BARD

FINAL REPORT
PROJECT NO. I-255-80

Asexual Embryogenesisin the Mango
(Mangifera Indica L.)

S. Gazit, R. J. Knight, Jr.

1989

000000115100

31358

31358 0

Standard BARD Cover Page for Scientific Reports

Date: 2.7.1989

-9. -7- 1989

BARD
P.O. BOX 6
Bet Dagan, ISRAEL

BARD project No. I 255-80

Title

Asexual embryogenesis in the mango

Investigators' Names (Principal listed first)	Investigators' Institutions
Shmuel Gazit	R.J. Knight, Jr.
A.R.O Volcani Center	USDA Subtropical Horticulture
Institute of Horticulture	Research Station
Bet Dagan, 50250, Israel	Miami, Fl. 33158, U.S.A.

Project's starting date: 9.1981

Type of Report: 1st Annual _____ 2nd Annual _____ Final X

S. Gazit
Signature
Principal Investigator

Yuval Eshdat
Dr. YUVAL ESHDAT
Head, Div. for Research Projects and Budgets
Agricultural Research Organization
Signature
Institution's Authorizing Official

630.72
BAR/GAZ
2nd copy

; 634.44 ; 581.166.2 ; 574.162 ; 581.33

B. TABLE OF CONTENTS

C. ABSTRACT

The main objectives of the research were: a. A survey of polyembryony in mango; b. to study the effect of self and cross pollination on fruit set and embryo development in polyembryonic mango cvs.

The occurrence of polyembryony was examined in 38 different cvs. In contrast with Horn's results we found a clear-cut difference between monoembryonic and polyembryonic cvs; the emergence of multiple seedlings from one seed was prevalent in polyembryonic cvs, and was absolutely absent in almost all monoembryonic cvs. In all polyembryonic cvs the occurrence of one seedling emerging from a seed was noted. In most cases it occurred in damaged polyembryonic seeds. However, genuine monoembryonic seeds were found in 5 out of 6 polyembryonic cvs which were examined. Ovules from 2 months old fruits were successfully cultured in modified solid Murashige and Skoog media. Precocious germination occurred from ovules of 5 cvs, 2-3 weeks later. No nucellus could be isolated from these ovules. Somatic embryogenesis occurred in nucellus excised from ovules of 5 cvs. Later, embryogenesis was induced in callus that was developed from nucellus taken from 2 polyembryonic cvs.

In almost all hand pollination experiments, initial fruit set (2-4 weeks after pollination) was minuscule. The results from a successful study indicated strongly that 'Turpentine' is self-incompatible, suggested that '13-1' may also be self-incompatible and that there may be a cross-incompatible reaction between '13-1' (female) and 'Turpentine' (male).

'Turpentine' and '13-1' trees were caged (with screen) individually and in pairs, during the flowering season. Results tend to support the conclusions that '13-1' is self-incompatible and cross-incompatible, as a female, with 'Turpentine'.

D. OBJECTIVES OF THE RESEARCH

1. To gather and document reliable data about the phenomenon of polyembryony in a wide range of monoembryonic and polyembryonic mango cultivars.
2. To study the effect of self and cross pollination (with monoembryonic and polyembryonic pollen) on fruit set, and embryo development and survival in polyembryonic cultivars.

E. SCIENTIFIC REPORT

E.1. Polyembryony Rate in Mango Seeds

Introduction

The phenomenon of polyembryony was found in mango more than 100 years ago (2, 15, 16). The occurrence of polyembryony in many mango cvs is well known to professionals working with mango. It has been used as an important descriptor of mango (11, 12, 13, 14, 15, 18). In many tropical countries polyembryonic cvs have been propagated cheaply from seeds. As a rule the seedlings produced come true to type (9, 11, 12, 13, 17). This uniformity of progenies is very advantageous in rootstock production (3, 9, 11, 12, 13). In Israel, polyembryonic rootstocks like 'Sabre', '4-9' and '13-1' are used almost exclusively in commercial propagation of mango (4, 12).

Though the phenomenon of polyembryony is of great importance in breeding and propagation, there is very little documented, detailed information about it. (1, 5, 6, 7, 8, 10, 17). The most extensive study was reported by Horn (5). He examined 7,780 germinating seeds from 200 cvs and reported that many monoembryonic cvs such as 'Alphonse', 'Mulgoa' and 'Sandersha' had a small percentage (1 to 13) of polyembryonic seeds. At the same time a high incidence of monoembryonic seeds was reported in known polyembryonic cvs. The rate was as high as 84.1% for 'Pico' and 56.1% for 'Cambodiana'. Horn's results are at variance with other findings (6, 7, 8, 15), and with data reported by nursery personnel. This scanty, conflicting information convinced us that a thorough study of these phenomena is warranted.

Material and Methods

Mature fruits were harvested, the fibrous bony endocarp separated from the flesh, and the seed then extracted. The papery translucent outer seed covering was removed. Most of the seeds were planted the same day they were extracted. Planting was sometimes delayed up to 3 days. In such a case, the seeds were kept moist in polyethylene bags. The seeds were planted in a well aerated, porous medium. In Israel a mixture of peat moss and "Kalkar" (granulated polystyrene) was used. The seeds were planted in small elongated containers (0.7 l) in a cooled hothouse (temp. fluctuated between 20 and 30 degrees C). Most seeds (95-99%) germinated. In Florida sphagnum moss was used as a medium, and seeds were sown in shallow plastic boxes (flats) prepared for the purpose.

Six weeks after planting the seeds were taken out of the medium. The number of individual seedlings (with one or more branches, and one taproot) was determined (1, 5). Though optimal conditions were provided for germination, we found that not all embryos had germinated. Some became brown and died, while others remained alive. We did not count ungerminated embryos, either dead or alive. Thus, our results fall short of the true number of embryos per seed. We discarded, and did not include in the results, seeds that did not sprout at all.

Results

The number of seedlings sprouting from one seed was determined for 41 different cultivars and selections (19 in Israel and 22 in Florida). Overall 5,245 germinating seeds were examined (4,742 in Israel and 503 in Florida). The results of the work carried out in Israel are presented in Table 1.1.

Table 1.1. Rate of polyembryony in germinating seeds from 6 monoembryonic and 13 polyembryonic mango cultivars in Bet Dagan, Israel*

Cultivar	No. seeds examined		Ave. no. plants per seed		% seeds with one plant	
	1981	1982	1981	1982	1981	1982
Alphonso	200	--	1.00	--	100.0	--
Dasheri	207	--	1.00	--	100.0	--
Haden	207	--	1.00	--	100.0	--
Irwin	221	--	1.00	--	100.0	--
Maya	195	--	1.00	--	100.0	--
Sandersha	203	--	1.00	--	100.0	--
Carabao	201	--	2.05	--	32.0	--
Colombo Kidney	--	19	--	2.20	--	37.0
Gedong	201	--	4.00	--	14.0	--
Mistikaui	180	201	3.99	4.87	12.0	2.0
Peach	200	162	4.83	3.93	3.0	3.0
Ruppin	207	42	1.01	1.00	99.0	100.0
Sabre	204	388	3.54	3.94	14.0	13.0
Turpentine	--	182	--	3.92	--	9.0
Warburg	192	162	2.25	2.39	32.0	25.0
4-9	--	118	--	4.27	--	3.0
8-16	157	--	2.93	--	15.0	--
13-1	148	341	3.28	2.87	7.0	13.0
14-12	204	--	2.81	--	8.0	--

*Seedlings were examined 6 weeks after sowing.

Not one of the 1,233 monoembryonic seeds (from 6 cvs) gave rise to more than one seedling per seed (Table 1.1). 'Ruppin', which had been considered as polyembryonic, was found to be almost 100% monoembryonic: out of 251 seeds tested only one sprouted more than one seedling. In contrast, the remaining 12 polyembryonic cvs performed as expected, producing more than one seedling per seed in the case of most seeds (63% - 98%). The average number of seedlings per seed ranged from 2.05 to 4.87. 'Carabao', 'Colombo Kidney' and 'Warburg' produced less seedlings per seed, compared to the other 9 cvs. The highest number of seedlings per seed encountered was 12 plants, from a single 'Peach' seed. Seeds from 6 cvs were tested during 2 consecutive years. The variation found between years was not pronounced.

The results of the work carried out in Florida are presented in Table 1.2. Only one seedling each emerged from seeds of known monoembryonic selections (HC3S-31, -33, -40, -41). The selection HC3S-56 showed a very weak tendency to produce more than one seedling per seed (Table 1.2). For the 17 polyembryonic cvs the average number of seedlings per seed ranged from 1.1 to 5.7. The highest number of sprouts (13) emerged from a 'Turpentine' seed. Anthracnose destroyed some emerging seedlings; this disease was especially prevalent during the second, more rainy season (Table 1.2). The lower number of seedlings per seed in the second season may be the result of this problem.

Certainly factors other than genotype exert a profound influence on the number of plants that germinate from a single seed, as data for 'Carabao' and M-20222 indicate (Table 1.2). In 1981, seeds of both these cvs gave rise to an average of 3.3 young plants whereas the next year both cvs averaged only 2.3 plants from a single seed. Furthermore there was a

great difference by years in the number of seeds that gave rise to only one plant: in 1981, only 4.3% of the 'Carabao' and 5.5% of the M-20222 seeds yielded a single seedling, whereas in 1982 21.4% of the 'Carabao' and 25.9% of the M-20222 seeds produced only one seedling. On the other hand, 2 different trees of 'Ono' gave fairly similar results in the year 1982, averaging 2.1 and 2.6 plants per seed, respectively, with 21.4 and 23.8% of the seed populations giving rise to one single plant (Table 1.2). Furthermore, the same clone in two different environments performed differently. Note that 'Carabao' in Israel in 1981 (Table 1.1) produced 2.05 plants per seed (vs. 3.3 in Florida) and 32% of its seeds produced only 1 plant (vs. 4.3% in Florida) whereas 'Sabre' in Israel (1983) averaged 3.94 plants per seed, vs. only 2.1 in Florida. 'Turpentine' in Israel in 1982 also averaged more plants per seed (3.92) than the highest reading for Florida that year, 3.8 (Tables 1.1 and 1.2).

The HC3S population, seedlings of HC3 open-pollinated, show segregation for the polyembryonic trait. Anecdotally, HC3 is derived from a cross of 'Haden' (monoembryonic) by 'Carabao' (polyembryonic) made by Edward Simmonds, an early director of the Miami station. 'Haden' traditionally is believed to result from a spontaneous cross of 'Mulgoba', its known seed parent, by 'Turpentine', the common seedling race widely grown in Florida in the late 19th century.

In Florida seedlings were observed in regard to their position from proximal (upper end of the seed) to distal (lower end) locations, and rated for vigor. A few generally were observed to be relatively large (rating 1), many of medium size (rating 2), and some of relatively small size (rating 3). The size of a particular plant appeared directly related to the amount of cotyledonal material that had broken off to form the segment

that fed that plant during the course of its development. In general (for most polyembryonic cvs), the larger, more vigorous seedlings appeared near the upper end of the seed, but results were not conclusive. In most cases seedlings tended to cluster at the upper end of the seed; this apparently was the result of where the nucellar buds appeared during the seed's early development (See E.5, below).

Table 1.2 Rate of polyembryony in germinating seeds from 4 monoembryonic and 17 polyembryonic mangos in Miami, Florida.

Cultivar	No. seeds examined	Ave. no. plants per seed	% seeds with one plant
1981:			
Carabao	23	3.3	4.3
M-20222	18	3.3	5.5
Turpentine	23	5.7	0.0
1982:			
HC3S-31	16	1.0	100.0
HC3S-33	17	1.0	100.0
HC3S-40	24	1.0	100.0
HC3S-41	15	1.0	100.0
Arumanis	27	1.7	44.4
Cambodiana	8	1.1	87.5
Carabao	14	2.3	21.4
Chino	9	2.8	11.1
Golek	6	1.5	50.0
HC3 (Parent)	11	1.5	63.6
HC3S-47	14	2.0	42.8
HC3S-50	20	1.3	80.0
HC3S-51	10	2.0	50.0
HC3S-56	21	1.05	95.2
HC3S-58	19	2.2	21.0
Heart	24	1.9	54.2
M-20222	27	2.3	25.9
Madoe	10	3.0	20.0
Ono (posn 1-1)	21	2.6	23.8
Ono (posn 2-7)	14	2.1	21.4
Sabina	8	2.0	25.0
Sabre	26	2.1	42.3
Turpentines:			
N2-1-7-2	28	2.6	25.0
N3-1-2-6	24	2.7	33.3
N4-1-1-10	26	3.8	11.5

Fig. 2.1. A 13-1 embryo (seedcoat removed).

2. Visual Estimate of Embryo Number

When the seedcoat is removed and the embryo or embryos are exposed it is possible to identify polyembryony (Fig. 2.1). We removed the seedcoat (a tedious chore) from ca. 750 seeds of 6 cvs used for the prior survey. We estimated the number of embryos per seed and then planted these seeds in labelled containers. Results are presented in Table 1.3.

We were surprised to find monoembryonic-like seeds in 5 of the 6 cvs tested. Seeds of 'Peach' only were consistently polyembryonic. In contrast, about 20% of the 'Carabao' seeds appeared monoembryonic (Table 1.3). After germination, we found in most cvs a high positive correlation between the visual estimate of embryo number per seed and the actual number of emerging seedlings. However, the results also show that no absolute accuracy can be achieved by a visual estimate. The clearest discrepancy can be seen in the seeds that we estimated to have only one embryo; of the 52 seeds in that category, 11 were eventually found to be polyembryonic. We checked carefully the 5 'Peach' seeds which were evaluated to be polyembryonic, but produced only one seedling per seed (Table 1.3). In every case, we found dead embryos in these seeds.

Discussion

Planting the seeds in light, well-aerated media provided optimal conditions for germination. The media enabled easy uprooting of the seedlings, thus facilitating reliable (1, 5) determination of their correct number.

Our results (Tables 1.1, 1.2, 1.3) show that, as a rule, there is a clear-cut difference between monoembryonic and polyembryonic mangos: seeds of monoembryonic mangos produced only one seedling plant per seed. This conclusion contradicts Horn's finding (5), but it is supported by the fact that no true multiple seedlings were found when thousands of monoembryonic seeds were planted for breeding purposes, both in Israel and Florida. Seeds of polyembryonic mangos usually produced more than one seedling per seed. However, in all cvs of this type tested, some seeds produced only one seedling. This phenomenon also was found by Horn (5). Our observations indicate that often this is the result of partial damage to a polyembryonic seed. In one of our studies (Table 1.3), 62% of 109 seeds with only one seedling, were identified earlier as polyembryonic. However, the occurrence of some genuine monoembryonic seeds is supported by our results (Table 1.3). This phenomenon was especially common in 'Carabao', where 17% of the seed were found, both visually and actually, to have only one embryo. In 4 other cvs this phenomenon also occurred, but was quite rare (in about 3% of the seeds). Only 'Peach' was found to produce 100% polyembryonic seeds.

The only cv that does not fit into this scheme is 'Ruppin' (HC3S-56 may be similar). 'Ruppin' had been identified as polyembryonic (11, 12). We found only one seed with more than one seedling out of 251 planted.

Table 1.3. The relation between visual estimate of embryos per seed and the actual number of seedlings emerged for 6 polyembryonic mango cultivars. Experiment performed in 1982 at Bet Dagan.

Cultivar	No. of seeds tested	Seeds grouped by visual estimate: No. embryos per seed	No. of seeds	No. of seeds with N seedlings: N=1	2	3	4	5	6	7	8	Correlation coef. est.: actual
Carabao	168	1	33	29	4							
		2	93	25	52	9	6	1				0.99
		3	40	3	20	12	3	2				
		4	2			1	1					
14-12	94	1	7	3	2	2						
		2	29	2	15	11	1					
		3	34	2	12	12	7	1				0.81
		4	23	1	6	11	5					
		5	1					1				
8-16	61	1	4	2		1		1				
		2	14	6	4	2	1	1				
		3	14	1	2	6	4	1				0.27
		4	18		5	3	5	5				
		5	11	1	1	9						
Gedong	112	1	4	3	1							
		2	36	6	12	11	7					
		3	48	1	5	14	10	10	3	3	2	0.74
		4	20			5	4	5	4	2		
		5	4			1	2	1				
Mistikawi	147	1	4	4								
		2	5	4	1							
		3	17	4	8	2	3					
		4	24	2	9	8	5					0.98
		5	35	5	1	8	11	6	1	3		
		6	23		2	7	3	5	4	2		
		7	22		1	5	2	7	2	4	1	
		8	17				3	6	6	1	1	
Peach	149	3	12	1	5	4	2					
		4	44	3	2	6	17	10	2	4		0.90
		5	39	1	3	4	8	8	10	4	1	
		6	28			2	2	4	11	6	3	
		7	16				4	5	3	2	2	
		8	10				1		2	3	4	

Oppenheimer, who observed a lot of 'Ruppin' seedling trees, found that they do not come true to type. It seems that 'Ruppin' is an exception to the rule, a monoembryonic cv that may rarely produce a polyembryonic seed. This conclusion means that 'Ruppin' should not be included anymore in the list of recommended polyembryonic mango rootstocks in Israel.

We found a great variation in the number of embryos and seedlings per seed, in seeds of the same cv (Table 1.3). This variation was noticed also in other studies (7, 15). It is believed that environmental conditions can influence the realization of the polyembryonic potential in Citrus. The same belief appears well conceived in the case of Mangifera.

Monoembryonic seeds of polyembryonic mango cvs are not necessarily zygotic. 'Warburg' can be safely propagated by seeds (11, 12), as almost all seedlings will be true to type. This indicates that the high percentage of single seedlings per seed (Table 1.1) are nucellar. The same argument can be applied to 'Carabao'. Though 32% of its seeds gave rise to only one seedling in Israel (Table 1.1), and 17% of its seeds were probably monoembryonic (Table 1.3), 'Carabao' is known to come true to type (9).

Literature Cited

1. Arndt, C. H. 1935. Notes on polyembryony and multiple shoots from the seed in Mangifera indica. Amer. J. Bot. 22:26-30.
2. Chaudhri, S. A. 1976. Mangifera indica -- mango, p. 403-447 in: Garner, R. J. and Chaudhri, S. A. (Eds.) The propagation of tropical fruit trees. Commonwealth Agric. Bureaux, Slough, England.
3. Belling, J. 1908. Report of the assistant in horticulture, Mango. p.110-125 in: Ann. Rep. Fla. Agric. Exp. Sta. for 1908.
4. Gazit S. and Kadman, A. 1980. 13-1, a new mango rootstock. HortSci. 15:669.

5. Horn, C. L. 1943. The frequency of polyembryony in twenty varieties of mango. Proc. Amer. Soc. Hort. Sci. 42:318-320.

6. Juliano, J. B. and Cuevas, N. L. 1932. Floral morphology of the mango (Mangifera indica Linn.) with special reference to the Pico variety from the Philippines. Philipp. Agriculturist 21:449-472.

7. Juliano, J. B. 1934. Origin of embryos in the Strawberry mango. Philipp. J. Sci. 54:553-561 (+ 3 plates).

8. Juliano, J. B. Embryos of the Carabao mango (Mangifera indica Linn.). Philipp. Agriculturist 25:749-760.

9. Knight, R. J. 1970. Polyembryonic mangos: their unrealized potential. Proc. Trop. Reg. Amer. Soc. Hort. Sci. 14:145-155.

10. Maheshwari, P. and Ranga-Swamy, N. S. 1958. Polyembryony and in vitro culture of embryos of Citrus and Mangifera. Indian J. Hort. 15:275-282.

11. Oppenheimer, C. 1947. The acclimatisation of new tropical and subtropical fruit trees in Palestine. Bull. Agric. Res. Sta. Rehovot, 44. pp. 183.

12. Oppenheimer, C. 1978. Subtropical fruit trees and their cultivation in Israel. Am Oved, Tel Aviv (in Hebrew). p. 447

13. Popenoe, W. 1920. Manual of tropical and subtropical fruits. Macmillan, New York. pp. 474.

14. Ruehle, G. D. and Ledin, R. B. 1956. Mango growing in Florida. Fla. Agric. Exp. Sta. Bull. 574.

15. Singh, L. B. 1960. The mango; botany, cultivation and utilization. Interscience Publ. Inc., New York. pp. 438.

16. Tisserat, B., Esau, E. B. and Murashige, T. 1979. Somatic embryogenesis in angiosperms. Hort. Rev. 1:1-72.

17. Webber, H. J. 1931. The economic importance of apogamy in Citrus and Mangifera. Proc. Amer. Soc. Hort. Sci. 28:57-61.

18. Young, T. W. and Sauls, J. W. 1981. The mango industry in Florida. Fla. Coop. Exten. Serv., IFAS, Univ. of Fla., Gainesville. pp. 70.

E.2. Anatomical Study of Early Polyembryony in '13-1' and 'Turpentine'

Introduction

A number of anatomical studies of polyembryony have been published (1, 2, 3, 4, 5, 6, 7). Before the present study, it had already been found that at anthesis dense nucellar cells already can be discerned at the micropylar end of the embryo sac. After the formation of endosperm, these cells start to divide rapidly, developing into adventitious embryos that protrude and enter into the embryo sac. We followed the polyembryonic developmental process in the 2 cvs ('13-1' and 'Turpentine') that we used for most of our experimental work in Israel.

Material and Methods

'13-1' and 'Turpentine' flowers and fruitlets were sampled near Bet Dagan, Israel and were fixed in FAA. Pistils, or ovaries, were excised, embedded in paraffin and cut serially in lengthwise sections of 12 μ thickness. Sections were stained with safranin and fast green.

Results

The development of the nucellar embryo was followed up to the age of 6 weeks. No significant differences were founed between '13-1' and 'Turpentine'. Results can be seen in Figs. 2.1, 2.2, 2.3, 2.4 and 2.5. A large number of nucellar embryos could be already seen at the age of 2-3 weeks (Fig. 2.2). At the age of 3-4 weeks the nucellar embryos began to be incorporated into the embryo sac. This was the last stage when the zygotic embryo could still be identified with certainty; at this stage it was surrounded completely by the endosperm, while the nucellar embryos were still located close to the micropylar nucellus wall. At the age of 4-6 weeks it was no longer possible to identify the zygotic embryo Figs. 2.3, 2.4, 2.5).

Fig. 2.3. Nucellar embryos (NE) in 3-4 weeks old '13-1' fruitlet (x 125).

Fig. 2.2. Early stage of nucellar embryos (NE) development. '13-1' fruitlets, 2-3 weeks old (x 125).

Fig. 2.1 Nucellar cells with pronounced large nuclei (NU) in '13-1' ovule at anthesis (x 125). (x

Fig. 2.5 Nucellar embryos in 5-6 weeks old '13-1' fruitlet (x 40).

Fig. 2.4. Nucellar embryos (NE) in 4-5 weeks old '13-1' fruitlet (x 125).

Discussion

The early development of nucellar embryos in '13-1' and 'Turpentine' mangos did not differ in any significant detail from prior reports (1, 2, 3, 4, 5, 6). The information obtained in this study was used in studying the effect of controlled hand pollination on subsequent development of young embryos (E.6).

Literature Cited

1. Belling, J. 1908. Report of the Assistant in Horticulture, Mango, p. 110-125 in: Ann. Rept. Fla. Agric. Exp. Sta. for 1908.
2. Juliano, J. B. 1934. Origin of embryos in the Strawberry mango. Philip. J. Sci. 54:553-561 (+ 3 plates).
3. Juliano, J. B. and Cuevas, N. L. 1932. Floral morphology of the mango (Mangifera indica Linn.) with special reference to the Pico variety from the Philippines.
4. Juliano, J. B. 1937. Embryos of the Carabao mango (Mangifera indica Linn.). Philipp. Agriculturist 25:749-760.
5. Sachar, R. C. and Chopra, R. N. A study of endosperm and embryo in mango Mangifera indica L. Indian J. Agric. Sci. 27:219-228.
6. Sturrock, T. T. 1967. Nucellar embryos of the mango. Proc. Fla. State Hort. Soc. 80:350-354.
7. Tisserat, B., Esau, E. B. and Murashige, T. 1979. Somatic embryogenesis in angiosperms. Hort. Rev. 1:1-72.

Plant Cell Reports (1982) 1: 264–266

Plant Cell Reports

E.3. Somatic Embryos from Cultured Ovules of Polyembryonic *Mangifera indica* L.

Richard E. Litz[1], Robert L. Knight[2] and Shmuel Gazit[3]

[1] University of Florida Agricultural Research and Education Center, Institute of Food and Agricultural Sciences, Homestead, FL 33031, USA
[2] United States Department of Agriculture, Subtropical Horticulture Research Unit, Miami, FL 33158, USA
[3] Department of Horticulture, Volcani Institute, Bet Dagan, Israel

Received June 1, 1982/September 27, 1982

Abstract

Ovules were aseptically removed from 2 month old fruits of 9 naturally polyembryonic cultivars and 1 monoembryonic cultivar of mango (Mangifera indica L.). Ovules were placed into culture on solid Murashige and Skoog medium that had been modified by the addition of half strength major salts and chelated iron, 6% sucrose, 400 mg/l glutamine, 100 mg/l ascorbic acid with or without the following growth regulators: 20% (v/v) CW, 1 or 2 mg/l BA. Somatic embryogenesis occurred from the nucellus excised from the ovules of 5 of the naturally polyembryonic cultivars after 1-2 months in culture. Somatic embryogenesis was not apparently affected by the growth regulator composition of the media; however, efficient somatic embryogenesis only occurred in liquid containing 20% CW.

Abbreviations: CW = coconut water, BA = benzyladenine

Introduction

The mango Mangifera indica L. is one of the most widely grown tropical fruit crops, and has been improved by selection from seedling populations for several thousand years (Lakshminarayana, 1980). Modern mango cultivars are either monoembryonic or polyembryonic. Maheshwari and Rangaswamy (1958) have demonstrated that adventitious embryos in polyembryonic mango cultivars are derived in vivo from densely cytoplasmic cells of the nucellus.

In order for tissue culture systems to be applied successfully to crop improvement programs, efficient in vitro methods for regenerating plants must exist. In vitro systems have been developed for agronomic and field crops such as the potato (Shepard, 1981) and sugar cane (Heinz, 1976) that have great potential for plant breeding. Woody plants have been difficult to mantipulate in tissue culture. Among the tree fruit crops, it has been possible to stimulate growth and proliferation of axillary buds and shoot tips of clonal apple varieties (Abbott and Whiteley, 1976), Prunus spp. (Tabachnik and Kester, 1977) and papaya (Litz and Conover, 1978). Somatic embryos have been induced in vitro from the nucellus of monoembryonic and polyembryonic Citrus spp. (Rangan et al., 1969, Kochba, et al., 1972). Similarly, somatic embryogenesis has been induced from ovule cultures of grape (Mullins and Srinivasan, 1976) and papaya (Litz and Conover, 1981; 1982).

The current work describes a procedure for the induction of somatic embryogenesis from cultured nucellus of 5 naturally polyembryonic mango cultivars.

Materials and Methods

Young mango fruits were harvested approximately 2 months after pollination from 10 mango cultivars in the germplasm collection of the U.S.D.A. Subtropical Horticulture Research Unit in Miami, Florida. Fruits were soaked for 20-30 minutes in 14.2 g/l Benlate in order to suppress anthracnose, and were set aside to dry. Following surface-sterilization in 20% (v/v) Clorox with 2-3 drops of Tween 20 for 20-25 minutes, the mango fruits were rinsed thoroughly in sterile distilled water. The ovules were aseptically removed, and were transferred to a modified Murashige and Skoog medium (1962), consisting of half strength major salts and chelated iron, 6% sucrose, 400 mg/l glutamine, 100 mg/l ascorbic acid, 0.8% Difco Bacto agar and with or without filter-sterilized 20% (v/v) CW or 1-2 mg/l BA in sterile plastic 60 x 15 petri dishes. The nucellus was removed from the cultured ovules between 1 and 3 weeks after culturing, and transferred onto the same medium. Proliferating cultures of somatic embryos were subcultured either on solid or in 30 ml liquid modified Murashige and Skoog medium containing 20% CW in 125 ml Erlenmeyer flasks maintained at 100 r.p.m. on a rotary shaker. The pH was adjusted to 5.7 with 1N HCl or NaOH prior to autoclaving at 121°C and 1.1 kg/cm^2 for 15 minutes. Filter-sterilized coconut water obtained from freshly picked 9-10 cm long immature coconuts was added to autoclaved media. The cultures were incubated in a growth chamber at 25°C with a 16 h photoperiod (1000 lux).

Results and Discussion

At the time of culture 2 months after pollination, the young seeds of the monoembryonic and polyembryonic mango cultivars were already well formed. The nucellus of cultivars of Cambodiana, Carabao, M20222, Turpentine N2-1-4-3 and Earliblush (monoembryonic) could not be identified or isolated. However, the nucellus in ovules from cultivars Chino, Sabre and Ono, and to a lesser extent Heart and Turpentine N2-1-7-2, was persistent and enlarged. Many adventitious embryos at the globular stage of development were associated with the nucellus of these cultivars (Figure 1). There was little bacterial or fungal contamination, although wounded tissue blackened quickly when not in direct contact with the growth medium.

Precocious germination of the existing embryos in cultured ovules of certain cultivars, i.e., Earliblush, Carabao, Cambodiana, M20222 and Turpentine N2-1-4-3 began to occur 2-3 weeks after culturing.

0721-7714/82/0001/0264/$ 01.00

Germinated plantlets had vigorous root and shoot systems (Figure 2).

Figure 1. Adventitious embryogenesis from enlarged nucellus of polyembryonic mango ovule, cultivar Chino. Globular embryos (arrow).

Figure 2. Precocious germination of polyembryonic mango cultivar, Camodiana.

Somatic embryogenesis occurred from the mass of enlarged and persistent nucellar tissue dissected from the ovules of cultivars Chino, Sabre, Ono, Heart and Turpentine N2-17-2 and subcultured on fresh medium. Somatic embryos at globular, heart and mature stages of development were observed (Figure 3). Somatic embryogenesis occurred on media containing CW, BA or no growth regulators. Continued proliferation of somatic embryos has been sustained upon subculture of differentiating nucellar tissue on medium with 20% CW; however the most efficient somatic embryogenesis occurred in liquid medium with CW (Figure 4).

Figure 3. Somatic embryogenesis from excised nucellus of mango ovule, cultivar Sabre. Three weeks after excision from ovule.

Figure 4. Efficient somatic embryogenesis from nucellar tissue of mango cultivar Chino in liquid medium with 20% CW.

The embryogenic response was cultivar-dependent (Table 1). Somatic embryogenesis has occurred in 5 of 9 naturally polyembryonic cultivars or seedling lines, and appeared to be related to the degree of polyembryony in the ovules at the time of culture. Somatic embryogenesis occurred from nucellus derived from 84% of surviving cultured ovules of cv. Chino, which were highly polyembryonic, but in the nucellar cultures of only 7% of surviving cultured ovules of cv. Heart, which were not highly polyembryonic.

Normal plantlet development from mature somatic embryos has not occurred following the transfer of somatic embryos to CW-free medium.

The regeneration of somatic embryos from nucellar tissue of certain naturally polyembryonic mango cultivars has been demonstrated. This process appears to resemble in vitro somatic embryogenesis from the

isolated nucellus of polyembryonic Citrus (Rangan et al., 1969; Kochba et al., 1972). In order for this technique to have practical significance, eg., mass production of disease-free mango rootstock or cultivar improvement, a method for efficiently regenerating plants from mature embryos must be demonstrated.

Table 1. In vitro response of excised Mangifera indica L. nucellus.

Cultivar	No. surviving ovules	Precocious germination	No. with somatic embryogenesis
Cambodiana	38	Yes	0
Carabao	47	Yes	0
Chino	51	No	43
Earliblush (monoembryonic)	23	Yes	0
Heart	56	No	4
M20222	36	Yes	0
Ono	43	No	16
Sabre	49	No	25
Turpentine N2-1-7-2	41	No	11
Turpentine N2-1-4-3	38	Yes	0

Acknowledgements

The authors are indebted to Ms. Carole Resciguo for her technical assistance. The support from a BARD agreement is also acknowledged by R. K. Knight and S. Gazit. Florida Agricultural Experiment Stations Journal Series No. 3902.

References

Abbott AJ, Whiteley E (1976) Sci Hortic 4:183-189
Heinz DJ (1976) Annl Rep Hawaiian Sugar Planters Assoc Exp. Stations: 9-11
Kochba J, Speigel-Roy P, Safran H (1972) Planta 106:237-245
Lakshminarayana S (1980) In: Nagy S, Shaw PE (eds), Tropical and Subtropical Fruits, AVI Publishing, Inc., Westport, Conn., pp 184-257
Litz, RE, Conover RA (1978) HortScience 13:241-242
Litz, RE, Conover RA (1981) Z Pflanzenphysiol 104:285-288
Litz, RE, Conover RA (1982) Plant Sci Lett 26:153-158
Maheshwari P, Rangaswamy NS (1958) Indian Jour Hort 15:275-282
Mullins, MG, Srinivasan C (1976) J Expt Bot 27:1022-1030
Murashige, T, Skoog F (1962) Physiol Plantarum 15:473-497
Rangan TS, Murashige T, Bitters WP (1969) In: Chapman HD (ed), Proc Int Citrus Symp, Univ. California, pp 225-229.
Shepard JF (1981) Ann Rev Phytopathol 19:145-166
Tabachnik L, Kester DE (1977) HortScience 12:545-547

Scientia Horticulturae, 22 (1984) 233—240
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

E.4. IN VITRO SOMATIC EMBRYOGENESIS FROM *MANGIFERA INDICA* L. CALLUS

RICHARD E. LITZ[1], ROBERT J. KNIGHT, Jr.[2] and SHMUEL GAZIT[3]

[1]*University of Florida, Agricultural Research and Education Center, Institute of Food and Agricultural Sciences, 18905 SW 280 Street, Homestead, FL 33031 (U.S.A.)*
[2]*United States Department of Agriculture, Subtropical Horticulture Research Unit, Miami, FL 33158 (U.S.A.)*
[3]*Department of Horticulture, Volcani Institute, Bet Dagan (Israel)*
Florida Agricultural Experiment Stations Journal Series No. 4216

(Accepted for publication 19 May 1983)

ABSTRACT

Litz, R.E., Knight, R.J., Jr. and Gazit, S., 1984. In vitro somatic embryogenesis from *Mangifera indica* L. callus. *Scientia Hortic.*, 22: 233—240.

The nucellus and globular adventitious proembryos were removed from 2-month-old fruits of mango (*Mangifera indica* L.) cultivars 'Ono' and 'Chino', and were cultured on sterile, solid Murashige and Skoog (MS) medium that had been modified as follows: half-strength major salts and chelated iron; 20% (v/v) coconut water (CW); 6% sucrose; 100 mg l^{-1} ascorbic acid and 400 mg l^{-1} glutamine. Embryogenic explants were sub-cultured after 4—6 weeks in liquid modified MS medium containing 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) instead of CW. Rapidly growing cultures were established and were sub-cultured monthly. Somatic embryogenesis was induced following sub-culture from MS medium with 2,4-D to MS without growth regulators and with or without activated charcoal (0.5%). Germination of somatic embryos appeared to be enhanced by 1 mg l^{-1} benzyladenine (BA); however, most of the germinating embryos became embryogenic.

Keywords: mango; somatic embryogenesis; tissue culture.

INTRODUCTION

Litz et al. (1982) have demonstrated that it is possible to stimulate the growth of somatic embryos in vitro from cultured ovules of naturally polyembryonic mango *(Mangifera indica* L.) cultivars. Somatic embryo development occurred from enlarged and persistent nucellar tissue that existed in certain polyembryonic mango cultivars 2 months after pollination. Globular somatic embryos had continued to develop from the nucellus at the time of culturing, and continued to develop for a few months.

The in vitro stimulation of growth of adventitious *Citrus* embryos from

0304-4238/84/$03.00

nucellus excised from developing seeds was reported by Rangan et al., (1968). Since then, it has been possible to induce somatic embryogenesis from callus derived from the nucellus of unfertilized ovules (Kochba et al., 1972) and from isolated nucellus of a number of *Citrus* cultivars (Mitra and Chaturvedi, 1972; Kochba and Spiegel-Roy, 1973). Success in the use of tissue-culture systems for improvement of *Citrus* has been dependent on the isolation and characterization of highly embryogenic nucellar callus (Spiegel-Roy and Kochba, 1980).

Following the successful stimulation of somatic embryos from some naturally polyembryonic mango cultivars (Litz et al., 1982), conditions for induction and growth of nucellar callus and for efficient somatic embryogenesis from this callus have been defined, and are described in this report.

MATERIALS AND METHODS

Immature mango *(Mangifera indica* L.) fruit of 'Chino' and 'Ono' were harvested from the germplasm collection of the U.S.D.A. Subtropical Horticulture Research Unit, Miami, Florida, approximately 2 months after pollination. Following the procedure of Litz et al. (1982), the fruit were initially soaked in Benlate solution (14.2 g l^{-1}) for 20—30 min in order to suppress anthracnose, then set aside to dry. After surface sterilization in 20% (v/v) Clorox for 25 min, the fruits were briefly rinsed with sterile distilled water. Immature seeds were aseptically removed, and cultured in 60 × 15 mm plastic petri dishes on sterile Murashige and Skoog (MS) medium (1962) modified as follows: half-strength major salts and chelated iron; 6% sucrose; 400 mg l^{-1} glutamine; 100 mg l^{-1} ascorbic acid; 20% (v/v) coconut water (CW); 0.8% Difco Bacto agar. The nucellus and globular adventitious proembryos were removed after 1—2 weeks and cultures were transferred to liquid medium in 125 ml Erlenmeyer flasks containing 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) instead of CW. Tissue cultures were sub-cultured monthly. After 3 months, the nucellar callus was transferred to liquid MS without growth regulators and with or without activated charcoal (0.5%) in order to induce somatic embryogenesis. Mature somatic embryos were transferred onto solid medium containing 0—5.0 mg l^{-1} benzylaminopurine (BA) together with one of the following: 100 mg l^{-1} casein hydrolysate; 100 mg l^{-1} malt extract; 10% CW.

The pH of all media was adjusted to 5.7 with 0.1 N HCl or KOH prior to autoclaving at 1.1 kg cm^{-2} and 120°C for 15 min.

Filter-sterilized CW derived from freshly harvested 9—10 cm immature coconuts was added to the medium after autoclaving. Cultures were maintained at 25°C with a 16-h photoperiod (1000 lux). Liquid cultures were maintained at 100 r.p.m. on rotary shakers.

RESULTS

Callus induction occurred from mango nucellar explants soon after transfer into liquid MS medium with 2,4-D. The callus grew rapidly and formed hard, spherical masses of different sizes, resembling pseudobulbils (Figs. 1 and 2). There was no visible evidence that differentiation occurred in the presence of 2,4-D. The medium and callus in some cultures became quite dark, probably due to the activation of certain oxidative enzymes. This was minimized through frequent sub-culturing. Following the transfer of callus into medium without 2,4-D and with or without activated charcoal, somatic embryogenesis began to occur 6—8 weeks later. Activated charcoal had a deleterious effect on subsequent embryogenesis, and most of the somatic embryos that resulted from these treatments died during the late heart-shaped stage of development. Numerous globular protuberances appeared from the callus masses or pseudobulbils (Fig. 3) in 30% of the flasks, with or without activated charcoal and irrespective of the cultivar. Smaller callus masses appeared to be more embryogenic. The protuberances rapidly became distinguishable as globular proembryos (Fig. 4) and subsequently mature embryos (Fig. 5). The entire callus appeared to be embryogenic. A high degree of uniformity was observed among mature somatic embryos. Developmental abnormalities such as polycotyledony or fasciation were rarely observed.

Fig. 1. Embryogenic nucellar callus of mango 'Chino' in liquid MS medium with 2 mg l^{-1} 2,4-D.

236

Fig. 2. Close up from Fig. 1 showing callus forming spherical pseudobulbils.

Fig. 3. Somatic embryogensis from mango 'Chino' nucellar callus following sub-culture from MS with 2,4-D to MS without growth regulators and with 0.5% activated charcoal. Initiation of globular embryos (arrow).

4

Fig. 4. Somatic embryogenesis from mango 'Ono' nucellar callus following sub-culture from MS with 2,4-D to MS without growth regulators. Globular embryos (arrow).

Fig. 5. Somatic embryogenesis from mango 'Chino' nucellar callus following sub-culture from MS with 2,4-D to MS without growth regulators. All stages of embryo development occur. Mature somatic embryos (arrow).

238

TABLE I

Effect of 4 supplements to plant-growth medium on the germination of mango somatic embryos. S = secondary embryo formation; G = precocious germination; CW = 10% (v/v) coconut water; CH = 100 mg l^{-1} hydrolysate; ME = 100 mg l^{-1} malt extract

BA (mg l^{-1})	O	CW	CH	ME
0	SG	SG		
0.5	S		SG	S
1.0	G	S	SG	
2.0	SG		SG	S
3.0		S		
4.0	S			
5.0				

Fig. 6. Secondary somatic embryos produced from the hypocotyl of a germinating somatic embryo of mango 'Ono'.

The mortality rate among mature somatic embryos was high (45%). This was characterized by gradual necrosis of the cotyledons. It was not possible to control this successfully by additives to the medium (Table I). Mature embryos began to germinate on solid MS medium with 1—2 mg

l^{-1} benzyladenine, although this formulation does not appear to be ideal. Normal root development together with elongation of the hypocotyls occurs, although plantlets were not successfully regenerated. Secondary somatic embryos developed from the hypocotyls and cotyledons of somatic embryos on most germinating embryos (Fig. 6).

DISCUSSION

Rangaswamy (1982) has emphasized the potential of the nucellus for in vitro studies involving woody plants. Despite the successful demonstration of in vitro somatic embryogenesis from *Citrus* nucellar callus, there have been only a few studies involving other economically important woody plants in which naturally occurring polyembryony occurs. According to Rangaswamy (1982), adventitious embryo development occurs from the nucellus in at least 16 plant families. Many important tropical fruit species are polyembryonic, including the mango.

Embryogenic mango callus appears to closely resemble the highly embryogenic *C. sinensis* 'Shamouti' ovular callus reported by Kochba et al. (1972). Button et al. (1974) demonstrated that this callus was composed of globular proembryos, at various stages of development, which were themselves also highly embryogenic. Spherical pseudobulbils were derived from enlarging globular proembryos. A similar pattern of development has also been described for somatic embryogenesis from *Vitis* nucellar callus (Srinivasan and Mullins, 1980) and from *Carica* ovular callus (Litz and Conover, 1983). The successful regeneration of mango plantlets from somatic embryos has not been obtained, possibly because of inadequacies of the growth-medium formulation, but more probably due to habituation of the mango suspension cultures as a result of prolonged exposure to 2,4-D.

The control of somatic embryogenesis and plant regeneration of mango should provide alternate strategies for cultivar improvement of this very important tropical fruit crop. Due to the long generational cycle of mango cultivars, conventional plant-breeding approaches to variety improvement are very difficult. Larkin and Scowcroft (1981) suggested that plant cell and tissue culture may generate discrete genetic variability, and that this variation could be useful in plant breeding. Increasing the rate of occurrence of useful variation or somatic mutation within existing mango clones in vitro could result in the more rapid development of new or improved mango cultivars. Likewise, the efficient regeneration of mango plantlets from somatic embryos might provide alternate methods for clonal propagation and exchange of disease-indexed germplasm.

ACKNOWLEDGEMENTS

The authors appreciate the excellent technical assistance provided by Carole Rescigno. The support of BARD Project I-255-80 is acknowledged by R.J. Knight, Jr. and S. Gazit.

240

REFERENCES

Button, J., Kochba, J. and Bornman, C.H., 1974. Fine structure of and embryoid development from embryogenic ovular callus of 'Shamouti' orange *(Citrus sinensis)*. J. Exp. Bot., 25:446—457.

Kochba, J. and Spiegel-Roy, P., 1973. Effect of culture media on embryoid formation from ovular callus of Shamouti orange (*Citrus sinensis*). Z. Pflanzenzucht., 69:156—162.

Kochba, J., Spiegel-Roy, P. and Safran, H., 1972. Adventive plants from ovules and nucelli in *Citrus*. Planta, 106:237—245.

Larkin, P.J. and Scowcroft, W.R., 1981. Somaclonal variation — a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet., 60:197—214.

Litz, R.E. and Conover, R.A., 1983. High frequency somatic embryogenesis from *Carica* suspension cultures. Ann. Bot., 51:683—686.

Litz, R.E., Knight, R.J. and Gazit, S., 1982. Somatic embryos from cultured ovules of polyembryonic *Mangifera indica* L. Plant Cell Rep., 1:264—266.

Mitra, G.C. and Chaturvedi, H.C., 1972. Embryoids and complete plants from unpollinated ovaries and from ovules of in vivo grown emasculated flower buds of *Citrus* spp. Bull. Torrey Bot. Club, 99:184—189.

Murashige, T. and Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant., 15:473—497.

Rangan, T.S., Murashige, T. and Bitters, W.P., 1968. In vitro initiation of nucellar embryos in monoembryonic *Citrus*. HortScience, 3:226—227.

Rangaswamy, N.S., 1982. Nucellus as an experimental system in basic and applied tissue culture research. In: A.N. Rao (Editor), Proc. COSTED Symp. on Tissue Culture of Economically Important Plants, COSTED and ANBS, Singapore, pp. 269—286.

Spiegel-Roy, P. and Kochba, J., 1980. Embryogenesis in *Citrus* tissue cultures. In: A. Fiechter (Editor), Advances in Biochemical Engineering, Vol. 16. Springer-Verlag, Heidelberg, pp. 27—48.

Srinivasan, C. and Mullins, M.G., 1980. High-frequency somatic embryo production from unfertilized ovules of grapes. Scientia Hortic., 13:245—252.

E.5. Identification of the Zygotic Embryo and its Location in the Polyembryonic Seed.

Introduction

In some polyembryonic cvs the sexual embryo does not develop at all, or degenerates and disappears (1, 5, 6, 12, 13, 15). Thus, it is not surprising that some cvs will come almost 100% true to type (2, 8, 9, 10, 12, 13, 17). However, a large number of polyembryonic cvs will produce a significant percentage of "off" type plants, which are of sexual origin. '13-1', which is the predominant rootstock in Israel (3), was found to produce from 10-15% zygotic plants under normal nursery practices. 'Turpentine', the main Floridian rootstock, also produces a significant percentage of "off" type plants.

When mango is propagated by seed it is of the utmost importance to be sure that all the plants will be identical to the mother tree. Nurserymen should discard "off" type plants, but not all zygotic seedlings can be identified morphologically at the nursery stage. Tammes reported that by discarding the third proximal part of the seed the zygotic embryo can be destroyed (14). Isozyme analysis has been used to identify zygotic embryos and seedlings in Citrus (16).

Work done in Israel

Material and Methods

82 germinating '13-1' seeds were uprooted 6 weeks after planting. All seedlings were tagged, their location on the seed noted, and then they were separated and planted in individual containers. 186 seedlings from the 82 seeds survived, and their leaves were sampled a year later. The isozyme analysis for PGI (Phosphoglucose isomerase) enzyme was performed in Dr. Degani's laboratory, using the standard methods developed for avocado (4).

In Florida, leaf samples from 28 seedlings of the polyembryonic cvs 'Kensington', 'Sabre', 'Cambodiana', and M-20222 were collected, and gas chromatograms were made from a steam distillate. Temperature at the start of the work was 50 degrees C, and this increased by 8 degrees per minute for 25 minutes, until 250 C was reached. The need to allow 25 minutes to analyze each sample was a definite constraint, considering the large number of seedlings that were available for analysis. Patterns from each sample were compared for uniformity, even though it was not possible to identify the individual peaks because we had no reference chemicals to aid in this effort. The hypothesis was that individuals showing similar chromatograms are likely to be of the same genotype.

Results

Of the 186 seedlings that were examined for their PGI zymogram, only 14 were found with a zymogram different from that of '13-1' (and the other 172 seedlings). Only one "off" type seedling was found in any one seed. The results are depicted in Fig. 5.1.

The zygotic seedling always emerged from the proximal part of the seed. However, all nucellar seedlings but one emerged too at the same part of the seed (Fig. 5.1). In a typical sprouting seed almost all seedlings emerged at the proximal end of the seed, close together (Fig. 5.2). In only one seed did the identified zygotic seedling emerge exactly from the typical location of the embryonal axis in the monoembryonic seed. Thus, the zygotic seedling could not be identified by the site of its emergence in '13-1' seeds.

Of 7 'Kensington' seedlings examined, none showed sufficient variation in the pattern of its gas chromatogram to be considered an "off" type. On the other hand, 2 of 14 'Sabre' seedlings compared with each other and with

a sample from the maternal tree, varied sufficiently from the rest to be considered "off" types (Table 5.1, Fig. 5.3).

Discussion

The analysis of one polymorphic enzyme for mango (PGI) revealed "off" zymogram patterns in 17% of 82 '13-1' seeds. We should consider this incidence as a lower limit for the rate of zygotic embryo occurrence in '13-1' seeds. We may expect that by the use of more than one polymorpyhic enzyme, a significantly higher incidence of zygotic seedlings will be found. Tammes (4) recommended discarding the proximal part of the seed in order to eliminate the zygotic embryo. Our results and observations (Figs. 5.1, 5.2) demonstrate that this operation also will eliminate almost all nucellar embryos in '13-1' seeds. We observed that seedlings of most polyembryonic cvs sprout in a pattern similar to that of '13-1'. Thus, Tammes conclusion may be true only for a small number of cvs, different from those with which we worked.

Isozyme analysis could and should be used for identification of the zygotic embryo and seedling. This will enable us to find out if indeed the zygotic embryo is small and the seedling is less vigorous than some of the nucellar ones (2, 12). We strongly believe that not all zygotic embryos are small and weak.

Gas chromatography appears to afford another way of identifying the zygotic seedling, but the length of time necessary to process each sample (25 minutes), exclusive of the time needed to collect and prepare each sample, makes this method too cumbersome to use on any but very small populations. Why the chromatogram of the parent plant of M-20222 did not closely match the pattern most common in its seedlings is an unanswered

question. In any case, such did not happen with 'Sabre', whose chromatogram closely matched that of 12 of the 14 'Sabre' seedlings examined.

Literature Cited

1. Belling, J. 1908. Report of the Assistant in horticulture, Mango. p. 110-125 in: Ann. Rep. Fla. Agric. Exp. Sta. for 1908.

2. Chaudhri, S. A. 1976. Mangifera indica -- mango. p.403-474 in: Garner, R. J. and Chaudhri, S. A. (Eds.) The propagation of tropical fruit trees. Commonwealth Agric. Bureaux, Slough, England.

3. Gazit, S. and Kadman, A. 1980. '13-1', a new mango rootstock. HortSci. 15:669

4. Goldring, A., Zamir, D., and Degani, C. 1985. Duplicated phosphoglucose isomerase genes in avocado. Theor. Appl. Genet. 71:491-494.

5. Juliano, J. B. and Cuevas, N. L. 1932. Floral morphology of the mango (Mangifera indica L.) with special reference to the Pico variety from the Philippines. Philipp. Agriculturist 21:449-472.

6. Juliano, J. B. 1934. Origin of embryos in the Strawberry mango. Philipp. J. Sci. 54:553-561 (+ 3 plates).

7. Juliano, J. B. 1937. Embryos of the Carabao mango (Mangifera indica Linn.) Philipp. Agriculturist 25:749-760.

8. Knight, R. J. 1970. Polyembryonic mangos: their unrealized potential. Proc. Trop. Reg. Amer. Soc. Hort. Sci. 14:145-155.

9. Oppenheimer, C. The acclimatisation of new tropical and subtropical fruit trees in Palestine. Bull. Agric. Res. Sta. Rehovot, 44. pp. 183.

10. Oppenheimer, C. 1978. Subtropical fruit trees and their cultivation in Israel. Am Oved, Tel Aviv. (in Hebrew). pp. 447.

11. Sachar, R. C. and Chopra, R. N. 1957. A study of endosperm and embryo in Mangifera indica L. Indian J. Sci. 27:219-228.

12. Singh, L. B. 1960. The mango: botany, cultivation and utilization. Interscience Publ. Inc., New York. pp. 438.

13. Sturrock, T. T. 1967. Nucellar embryos of the mango. Proc. Fla. State Hort. Soc. 80:350-354.

14. Tammes, P. M. L. 1965. Vegetative propagation from polyembryonic mango seeds. Chron. Hort. 5(3):64.

15. Tisserat, B., Esau, E. B. and Murashige, T. 1979. Somatic embryogenesis in angiosperms. Hort. Rev. 1:1-72.

16. Torres, A. M. 1983. Fruit trees. in: Tanksley, S. D. and Orton, T. G. (Eds.) Isozymes in plant genetics and breeding. Elsevier Sci., Amsterdam. p. 401-421.

17. Webber, H. J. 1931. The economic importance of apogamy in Citrus and Mangifera. Proc. Amer. Soc. Hort. Sci. 28:57-61.

Table 5.1. Probable zygotic seedlings among mango seedling populations as determined by uniformity of gas chromatograms. Experiment performed in 1982 in Miami.

Cultivar	No. of seedling plants examined	No. of seeds from which these plants sprouted	No. of plants with similar gas chromatograms	No. of plants with different gas chromatograms
Sabre (parent)	--	--	1	0
Sabre	14	6	12	2
Kensington	7	6	7	0
M-20222 (parent)	--	--	0	1 [1/]
M-20222	4	n.r.	3	1
Cambodiana (parent)	--	--	1	0
Cambodiana	3	n.r.	3	0

1/ Chromatogram of parent differed from those of all 4 seedlings, but chromatograms of 3 out of 4 seedlings matched well.

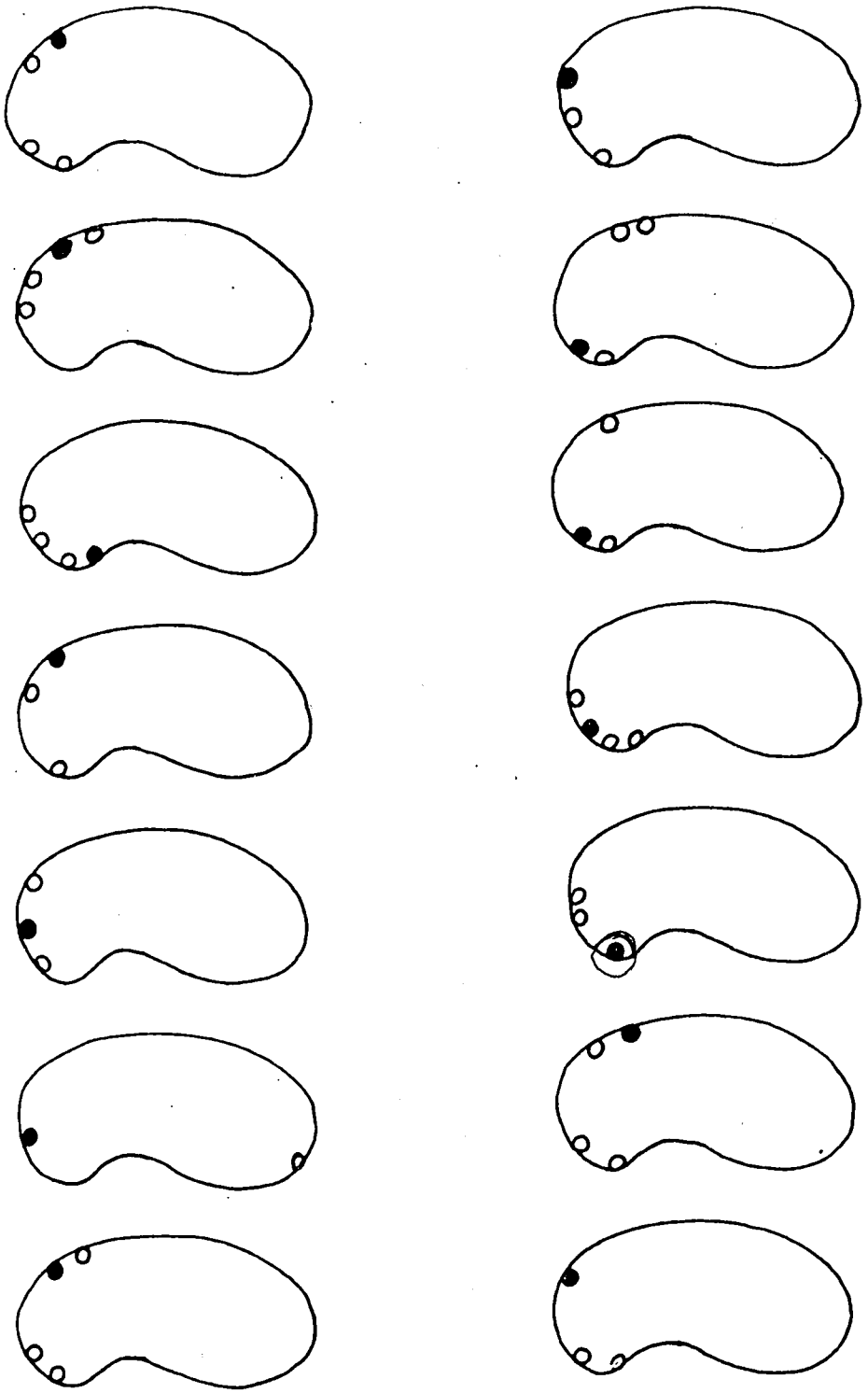

Fig. 5.1. The original location of emergence of each seedling in 14 '13-1' seeds that were found to have "off" type (zygotic) PGI zymograms. (o = nucellar, ● = zygotic, ⊖ = location of embryonal axis in monoembryonic seeds.

Fig. 5.2. Typical germinating '13-1' seed.

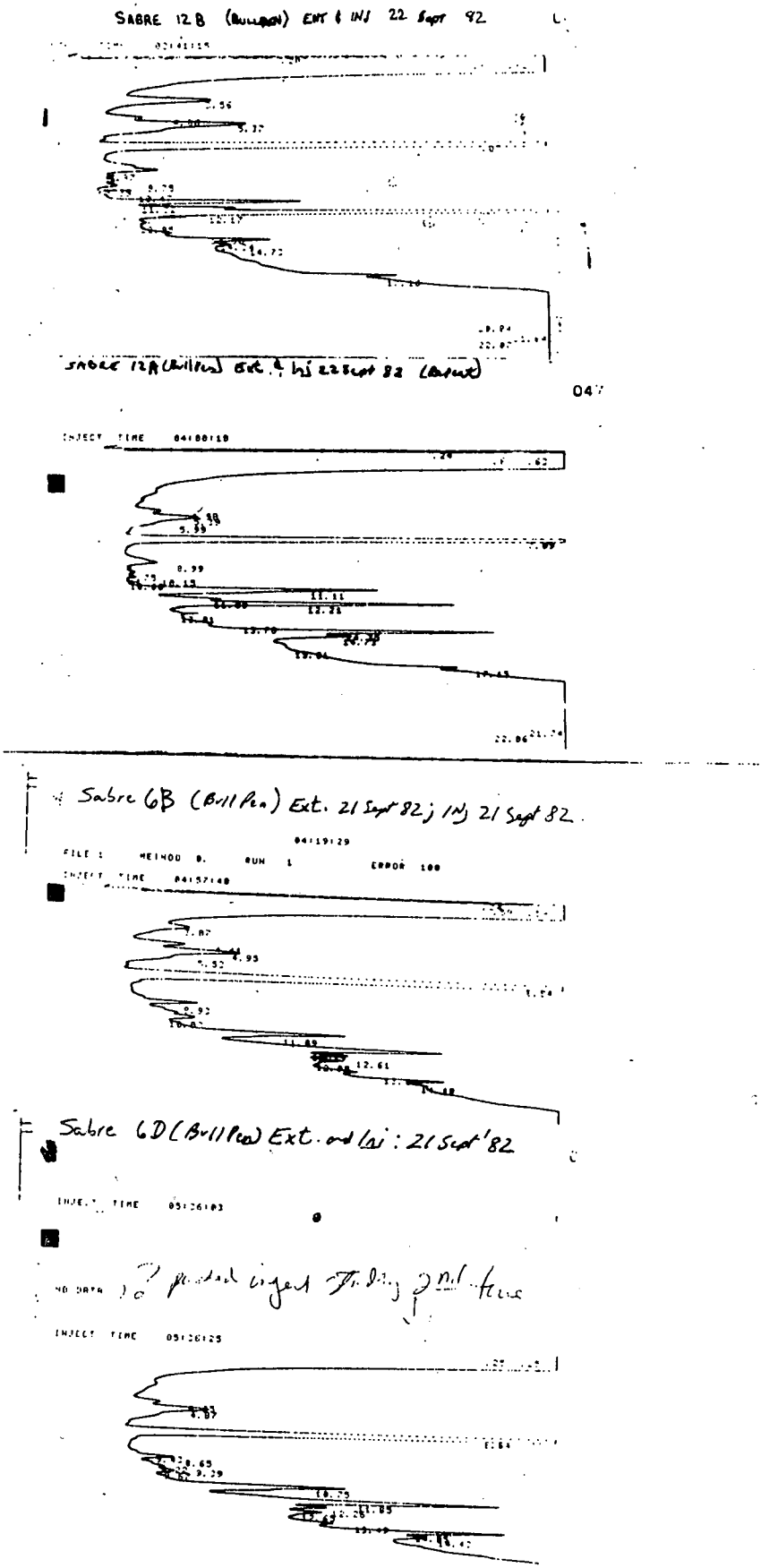

Fig. 5.3 Gas chromatograms from 4 seedlings of 'Sabre', one (Sabre 12B) of "off" type, the other 3 of "normal", similar patterns.

E.6. Self and Cross Pollination in Relation to Fruit Set and Polyembryony.

Introduction

A mature mango tree normally bears several million flowers (4). A considerable percentage of these (30-99) are staminate (11). The final fruit set from hermaphroditic flowers in nature is minuscule, about 0.01% to 0.1% (4, 11, 15). Inadequate pollination is one of the factors blamed for this low set. Hand pollination has been used in breeding and pollination studies (3, 8, 11, 12, 15, 16). Initial set was found to be increased greatly by hand pollination. Young reported a 21-fold increase in Florida (15, 16). Singh, R. N. (12) reported 5% - 10% set at the "pea" stage; Gunjate et al. (3) reported 14-28% set. Ram et al. achieved 5% to 46% set of 28-day-old fruitlets of 'Dashehari', 3% - 28% set of 25-day-old fruitlets in 'Chausa', and 2% to 14% set of 25-day-old fruitlets in 'Langra'.

Final set after hand pollination was much smaller than the initial set. Young obtained only 0.35% set out of 12,703 'Haden' pollinations (15). Singh, R. N. reported 0.38% set in 'Dashehari' and 0.0% in 'Langra' (12). Recent studies report much higher sets; 0.3% to 2.6% for 'Alphonso' (3). Ram reported even higher rates of success, 2.0% to 10.0% in 'Dashehari' and 1.0% to 8.0% in 'Chausa' (8). Hand pollination studies revealed the existence of post-zygotic incompatibility in mango (8, 10, 13). In addition to self-incompatibility, cross-incompatibility was found in some specific combinations. Most Floridian commercial cvs are apparently self fruitful, as they will produce good crops when planted in pure stands. In Israel caged 'Maya' trees (with beehives) were found to set and carry a full crop, while the fruit set of caged 'Haden' trees was

only 5.0% of that of open-pollinated trees (1). No pollination studies were carried out with polyembryonic cvs.

Endosperm and embryo are formed after pollination with incompatible pollen in the mango, but 4-5 weeks later these tissues degenerate, then the fruitlet will drop (8, 10). We raised the possibility that in polyembryonic cvs the incompatibility reaction should not include the nucellar embryos; thus, self-pollination of incompatible cvs will result in seeds having only nucellar embryos, while cross-pollination will produce seeds having also the zygotic embryo.

Material and Methods

1. Hand Pollination studies

Open pollination was prevented either by caging trees (without pollinators) or by bagging inflorescences. Pollinated flowers were tagged. Stamens with dehisced anthers were used for pollination. The pollen-laden anther was used to brush lightly the stigma. Mango flowers persist for 4-6 days before their petals shrivel and die. The color of the petals indicates the flower's age (days from opening). On the first day the petals tend to be pale yellow; in a few days they become deep yellow with bands of pink and red. The color changes are slightly different in different cvs (7). We took note of the changes in the petal color of the cvs we were working with. Good receptivity of the mango stigma was found to occur during the first 1-2 days after flower opening (7, 9, 11). To be on the safe side, we pollinated only flowers that were judged, by their petal color, to be no more than one day old. Pollen tube growth was determined in hand pollinated and open pollinated flowers. Flowers were sampled 24-48 hr after pollination, or after anthesis. The flowers were put into ethanol: acetic acid (2:1) fixing solution. Pistils were separated from the

flowers and cleared for 7-8 hr in 8N NaOH, rinsed in water, stained for 5 min. with 0.2% analin blue (dissolved in 0.1 N K3 PO4) and mounted in glycerin (7, 14). Microscopic observations were made with a Leitz Ortholux microscope. The UV source was a 200w high pressure mercury lamp. The transmission filters were: 2 mm UG, + 4 mm BG 38, and the suppression filter was K 460.

2. Caged Trees

Mature trees were caged with 15 mesh nets either individually (Fig. 6.1) or in pairs. Bees were used as the pollinating agent in Israel, while blowflies (Fam. Calliphoridae) were employed in Miami.

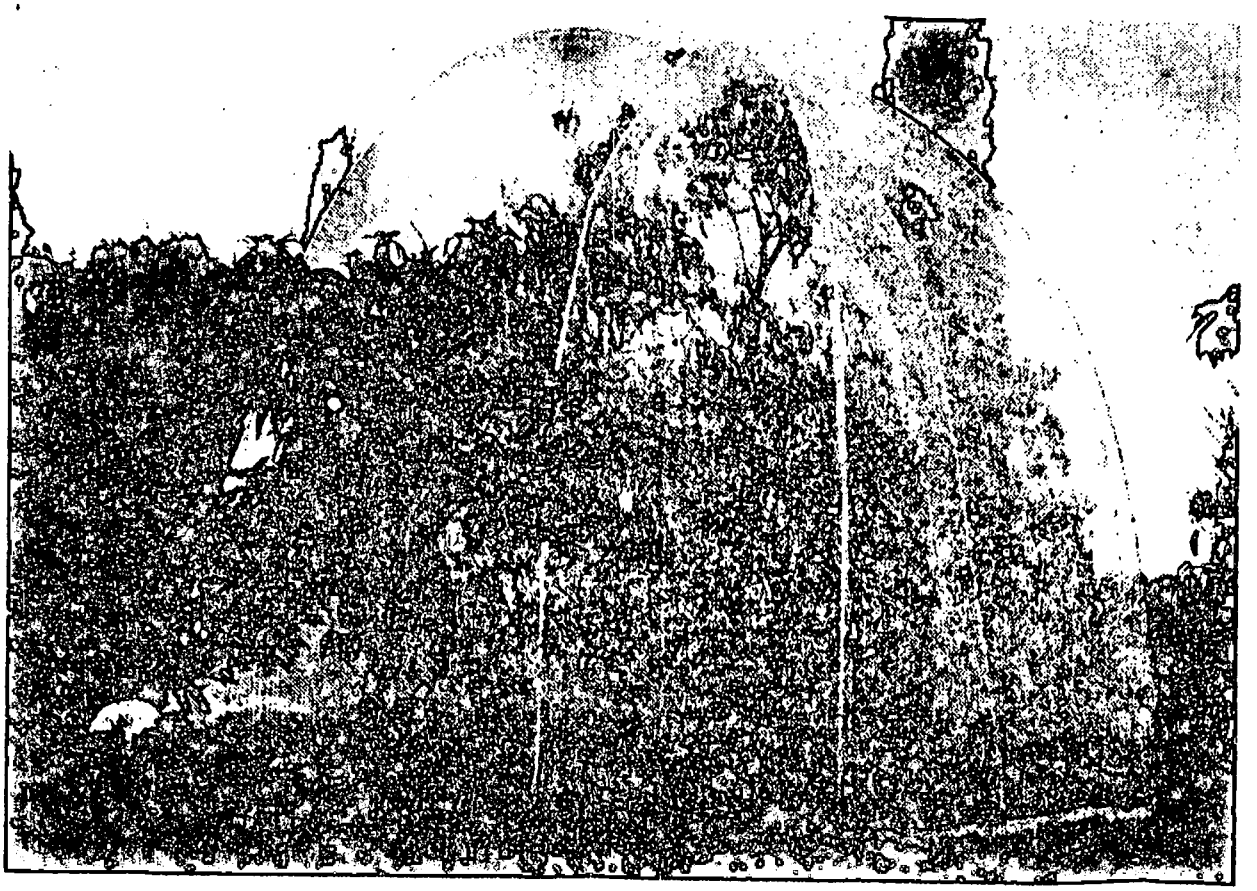

Fig. 6.1. Caged individual tree.

Results

Methodological Studies in Hand Pollination (conducted in Israel)

Hand pollination of mango flowers was found to be very tedious, time consuming and an unrewarding task. The flowers are small and difficult to tag. It is impossible to check with the naked eye, if the scarce pollen was indeed deposited on the tiny stigma. In order to find out if we were successful in pollinating flowers in the orchards, we compared several times the results of orchard pollination and the pollination of detached flowers (their stems inserted in agar in a petri dish) under a stereomicroscope in the laboratory. 24 hours after pollination, pollen germination and pollen tube growth were determined. In all of these comparisons we did not find any significant differences between lab pollinated and orchard pollinated flowers. (For example, on 11.5.83 successful pollination was noted in 68% of 150 orchard pollinated flowers and in 76% of 114 lab pollinated flowers. The difference between the two groups was found to be statistically insignificant). These results assured us that we were indeed depositing pollen on the stigmas in the orchard. We found that thinning out all unpollinated flowers, at all stages of development, did not improve fruit set (Fig. 6.2). Thus, we did not thin flowers but removed all fruitlets from the hand pollinated inflorescences.

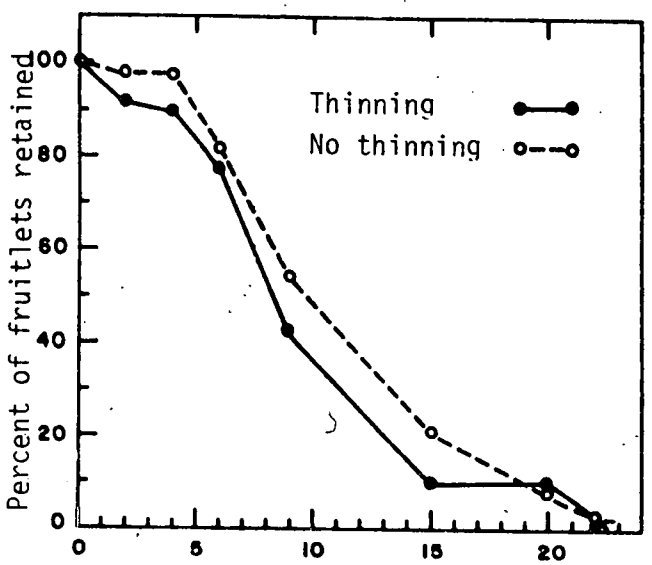

Fig. 6.2. Effect of thinning out all unpollinated flowers on retention of pollinated flowers and fruitlets. (6 inflorescences used for each treatment, 6-10 flowers pollinated on each inflorescence.)

2. Variation in the Rate of Naturally Successful Pollinations during the Flowering Season in Israel.

In the first season we started our hand pollination experiments rather late, at the end of April. To our great dismay when we looked for pollen germination we could not find any pollen on pollinated stigmas. As ungerminated pollen will be washed away during the preparatory stage, we surmised that either the pollen or the stigma (or both) were defective. At that time we were still unsure about the effectiveness of our method of pollination. We observed apparent open pollination taking place, as pollinating insects (bees, flies etc.) were visiting mango flowers. We sampled open pollinated flowers and found, again, no pollen germination. These unexpected findings prompted us to conduct a survey of the rate of

open pollination in the orchard, during two consecutive years. The results of this survey are presented in Table 6.1.

Table 6.1. Percentage of open pollinated mango flowers that were found to be successfully pollinated (germinating pollen tubes traversing at least the upper third of the style) in 1982 and 1983. Survey conducted in orchards near Bet Dagan, Israel.

Sampling period	Cultivars checked	No. of flowers examined	% of flowers successfully pollinated
21-23.4.82	13-1, Maya, Haden	156	0
25.4-6.5.82	" , " , " .	405	9
12.5-18.5.82	13-1	298	53
31.3-29.4.83	13-1, Turpentine, Haden, Keitt, Maya, Irwin, Palmer.	1443	2
1.5-6.5.83	13-1, Turpentine, Haden, Keitt, Maya, Palmer, Irwin.	764	10
10.5-3.6.83	Turpentine, Haden, Irwin, Keitt, Maya, Palmer.	1057	41

Both monoembryonic cvs ('Haden', 'Irwin', 'Keitt', 'Maya', 'Palmer') and polyembryonic cvs ('Turpentine', '13-1') were sampled. No consistent differences could be noted between the two groups. In both years we found that almost no successful pollination occurred at the first part of the flowering season. Consistently high rates of successfully pollinated flowers were found only in May and early June, during the last 1-3 weeks of the flowering season (Table 6.1). As could be expected, there was a

general trend of warming with the season. However, there had been some warm days and nights during the early flowering period. Thus, good weather conditions for mango pollination occurred also during the period when pollen did not germinate on the stigmas of flowers in the orchard. We made an anatomical study of the mature anthers and pistils, and found a very high rate of defective pollen and ovules at the period when pollination was not successful. We believe that the long period with low minima in March and early April (temperatures at night often dropped to 4-8 C) had a long-term detrimental effect on the reproductive organs.

As a result of this survey, we conducted all of our hand pollination experiments only during the last part of the flowering season, when we could find consistently successful pollination in open pollinated flowers.

3. Hand Pollination Studies in Israel.

In May, 1982, 1252 flowers were pollinaterd on 5 polyembryonic cvs ('Peach', 'Sabre', 'Turpentine', '4-9', and '13-1'). Self and cross pollinations were performed in all cvs. In '13-1' we also compared the effect of foreign monoembryonic pollen ('Maya') to that of foreign polyembryonic pollen ('Turpentine').

Four weeks after pollination only 10 fruitlets survived (0.8% initial set). All surviving fruitlets resulted from cross pollination.

Several additional pollination experiments gave similar negligible initial fruit set. We got useful information from only one pollination experiment. At the end of May (19-23.5) in 1983, 2830 'Turpentine' and '13-1' flowers were hand-pollinated. Self-pollination and cross-pollination, with both monoembryonic pollen and polyembryonic pollen, were performed. The two cvs were located at two sites: '13-1' at Zrifin Experimental Station and 'Turpentine' at the Faculty of Agriculture,

Rehovot. Results are presented in Table 6.2.

Table 6.2. Initial fruit set, 25 days after pollination, of '13-1' and 'Turpentine' flowers.

Cultivars female	Cultivars pollen donor	No. flowers pollinated	No. fruitlets total	No. fruitlets with seeds	% fruitlets set total	% fruitlets set with seeds
13-1	13-1 (self)	500	3	1	0.60	0.20
"	Maya	500	9	7	1.80	1.40
"	Turpentine	500	2	1	0.40	0.20
Turpentine	Turpentine (self)	480	6	3	1.25	0.63
"	Maya	440	31	22	7.05	5.00
"	13-1	410	62	55	15.12	13.41

All of the 25-day-old fruitlets from this experiment were harvested, fixed, embedded in paraffin, cut with a microtome and stained. A few of the fruitlets were ruined during this procedure. The results of the anatomical study are presented in Table 6.3.

Table 6.3. Percent of 25-day-old '13-1' and 'Turpentine' fruitlets with healthy normal embryos and the average number of embryos per seed, found in an anatomical study of fruitlets set after self- and cross-pollination.

Cultivars female	pollen donor	No. fruitlets examined	with normal embryo	% normal fruitlets	Ave. no. of embryos per normal seed
13-1	13-1 (self)	1	1	100.0	6.0
"	Maya	7	4	43.0	6.0
"	Turpentine	0	--	--	--
Turpentine	Turpentine (self)	6	1	17.0 A	6.0
"	Maya	31	22	71.0 B	3.6 a
"	13-1	59	55	93.0 C	4.6 a

A great difference in the amount of seeded fruit set by 13-1 was found between flowers pollinated by 'Maya'pollen (1.4%) and flowers pollinated by 'Turpentine' and '13-1' pollen (0.2%). Though these results may hint that '13-1' is self incompatible and that it is also cross-incompatible with 'Turpentine', the low set does not permit us to state this with full confidence. The much higher initial set on 'Turpentine' (6% of seeded fruitlets) allows us to state more strongly that 'Turpentine' is self-incompatible. The calculated set of fruits with normal embryos (Tables 6.2 and 6.3) was 0.2% after self-pollination, 5% after pollination with 'Maya' pollen and 14% after pollination with '13-1' pollen. The number of embryos per seed was lower in fruitlets resulting from pollination by the monoembryonic cv 'Maya', but the difference was not significant.

4.Hand Pollination Studies in Florida

In 1982 most mango cvs did not flower at all (the result of an unusually warm winter). Two monoembryonic selections (11-25 and 16-18) were hand pollinated (selfed or crossed with polyembryonic 'Ngowe'). None of the self-pollinated flowers set fruit, while of the cross-pollinated flowers, 2% survived to set fruitlets.

The bloom in 1983 was very heavy and 3,780 flowers were hand-pollinated. Unfortunately, the rainfall was unprecedentedly heavy and the mango flowers were constantly wet. This weather may have had a detrimental effect on fruit set. Seven polyembryonic and 4 monoembryonic cvs were pollinated. Results are presented in Table 6.4. Four to six weeks after pollination the rate of fruitlet retention was dismally low (0.13%). Three fruit from this series of pollinations reached maturity, the seeds were planted, and the resultant trees are currently growing in the field at Miami. Fruitlets set after hand pollination in 1983 and 1984 were sampled 14-28 days later, fixed in FAA and prepared for anatomical study (see E.2). Fifty in Miami and about 100 were examined in Israel. Close to 100% were found to be defective, either lacking endosperm and embryo or having degenerate seed tissues.

The year 1985 was a good one for mango in Florida. 1527 hand pollinations were performed on 4 polyembryonic cvs. Results are presented in Table 6.5. From this table we can see that even during a good season, with a heavy mango crop, the rate of success from hand pollination was very low.

Table 6.4. Results of hand pollination of mango at Miami, spring 1983. (P = polyembryonic, M = monoembryonic)

Combination	No.	No. set at 4-6 weeks	Seed matured (%)	
13-1 (P), unpollinated check	65	0		--
13-1 x self	147	0		--
13-1 x Keitt	151	0		--
Cambodiana (P), unpollinated check	47	0		--
Cambodiana x self	79	0		--
Cambodiana x Tommy Atkins	72	0		--
East Indian (P) x self	109	0		--
East Indian x Keitt	106	0		--
Harumanis (P), unpollinated check	40	0		--
Harumanis x self	74	0		--
Harumanis x 13-1	76	0		--
Harumanis x Keitt	108	0		--
Kensington (P), unpollinated check	23	0		--
Kensington x self	62	0		--
Kensington x Keitt	35	0		--
Kensington x Tommy Atkins	36	0		--
M-20222 (P), unpollinated check	265	0		--
M-20222 x self	493	0		--
M-20222 x Keitt	424	0		--
M-20222 x Tommy Atkins	72	0		--
M-20222 x 13-1	75	0		--
Ono (P), unpollinated check	30	0		--
Ono x self	62	1	1	(1.6)
Ono x Tommy Atkins	32	1*	0	(0.0)
Haden (M), unpollinated check	60	1**	0	(0.0)
Haden x self	118	0		--
Haden x Tommy Atkins	200	0		--
Keitt (M), unpollinated check	204	0		--
Keitt x self	502	1	1	(0.2)
Keitt x Vanraj	256	0		--
Keitt x 13-1	129	0		--
Keitt x M-20222	68	1	1	(1.5)
Tommy Atkins (M), unpollinated check	43	0		--
Tommy Atkins x self	114	0		--
Tommy Atkins x Haden	29	0		--
Tommy Atkins x 13-1	57	0		--
Vanraj (M) x Keitt	34	0		--
Totals (excluding checks):	3720	5	3	(0.08)

*Embryo aborted, failed after growth started. **No embryo was apparent.

Table 6.5. Results of hand pollination studies at Miami, spring 1985.

Combination	Number	Initial set		Final set	
		No.	%	No.	%
M-20222 x self	134	1	0.75	0	--
M-20222 x Tommy Atkins	187	1	0.53	0	--
Kensington x self	128	0	0.0	--	--
Kensington x Tommy Atkins	182	0	0.0	--	--
Colombo Kidney x self	85	1	1.2	1	1.2
Colombo Kidney x Tommy Atkins	207	2	1.0	2	1.0
13-1 x self	378	0	0.0	--	--
13-1 x Tommy Atkins	316	0	0.0	--	--
Totals:	1527	5	0.3	3	0.2

5. Studies made with Caged Trees in Israel

Two separate experiments were conducted. The first experiment was carried out in Zrifin Experimental Station, on '13-1' trees. For 2 consecutive years (1982, 1983) the same 8 trees were caged during the flowering season. Four trees were caged without bees and 4 with a beehive. Four uncaged trees were used as open-pollinated controls. The second year, 5 additional '13-1' trees were caged, each with a small tree of 'Turpentine' and a beehive. The rate of successful pollinations was found to be good (30-60%). Results are presented in Table 6.6.

Table 6.6. Number of mature fruits harvested from '13-1' trees caged in 1982 and 1983 with and without a beehive, from '13-1' and 'Turpentine' trees caged together with beehives, and from uncaged trees.

Treatment / Tree	Uncaged '13-1'		Caged '13-1' without beehive		Caged '13-1' with beehive		Caged pairs with beehive	
							'13-1'	Turpentine
	1982	1983	1982	1983	1982	1983	1983	1983
1	56	10	20	2	36	1	3	60
2	76	63	17	12	11	1	15	73
3	62	13	16	34	13	7	17	92
4	76	214	16	0	4	7	19	127
5								186
Ave for 1982	68 A		18 B		16 B			
Ave for 1983		75		12		4	14	108

The second experiment was carried out in 1983, at the Experimental Farm of the Faculty of Agriculture at Rehovot, with small 'Turpentine' trees. Eight trees were caged alone, with and without a beehive. Results are presented in Table 6.7.

Production of caged '13-1' trees in 1982 was about 25% and in 1983 was about 10% of the production of uncaged trees. The presence of bees and a pollenizer did not have any significant effect (Fig. 6.6). The heavy production on the small 'Turpentine' trees showed that caging by itself did not have a detrimental effect on fruit set.

Table 6.7. Number of mature fruits harvested from 'Turpentine' trees caged with and without a beehive, and from uncaged trees.

Treatment / Tree.	Uncaged	Caged	
		without a beehive	with a beehive
1	125	10	34
2	165	16	37
3	128	39	41
4	214	128	265
Ave	158 A	48 C	94 B

The production of caged 'Turpentine' trees compared to that of uncaged, open-pollinated trees was significantly lower, 59% with bees and 30% without bees.

Unexpectedly, in both experiments caged trees devoid of bees set significant numbers of fruits. This may indicate that the isolation of the caged trees was not perfect. Unfortunately, in 1983 we could not use isozyme analysis to check this suspicion.

Discussion

We encountered a lot of unexpected difficulties in our pollination work. As a result, our goals were only partially achieved. High initial set (5%-45%) was achieved by hand pollination in India (3, 8, 11). A massive fruitlet drop reduced the set considerably, later. We planned to harvest the fruitlets ahead of this massive drop, 3-4 weeks after pollination, and to examine them anatomically. A similar approach has been

used in avocado (2). However, in most of our hand pollination experiments the negligible initial set foiled this plan. Young claimed that the cool weather prevailing in Florida during the period of development of the reproductive organs (pollen and ovule) has a detrimental effect on them (17). Our difficulties may be explained by large-scale damage to the reproductive organs.

The results of the only successful hand pollination experiment (Tables 6.2, 6.3) strongly indicate that 'Turpentine' is self-incompatible and that '13-1' pollen appreciably increases its initial set. The low set on '13-1' allows us only to suggest that '13-1' may also be self-incompatible and that 'Turpentine' pollen may elicit a cross-incompatibility reaction. The evidence from the caged '13-1' trees (Table 6.6) supports the speculation that this cv is self-incompatible and that as a female it may be cross-incompatible with 'Turpentine'. The evidence from caged 'Turpentine' trees (Tables 6.6, 6.7) strongly supports the conclusion that '13-1' is an effective pollenizer for 'Turpentine', and may suggest that 'Turpentine' is not fully self-fruitful. The unexpected set that consistently happened in caged '13-1' and 'Turpentine' trees devoid of pollinating bees (Tables 6.6, 6.7) was found also in monoembryonic 'Haden' trees (9). It may be the result of wind pollination (5, 6) or the work of very small pollinating agents (such as thrips) that can easily pass through 15-mesh screen. Isozyme analysis of the seed tissues may resolve the mystery and tell us if foreign pollen was able to reach the caged trees.

Literature Cited

1. Dolev, M. 1979. Self and cross pollination and their effects on fruit set in some mango varieties. M. Sc. Thesis, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel (in Hebrew).

2. Gazit, S. and Gafni, E. 1986. Effect of hand pollination with different pollen donors on initial fruit set in avocado. Israel Agrisearch 1:3-17 (Hebrew with English summary).

3. Gunjate, R. T., Jorwekar, D. P. and Lad, B. L. 1983. Pollination, fruit set and fruit drop in Alphonso mango. J. Maharashtra Agric. Univ. 8:168-170.

4. Oppenheimer, C. 1978. Subtropical fruit trees and their cultivation in Israel. Am Oved, Tel Aviv (in Hebrew). pp. 447.

5. Popenoe, W. 1917. The pollination of the mango. USDA Bur. Plant Introd. Bull. 542

6. Popenoe, W. 1920. Manual of tropical and subtropical fruits. Macmillan, New York. pp. 474.

7. Rahat, M. 1970. Flowering, pollination and fruit set in the 'Haden' mango. M. Sc. Thesis, Faculty of Agricujlture, The Hebrew University of Jerusalem. Rehovot, Israel (in Hebrew).

8. Ram, S., Bist, L. D., Lakhanpal, S. C., and Jamwal, I. S. 1976. Search for suitable pollenizers for mango cultivars. Acta Hort. 57:253-263.

9. Roizman, Y. 1984. The involvement of different factors in the process of pollination, fruit set and embryo development of monoembryonic and polyembryonic mango varieties. M. Sc. Thesis, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel (in Hebrew).

10. Sharma, D. K. and Singh, R. N. 1972. Investigations on self-incompatibility in Mangifera indica L. Acta Hort. 24:126-130.

11. Singh, L. B. 1960. The mango; botany, cultivation and utilization. Interscience Publ. Inc., New York. pp. 438.

12. Singh, R. N. 1954. Studies in floral biology and subsequent development of fruit in the mango (Mangifera indica L.) varieties Dashehari and Langra. Indian J. Hort. 11:69-88.

13. Singh, R. N. and Sharma, D. K. 1972. Studies on some pollination problems in mango. Acta Hort. 24:134-138.

14. Tomer, E. and Gottreich, M. 1975. Observation on the fertilizastion process in avocado with fluorescent light. Euphytica 24:531-535.

15. Young, T. W. 1942. Investigations of the unfruitfulness of Haden mango in Florida. Proc. Fla. State Hort. Soc. 55:106-110.

16. Young, T. W. and Ledin, R. B. 1954. Mango breeding. Proc. Fla. State Hort. Soc. 67:241-244.

17. Young, T. W. Mango research on sandy soils. Proc. Fla. Mango Forum 1957:27-30.

E. 7. Effect of Growth Regulators on Fruit Set

Introduction

Sprays with a long list of growth regulators were reported to increase mango fruit set and to decrease fruit drop (1, 2). We hoped to use growth regulators both to increase initial set and to influence the formation and survival of the zygotic and nucellar embryos.

Materials and Methods

The following growth regulators were used:

Gibberelic acid (GA3) at 250 and 1250 ppm;

Gibberellins A1 and A7 at 250 ppm;

Naphthalenacetic acid (NAA) at 25, 100 and 250 ppm;

2,4,5-trichlorophenoxyproprionic acid (2,4,5-T.P.) at 20, 100 and 250 ppm;

Benzylamino purine (BAP) at 25 and 100 ppm;

GA3 250 ppm + NAA 100 ppm;

GA3 250 ppm + NAA 100 ppm + BAP 25 ppm.

Triton X-100 at 0.05% was included in all solutions.

Results

Inflorescences were sprayed on 3 polyembryonic cvs ('Gedong', 'Peach' and '13-1') and 2 monoembryonic cvs ('Haden' and 'Maya'). Flowers at anthesis were labelled on the day of spraying.

The results were a complete disappointment. None of the treatments were found to increase the set or to prevent dropping of fruitlets. At the end of the flowering season, not one treatment showed any positive effect.

Discussion

We hoped that growth regulators would be able to prevent the massive

drop of seeded fruitlets. Our discouraging results with auxins, gibberellins and cytokinin sprays, coupled with the consistent minuscule set of seeded fruitlets from hand pollination, convinced us to abandon this futile approach.

Literature Cited

1. Ram, S. 1983. Hormonal control of fruit growth and fruit drop in mango cv Dashehari. Acta Hort. 134:169-178.

2. Singh, L. B. 1960. The mango; botany, cultivation and utilization. Interscience Publ. Inc., New York. pp. 438

F. Description of Cooperation

Information about research techniques, difficulties encountered, results and ideas were freely exchanged between the two cooperating scientists. Dr. Gazit went to Miami in 1984 and Dr. Knight's visit to Bet Dagan and Rehovot in 1985, provided ample opportunity for a wide range of discussions on the research. About 100 fruitlets, set after hand pollination in Miami, were processed and anatomically examined in Rehovot.

G. Evaluation of Research Achievements

1. Reliable data on the phenomenon of polyembryony in a large number of monoembryonic and polyembryonic mango cvs were amassed. This information corrects some erroneous information found in the literature. All monoembryonic cultivars produced 100% monoembryonic seeds. Thus, no nucellar seedlings should be expected from this group. 'Ruppin' previously was recommended as a polyembryonic rootstock. We found this cv to be practically monoembryonic. Some genuinely monoembryonic seeds were observed in several polyembryonic cvs. They should be checked to determine whether they are of nucellar or zygotic origin.

2. Mango ovules were successfully cultured on solid modified Murashige and Skoog media. When the ovules were at a stage in which no nucellus could be identified, precocious germination occurred. This technique may be used to maximize the rate of embryo germination and survival.

3. Somatic embryogenesis was achieved from cultured nucellus of polyembryonic cvs. This may lead to wide-scale vegetative propagation by tissue culture.

Tissue culture work had not been included in the original research

plan. However, when faced in 1982 with a very scanty fruit set in Miami we decided to use the small number of fruitlets in the most effective way. Dr. Litz from the University of Florida, at Homestead, was ready to collaborate and went to work with the plant material that Dr. Knight supplied.

4. Isozyme analysis was used successfully to identify the zygotic seedlings from tagged '13-1' seed "families". The location of the emerging zygotic seedling was found not to differ from that of its emerging nucellar siblings. We found almost all seedlings, zygotic as well as nucellar, to emerge from the proximal part of the seed. Thus, Tammes' suggestion to cut off the proximal part of the seed, in order to get rid of the sexual embryo, will result in destroying all nucellar embryos as well, in most '13-1' seeds.

5. Dismal results from hand pollination let to a study of factors affecting successful mango pollination in Israel. We were surprised to find that in the coastal plain of Israel, successful pollination (and fruit set) occur only at the end of the flowering season. This dismal situation emphasizes the urgent need to develop methods to delay the flowering season.

6. Our studies (hand pollination with anatomical examination of the resulting young fruitlets, and of fruitlets set on caged trees) indicate that both 'Turpentine' and '13-1' are self-incompatible and that though '13-1' is apparently a good pollenizer for 'Turpentine', 'Turpentine' is apparently a poor pollenizer for '13-1'. These conclusions have great importance for breeding new rootstocks, and for seed production of these leading rootstocks in Israel and Florida.

H. List of Publications

1. Gazit, S. and Roizman, Y. 1989. Factors responsible for inadequate successful pollination under subtropical climatic conditions. (A paper to be delivered at the 3rd Int. Mango Symposium and published in Acta Hort.)

2. Litz, R. E., Knight, R. and Gazit, S. 1982. Somatic embryos from cultured ovules of polyembryonic Mangifera indica L. Plant Cell Rep. 1:264-266.

3. Litz, R. E., Knight, R. and Gazit, S. 1984. In vitro somatic embryogenesis from Mangifera indica L. callus. Scientia Hortic. 22:233-240.