Microsatellite Markers for the Genus *Cucurbita*

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Introduction

Microsatellites (SSRs) as markers are advantageous over other marker types because of their high abundance, co-dominance, high polymorphism and ready transferability between populations and even species. SSRs as anchored markers are especially useful for map construction.

In a former project we created a *C. pepo* map using an inter-subspecies F2 population of an oil-pumpkin x crook-neck cross. In this map no SSR markers were included. Meanwhile, in a pilot project, we have developed the first 18 *Cucurbita* SSR markers (STIFT et al. 2004). Inspired by the experiences we have collected in this project, we decided to develop a large number of *Cucurbita* SSRs to

(1) stabilize the *C. pepo* map,

(2) create two *C. moschata* genetic maps,

(3) compare the *C. pepo* map with the *C. moschata* map.

Materials and methods

Plant materials for SSR development

C. pepo subsp. *pepo*, Styrian oil-pumpkin

C. moschata cultivar Soler (Puerto Rico)

SSR development procedure

After nebulized to small fragments, the genomic DNA was end-polished by Mung bean nuclease (MBNase) and ligated with adaptors. Adaptor ligated DNA fragments were hybridized with biotinylated SSR oligos, (GA)13, (CA)13, (CAT)8, (CCA)8, (CAA)8, (GAA)8, and captured by streptavidin coated magnetic beads (Dynal). Captured DNA was amplified with adaptor specific primers and a partial genomic insert library was made. Clones with an insert were sequenced with a MegaBace 1000. Primers were designed for di-nucleotide motifs having at least 7 repeats, and for three nucleotide motifs having at least 5 repeats.

Primer test and polymorphism assessment

To find out the proper working conditions of the primers, a touch-down PCR was performed. To assess polymorphism a tester set composed of three *C. moschata* (Nigerian Local, NL, Waltham Butternut, WB, a hull-less Chinese line, ZHOU), 1 *C. ecuadorensis*, 8 *C. pepo* genotypes, representing the 8 cultivar groups (PARIS 1986), were used.

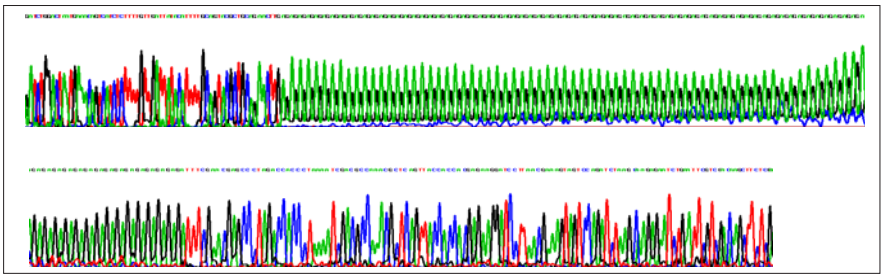

Figure 1: **Sequence of an SSR containing more than 69 GA repeats (arrow) in *C. pepo*. Fragment was sequenced from both ends, due to overlapping of the SSR sequence in the two sequencings exact repeat number can not be determined. Total fragment length is ca. 270 bp.**

Results on SSR development

In *C. pepo*, until now 1056 clones were sequenced, 434 clones included SSRs of at least 4 repeats, enrichment efficiency was 41.2%, 59 clones (5.6%) had SSRs too close to the end of the fragment; 230 primer pairs were designed, i.e. 53% of the SSR containing sequences.

In *C. moschata*, 1344 clones were sequenced, 610 clones included SSRs of at least 4 repeats, enrichment efficiency was 45.4%, 35 clones (2.6%) included SSRs too close to the end, 342 primer pairs were designed (56%).

Up to now 384 primer pairs were synthesized and tested by touch-down PCR at a 54°C final annealing temperature. 346 (90.1%) produced bands of expected size.

80 primer pairs of *C. pepo* were tested so far using the tester set, 54 of them showed polymorphism (67.5%), 9 were difficult to evaluate. On average, each primer pair produced 3.3 alleles (*Figure 1*).

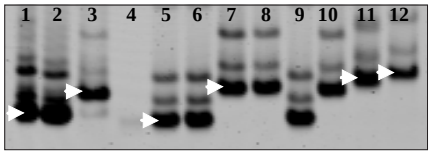

Figure 2: **The SSR marker CPGA23 detected 6 (arrows) alleles in 12 different *Cucurbita* genotypes: 1: NL, 2: WB, 3: ZHOU, 4: *C.ecuadorensis*, 5: Zucchini, 6: Cocozelle, 7: Staightneck, 8: Crookneck, 9: Oil pumpkin, 10: Scallop, 11: Acorn, 12: Vegetable Marrow**

References

PARIS, H., 1986: A proposed subspecific Classification for *Cucurbita pepo*. Phytologia 61, 133-138.

STIFT, G., A. ZRAIDI and T. LELLEY, 2004: Development and Characterization of microsatellite Markers (SSR) in *Cucurbita* Species. CGCR 27, (in press).

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