 species, but only two were polymorphic within and between species (CMTGN17 and CMAGN73).

Results agree with previous studies in Cucurbitaceae and other families, indicating limited conservation of SSR *loci* between *Cucumis* and *Cucurbita*. Despite this limited success, the selected SSR markers were informative and allow us to reach certain conclusions about the analysed germplasm. In *C. maxima* a similar level of variation was detected among the Spanish landraces and the South American accessions. However, accessions of *C. moschata* from South and Central America displayed a higher level of variation than Spanish landraces. *C. pepo* was the most variable species and alleles unique to subspecies were detected.

The density of EST-SSR markers in the melon genetic map is currently being increased using a large collection of ESTs obtained in the Spanish Melon Genome Initiative (2). The usefulness of these EST-SSRs for genetic studies of *Cucurbita* is currently being investigated.

This research was supported by the projects INIA RF03-003 and RF2004-00003-00-00

Literature Cited:

1. Danin-Poleg, Y., N. Reis, G. Tzuri, and N. Katzir. 2001. Development and characterization of microsatellite markers in *Cucumis*. *Theoretical and Applied Genetics* 102:61-72.
2. Fernandez-Silva, I., J. Blanca, B. Picó, F. Nuez, P. Arus, and A.J. Monforte. 2006. Mapeo de marcadores microsatélites a partir de librerías de ESTs de melón (*Cucumis melo*) mediante genotipado selectivo. *Actas de Horticultura* 54:109-110.
3. Ferriol, M., B. Picó, and F. Nuez. 2003. Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. *Theoretical and Applied Genetics* 107:271-282.
4. Gonzalo, M.J., M. Oliver, J. García-Mas, A. Monfort, A. Dolcet-Sanjuan, N. Katzir, P. Arus, and A.J. Monforte. 2005. Simple-sequence repeat markers used in merging linkage maps of melon (*Cucumis melo* L.). *Theoretical and Applied Genetics* 110:802-811.
5. Katzir, N., Y. Danin-Poleg, G. Tzuri, Z. Karchi, U. Lavi, and P.B. Cregan. 1996. Length polymorphism and homologies of microsatellites in several Cucurbitaceae species. *Theoretical and Applied Genetics* 93:1282-1290.
6. Katzir, N., Y. Tadmor, G. Tzuri, E. Leshzeshen, N. Mozes-Daube, Y. Danin-Poleg, and H.S. Paris. 2000. Further ISSR and preliminary analysis of the relationships among accessions of *Cucurbita pepo*. *Acta Horticulturae* 510:433-439.
7. Paris, H.S., N. Yonash, V. Portnoy, N. Mozes-Daube, G. Tzuri, and N. Katzir. 2003. Assessment of genetic relationships in *Cucurbita pepo* (Cucurbitaceae) using DNA markers. *Theoretical and Applied Genetics* 106:971-978.

Table 1. SSR loci, derived from two *Cucumis* species, which detected polymorphism between or within species in a collection of *Cucurbita* germplasm.

SSR locus	WS ^z			LG ^y	BS ^x	Origin	Cm/Cs ^w	Ref.
	<i>C. maxima</i>	<i>C. moschata</i>	<i>C. pepo</i>					
CSTCC813	1/nt	2/nt	2/ni	G12	NO	Cucumber cDNA library	ns/2	1,7,4
CMTC51	3/nt	4/nt	2/1-null	G4	YES	Non-coding region of EMBL CMACO1	1/2	1,6,7,4
CMTC123	3/nt	1/nt	3/nt	G12	YES	Melon genomic library	2/1	1,4
CMAG59	1/1	1-null/1	2-null/3	G1	YES	Melon genomic library	5/ns	5,6,7,4
CMGA172	1/nt	2/nt	1/nt	G9	NO	Melon genomic library	3/2	1,4
CMTC168	1/nt	1/nt	1-null/nt	G10	YES	Melon genomic library	4/ns	1,4
CMTGN17	1/ni	3/ni	2-null/ni	nm	YES	Melon genomic library	ni	7
CMAGN73	1/ni	1/ni	3/ni	G10	YES	Melon genomic library	ni	6,4

^z Variability within species, the first number indicates the number of alleles identified in our assay (null, null allele), and the second number the alleles previously reported (nt: not previously tested, ni: previously reported as polymorphic, but without information about the number of alleles)

^y Linkage group according to Gonzalo et al. (4), nm: not mapped

^x Variability between species

^w Number of alleles reported in *Cucumis melo* and *Cucumis sativus*, ns: no signal, ni: no information

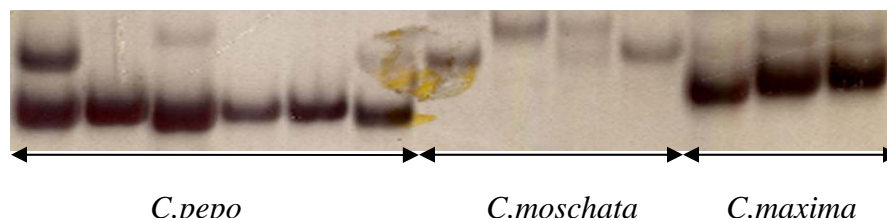


Figure 1. Example of the SSR locus CMTC51 that detects different alleles in *C. maxima*, *C. moschata* and *C. pepo*.