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Enzyme Polymorphism in Mango

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Abstract. **Forty-one *(Mangifera indica* L.) cultivars were characterized electrophoretically using the isozyme systems aconitase, isocitrate dehydrogenase, leucine aminopeptidase, phosphoglucose isomerase, phosphoglucomutase, and triosephosphate isomerase. The outcross origin of some of the mango cultivars was supported by the isozymic banding patterns. Reported parentage of some other cultivars was not consistent with their isozymic banding patterns.**

The mango is one of the most popular fruits in the tropics (Lakshminarayana,. 1980). The nearly 14 million tonnes of mango world production in 1984 (FAO, 1984) indicates that this crop is the fifth most important fruit crop after grape, *Muss, Citrus,* and apples.

There are hundreds of mango cultivars, of which only some 25 to 40 are of commercial importance (Chadha and Pal, 1986). Commercially grown cultivars have been identified on the basis of leaf, panicle, fruit, and stone characteristics; however, these characters may change with environmental conditions (Lakshminarayana, 1980). Furthermore, the actual identity of some cultivars is still in question, because similar cultivars grown in different areas often have various names (Lakshminarayana, 1980). Having effective means of cultivar identification and verification, therefore, is important. Reliable gene markers should be of great value in this regard.

In recent years, enzyme polymorphism has been used successfully to identify cultivars in various fruit species, including avocado (Goldring et al., 1985), apple (Weeden and Lamb, 1985), loquat (Degani and Blumenfeld, 1986), cherimoya (Ellstrand and Lee, 1987), and pineapple (DeWald et al., 1988). Enzyme polymorphism has also been used to distinguish hybrids from selfs (Degani and Gazit, 1984; Degani et al., 1989; Goldring et al., 1987; Torres and Bergh, 1978; Vrecenar-Gadus and Ellstrand, 1985) and zygotic from nucellar seedlings in citrus (Roose and Traugh, 1988; Torres et al., 1982). Isozymes, as genetic markers, have been proven to be reliable, consistent, and essentially unaffected by environmental conditions (Bailey, 1983; Torres and Bergh, 1980). However, isozymes can be affected by stage of development and tissue used for extraction (Feret and Bergmann, 1976). Mango leaf isozymes of esterases, aspartate aminotransferase, acid phosphateses, and alkaline phosphatases were used to detect possible genetic variation among individuals of so-called clones (Gan et al., 1981). However, enzyme polymorphism in mango has not been examined systematically.

The objective of the present study was to characterize a wide range of mango cultivars using enzyme polymorphism. The enzyme systems examined included: phosphoglucose isomerase (PGI; EC 5.3.1.9), triosephosphate isomerase (TPI; EC 5.3.1.1), leucine aminopeptidase (LAP; EC 3.4.11.1), NADP-specific isocitrate dehydrogenase (IDH; EC 1.1.1.42), phosphoglucomutase (PGM; EC 2.7.5.1), and aconitase (ACO; EC 4.2.1.3). Starch was selected as the preferred medium for electrophoresis because enzyme extraction and preparation for starch gel electrophoresis are simple and the apparatus required is inexpensive.

Materials and Methods

Leaf samples were taken from the mango collection at the Hebrew Univ., Rehovot, Israel. Forty cultivars of mango were selected from this collection for analyses. 'Mulgoba' (two sources) and 'Carabao' leaf samples were obtained from Miami, Fla.

Electrophoresis was performed using starch gel (Sigma starch hydrolyzed). The composition of the gel and the electrode buffers used are listed in Table 1. Starch gels (12.3%) were poured into molds 0.6 cm deep, 15 cm wide, and 17.5 cm long. For aconitase detection, gels were prepared with the inclusion of 4% sucrose. For isozymic analyses, 6 × 6-mm pieces of leaves were crushed thoroughly in 80 µl of the extraction buffer (Degani and Gazit, 1984). Samples of the extracts were absorbed onto 4 × 6-mm Whatman 3 MM paper wicks and inserted into gels. Wicks dipped in bromophenol blue dye solution were also inserted to visualize the migration of the front. After loading, the gels were placed in a 4C incubator and run at 25 mA for 30 min. The wicks were then removed and electrophoresis was continued at constant current and 300-V limit for a period that depended on the buffer used (Table 1). When the run was complete, gels were sliced horizontally to yield slices 2 mm thick that subsequently were stained by the agar overlay method for aconitase (Soltis et al., 1983), isocitrate dehydrogenase (Soltis et al., 1983), leucine aminopeptidase (Degani et al., 1986), phosphoglucose isomerase (Goldring et al., 1985), phosphoglucomutase (Soltis et al., 1983), and triosephosphate isomerase (Goldring et al., 1987). For each enzyme, electrophoresis was repeated three times, from three leaf samples. R_f values (distances traveled by the bands divided by the disance traveled by the bromophenol blue dye front) were calculated as average migration distance based on the three leaf samples.

Results and Discussion

Differences in banding patterns among cultivars were observed in all the enzyme systems studied. Repeated sampling of the leaf tissue invariably gave the same set of isozyme phenotypes. The isozyme banding patterns in the systems used appear to be independent of the season, since periodic sampling of leaf tissue during the year gave consistent isozyme phenotypes.

Two well-resolved zones of PGI activity were detected. The fast-migrating zone (most anodal) was monomorphic in all cultivars studied and was designated PGI-1. The slow-migrating zone of activity was polymorphic and was designated as PGI-2. Two phenotypes were observed for PGI-2: one possessed the

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Table 1. Buffer composition and electrophoretic conditions used to resolve mango isozymes.

Enzyme resolved	Electrode buffer	Gel buffer	Running conditions[z]
PGI TPI PGM	0.3 M sodium borate, pH 8.2	0.076 M Tris, 0.005 M citric acid, pH 8.7	50 mÅ/4 hr
LAP	0.04 M citric acid adjusted with *N*-(3-aminopropyl) morpholine to pH 6.1	1 Electrode buffer : 19 water	35 mÅ/2.5 hr
IDH	0.04 M citric acid adjusted with *N*-(3-aminopropyl) morpholine to pH 8.0	1 Electrode buffer : 19 water	10 mÅ/19 hr
ACO	0.3 M sodium borate, pH 8.2	0.0114 M Tris, 0.00178 M citric acid, pH 7.6	50 mÅ/3 hr

[z]The periods indicated are those during which the bromophenol blue front had migrated a distance of ≈8 cm from the origin.

fast-migrating band labeled aa, while the other exhibited triple-banded PGI-2 phenotype ab (Figs. 1 and 2). The slow-migrating bb phenotype of PGI-2 was not found among the cultivars studied (Table 2). Weeden and Gottlieb (1979, 1980) have demonstrated that, in some plants, the more anodal form (PGI-1) is the chloroplast-specific enzyme, whereas the PGI-2 set of enzymes are cytosolic in origin.

One zone of enzyme activity was observed when gel slices were assayed for TPI activity. Three TPI phenotypes were easily distinguished in this polymorphic zone: fast-migrating one-banded phenotype aa; slow-migrating one-banded phenotype bb, and three-banded phenotype ab (Fig. 1).

The structures of PGI and TPI have been shown to be dimeric in several plant species (Goldring et al., 1985, 1987; Gottlieb, 1981; Weeden and Lamb, 1985). The observed banding patterns of mango PGI-2 and TPI indicated for each of these enzymes a dimeric structure coded by two alleles a and b.

For IDH, a single zone of enzyme activity was observed, consisting of one-banded phenotypes (aa, bb, or cc) and three-banded phenotypes (ac and ab) (Figs. 1 and 2). The one-banded phenotype, bb, was exhibited only by 'Yotvata 191'. Apparently, IDH mango isozymes are specified by one gene having three alelles a, b, and c.

With PGM, clear and consistent bands appeared in the fastest-migrating zone of enzyme activity, i.e., PGM-1. PGM-1 was polymorphic and exhibited three single-banded and three double-banded patterns. The single-banded phenotypes were designated aa, bb, and cc, and the double-banded phenotypes ab, ac, and bc (Figs. 1 and 2).

Electrophoresis of the LAP enzymes exhibited two zones of activity, LAP-1 and LAP-2. LAP-1 was polymorphic, showing either single- or double-banded patterns (Figs. 1 and 2). All cultivars studied had the aa phenotype, except for two that had the ab phenotype; none had the bb phenotype (Table 2). The LAP-2 zone consisted of two invariant bands of activity in all cultivars studied.

LAP and PGM are known to be monomeric enzymes (Goodman and Stuber, 1983; Gottlieb, 1981; Torres et al., 1978), and, in the present study, LAP-1 and PGM-1 behaved electrophoretically as such.

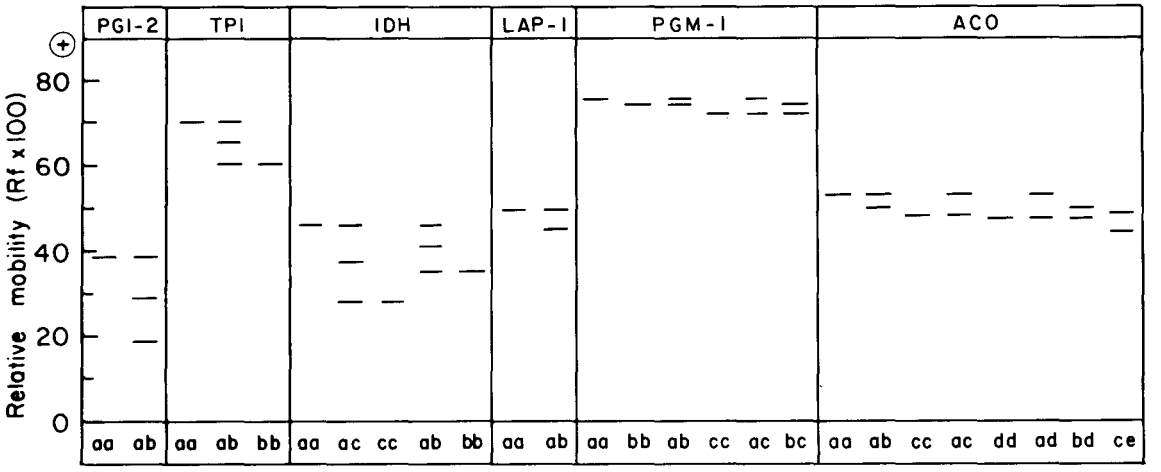

Fig. 1. Schematic zymograms of representative phenotypes for PGI, TPI, IDH, LAP, PGM, and ACO isozymes in mango. Relative mobility ($R_f \times 100$) on the left; O = origin. All enzymes migrated anodally (toward the top of the figure).

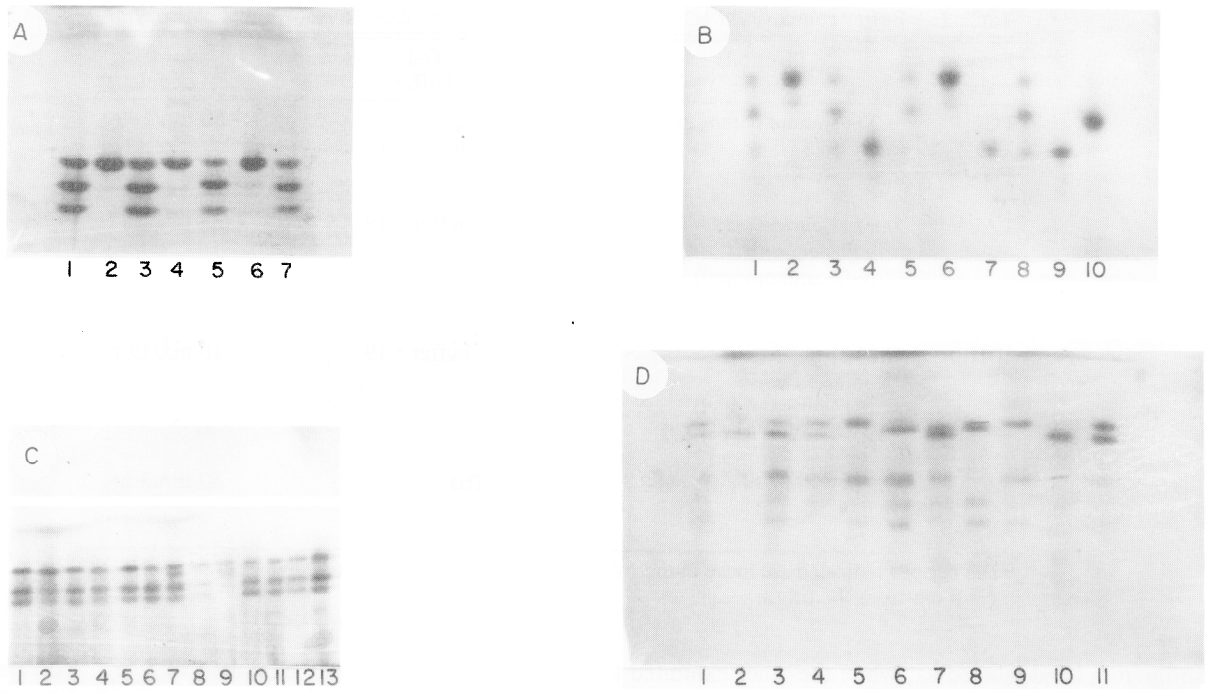

Fig. 2. Variation in **(A)** PGI, **(B)** IDH, **(C),** LAP, and **(D)** PGM isozymes in mango leaf extracts. The sequence of cultivars for PGI is: 1) 'Haden', 2) 'Pairi', 3) 'Palmer', 4) 'Warburg', 5) 'Kent', 6) '13-1', 7) 'Edward'. The sequence for the IDH gel is: 1) 'Haden', 2) 'Carabao', 3) 'Gumera', 4) 'Keitt', 5) 'Turpentine', 6) 'Pico', 7) 'Maya', 8) 'Jubilee', 9) 'Irwin', 10) 'Yotvata 191'. The sequence for the LAP gel is: 1) 'Turpentine', 2) '13-1', 3) 'Sabre', 4) 'Gumera', 5) 'Ein-Yahav 4', 6) 'Ein-Yahav 5', 7) 'Yotvata 8', 8) 'Yotvata 142', 9) 'Yotvata 191', 10) 'Warburg', 11) 'Carabao', 12) 'Pico', 13) 'Yasmin'. The sequence for the PGM gel is: 1) 'Nimrod', 2) 'Brindibani', 3) 'Tommy Atkins', 4) 'Edward', 5) 'Haden', 6) 'Carabao', 7) 'Yotvata 191', 8) 'Yotvata 8', 9) 'Irwin', 10) 'Pairi', 11) 'Tahar'.

One set of aconitase isozymes was detected in mango leaf extracts (Fig. 1). Three homozygous phenotypes were observed: aa, cc, and dd, in order of decreasing relative mobility. The dd phenotype exhibited a mobility (R_f = 0.48) only slightly slower than-the cc phenotype (R_f = 0.49), and could be mistaken for the latter if standards had not been run on the same gel. Five heterozygous phenotypes of aconitase were detected; i.e., ab, ac, ad, bd, and ce. The bb phenotype, with slightly faster mobility (R_f = 0.51) than the cc phenotype (R_f = 0.49), was not detected among the cultivars studied.

At the Ein-Yahav selection plot of mango seedlings tested for tolerance to calcareous soils, the cultivars Ein-Yahav 4 and Ein-Yahav 5 were selected. These cultivars are morphologically indistinguishable, consistent with their recorded origin from the polyembryonic 'l-7' mother-tree. Indeed, when examined by isozyme analysis, 'Ein-Yahav 4', 'Ein-Yahav 5', and '1-7' exhibited identical banding patterns in all enzyme systems tested.

'Pico' and 'Carabao' are morphologically very similar. However, they can be easily distinguished on the basis of their different banding patterns at PGM-1—'Pico' possessing the cc phenotype and 'Carabao' the bb phenotype.

The accepted hybrid origin of some mango cultivars can be supported or refuted on the basis of their isozyme phenotypes. 'Edward' was reported to be a cross between 'Haden' and 'Carabao' (Lynch and Krome, 1951; Ruehle and Ledin, 1960); however, isozyme evidence contradicts the commonly accepted origin of 'Edward' from 'Haden' and 'Carabao' (Table 2). At PGM-1, 'Haden' is aa and 'Carabao' is bb; hence, such a combination could not account for the 'Edward' ac phenotype (Fig. 2). To validate this conclusion, we carried out isozymic analysis of 'Carabao' leaf samples obtained from Florida. We found that the isozymic banding patterns of 'Carabao' from Florida were identical to those of 'Carabao' from Israel (Table 2), thereby excluding the possibility of 'Carabao' being a parent of 'Edward'.

'Keitt' is assumed to be a seedling of 'Mulgoba' (Lynch and Krome, 1951); however, the banding patterns at IDH, where 'Mulgoba' possesses the ab phenotype and 'Keitt' the cc phenotype, refute the hybrid origin of 'Keitt' from 'Mulgoba'.

'Givatayim' was believed to be an offspring of the polyembryonic cultivar 13-1. Comparison of the aconitase banding pattern of these two cultivars (Table 1) revealed that, while 'Givatayim' exhibited the homozygous phenotype aa, '13-1' exhibited the homozygous phenotype cc. This result demonstrated that 'Givatayim' could not have originated from '13- 1'.

'Tahar' is considered to be an offspring of 'Irwin' (Slor and Gazit, 1982). Comparison of the isozymic banding patterns of these two cultivars shows that, while 'Irwin' exhibits the homozygous phenotype at IDH (cc) and at PGM-1 (aa), 'Tahar' exhibits the heterozygous phenotype (at) in both systems. This confirms that 'Tahar' originated from 'Irwin', probably by cross-pollination.

The cultivars 20-1 and 20-26 are offspring of 'Kent' (E. Tomer and S. G., unpublished results). Comparison of the TPI banding pattern of 'Kent' (bb) with that of '20-1' (ah), and the IDH banding pattern of 'Kent' (cc) with that of '20-26' (at) (Table 2), confirms that '20-1' and '20-26' are progeny of 'Kent', resulting from outcrossing.

'Haden' is known to be a seedling of 'Mulgoba', and 'Zill' to be a seedling of 'Haden' (Fairchild, 1951; Lynch and Krome, 1951). The isozymic banding patterns of 'Haden' and 'Zill' (Table 2) support their origin from 'Mulgoba' and and 'Haden', respectively, by cross-pollination.

Table 2. Isozyme phenotype for six polymorphic isozymes in 41 mango cultivars.

Cultivar	Isozyme					
	PGI-2	TPI	IDH	LAP-1	PGM-1	ACO
	Monoembryonic cultivars					
Brindibani	aa	bb	cc	aa	cc	ac
Edward	ab	ab	ac	aa	ac	ac
Gailour	aa	bb	ac	aa	cc	ac
Haden	ab	ab	ac	aa	aa	ac
Irwin	ab	ab	cc	aa	aa	cc
Jubilee	ab	aa	ac	aa	ab	ac
Keitt	ab	ab	cc	aa	ac	ad
Kent	ab	bb	cc	aa	ac	ac
Lili	ab	ab	cc	aa	cc	ac
Maya	ab	aa	cc	aa	ac	cc
Mulgoba 1[z]	aa	bb	ab	aa	ab	ac
Mulgoba 2[y]	aa	bb	ab	aa	ab	ac
Nimrod	aa	bb	cc	aa	ac	ce
Pairi	aa	bb	cc	aa	cc	ac
Palmer	ab	ab	cc	aa	ab	cc
Tahar	ab	ab	ac	aa	ac	cc
Tommy Atkins	ab	bb	cc	aa	ac	ad
Van-Dyke	ab	ab	cc	aa	ac	ac
Zill	aa	bb	ac	aa	ac	cc
Zillate	ab	bb	cc	aa	cc	dd
1-5	ab	ab	cc	aa	ac	cc
6-6	aa	aa	cc	aa	aa	cc
16-36	ab	ab	cc	aa	aa	cc
20-1	aa	ab	cc	aa	cc	cc
20-26	ab	bb	ac	aa	ac	cc
	Polyembryonic cultivars					
Carabao	aa	bb	aa	aa	bb	ce
Carabao 1[x]	aa	bb	aa	aa	bb	ce
Carabao 2[w]	aa	bb	aa	aa	bb	ce
Ein-Yahav 4	ab	ab	ac	aa	ac	ab
Ein-Yahav 5	ab	ab	ac	aa	ac	ab
Givatayim	ab	bb	ac	aa	ac	aa
Gumera 1	ab	ab	ac	aa	aa	cc
Pico	aa	bb	aa	aa	cc	ce
Sabre	aa	bb	cc	aa	ac	bd
Turpentine Miami	ab	ab	ac	aa	ac	ab
Turpentine Zill	ab	ab	ac	aa	ac	ab
Warburg	aa	bb	ac	aa	cc	ce
Yasmin	aa	ab	ac	aa	ac	cc
Yotvata 8	aa	bb	ac	ab	ab	ac
Yotvata 142	aa	bb	ac	aa	cc	ce
Yotvata 191	aa	bb	bb	ab	bc	ac
1-7	ab	ab	ac	aa	ac	ab
4-9	ab	ab	ac	aa	ac	ab
13-1	aa	ab	ac	aa	ac	cc

[z]Mulgoba 1 from USDA M4502 (PI 159045; N-3-1-3-3). M = Miami; PI = Plant introduction number; N = Number of tree.
[y]Mulgoba 2 from Fairchild Tropical Garden.
[x]Carabao 1 from USDA M 4336 (PI 024927; N-2-1-9-1).
[w]Carabao 2 from USDA M 4502 (PI 80856; N-3-1-3-7).

The use of isozymes as genetic markers should be of great value in mango breeding programs where both parents are known. Isozymes are advantageous because they are expressed in young tissue as co-dominants, permitting early selections between desirable individuals (selfed or crossed progeny). In Israel, isozymes are now used routinely for this purpose in the ongoing avocado breeding program. Mango isozymic analysis could be of further use in distinguishing nucellar from zygotic seedlings, as has been done successfully in citrus.

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