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ARE POLYEMBRYONIC MANGOS DEPENDABLE SOURCES OF NUCELLAR SEEDLINGS FOR ROOTSTOCKS?

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Abstract. **Five isozyme systems were used to detect zygotic seedlings in five polyembryonic cultivars of mango. Significant differences were found between varieties (chi-square = 35.53, $p < .001$) for the number of zygotic and nucellar seedlings detected. The range in the number of offtypes was from 0 in '13-1' to 64% in 'Golek'. The frequency in 'Sabre' was 4% and in 'Turpentine' and 'Madoe' was 24% and 36%, respectively. Three of eight rootstock mother trees of 'Turpentine' were determined to be offtypes.**

Polyembryony is common in mango cultivars derived from southeast Asian ancestry (Singh, 1960). Polyembryonic cultivars are propagated from seed and generally come true to type (Knight, 1970). Mango rootstocks are commercially propagated from open-pollinated seed of polyembryonic cultivars. Progeny arising from the open-pollinated seed usually produce uniform populations of nucellar seedlings that are advantageous for rootstock production.

Most polyembryonic cultivars occasionally produce morphologically "offtype" plants which presumably are zygotic in origin. These zygotic seedlings are undesirable as rootstocks because of their unknown effect on the scion. The frequency of offtypes varies among cultivars. The primary rootstock used for commercial plantings in Israel, '13-1', produced between 10 and 15% morphologically offtype plants under commercial nursery conditions (S. Gazit, personal communication).

Isozymes have been used as biochemical markers to distinguish zygotic from nucellar seedlings in citrus (Moore and Castle, 1988). Isozymes have advantages over other detection methods because they are codominantly inherited and free from environmental effects. Furthermore, the analysis is nondestructive, and the assay is simple.

Gazit and Knight (1989) used one enzyme system, glucose-6-phosphate isomerase (GPI), and gas chromatography to detect zygotic plants among open-pollinated seedling populations from polyembryonic mango cultivars. Gas chromatograms were too cumbersome for large numbers to be analyzed; the isozyme system proved to be simple, reliable, and cost effective.

Several enzyme systems have been used in systematic characterization of a diverse array of mango cultivars. Degani et al. (1990) found polymorphisms for six enzyme systems. Genetic inferences were made from gel isozyme patterns with a total of 6 loci and 17 allelomorphs identified.

The use of a single enzyme system would result in offtype plants being missed if the zygotic plant had the same allelic configuration as the maternal parent. The use of other enzyme systems in addition to GPI should enhance the detection of zygotic seedlings among rootstock populations derived from polyembryonic cultivars. This investigation was initiated to determine the frequency of zygotic plants among open-pollinated seedling populations of five polyembryonic rootstock populations using five enzyme systems.

During the 1989 mango fruiting season (June-August), 25 seeds were collected from each of the following rootstock varieties: '13-1', 'Madoe', 'Sabre', 'Golek', and 'Turpentine'. These plants are part of the National Clonal Germplasm Repository for Subtropical, Tropical Fruit, Sugar, and Beverage Crops located at Miami, Florida (NCGR-Miami). Seed were collected from a single tree for all varieties except 'Turpentine'. Seed for the 'Turpentine' rootstock was collected from eight different mother trees and bulked. Seed were germinated in flats and transferred to single pots after seedling emergence. Commercial nursery and NCGR-Miami standard practice involves removing all but the most vigorous seedling germinating from a single seed. The rootstocks studied are part of a scion/rootstock interaction study where effect of rootstocks on commercial production is being investigated. We were not interested in the total number of nucellar vs. zygotic seedlings produced from each seed. Zygotic embryos that give rise to the most vigorous shoots from a single seed are ultimately used as rootstocks in commercial groves. It is important to know if the rootstock is zygotic or nucellar in origin because the rootstock may affect fruit yield and tree vigor.

To estimate the frequency of zygotics occurring within the most vigorous seedlings of five rootstock seedling populations, five isozyme systems previously described by Degani et al. (1990) were used. The material for isozyme analysis was collected in December, 1989 from immature leaf tissue of each of the 125 seedlings. After collection, 400 mg sections were cut from lamella tissue and ground in a chilled mortar in 2 ml of crushing buffer (Degani et al., 1990). The ground tissue and buffer were collected and centrifuged at 3,000 × g for 10 minutes. Wicks (Whatman 3 mm chromatography paper 3 mm × 6 mm) were dipped into the supernatant and stored at –70 C.

Starch gels were prepared by mixing 11% (w/v) of hydrolyzed starch, (Connaught Laboratories Ltd, Ontario, Canada), with gel buffer following the procedure of Marty et al. (1984). The starch solution was poured into a Plexiglas gel form (18.5 × 16.2 × 0.6 cm), allowed to cool, and placed into a chromatography chamber at 4 C. Wicks were placed into the gels no more than ten minutes prior to use. Electrophoresis was conducted using the buffer systems and procedures of Degani et al. (1990) and was continued for the recommended time or, if the system formed a front, until the front had migrated 8 to 10 cm. Gels were usually cut into three slices and stained for three different enzymes. The gels were trimmed to remove the portion anodal to the front. Stains were prepared according to protocol as described by Soltis et al. (1983) for the following enzymes: isocitrate dehydrogenase (EC 1.1.1.42) (IDH), leucine aminopeptidase (EC 3.4.11-) (LAP), glucose-6-phosphate isomerase (EC 5.3.1.9) (GPI), phosphoglucomutase (EC

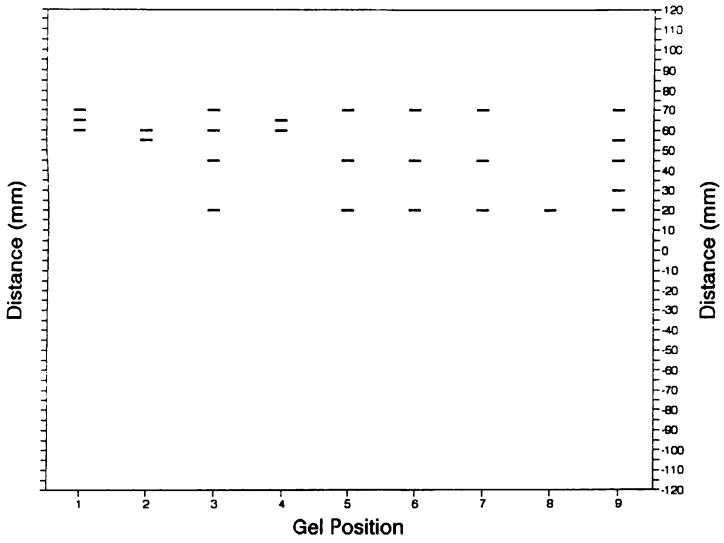

Fig. 1. Isozyme banding patterns for GPI-2 phenotypes observed in rootstock varieties and seedlings: 1) 'Madoe' (AB). 2) 'Madoe' RSP 1 (v). 3) 'Madoe' RSP 11 (w). 4) 'Madoe' RSP 18 (v). 5) '13-1' (AC). 6) 'Turpentine' (AC). 7) 'Turpentine' RSP 2 (AC). 8) 'Turpentine' RSP 9 (CC). 9) 'Golek' RSP 13 (x).

2.7.5.1) (PGM) triosephosphate isomerase (EC 5.3.1.1) (TPI).

Isozyme phenotypes were consistent with previous reports in mango (Degani et al., 1990), with the exception of GPI (Fig. 2). GPI generally has two loci which occur in most diploid plants. One is localized in the chloroplast, the other in the cytosol. The enzyme is dimeric, but only polypeptides with the same subcellular localization associate (Gottlieb, 1977; Weeden and Gottlieb, 1979). In mango, two staining regions are apparent. The fast-migrating zone (most anodal) is labelled GPI-1, and is monomorphic. The slow-migrating zone, labelled GPI-2, is polymorphic and six or more phenotypes were observed: AA (one fast migrating band); AB, the triple banded phenotype that was not seen by Degani et al. (1990) which may indicate another allele at this locus; the AC phenotype analogous to Degani et al.'s (1991) AB; and the CC phenotype. In addition to these patterns, several five-banded, four-banded, and two-banded patterns were seen in single seedlings from 'Golek' and 'Madoe' (Fig. 2). These patterns are not easily explained using a single-locus model. This enzyme may be a two-locus system similar to that found in avocado by Goldring et al. (1985) which would explain these banding patterns. Mango is believed to have an allopolyploid origin (Singh, 1960), therefore duplicated loci may occur.

All other enzyme systems, IDH, LAP, PGM, TPI gave the same banding patterns reported by Degani et al. (1990). Under our conditions, PGM resolved better in Tris/citric acid pH 6.7 buffer than in Poulik buffer. The phenotypes we observed are in agreement with those of Degani et al. (1990) even though the buffer systems differed.

The seedlings from each rootstock were classified as maternal type or offtype based on the isozyme phenotypes (Table 1). A contingency table was calculated and chi-square value estimated (Snedecor and Cochran, 1967) to determine whether observed differences in offtype frequencies were significant. With chi-square = 35.53 (4 df and $P < 0.001$), significant differences existed between cultivars. The range of variation was from 0 offtypes in '13-1' to 16 of 25 in 'Golek' (Table 1). The frequency of offtypes reported for '13.1' in Israel is from 10 to 15%. We did not find offtypes among the 25 seedlings of '13-1'. This may be due to the small sample size or to cross incompatibility with surrounding varieties, or to the relative vigor of '13-1's nucellar vs. its zygotic embryos. The frequency of offtypes in 'Sabre' is also very low, 4%. The frequencies in 'Turpentine' and 'Madoe' were high, 24% and 36%, *respectively*.

The number of heterozygous loci differed among the rootstock varieties. 'Turpentine' and '13-1' are heterozygous at four loci, 'Madoe' is heterozygous at two, and 'Golek' and 'Sabre' heterozygous at one. The probability of detection of self-pollinated individuals was higher in '13-1' and 'Turpentine' seedlings, because of their relatively higher degree of heterozygosity, than in the other three varieties. Among seedlings of '13-1', no outcrossed or self-pollinated individuals were detected. 'Turpentine' produced three plants that resulted from cross-pollination and three that could have resulted from self-pollination. Among offtypes of 'Madoe' all resulted from cross-pollination while among offtypes of 'Golek', ten were from cross-pollination and six could have resulted from selfing. The single offtype seedling detected in 'Sabre' was from cross-pollination (Table 1). The detection of seedlings resulting from self-pollination may be underestimated in 'Golek', 'Madoe' and 'Sabre' due to the low number of heterozygous loci.

Mango is polygamous and some flowers are unisexual (staminate) while others are bisexual, both types being produced in the same panicle. Self- and cross-incompatibilities are known to exist in mango; however, the mechanism and degree of incompatibility are not understood. 'Turpentine' and '13-1' were found to be self-incompatible in pollination studies (Gazit and Knight, 1989); therefore, it is unlikely that any of the seedlings from 'Turpentine' or '13-1' resulted from self-pollination. The fact that offtype seedlings of 'Madoe' could not have resulted from selfing may indicate that this cultivar is also self-incompatible, but information on self- and cross-incompatibilities of 'Madoe', 'Golek', and 'Sabre' does not exist.

'Turpentine' seed that produced the seedlings reported in Table 1 were collected from eight different trees. Isozyme phenotypes of the mother trees revealed three trees that have different patterns than those reported by Degani et al. (1990) (Table 2). 'Turpentine' was carried from this station to Israel in 1980. The distribution record indicates that 200 seeds were collected from M-26463 (local accession number). The isozyme phenotypes recorded by Degani et al. (1990) match our phenotypes for that tree. Four of the trees in Miami were planted in 1932 and were given the same local number, M-30602. Their source is unknown and all offtype 'Turpentine' trees are from this introduction. There is no consistent isozyme banding pattern among the three trees of 'Turpentine' that differ from the common phenotype. We conclude from this evidence that the true 'Turpentine' is the most common pattern observed. The identification of this mixture of isozyme phenotypes of mother trees is an example of how this procedure can be used to help certify rootstock mother trees. The offtype seedlings of 'Turpentine' (Table 1) do not have the isozyme phenotypes of any of the three offtype mother stocks and are therefore considered zygotic.

Clearly, the incidence of offtype rootstocks is important to nurserymen and to the growers who buy grafted trees

Table 1. Maternal parent, number of seedlings, percent offtypes, and isozyme phenotypes of parents and offtype seedlings for five polyembryonic mango cultivars.*

Maternal parent/seedling	Number of seedling	Percent offtypes	Isozyme phenotypes					
			GPI-2	ICDH	LAP	PGM-1	TPI	SOURCE
13-1	25	0	AC	AC	AA	AC	AB	
SABRE	25	4	AA	CC	AA	AB	AA	
SABRE RSP 24						AC		z
TURPENTINE	25	24	AC	AC	AA	AC	AB	
TURP RSP 1			AA	AA	AB		BB	z
TURP RSP 7			AA				AA	y
TURP RSP 8			AA					y
TURP RSP 9			CC	CC			BB	y
TURP RSP 10			AA		AB			z
TURP RSP 11			AA		AB		AA	z
MADOE	25	36	AB	CC	AA	CC	BB	
MADOE RSP 1			v				AB	z
MADOE RSP 2							AB	z
MADOE RSP 3							AB	z
MADOE RSP 4							AB	z
MADOE RSP 5							AB	z
MADOE RSP 10			AC	AC			AB	z
MADOE RSP 11			w	AC			AB	z
MADOE RSP 18			v	AC				z
MADOE RSP 25			AA	AC				z
GOLEK	25	64	AB	AC	AA	BB	AA	
GOLEK RSP 1			AB					y
GOLEK RSP 2			AC			AB		z
GOLEK RSP 4			AA					y
GOLEK RSP 5			AC	CC		AB		z
GOLEK RSP 6			AC					z
GOLEK RSP 7				AA				y
GOLEK RSP 8			x		AB	AB		z
GOLEK RSP 9						AB		z
GOLEK RSP 10						AB		z
GOLEK RSP 11						AB		z
GOLEK RSP 13			x	CC				z
GOLEK RSP 18			x					z
GOLEK RSP 21				CC				y
GOLEK RSP 23				AA				y
GOLEK RSP 24			x					z
GOLEK RSP 25				CC				y

Chi square = 35.53 ($p < .001$) for comparison of frequencies of off-type seedlings between the five polyembronic rootstock varieties.
RSP Rootstock plant number.
v Two-banded phenotype.
w Four-banded phenotype.
x Five-banded phenotype.
y Resulted from self- or cross-pollination.
z Resulted from cross-pollination.
* Phenotypes not designated were identical to parental type.

Table 2. Isozyme phenotypes of 'Turpentine' rootstock mother trees.

Clone	Miami #	Location	GPI	PGM	ICDH	LAP	TPI
TURPENTINE	5158	N2-1-4-6	AC	AC	AC	AA	AB
TURPENTINE	6878	WB3-20-12	AC	AC	AC	AA	AB
TURPENTINE	26463	N2-1-3-2	AC	AC	AC	AA	AB
TURPENTINE	30602	N2-1-3-1	AC	AC	CC[z]	AA	AB
TURPENTINE	30602	N2-1-4-3	AA[z]	AA	CC[z]	AA	AB
TURPENTINE	30602	N2-1-5-8	AC	AC	AC	AA	AB
TURPENTINE	30602	N3-1-7-2	AC	AA[z]	AC	AA	AA[z]
TURPENTINE	34171	N4-1-1-10	AA	AC	AC	AA	AB
TURPENTINE (ISRAEL)			AC	AC	AC	AA	AB

[z]Offtypes.

for orchard planting. Thus, '13-1' and 'Sabre' under south Florida conditions are more dependable sources of uniform rootstocks than 'Turpentine', and much more dependable than 'Madoe' or 'Golek'. Neither '13-1' nor 'Sabre', however, has yet been evaluated for rootstock potential in south Florida. The only mango of the group that has been used commercially here to date is 'Turpentine'; the test currently in progress is expected to indicate whether any of the other four has commercial rootstock potential for Florida.

Significant differences were observed between polyembryonic cultivars for the number of zygotic seedlings produced for rootstocks under our conditions. Isozymes are useful in mother tree identification when phenotypes are known, as has been demonstrated here. The zygotic seedlings identified in this study will be evaluated as rootstocks to see if they affect growth habit and productivity of the scion cultivar.

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THE 'PARVIN' MANGO

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Abstract. **The 'Parvin' mango originated in the 1940's at Bradenton, Florida as a seedling of 'Haden'. It first attracted attention because of its attractive color, firm flesh, and relative freedom from anthracnose disease. The fruit is ovoid with a smooth, regular surface. Fruit weight is 450 to 690 g, with an average weight of about 560 g. The pulp is dark yellow and firm, with a mild, sweet, and pleasant flavor. The fruit is resistant to handling damage, with an unusually long postharvest life. A few commercial growers planted 'Parvin' in the 1950's, but the cultivar never became popular in this state. An important defect of 'Parvin' is the tendency to produce seedless fruits, or "nubbins". The fruit also has internal breakdown at maturity in some years. 'Parvin' was introduced to Puerto Rico long ago and is well regarded there. This cultivar appears to respond well to the initiation of off-season bloom by chemical treatment in tropical climates, suggesting possibilities for production of fruit outside of the normal season.**

Florida has been a center of origin for many mango cultivars over the last 60 years (Lynch and Mustard, 1955; Young and Sauls, 1979). The diverse group of mango cultivars originating in Florida is the result of the importation and mixing of genetic material from many different regions of the world, combined with a vigorous effort of evaluation and testing conducted by both professionals and amateurs within the state. Florida mango cultivars have had a significant impact on world mango production; namely, 'Haden', 'Keitt', 'Tommy Atkins', and 'Van Dyke' which have become commercially successful in Florida and throughout the world. However, there are many other cultivars originating in Florida that have not been commercially successful on a large scale, but may hold commercial promise for the future. The success or failure of Florida mango cultivars in commercial production is largely dependent upon the environmental conditions of a particular region, and the demands of production for an intended market.

The objective of this paper is to describe the 'Parvin' mango, which is not grown extensively in Florida, but has promise as an important cultivar in tropical America.

History

'Parvin' originated from a 'Haden' seed planted in the 1940's at the residence of Mr. Clint Parvin, Bradenton, Florida. The pollinating parent is not known. The fruit first attracted attention because of its attractive color and its relative resistance to anthracnose infection. 'Parvin' received a positive recommendation from the Variety Committee of the Florida Mango Forum in the 1940's. 'Parvin' trees were propagated in the 1950's and some commercial growers in Florida planted this cultivar, but it never became popular in Florida. Trees were taken to Puerto Rico and have met with greater commercial success there.

Description

'Parvin' trees are vigorous and form a rounded, dense canopy. Trees generally begin to produce in 3 to 4 years after planting in the field and are consistent bearers pro-