Comparative mapping of *Cucurbita moschata* and *C. pepo* using SSR markers

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**Keyword**: Microsatellite, molecular mapping, *Cucurbita*, hull-less seed, green rind

**Abstract**

The first SSR-based genetic linkage map of *C. moschata* was created by integrating the maps of two F\textsubscript{2} populations with one common parent developed from the crosses Waltham Butternut (WB) × Nigerian Local (NL) and ZHOU (a hull-less type) × WB. This integrated *C. moschata* map comprises 205 SSR markers and 2 morphological traits (*Gr* and *n*). The map is composed of 27 linkage groups with a marker density of 7 cM. Comparison of the *C. moschata* map and the published *C. pepo* map revealed a high level of macro-synteny. Seventy-one common SSR markers between *C. moschata* and *C. pepo* are located in homologous linkage groups. These markers representing orthologous loci in general have conserved orders and similar genetic distances. Four SSR markers were mapped in non-homologous linkage groups. They may represent different members of multi-gene families, chromosomal rearrangements and paralogs. The comparative mapping of *C. moschata* and *C. pepo* reported here will be useful for further studies on *Cucurbita* evolution, gene isolation, and breeding.

**INTRODUCTION**

*C. pepo* (2n=2x=40) and *C. moschata* (2n=2x=40), two species of the genus *Cucurbita*, are economically important crops worldwide (Robinson and Decker-Walters 1997, Loy 2004). Genetic mapping in the genus *Cucurbita* was hampered by the lack of available sequence specific markers. Based on anonymous RAPD and AFLP markers, two maps using inter-specific crosses between *C. pepo* and *C. moschata* (Lee et al. 1995; Brown and Myers 2002) and one map of *C. pepo* (Zraidi et al. 2007) were developed, which did not allow comparative analysis between species. This, however, became possible after the development of a large number of SSR markers specific for *Cucurbita* (Gong et al. 2008), which allowed updating the *C. pepo* map published by Zraidi et al. (2007). Well developed SSR maps are of great value for comparative genetics and to accelerate marker-assisted selection (MAS). Genome sequence information in *Cucurbita* is very limited yet; the comparative mapping of *C. moschata* and *C. pepo* may contribute to future *Cucurbita* genomic studies.

**MATERIALS AND METHODS**

*Cucurbitaceae* 2008, Proceedings of the IX\textsuperscript{th} EUCARPIA meeting on genetics and breeding of *Cucurbitaceae* (Pitrat M, ed), INRA, Avignon (France), May 21-24\textsuperscript{th}, 2008
Two *C. moschata* F₂ populations with one common parent, Waltham Butternut (WB, ♀) x Nigerian Local (NL, ♂), ZHOU (♀) x Waltham Butternut (WB, ♂), each derived from a single F₁ fruit and consisting of 94 plants, were used for *C. moschata* mapping. ZHOU is a Chinese hull-less *C. moschata* genotype (Zhou 1987). DNA was extracted following a slightly modified protocol of Promega’s Wizard Genomic DNA Purification Kit (Promega Corp., Madison, USA). Two qualitative gene loci, *n* (hull-less or naked seeds) and *Gr* (Green rind) (Zhou 1987; Paris and Brown 2005) showing clear segregation in the F₂ populations were scored.

The newly obtained 500 *Cucurbita* SSR markers were screened for polymorphism. PCR and electrophoresis conditions were as described in Gong et al. (2008). JoinMap® version 3.0 (Stam 1993; Van Ooijen and Voorrips 2001) was used for linkage analysis and map calculations. Linkage groups were determined using a LOD threshold of 3.0. Map construction was performed using the Kosambi mapping function with JoinMap parameter settings as follows: Rec=0.4, LOD=1.0, Jump=5.

Map integration of the two *C. moschata* populations, was made by those groups with common markers, using JoinMap® version 3.0. The same parameters as for the individual maps were employed for the integrated map. The integrated *C. moschata* map and the previous SSR-based genetic map of *C. pepo*, which was based on the F₂ population of O5 × CN (Gong et al. 2008), were aligned to make a comparative map.

**RESULTS AND DISCUSSION**

The maps of the two *C. moschata* populations were built separately by JoinMap. The 62 common SSR markers provided the bridge between the two *C. moschata* populations with a common parent (WB), which allowed us to construct an integrated map. The resulting integrated map was composed of 205 SSR markers and 2 morphological traits (*Gr* and *n*). Of the 205 SSR markers 146 originated from *C. moschata* and 59 from *C. pepo*. It covers 1445.7 cM with 27 linkage groups and a marker density of 7 cM.

The comparative alignment between *C. moschata* and *C. pepo* was based on 75 common SSRs, 55 developed from *C. moschata* and 20 from *C. pepo*. The published *C. pepo* map contained 20 linkage groups. Based on map comparisons, 26 linkage groups of *C. moschata* map were combined to 20 larger groups and one linkage group remained unaligned. This 20 pairs of homologous linkage groups, each pair sharing 1-8 common SSRs, may represent the 20 chromosomes of haploid *Cucurbita* genome. Seventy one common SSRs are orthologous loci; in most cases they have the same order and similar map distance. Hence, the comparative map clearly proved high level of macro-synteny between *C. moschata* and *C. pepo*. It reflects a close phylogenetic relationship between *C. moschata* and *C. pepo* as indeed was inferred from the mitochondrial *nad1* dehydrogenase intron 2 region between exons B and C (Sanjur et al. 2002). A further indication of the close genetic relationship is the high transferability rate (~90 %) of SSR markers between the two species. When *C. pepo* and *C. moschata* diverged from each other remains unknown. However, the high level of macro-synteny between them indicates that they experienced a fairly slow chromosomal evolution since their speciation.

Four common SSRs, CMTp260, CMTp61, CMTm68 and CMTm255, were located in four non-homologous groups. In addition, a small inversion between LGp12 and LGm9 was found. The very low success rate in obtaining hybrid seeds
between *C. pepo* and *C. moschata* suggests divergence between the two genomes. These discrepancies can be taken as a further indication of chromosome rearrangement. CMTp260 produces in both species one monomorphic and one polymorphic band. CMTp61 produced one polymorphic band in *C. pepo* and two bands in *C. moschata* with one being monomorphic, while the other was polymorphic. It suggests that CMTp260 and CMTp61 represent duplicate loci located on different chromosomes, they are paralogs. A BLAST search of CMTp260 revealed a 69% identity with *C. moschata* gene CmATS1 at E = 3E^-21 (NCBI), which may determine the chilling tolerance of plants. At present, two isogenes of CmATS1 have been identified (Nishida et al. 2000). The remaining two SSRs (CMTm255 and CMTm68) produced in both species only one polymorphic band. This may suggest the occurrence of translocation during the evolution. Some other small discrepancies in marker orders could be considered due to mapping imprecision, rather than due to chromosome rearrangements.

![Figure 1](image)

Figure 1. An example of homologous linkage groups in comparative map between *C. moschata* and *C. pepo*. Common markers between homologous groups are connected by broken lines. The names of *C. pepo* LGs start with “LGp”; the *C. moschata* LGs start with “LGm”. Linkage group bars of *C. pepo* are shown in white, that of *C. moschata* in black. The non-orthologous marker is underlined. Marker distances are given in cM.

Weeden (1984) put forward the hypothesis of paleopolyploid origin of the genus *Cucurbita* based on isozyme studies. Polyploidy may lead to meiotic pairing disturbances to translocation among homoeologous chromosomes (Lagercrantz, 1998). However, if *C. pepo* and *C. moschata* were of a paleopolyploid origin, such a high level macro-synteny requires a satisfactory explanation. A strong diploidization long before speciation, could have taken place, therefore the characters of polyploidy may not be easily detected by SSR marker. But it is also possible that the rearranged
segments in the chromosomes are relatively small compared to the large conserved segments, thus it is impossible to be detected at the present level of map density. The different genome sizes of *C. pepo* (1C ≈ 0.432pg) and *C. moschata* (1C ≈ 0.354pg) (Sisko et al. 2003) might also be a reason for the difficulty to detect discrepancies at a limited level of sequence information. The high level of macro-synteny between *C. moschata* and *C. pepo* revealed in this study will enable easy transfer of genes and markers tightly linked to important traits and may facilitate the construction of a reference map for the whole *Cucurbita* genus.

**ACKNOWLEDGEMENTS**

This research is financially supported by the Austrian Science Fund (No: P19662-B16)

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