

Molecular Genetic Marker Comparison in Winter Squash (*Cucurbita maxima*)

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Rationale

~ The Plant Genetic Resources Unit (PGRU) in Geneva, New York is part of the U.S. National Plant Germplasm System (NPGS). The mission of the PGRU is to safeguard, evaluate and utilize seed and clonal crops of temperate zone vegetables and fruit. Our primary interest is in the conservation of genetic diversity within the vegetable seed crops. This study focuses on winter squash, *Cucurbita maxima* Duch.

~ The objective of this investigation is to identify reliable molecular markers for use in estimating genetic polymorphism within *C. maxima* to describe geographical variation within this species. Two types of molecular markers were used to test for genetic diversity within accessions of *C. maxima*, simple sequence repeats (SSRs) and sequence-based amplified polymorphisms (SBAPs). The SSRs used in this study were selected from previous work with *C. maxima* and/or other closely related species: melon and cucumber (Chiba, et al 2003, Katzir, et al 1996).

~ SSR markers have been widely used for molecular genotype analysis, crop germplasm evaluation and comparisons (Hokanson, et al 1998). SBAPs are 17 or 18 base pairs long and are designed to preferentially amplify random DNA sequence within coding regions of the genome. The SBAPs primers tested were developed from *C. maxima*. Little is known about the genetic diversity within this species, with very few published reports regarding molecular genetic markers for *C. maxima* (Ferriol, et al 2003).

Materials and Methods

~ Twenty accessions (unique seed populations), four each from South America, North America, Europe, Asia, and Africa were sampled, two plants per accession, for genotyping (Table 1).

~ SSR primers were: CMT168, CMT160a&b, CSAT425 (Danin-Poleg et al, 2000), CSWCT12A, CSWCT13B, CSWCTG08 (Fazio et al, 2002).

~ Approximately 50 to 100 mg of tissue was collected from each of two plants of 20 accessions. DNA was extracted using a CTAB extraction protocol (Colosi and Schaal, 1993) and Genogrinder to expedite the extraction process. DNA samples were then cleaned using Epicenter Technologies MasterPure™ Plant Leaf DNA Purification Kit to remove PCR inhibiting contaminants.

~ DNA was quantified using a Hoechst dye assay (Latt and Stetten, 1976) on a TECAN Spectrafluor plate reader.

~ SSR markers were amplified in a 25 ul PCR reaction containing 10x PCR buffer, 10 mM dNTPs, 1.5 to 2.5 mM MgCl₂, 5 units Taq polymerase, 4 pmoles of each SSR primer and 40 ng of genomic DNA. Annealing temperatures from 45° C – 60° C in 20 increments were tested. The thermoprofile for the PCR reaction was: 94°C for 6 minutes, then 35 cycles of 94°C for 1 minute, 45° to 60°C for 1 minute, and 72°C for 1 minute. The samples were held at 72°C for 10 minutes for final extension. SSR genotypes were visualized on 2% agarose gel, 0.5x TBE, 110 volts, 0.05 mA for 45 minutes and scored as + or – based on presence or absence of a band.

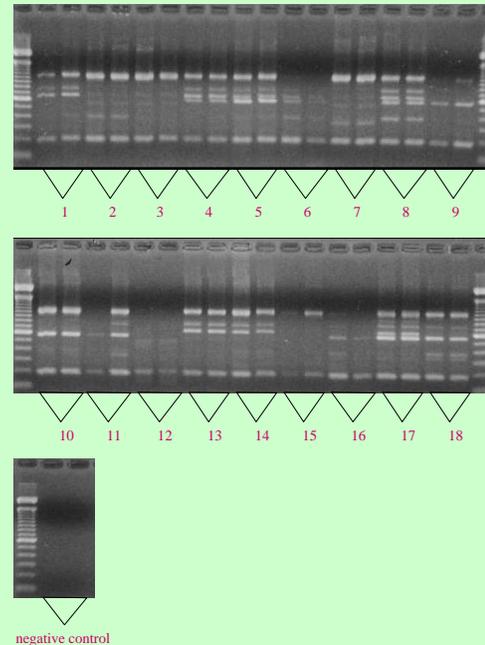
~ SBAPs primer reactions were in 50 ul volumes using the same final concentrations of components as listed above for the SSR reactions. The thermoprofile for the SBAPs primer reaction was: 94°C for 5 minutes, then 5 cycles of 94°C for 1 minute, 35°C for 1 minute, and 72°C for 2 minute, followed by 30 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 5 minutes (Ferriol, et al 2003).

~ SBAP reaction products were cleaned to eliminate salt and dNTP residue using BioEdge Systems QuickStep 2™ PCR clean-up kit. Genotypes were visualized on 2% agarose gel, 0.5x TBE, 95 volts, 0.04 mA for 55 minutes and scored as + or – based on presence or absence of a band.

Table 1. Twenty *C. maxima* accessions genotyped using SSRs and SBAPs.

ID	Common name	Country
G 23728	Chiquie	Argentina
PI 560953	Zapallito Amargo	Argentina
PI 560948	Zapallo	Bolivia
PI 390616	-	Peru
PI 298036	-	Austria
PI 193771	-	Ethiopia
PI 414906	Arka Suryamukhi	India
G 9187	Ucike Kuri	Japan
PI 548967	-	Malta
G 29515	-	Nepal
G 29646	-	Nigeria
PI 512797	-	Spain
PI 169455	Kara Kabak	Turkey
G 26694	Buttercup	United States
G 27743	Big Max	United States
G 28224	Sweet Mama	United States
G 32304	Mountaineer PS Hubbard	United States
PI 490349	Kamon	Upper Volta
PI 357896	Kumanovska	Yugoslavia
PI 526246	-	Zimbabwe

Figure 1. SBAPs primers ME7 X EM1 tested in replicated PCR reactions.



Results

The six SSR markers tested performed poorly over a range of conditions: annealing temperature from 45°C to 60°C in 2° increments and 1.5 to 2.5 mM MgCl₂. Marker CMT168 amplified, but this was not highly reproducible. None of the other five markers amplified (results not shown).

Banding patterns obtained using SBAPs markers appeared to be highly reproducible (Fig.1). We observed polymorphism among individuals, and within and among accessions.

The SSR molecular genetic markers developed in melon and cucumber species were not promising. It is possible the primer binding sites have diverged so much between species that the primer did not effectively bind any longer. It may be possible to further optimize them in *C. maxima*.

Preliminary analysis of SBAPs banding patterns showed no evidence of population structure. Identical genotypes were spread among the continents.

Due to their high reproducibility and high levels of polymorphism, SBAPs appear to be promising markers for studying genetic diversity in our *C. maxima* collection.

References

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