Segregation Distortion and Linkage of Mango Isozyme Loci

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Abstract. The inheritance of five polymorphic enzyme systems, aconitase (ACO), isocitrate dehydrogenase (IDH), phosphoglucose isomerase (PGI), phosphoglucosmutase (PGM), and triosephosphate isomerase (TPI), was studied in selfed progenies of four mango (*Mangifera indica* L.) cultivars and selections. Only in 'Haden' did the allozymes of all of the studied loci segregate in the expected Mendelian ratios. Distorted segregations were present in the other cultivars at some loci; three of the five analyzed in 'Edward' showed distorted segregations, as did two of three loci in '13/1', and both loci in '21/6'. The distorted ratios in 'Edward', a descendant of 'Haden', did not appear to be associated with gametic selection because pollen viability in both of these cultivars was high. The five enzymic loci were not linked to one another in 'Edward', '13/1', or '21/6'. In 'Haden', however, *Pgi-2* and *Aco* were linked, with a distance of about 19.4 map units.

Recently, Degani et al. (1990, 1992) detected several polymorphic enzyme systems in mango and used them for the systematic characterization and parentage analysis of various cultivars. These isozyme systems enabled the identification of zygotic seedlings in the progeny of polyembryonic cultivars (Degani et al., 1993; Schnell and Knight, 1992; Truscott, 1992).

Polyembryony in mango was first recorded by Schacht in 1859 (Belling, 1908). Seeds of polyembryonic mango trees characteristically contain several nucellar embryos, but may also contain a zygotic embryo. Polyembryonic cultivars are preferred as rootstocks because of the uniformity of their progeny. Zygotic seedlings are generally considered undesirable for this purpose because their performance is unpredictable (Anderson et al., 1991). However, whereas nucellar seedlings are preferred for the propagation of mango rootstocks, the breeder is generally interested in sexual seedlings for the selection of new improved rootstocks.

The genetic control of the polyembryony trait in mango is not yet understood. To study the inheritance of polyembryony in mango, selfed progenies of several monoembryonic and polyembryonic cultivars were produced. Isozyme analysis was used to check the assumption that caging is effective in ensuring selfed progeny, and to identify zygotic seedlings in the progeny of the polyembryonic cultivar. The isozyme data facilitated a study of the genetic control of five isozyme loci in

mango. The usefulness of isozymes as trait markers is enhanced if they are distributed throughout the genome, rather than clustered on a few chromosomes (Tanksley and Rick, 1980). Linkage groups were therefore identified by assessing cosegregation of pairs of loci and the segregation ratios of individual loci concurrently (Lee and Ellstrand, 1987). The present study reports the inheritance patterns and linkage relationships of five isozyme loci in mango.

Materials and Methods

Four mango cultivars and selections were studied: 'Edward', 'Haden', '13/1', and the selection '21/6'. 'Edward' is a monoembryonic cultivar, thought to be a cross between 'Haden' (female) and the polyembryonic 'Carabao' (male) (Campbell, 1992). The role of 'Carabao' as the male parent of 'Edward' has recently been refuted on the basis of isozyme and DNA fingerprint analyses, which, nevertheless, do support 'Haden' as the female parent (Adato et al., 1995; Degani et al., 1990). 'Haden' is a monoembryonic seedling of the monoembryonic 'Mulgoba'; isozyme and DNA fingerprint analyses suggest that the polyembryonic 'Turpentine' may be the pollen parent (Adato et al., 1995; Campbell, 1992). '13/1' is a polyembryonic cultivar (Gazit and Kadman, 1980), and '21/6' is a monoembryonic offspring of '13/1'.

Selfed progeny were obtained by enclosing single trees in cages made of 15-mesh nets (openings of 1.5 × 1.5 mm) at The Hebrew Univ.'s experimental farm in Rehovot, Israel, and at the Besor Experimental Station (West Negev, Israel). A beehive was placed in each cage during flowering. Mature fruit were collected, and seeds were planted at the Volcani Center in 750-mL plastic pots containing peat

moss mixed with polystyrene flakes (1:1, v/v) and a slow-release fertilizer. Seedlings were grown in a greenhouse (18 °C night/28 °C day) and watered daily. Isozyme analysis was used to check the assumption that caging is effective in ensuring selfed progeny (Degani et al., 1992), and to identify zygotic seedlings in the progeny of the polyembryonic cultivar (Degani et al., 1993).

The inheritance of the following polymorphic enzymes was studied: aconitase (ACO; EC 4.2.1.3), isocitrate dehydrogenase (IDH; EC 1.1.1.42), phosphoglucose isomerase (PGI; EC 5.3.1.9), phosphoglucosmutase (PGM; EC 2.7.5.1), and triosephosphate isomerase (TPI; EC 5.3.1.1). Extraction of enzymes from leaves, horizontal starch–gel electrophoretic procedures, and isozyme-staining techniques were according to Degani et al. (1990). The computer program LINKAGE-1 (Suiter et al., 1983) was used to test for single-locus segregation and possible linkage between various loci.

Panicles from mango trees at The Hebrew Univ.'s experimental farm and at the Besor Experimental Station were collected during the morning hours and held in an incubator at 20 °C until pollen was shed. Pollen viability was determined by microscopic examination after dissecting mature anthers in 2% acetocarmine. Regularly shaped, darkly stained pollen grains were considered viable.

Results and Discussion

In the progeny of three mango cultivars that were homozygous for one or more isozyme loci ('Haden' for *Pgm-1*; '21/6' for *Pgi-2*, *Aco*, and *Idh*; '13/1' for *Pgi-2* and *Aco*), no heterozygous offspring were found. This result supports the conclusion that the progenies obtained from the caged trees were, indeed, selfed. In the polyembryonic '13/1', zygotic seedling identification was based on the loci *Idh*, *Pgm-1*, and *Tpi*, for which this cultivar is heterozygous. Seedlings homozygous for any of these loci were identified as zygotic. With these three heterozygous loci there was an 88% probability of detecting the zygotic seedlings (Torres et al., 1982).

When the cultivar was heterozygous for a certain locus, Mendelian segregation was expected among the progeny. In progeny tests of 'Haden', the five polymorphic isozyme loci segregated in typical Mendelian ratios (Table 1). Deviations from the expected segregation ratios were observed for the progenies of the other three cultivars. Three loci, *Tpi*, *Pgm-1*, and *Idh*, differed significantly ($P < 0.05$) from Mendelian ratios in at least two cultivars (Table 1). The progeny of 'Edward' showed a deficiency of homozygous genotypes aa and bb in *Tpi*, and a striking absence of the aa genotype in *Idh*. In *Pgm-1*, segregation was also skewed. In contrast, for *Pgi-2* and *Aco*, segregation patterns were as expected. Deviations at enzymic loci from the expected segregation ratios have been reported for various fruit trees, e.g., *Annona* (Lee and Ellstrand, 1987), *Citrus* (Torres et al., 1985), *Corylus* (Rovira et al., 1993), *Persea* (Degani et al., 1986; Torres et al., 1986), and *Vitis* (Parfitt and Arulsekar,

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Table 1. Single locus goodness-of-fit to the 1:2:1 ratio at five polymorphic isozyme loci in mango progenies.

Cultivar/ line	Locus	No. zygotic seedlings	Parental genotypes	Progeny genotypes	χ^2	P
Edward	<i>Tpi</i>	55	ab	9aa:42ab:4bb	16.20	0.0004
21/6		180	ab	27aa:120ab:33bb	20.40	0.000037
13/1		42	ab	5aa:27ab:10bb	4.62	0.099
Haden	<i>Pgi-2</i>	26	ab	6aa:12ab:8bb	0.46	0.793
Edward		55	ab	9aa:31ab:15bb	2.20	0.478
Haden		26	ab	4aa:14ab:8bb	1.38	0.500
Edward	<i>Pgm-1</i>	55	ac	14aa:37ac:4cc	10.20	0.006
21/6		180	ac	68aa:88ac:24cc	21.60	0.0002
13/1		42	ac	17aa:20ac:5cc	6.95	0.030
Edward	<i>Aco</i>	55	ac	13aa:28ac:14cc	0.05	0.973
Haden		26	ac	9aa:15ac:2cc	4.38	0.111
Edward		55	ac	0aa:38ac:17cc	18.53	0.00009
13/1	<i>Idh</i>	42	ac	2aa:10ac:30cc	48.86	0.00000
Haden		26	ac	4aa:16ac:6cc	1.69	0.429

Table 2. Linkage analysis between pairs of mango isozyme loci.^a

Cultivar/ line	No. zygotic seedlings	Locus pair	χ^2	P	Recombination value $\pm$ SE
Edward	55	<i>Tpi/Pgi-2</i>	4.866	0.301	0.388 $\pm$ 0.06
		<i>Tpi/Aco</i>	2.702	0.608	0.422 $\pm$ 0.06
		<i>Pgi-2/Pgm-1</i>	7.854	0.097	0.309 $\pm$ 0.05
		<i>Pgi-2/Aco</i>	5.050	0.281	0.482 $\pm$ 0.06
		<i>Pgi-2/Idh</i>	2.329	0.675	0.391 $\pm$ 0.06
		<i>Pgm-1/Aco</i>	1.663	0.797	0.476 $\pm$ 0.06
		<i>Aco/Idh</i>	0.055	0.996	0.499 $\pm$ 0.06
		<i>Tpi/Pgm-1</i>	9.554	0.048	0.418 $\pm$ 0.07
13/1	42	<i>Tpi/Idh</i>	4.853	0.302	0.370 $\pm$ 0.07
		<i>Tpi/Pgi-2</i>	7.331	0.119	0.429 $\pm$ 0.09
Haden	26	<i>Tpi/Idh</i>	3.904	0.419	0.499 $\pm$ 0.09
		<i>Tpi/Aco</i>	4.537	0.338	0.419 $\pm$ 0.09
		<i>Pgi-2/Idh</i>	3.375	0.497	0.458 $\pm$ 0.09
		<i>Pgi-2/Aco</i>	15.393	0.004	0.194 $\pm$ 0.06
		<i>Idh/Aco</i>	1.709	0.789	0.499 $\pm$ 0.9

^aOnly those pairs in which at least one or both loci segregated normally were included in the analysis (Bailey, 1961).

1989). Such segregation distortions are often due to chance (Wendel and Parks, 1982). In other cases, they can be caused by several mechanisms, including linkages between isozyme markers and genes that affect gametic or zygotic selection (Degani et al., 1986; Wendel and Parks, 1984; Zamir and Tadmor, 1986), linkages to lethal alleles (Cousineau and Donnelly, 1992; Parfitt and Arulsekhar, 1989), and elimination of the chromosome piece carrying these isozyme markers (Vaillancourt and Slink, 1992).

'Haden' has been reported to be the female parent of 'Edward' (Campbell, 1992). However, in 'Haden', *Pgi-2* and *Aco* are linked (Table 2), whereas this linkage was not detected in 'Edward'. A possible explanation for such a discrepancy between two closely related cultivars is that they differ from one another by a chromosome rearrangement that split *Pgi-2* from *Aco* in 'Edward'. Such a hypothetical rearrangement would have to be quite small because it had no effect on the segregation pattern of either of these two genes (Table 1). Another indication of the minuteness of this presumed rearrangement is reflected in the pollen stainability in 'Haden' and 'Edward'. Normally, chromosomal rearrangements in the heterozygous condition reduce gamete viability by nearly 50% (Sybenga, 1972). Pollen stainability in 'Edward' and 'Haden' was high, with no significant differ-

ences between them (95% and 93%, respectively).

The loci *Tpi* and *Pgm-1* in both 'Edward' and '21/6', and *Idh* in 'Edward' and '13/1', were significantly skewed from the expected Mendelian ratio, possibly due to gametic or zygotic selection (Degani et al., 1986; Wendel and Parks, 1984; Zamir and Tadmor, 1986). However, the pollen viability study revealed high pollen stainability in 'Edward', '21/6', and '13/1' (95%, 89%, and 85%, respectively), making it unlikely that the deviation from the expected Mendelian ratio was due to gametic selection.

The chromosome number in mango is $2n = 40$, and, at meiosis, 20 bivalents are formed, with no univalents or quadrivalents being present. Therefore, mango is believed to be an amphidiploid (Mukherjee, 1950). Genetically, it is expected to behave as a diploid, and the loci are expected to exhibit Mendelian segregation. The observed segregation pattern provides further insight into how heterozygosity has been maintained in these loci. A heterozygous phenotype can result from three genetic circumstances: 1) Different alleles are present at each locus (aa - AA) and, consequently, heterozygosity is permanent, which obviously was not the case, because all the heterozygous loci segregated. 2) Both loci are heterozygous (Aa - Aa), segregating independently in a dihybrid fashion, 1:14:1. This was not the case

either, even with loci exhibiting deviation from the 1:2:1 ratio. The χ^2 values for 1:14:1 segregation were even higher than for the 1:2:1 segregation. 3) Only one locus is heterozygous. This case was supported by the segregation pattern, but we were unable to determine whether the other locus possessed similar alleles or was inactive due to silencing or mutation.

Mango genes are arranged in 20 linkage groups (chromosomes). Recognition of linkage relationships between isozyme loci may help in mango breeding. The five enzyme loci studied here do not appear to be linked to one another in 'Edward' and '13/1'. In 'Haden', however, the loci *Pgi-2* and *Aco* were linked, with a distance of ≈ 19.4 map units between them (Table 2). To the best of our knowledge, this is the first report of a linkage between two loci in mango.

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