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Identification of Two Linkage Groups in *Cucurbita palmata* Using Alien Additions Lines

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Abstract. **Isozyme phenotypes were used to identify two (P2 and P3) of the six monosomic alien addition lines that have been isolated from a *Cucurbita moschata* x *C. palmata* hybrid. Phenotype P2 displayed the *C. palmata* fumarase isozyme, whereas P3 exhibited two glucose phosphate isomerases and an aspartate aminotransferase derived from *C. palmata*. P2 also possessed the hard rind trait characteristic of *C. palmata*. Both the biochemical and the morphological phenotypes were inherited in a non-Mendelian fashion, and no recombination was observed within either the P2 or P3 set of characters. It was concluded that the loci coding fumarase and hard rind were situated on the alien chromosome in P2 trisomics and that the other three loci were on a 2nd *C. palmata* chromosome possessed by the P3 line. The loci responsible for other *C. palmata* isozymes either were not expressed or were not located on any of the five *C. palmata* chromosomes represented in the alien addition lines.**

Monosomic alien addition lines contain a normal diploid genome of one species plus an extra "trisomic" chromosome from a related species. These lines can represent a useful intermediary step in interspecific gene transfer and can be used as a method to overcome introgression barriers. The introduction of rust resistance into wheat (20), nematode resistance into beets (14), and the transfer of oat stem rust resistance to cultivated oats (5) have been accomplished by this approach. This technique may prove useful in squash breeding, where sterility and a lack of chromosome homology in the interspecific hybrid have been obstacles to the transfer of the disease resistance, drought tolerance, and perennial habit of *Cucurbita palmata* to 'Butternut' squash, *C. moschata* (2).

Bemis (3) first generated a *Cucurbita* alien addition line by initially crossing the amphidiploid hybrid of *Cucurbita moschata* (Duch. ex Lam.) Duch. ex Poir. ($2n = 40$) x *C. palmata* S. Wats. ($2n = 40$) with *C. moschata* to produce an autoallotriploid. Another backcross to *C. moschata* resulted in segregation for aneuploids, including trisomics with 40 chromosomes from *C. moschata* and one from *C. palmata*. At least six different, fertile trisomic lines were recovered in this manner. These were classified into six phenotypes, P1 to P6 (8, 9). It could not be determined if each phenotypic group contained more than one trisomic genotype, because the chromosomes of *C. palmata* are small and morphologically indistinguishable. Inheritance of the alien chromosome was found to occur only through the female germ line, with transmission rates of 5–40% when selfed or when pollen from disomic plants was used (8). Identification of trisomic plants in a segregating progeny proved to be an onerous task; chromosome counts were tedious due to the small size and large number of chromosomes, and morphological differences between a trisomic plant and the normal diploid were minor or expressed only in the fruit.

Isozyme phenotypes can be used to identify trisomic plants, either through gene dosage effects (6, 16, 22) or by the presence of one or more novel activity bands (18). This approach has been particularly useful in mapping studies because entire chromosomes can be tested for the presence or absence of a locus. The technique fails only if expression of the locus under investigation is suppressed by dosage compensation or properties of the foreign cytoplasm. Such cases appear to be rare (4), and the approach has been successfully applied in wheat and its relatives (1, 10), maize (18), barley (1, 17), tomato (7), and other plant species (12). By testing for the expression of *C. palmata* loci in the alien addition lines, we hoped to find biochemical markers that could be conveniently used to distinguish the trisomic plants and to identify linkage groups corresponding to specific chromosomes in *C. palmata*.

Seeds for all six trisomic types were provided by W.P. Bemis. Plants, including *C. moschata* and *C. palmata* controls, were grown to maturity in the greenhouse and field plots at Geneva, N.Y. Each plant was tested for isozyme phenotype and screened for morphological features characterizing the different trisomic types (9). The P2 and P3 trisomics identified were self-pollinated and the resulting progenies surveyed for isozyme phenotypes. Young leaf tissue (about 2 cm^2) was extracted, and electrophoresis was performed as described previously (11). After electrophoresis, the gels were cut horizontally into five slices. The top slice was discarded and each of the remaining slices was immersed into 25 ml of appropriate assay mix. Slices from the Tris/borate gel were stained for leucine aminopeptidase (LAP),

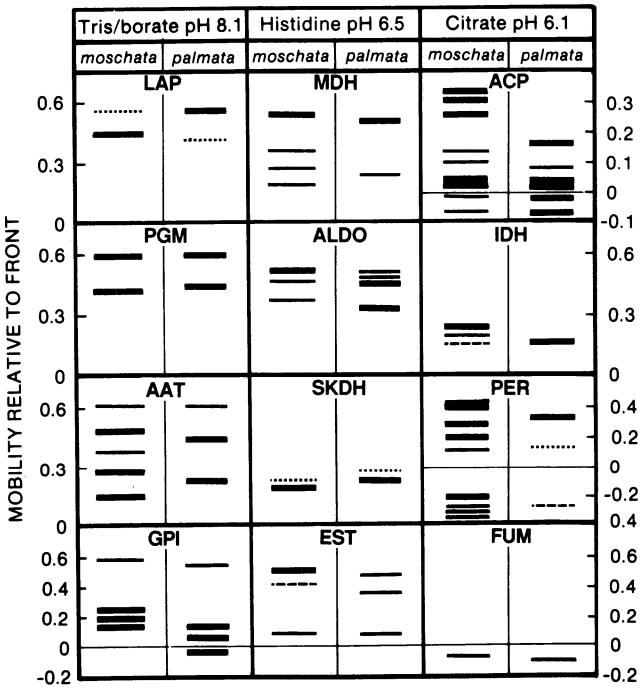

Fig. 1. Diagrammatic representation of isozyme phenotypes observed in diploid *C. moschata* and *C. palmata* parental lines. Anode is toward top of figure in all cases.

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Table 1. Summary of number of plants screened, number of trisomic plants identified, and number of trisomic plants exhibiting isozyme bands apparently derived from *C. palmata*.

Trisomic group of parent	No. plants examined	No. trisomics found	No. trisomics exhibiting *C. palmata* isozymes
P1	113	21	0
P2	13	4	4
P3	30	5	5
P4	25	2	1[z]
P5	21	0	0
P6	69	2	0

[z]Exhibited group P3 isozyme phenotype.

aspartate aminotransferase (AAT), glucose phosphate isomerase (GPI), and phosphoglucomutase (PGM). Slices from the histidine gel were stained for shikimic dehydrogenase (SKDH), esterase (EST), malate dehydrogenase (MDH), and aldolase (ALDO). Slices from the pH 6.1 gel were assayed for fumarase (FUM), peroxidase (PER), isocitrate dehydrogenase (IDH), and acid phosphatase (ACP). Assays were performed using standard recipes (21, 23).

Repeatable differences were observed in the isozyme phenotypes of the two parental species in each of the 12 systems reported (Fig. 1). In a number of the systems studied (FUM, GPI, SKDH, EST, LAP, and PGM) the same number of bands were observed for each species after electrophoresis of extracts, but the relative position of at least one of the bands differed. In the other isozyme systems, the zymograms of the two species differed not only in position but also in number of bands, Estimation of the number of loci involved was difficult in many systems. Based on previous genetic investigations on *Cucurbita* isozymes (13, 24, 26, 27), at least one locus was responsible for the observed phenotypic difference for LAP, two for MDH, one for ACP, one for PGM, one for ALDO, two for AAT, one for SKDH, one for PER, two for GPI, one for EST, and one for FUM. In total, at least 14 isozyme loci differed between *C. moschata* and *C. palmata* and could be used as genetic markers in our study.

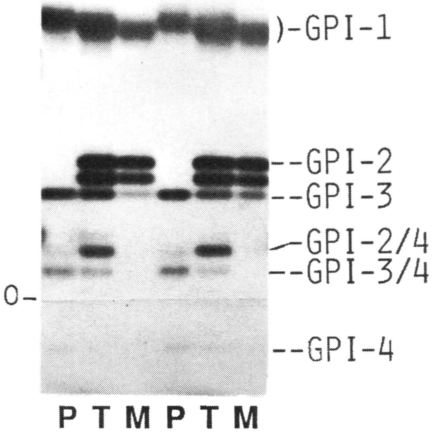

Fig. 2. Glucose phosphate isomerase isozyme phenotypes for *C. palmata* (P), *C. moschata* (M), and the P3 trisomic (T). The homodimeric forms are marked as GPI-1, GPI-2, GPI-3, and the cathodally migrating GPI-4. Two intergenic heterodimers are noted as GPI-2/4 and GPI-3/4. A third intergenic heterodimer between GPI-2 and GPI-3 is not labeled. The position of the origin is designated by the letter 'o'. The anode is toward top of photograph.

The number of plants examined and the number of trisomic plants found for each type is given in Table 1. In the P1 progeny, all of the 21 plants identified as trisomics exhibited an isozyme phenotype identical to that of *C. moschata*. A broad band of fumarase activity for P2 trisomics overlapped both the fumarase isozyme from *C. palmata* as well as that of *C. moschata*. Thus, the phenotype corresponded to that expected for a hybrid individual containing fumarase genes from both species. Every plant with this "hybrid" phenotype also was identified as a group P2 trisomic using morphological criteria, including the lignified, hard fruit rind. This trait is derived from *C. palmata* and is dominant to the soft rind of *C. moschata*. Hard rind is governed by a single dominant gene in *C. pepo* (15, 19) and *C. andreana* (28). It is possible that a single dominant gene also controls the hard rind character in the P2 trisomics and that this gene is on the same chromosome as the locus specifying the FUM isozyme.

A different isozyme phenotype was observed in group P3 trisomics. Each plant identified as a P3 trisomic by morphological characters possessed unique AAT and GPI phenotypes. An activity band corresponding to the slowest migrating AAT isozyme in the *C. palmata* phenotype was present in P3 extracts. An additional activity band with a mobility intermediate between the *C. palmata* isozyme and the slowest migrating *C. moschata* isozyme was also present. This latter band was apparently the intergenic hybrid dimer formed between the subunits of the slowest migrating isozyme from the respective species. The GPI phenotype (Fig. 2) showed even more differences from the *C. moschata* pattern. The *C. palmata* GPI-1 isozyme was evident as a broadening of the most anodal zone of activity. Two other activity bands from the *C. palmata* GPI phenotype were also present in P3 extracts, the cathodal GPI-4 and the most slowly migrating anodal band (GPI-3/4). As was the case for the AAT phenotype, P3 also exhibited a unique GPI band (marked GPI-2/4 in Fig. 2). This band most likely represented the dimeric combination of GPI-2 and GPI-4 subunits. Although the *C. palmata* GPI-1 and GPI-4 isozymes were clearly expressed in the P3 trisomic, we could not conclusively determine if the *C. palmata* GPI-3 homodimer was also present. Its position would overlap that of GPI-3 from *C. moschata*, and thus its presence would not produce a unique band in the trisomic phenotype. The significantly greater intensity of the GPI-2/4 band relative to the GPI-3 and GPI-3/4 bands in the trisomic (Fig. 2) indicates that the *C. palmata* GPI-3 is not being synthesized; however, further linkage analysis of the genes coding the several GPI isozymes will be necessary to verify this interpretation.

Two plants from different P4 trisomic parents were identified as trisomics using morphological characters. One of these plants displayed the same AAT/GPI variant phenotype as was seen in the group P3 trisomics. The other gave a normal *moschata* phenotype. These results suggest that the original group of P4 trisomics contained at least one P3 trisomic. The misclassification probably was due to difficulty in distinguishing between trisomic lines using morphological features. Further support for this interpretation was obtained from the trisomic parent with the P3 AAT/GPI phenotype had a transmission rate of 41%. This rate was similar to that observed for P3 trisomics (30–40%) but considerably greater than the 21% reported for the other P4 trisomic (8).

Despite screening 21 progeny of a known P5 trisomic, we were unable to identify a plant expressing the appropriate morphological phenotype of a P5 trisomic. The apparent lack of a P5 trisomic plant among the progeny tested prohibits conclusions regarding the isozyme phenotype of this trisomic. We did not observe *moschata* x *palmata* hybrid isozyme phenotypes among the P6 progeny tested.

Self-pollination of the identified P2 and P3 trisomic plants gave progenies segregating for isozyme phenotype. About 10% of the P2 progeny exhibited the hybrid FUM phenotype, while the remaining plants possessed a normal *C. moschata* isozyme phenotype for all enzyme systems examined. Progenies from P3 trisomics gave similar results, with about 20% of the plants tested showing the additional activity bands in the AAT and GPI systems. The distorted segregation ratios, the apparent lack of recombination between isozyme loci, and the absence of a homozygous *C. palmata* phenotype (e.g., a phenotype containing only the *C. palmata* FUM isozyme) in progenies of trisomic plants, indicate that the loci coding the *C. palmata* isozymes are located on the alien chromosome and have not become incorporated into the *C. moschata* genome by chromosomal rearrangements. Therefore, we can define two distinct chromosomal linkage groups in *C. palmata*. The chromosome associated with P2 trisomics contains the locus coding fumarase subunits and possibly a gene homologous to the hard rind, *Hr*, gene in *C. pepo*. The 2nd linkage group, including the loci specifying subunits of at least two GPI isozymes and an AAT isozyme, is on the *C. palmata* chromosome in P3 alien addition lines. A linkage between

genes coding GPI and AAT isozymes also has been observed in *C. pepo* (27), *C. maxima*, and *C. ecuadorensis* (26), suggesting that this linkage group has been conserved within much of the genus.

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Determining the Interspecific Origins of Clones Within the 'Saba' Cooking Banana Complex

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Abstract. **Leaf tissue extracts of *Musa acuminata* Colla diploid subspecies *malaccensis*, diploid *M. balbisiana* Colla, the triploid dessert banana cultivar 'Valery', the interspecific hybrid (*acuminata* × *balbisiana*) cooking banana cultivars 'Chato' and 'Pelipita' and the triploid putative *balbisiana*-derived cultivars 'Saba', 'Saba Puti', and 'Cardaba' were subjected to isozyme analysis for four different enzymes. Isozyme banding patterns of the interspecific hybrids were generally additive and were a composite of the species-specific forms of each enzyme. Banding patterns for shikimate dehydrogenase, malate dehydrogenase, peroxidase, and phosphoglucomutase indicate that 'Saba', 'Saba Puti', and 'Cardaba' are *acuminata* × *balbisiana* hybrids.**

Intraspecific *Musa acuminata* (A genome donor) and interspecific *M. acuminata* × *M. balbisiana* (B genome donor) hybridizations occur frequently (11). *Acuminata* × *acuminata* hybridizations, in conjunction with female restitution, yield triploid dessert-type (AAA) bananas. AB diploids yield plantains (AAB) and cooking or Bluggoe-type bananas (ABB) when backcrossed to *acuminata* or *balbisiana*, respectively (11). In this latter case, the occurrence of female restitution again results in triploid progeny. The genomic constitution of hybrid clones is commonly estimated by examination of dosage effects on species-specific morphological markers (12).

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In contrast to the widespread occurrence of parthenocarpy and triploidy within *M. acuminata*, these characteristics have not been well-documented within *M. balbisiana* despite repeated attempts to do so. Several investigators (1, 2, 10, 13, 15) have suggested that parthenocarpic 'Saba' clones, which originate in the Philippines, are BBB types. The 'Saba' cooking bananas had previously been classified ABB by Simmonds (11). When individual clones within this group are classified for their genomic group on the basis of morphological markers, they appear to be closely related to *M. balbisiana*. In addition, Rivera (10) examined members of this group for peroxidase and polyphenoloxidase polymorphisms and concluded that several 'Saba' clones were BBB.

In this study, we analyzed three members of the 'Saba' group of clones previously classified (10) as triploid *balbisiana* ('Saba', 'Saba Puti', and 'Cardaba') for four species-specific (6) isozyme markers in order to clarify their probable diploid species ancestry.