

RAPD Markers Linked to Morphological and Disease Resistance Traits in Squash

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Molecular markers linked to phenotypic traits can be useful to breeders. The most useful types of markers are those which allow for selection in seedlings instead of mature plants, or which permit selection for disease resistance without inoculating the entire segregating population. Sometimes markers are developed for a specific trait of interest using bulked segregant analysis or near-isogenic lines. However, markers linked to morphological and disease resistance traits are often detected while constructing genetic maps. Saturated or nearly saturated genetic maps exist for a number of vegetable species, including cucumber and melon. *Cucurbita* has not been extensively mapped; the map published by Lee et al. (2) contained only five linkage groups and no morphological traits. The Random Amplified Polymorphic DNA (RAPD) marker – trait linkages reported here are part of a larger RAPD map of an interspecific *Cucurbita pepo* x *C. moschata* cross. The entire map will be published elsewhere.

RAPD markers are useful for making genetic maps in minor crops, as they are much less costly than Restriction Fragment Length Polymorphisms (RFLPs) or Simple Sequence Repeats (SSRs). However, they do have some recognized drawbacks. They are dominant markers. Reproducibility can be poor, particularly of complex banding patterns. Perhaps most significantly, RAPD markers cannot be readily transferred between populations, as they are identified by size rather than sequence. Thus RAPDs are most useful for marker assisted selection when they are tightly linked to a disease resistance gene or other trait which has been transferred from a single donor line into many different populations. Many of the difficulties with the reproducibility of markers can be solved by sequencing the RAPD band and creating a longer, more specific primer that identifies a Sequence Characterized Amplified Region (SCAR).

Material and Methods: *Plant material.* The mapping population consisted of 162 BC₁ individuals from a cross between a commercial yellow straightneck (*C. pepo*) inbred and *C. moschata* ‘Nigerian Local’. The yellow squash was the

recurrent parent. ‘Nigerian Local’ is resistant to zucchini yellow mosaic virus (ZYMV) and has been much used as a source of resistance in summer squash. The yellow squash inbred is resistant to powdery mildew. Morphological traits included precocious yellow fruit (the *B* gene), fruit flesh color, the intensity of fruit color, warts, mottled leaves, fruit shape, and spiny stems. The population segregated for resistance to the two diseases and for the morphological traits described above.

Assessment of Phenotype. Plants in the mapping population and five individuals from each parental line were grown to maturity in the greenhouse in Corvallis, Oregon from September through January, 1999-2000. All morphological data were collected using simple observation. Powdery mildew resistance was determined based on the severity of symptoms after extensive natural infection. Attempts were made to control the mildew only on the ‘Nigerian Local’ plants, which were known to be extremely susceptible. Virus inoculation with ZYMV was done on mature plants using a high-pressure paint sprayer to apply the inoculum directly to the growing points. We inoculated mature plants so that the virus symptoms would not interfere with data collection on the other morphological traits. One month after inoculation plants were visually rated for virus symptoms and tissue was collected for Enzyme Linked Immunosorbant Assay (ELISA) testing.

DNA Extraction and RAPD Analysis. DNA was extracted from newly expanded true leaves using a CTAB –chloroform:isoamyl method (1). Following extraction the DNA was further purified by running each sample through a spin column re-precipitating the DNA (Genemate PCR Pure kit). This was done to remove salts and other compounds that were interfering with the Taq polymerase; it permitted us to reduce the amount of Taq in the reaction by 50%. DNA was quantified with a DyNAQuant fluorometer (Hoechst) and working solutions were standardized at 5 ng/ul DNA.

Parents were screened with 378 10-mer primers from Operon and the University of British Columbia.

Those primers that had strong bands present in Nigerian Local but not in the yellow squash were used to amplify the mapping population. Because the mapping population was the BC₁ to the yellow squash parent, bands present in that parent did not segregate in the progeny.

Reaction mixtures contained 30 ng of plant genomic DNA, 1.5 mM MgCl₂, 0.1 mM dNTPs, 3 pmole of primer (Operon), 0.5 units of Taq DNA polymerase (Promega), and 1X Taq buffer in a total volume of 15 ul. The amplification program was 2 min. at 94°C followed by 5 cycles of 5 seconds at 94°C, 1 min. at 37°C, 30 sec. at 54°C, a 7 min. ramp to 72°C, and 2 min. at 72°C and 30 cycles of 5 seconds at 94°C, 1 min. at 37°C, 30 sec. at 54°C, 2 min. at 72°C ending with 15 min. at 72°C. Because of the size of the population (162 BC₁ individuals, 5 samples from each parental line, and parental and H₂O controls, for a total of 178 samples) it was necessary to amplify the population in two batches for each primer. Parental and H₂O controls were included in each batch, to enable us to detect any lack of repeatability between batches. In the interest of speed, four thermocyclers (an MJR-100, a Perkin-Elmer 9600 and two Perkin-Elmer 9700s) were used, but both batches for each primer were run on the same machine. In addition, the amplification programs were adjusted to be as identical as possible on the four machines. The PCR products were separated on 1.5% agarose (Seakem LE from FMC or Ultrapure from Gibco) in 0.5% TBE buffer. Size was determined with a 100-bp ladder (Promega). The gels were stained with ethidium bromide and recorded on Polaroid 667 film. Only strong, repeatable bands were scored.

Results: The yellow squash parent had smooth, intense yellow preanthesis fruit, which matured golden yellow. Peduncles were yellow and the fruit flesh was white. Leaves were uniform green. It was resistant to powdery mildew, but susceptible to ZYMV. The Nigerian Local parent had warty, intense black-green fruit and white flesh. Other plants in the population had white fruit or mottled dark- and light-green fruit. The leaves were mottled with silver. It was resistant to ZYMV but highly susceptible to powdery mildew. The F₁ plant had warty fruit, which was bicolor for intense yellow/intense green, and a green peduncle. The BC₁ progeny segregated for bicolor, peduncle color, color intensity, orange or

white flesh, wartiness, mottled leaves, powdery mildew susceptibility and ZYMV susceptibility.

The morphological traits associated with markers were two affecting fruit color (including the economically important precocious yellow *B* gene), mottled leaf, and a virus resistance gene complementary to ZYMV resistance (*Zym*). RAPD markers are identified by the primer and the size of the band in base pairs. Map units are Kosambi centimorgans.

The precocious yellow gene (*B*) from the yellow squash parent was linked in repulsion to Operon I10₁₇₀₀ at 27.1 cM. This linkage is somewhat questionable both because it is loose, and because I10₁₇₀₀ and the RAPD markers most closely linked to it show distorted segregation, while *B* segregated 1:1 as expected. Operon H14₆₀₀ and UBC 489₁₂₀₀ flanked the dominant mottled leaf allele (*M*) from Nigerian Local. H14₆₀₀ is 13 cM from *M*, and 489₁₂₀₀ is 16.3 cM away. The actual linkages may be tighter, as the mottle-leaf trait was under-expressed in our low-light greenhouse. A gene controlling the intensity of mature fruit color is flanked by Operon B14₇₅₀ 17.8 cM away on one side and Operon G17₇₀₀ 9.7 cM away on the other. Both markers are linked to the fruit color gene in repulsion. A complementary gene for ZYMV resistance is 26.7 cM from Operon L14₁₀₅₀. They are linked in coupling. The primary resistance gene *Zym* is epistatic to this gene, whose phenotype is a positive ELISA score for a symptomless plant one month after inoculation with ZYMV. We are currently conducting progeny tests to further understand this new gene. Allelism tests have not been done to determine its relationship to *Zym-2* and *Zym-3* (3). Other genes which have been mapped but remain unlinked are powdery mildew resistance (*pm*), orange fruit flesh, *Ep*, *Zym*, and warts (*Wt*).

Discussion: These five marked loci are only a beginning. They are not likely to be of great use in marker assisted selection because of the poor transferability of RAPDs, linkages in repulsion to the traits in *C. pepo*, and loose linkages. However, we hope to add additional markers to the squash map, including SSR markers, which are highly transferable. Progeny testing currently under way will tell us more about the new ZYMV resistance locus, and bulked segregant analysis may let us find markers which are more tightly linked. These could

be converted to SCARs for use in marker-assisted selection, as could the markers linked to leaf mottle.

Literature Cited

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