The Use and Development of Molecular Breeding Tools in *Cucurbita*: A Literature Review

Rebecca N Brown
Department of Horticulture, Oregon State University, Corvallis, OR 97331

Tools for molecular mapping and marker-assisted selection are being increasingly applied to the genus *Cucurbita*. However, much of the work which has already been done has remained unpublished, or has been published in journals of limited circulation, such that many who work with *Cucurbita* may not be aware of it. This review of the literature to date is intended to provide some background for current work.

The development of molecular tools for use in breeding *Cucurbita* species is still in the early stages, particularly when compared to cereal crops such as corn and wheat, and other vegetable crops such as tomatoes and lettuce. Even the *Cucumis* species *C. melo* and *C. sativus* are far ahead of *Cucurbita*. One reason for the late development of molecular tools in *Cucurbita* is that all species have twenty pairs of relatively short chromosomes. Using flow cytometry, Arumuganathan and Earle (1) determined that the haploid genome of zucchini (*C. pepo*) is approximately 500 million base pairs long. A typical nucleus (2n) contains 1.04 – 1.08 picograms of DNA. Most morphological traits appear to be unlinked, and many markers are required to adequately map the genome. *Cucurbita* species are of limited economic significance in developed countries, and most *Cucurbita* researchers work on several crops. The large size of the plants makes them ill-suited to genetics studies, and some types require a very long growing season.

No linkage map of any *Cucurbita* species existed prior to the development of molecular mapping. In the 1980s the use of isozymes revealed that *Cucurbita* is an ancient allotetraploid (28). Isozymes also provided a marker linked to one of the complementary genes responsible for the expression of WMV resistance in crosses between *C. maxima* and *C. ecuadorensis* (27).

Many of the species in *Cucurbita* can be successfully crossed, particularly if embryo rescue is used. However, the F1 and later generations of the interspecific crosses are frequently sterile or exhibit reduced fertility (22). Isozymes have been used to determine if the reduced fertility was a result of chromosomal rearrangement in crosses between *C. maxima* and *C. ecuadorensis*. Wall and Whitaker (25) examined the inheritance of leucine aminopeptidase and esterase isozymes in a set of *C. ecuadorensis* x *C. maxima* crosses and concluded that the chromosomal structure of the two species differed in the region of the esterase locus. Weeden and Robinson (26) examined the inheritance of twenty isozymes in the cross *C. maxima* x *C. ecuadorensis*. They used their data to build the first map of *Cucurbita*. It was based on the F2 of the cross *C. maxima* x *C. ecuadorensis*, and contained 11 isozyme loci in five linkage groups. From the isozyme map Weeden and Robinson (26) were able to determine that the significant decrease in fertility of the F2 and backcross generations of crosses between *C. maxima* and *C. ecuadorensis* was not a result of minor chromosomal rearrangements.

Restriction Fragment Length Polymorphisms (RFLPs) have not been much used in *Cucurbita* because of the time and expense involved in creating the probes. However, ribosomal (8, 24) and chloroplast DNA has been studied, and the chloroplast genome has been mapped (20). The chloroplast map was constructed using gene-specific probes from other species; this is possible because the chloroplast genome is highly conserved. Wilson et al. (29) used restriction enzymes and cloned chloroplast fragments to study the chloroplast DNA diversity of 15 species of *Cucurbita*. Their conclusions agreed with those obtained through interspecific crossing studies and standard taxonomy. They also determined that the annual *Cucurbita* species evolved from the perennial species. Havey et al. (10) used RFLPs to study the transmission of the chloroplast and mitochondrial genomes in cucurbits. They concluded that both organelle genomes were maternally transmitted in *Cucurbita*, unlike in *Cucumis* where the mitochondrial genome is paternally transmitted.

Lee et al. (17) developed a RAPD map of an F2 population of a *C. pepo* x *C. moschata* interspecific hybrid. They screened the parents with 70 10-mer primers from the University of British Columbia (UBC 501-570); 15 of the primers were polymorphic between the parents. These fifteen primers were used to amplify DNA from 40 F2 individuals, resulting in 58 RAPD bands. Forty-seven reproducible markers were used to build the map; 28 markers were mapped into five linkage groups. No morphological traits or other types of markers were included on the map. The map of Lee et al. (17) was small, based on a small number of progeny, and was published in the Korean Journal of Horticulture, with the result that it is not well known in the *Cucurbita* community. Furthermore, they selected arbitrary identifiers for the markers on their map, rather than following the standard practice of identifying the markers by the primer and the band size in base pairs. This further prevents comparisons between their map and other *Cucurbita* maps. The map of Lee et al (17) is currently the only published molecular map of *Cucurbita*. Data were collected for a random amplified polymorphic DNA (RAPD) map on a *C. maxima* x *C. ecuadorensis* population, but the map was never published (N. Weeden, personal communication).

RAPDs and other molecular marker technologies have been used to do DNA fingerprinting analysis within and between *Cucurbita* species. Jeon et al. (11) used RAPDs to distinguish among Korean cultivars of *C. pepo* and *C. moschata*, while Youn et al. (30) used RAPDs to study the genetic relationships among South Korean landraces of *C. moschata*. Stachel et al. (23) used RAPDs to estimate genetic diversity among commercial inbred lines of Austrian oilseed pumpkin, *C. pepo* var. *styriaca*. Gwanama et al (9) used RAPDs to determine the genetic variability present in the *C. moschata* landraces of south-central Africa. Baranek et al. (2) used RAPDs to study the genetic diversity within and between species of *C. pepo*, *C. moschata*, and *C. maxima*. All the researchers found RAPDs to be effective for determining the relatedness of different *Cucurbita* accessions. Katzir et al. (13) used microsatellite-anchored sequences as primers (ISSR) to classify cultivars of *C. pepo*. No microsatellites specifically designed for *Cucurbita* have been published, but polymorphisms among *Cucurbita pepo* accessions have been detected using SSRs developed for *Cucumis* (14, 15).

Polymorphism levels in *Cucurbita* are moderate. Stachel et al. (23) found 116 polymorphic markers by screening a set of 20 inbred lines of oilseed pumpkin (*C. pepo* var. *styriaca*) with 34 RAPD primers. Baranek et al.(2) found 42.5% marker polymorphism among six Austrian *C. pepo* genotypes. Katzir et al. found that 14% of the *Cucumis* SSR primers (15) and 82% of ISSR markers (13) were polymorphic among cultivar groups of *C. pepo*. Youn et al. (30) found that 18.6% of markers were polymorphic among *C. moschata* landraces. Brown and Myers (4) found 14% marker polymorphism between temperate and tropical *C. moschata* lines. Baranek et al. (2) found 64.1% marker polymorphism among a geographically disparate collection of *C. moschata* accessions. They found 55.9% marker polymorphism among a disparate collection of *C. maxima*.

Marker assisted selection is little-used in *Cucurbita* breeding. Brown and Myers have identified RAPD markers linked to several morphological traits in an interspecific cross between a *C. pepo* summer squash and *C. moschata* 'Nigerian Local' (see article in this issue) but they are not yet in a form that will be directly useful to breeders. Two groups have been working on identifying molecular markers linked to ZYMV resistance from 'Nigerian Local' introgressed into *C. pepo* (2, 19) but no markers have been published.

A number of *Cucurbita* genes have been characterized at the molecular level and cloned. However, this work has been done entirely by molecular geneticists interested in the control and functioning of pathways common to many plants. Thus the genes cloned have been of little direct use to squash breeders. Researchers in the laboratory of G. A. Thompson at the University of Arizona have studied and cloned the genes encoding important proteins in the phloem transport system of *C. maxima* (3, 6, 18). Other recently cloned genes in *Cucurbita* include a calcium-dependent protein kinase from zucchini (7), a class-3 chitinase (16), a glyoxysomal malate dehydrogenase (12), and an anionic peroxidase (5). Cloning of these types of pathway-controlling genes from *Cucurbita* has been greatly facilitated by the availability of probes and sequence information from other species where genes with the same function have already been cloned.

It is apparent from the literature that molecular genetics is just beginning in *Cucurbita*. As the genome is mapped, it should be possible to identify markers for many useful traits. Markers could be particularly useful for tagging the complementary virus-resistance genes, such as *Zym-2* and *Zym-3* (21) and ensuring that they are transferred during backcrossing. Molecular maps will also allow further investigation into the evolution and species relationships in *Cucurbita*, and between *Cucurbita* and other cucurbits.

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