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Differential expression of polyembryony in certain mango genotypes

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ABSTRACT

In the present study, an attempt was made to understand the expression of polyembryony in 21 polyembryonic genotypes of *Mangifera indica* and two related species namely *M. zeylanica* and *M. odorata.* The results revealed the significant variations in the number of segments per kernel, number of seedlings arising from a kernel and the vegetative growth of the seedlings. The Chengavarikai genotype (9.25) had the highest number of segments in kernel followed by Turpentine (7.33) while the least (2) were recorded in Kitchener. Maximum number of seedlings emerged per kernel in Moreh (2.86) followed by Moovandan (2.82) whereas *M. odorata* (1.20) recorded the least followed by Vattam (1.27) and Kitchener (1.29). All season (29.41) was fastest in germination followed by Moovandan (30.25); on the other hand, Carabao needed the maximum number of days (45.12) for germination. Highest germination was recorded in All season (94.29) followed by Moovandan (88.89), whereas the lowest was in *M. zeylanica* (6.90) followed by Starch and Prior (10.00). No variations in colour of emerging leaf was observed among the progeny of each genotype. Plant height, rate of leaf emergence, seedling girth and vigour index were more in Moovandan, Bappakai, All season and Gomavu while Peach, Prior, Corabao, *M. zeylanica*, and Vellaikolumban were less vigorous. The earlier emerging seedlings were more vigorous than later emerging ones within each genotype. No xenic effect was observed on expression of polyembryony in controlled pollination studies involving monoembryonic genotypes as male and *vice versa*.

Key words: *Mangifera indica* L.*,* polyembryony, zygotic embryo, xenia

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the choicest fruits cultivated in tropical and sub-tropical regions of the world. The mango has been classified as monoembryonic types (Indian types) and polyembryonic types (South East Asian types) based on the number of embryos present in a seed (Mukherjee, 12). In 'monoembryonic' mango, seed contains a single embryo that develops from the zygote, giving only one seedling. In some varieties, multiple embryos develop in an individual seed, and consequently many seedlings are produced by a type of apomixes, wherein autonomous development of supernumerary embryos occur in the ovule. Polyembryony was first reported by Leeuwenhoek in citrus as early as 1719. Subsequently, this phenomenon has been reported in 59 families, 158 genera and 239 species. Among fruit crops, polyembryony occurs in citrus (Frost, 4), mango (Sachar and Chopra, 17) and strawberry (Lebegue, 10). The occurrence of polyembryony depends on species and varieties; in other words not all species of a genus exhibit polyembryony, and not all varieties in a species show polyembryony, and the reason could be genetic. Adventitious embryo development from nucellus commonly observed in mango is helpful for clonal propagation (Kobayashi *et al.,* 7). Many mango varieties especially those grown along sea coast have polyembryony in India. Various workers observed that the number of seedlings that can be raised from a single polyembryonic seed is much less than the number of embryos contained in the seed. The criteria for consideration of polyembryony based on the number of seedlings emerging from single stone followed in most of the earlier studies may not be always appropriate as not all of the many embryos present grow up to seedling due to several factors; hence, the polyembryony could be better assessed based on number of segments that represents the embryos per kernel. In citrus, neither the seed setting nor the formation of the nucellar embryos takes place without pollination of flower. The development of nucellar embryos is induced by fertilization (Koltunow, 8). Polyembryony is also reported to be affected by the type of pollinators, pollen viability, plant nutrition, temperature, environmental and soil humidity. Therefore, any factor that affects pollination, fertilization or seed development could also affect the embryo number per seed .

Identification of nucellar and zygotic seedlings at juvenile stage using morphological traits is extremely difficult, though the seedlings possessing the true to type characters of mother plant would be nucellar in origin, while the seedling showing variation would

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be zygotic. There is considerable variation among zygotic seedlings and their visual recognition on the basis of height, leaf size, thorn length, petiole length and stem diameter is extremely difficult. The zygotic seedling is weakest in polyembryonic mango seed, because it probably getting degenerated due to vigorous growing seedlings from nucellar embryos (Sachar and Chopra, 17). For propagation of mango, it is important to use nucellar seedlings which are true to the type and uniform (Sturrock, 20). Nucellar seedlings also give the beneficial traits of clonal rootstocks with the added advantages of taproot system providing better soil anchorage and drought tolerance compared to clonally propagated rootstocks obtained by other vegetative means. Nucellar seedlings of mango varieties vary in their salinity tolerance (Nimbolkar *et al.,* 13), and affecting scion vigour. In the present study, efforts were made to understand differential expression of polyembryony in open pollinated seeds of 21 polyembryonic genotypes of *Mangifera indica* and two related species namely *M. zeylanica* and *M. odorata,* and to examine the effect of pollen from certain monoembryonic varieties on the polyembryonic genotypes and *vice versa* for the expression of polyembryony through controlled crossing of flowers.

MATERIALS AND METHODS

An experiment was conducted at ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bengaluru-560089, located at 13 °N and 17°37′ E, 890 m above mean sea level (MSL). The mature fruits were collected from open-pollinated trees of twenty-one known polyembryonic genotypes of *Mangifera indica* as well as two related species of *Mangifera viz*., *M. zeylanica*, *M. odorata* (Table 2). One hundred stones from each genotype were extracted, washed, dried (under shade), disinfected with carbendazim 0.1% treatment, de-husked and sown in nursery bed after counting the visible number of segments on the kernels. Observations on days to germination and the seedlings emerged per kernel up to 45 days after sowing were noted, and the germination % was calculated. Number of seedlings per kernel were individually counted after uprooting at the time of transplanting, after 45 days of germination. The seedlings from each kernel were separated and all the seedlings from each stone were transplanted into polythene bags of size 6”×8” containing mixture of soil, sand and FYM in 1:1:1 ratio. The experiment was arranged in randomized block design with three replications, and in each replication, 10 plants were selected for recording the growth data. The growth parameters were recorded at an interval of 30 days up to 180 days. Plant height was measured using meter scale. Plant girth (mm) was measured using ‘Vernier caliper; rate of leaf emergence was counted manually; colour of emerging leaf determined using RHS colour chart and vigour index-I was calculated at 180 days [Germination (%) × seedling height]. An illustration of the polyembryony and monoembryony is depicted in Figure 1 A and B.

For studying the xenic effect in mango, controlled pollination was done in two polyembryonic genotypes namely Vellaikolumban and Olour as female and seven monoembryonic cultivars as a male parents and *vice versa* (Table 1). For controlled hand pollination, crosses were made following hand emasculation and pollination (Mukherjee *et al.,* 11; Dutta *et al.,* 3). The statistical analysis of data was done using web-based agricultural statistics software package (WASP 2.0) developed by ICAR - Central Coastal Agricultural Research Institute, Goa.

RESULTS AND DISCUSSION

The results revealed that Chengavarikai had the most segments per kernel (9.25) followed by Turpentine (7.33) while the least was in Kitchener (2 segments / kernel as the kernel looked like monoembryonic but produced multiple seedlings). Most seedlings per stone emerged in the Moreh (2.86) followed by Moovandan (2.82) whereas *M. odorata* recorded the least (1.20) followed by Vattam (1.27) and Kitchener (1.29) (Fig 2). The results revealed significant variation on the extent of polyembryony in terms of number of segments per

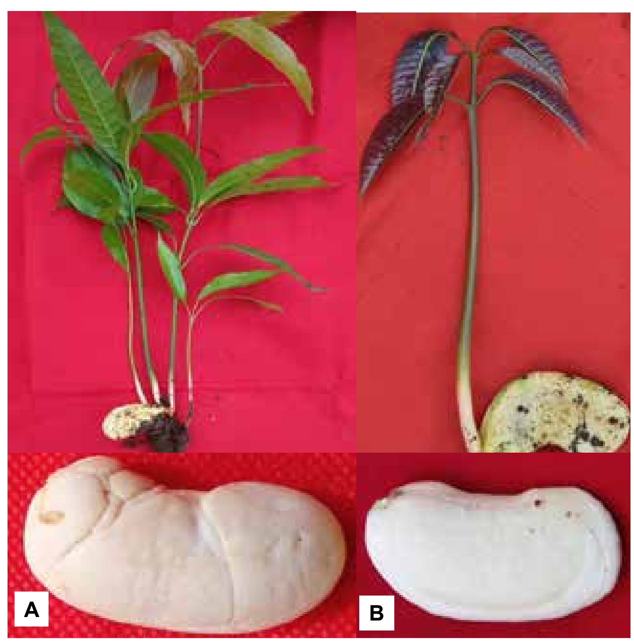

Fig. 1. (A) Polyembryonic genotype Turpentine and (B) Monoembryonic cv. Totapuri.

Table 1. Polyembryonic and monoembryonic cross combinations used for studying the xenic effect on expression of polyembryony

1	Vellaikolumban × Alphonso	Olour × Alphonso	Totapuri × Olour
2	Vellaikolumban × Dashehari	Olour × Dashehari	Totapuri × Vellaikolumban
3	Vellaikolumban × Totapuri	Olour × Totapuri	
4	Vellaikolumban × Banganapalli	Olour × Banganapalli	
5	Vellaikolumban × Neelum	Olour × Neelum	
6	Vellaikolumban × Rumani	Olour × Rumani	
7	Vellaikolumban × Amrapali	Olour × Amrapali	

Table 2. Days to germination, germination percentage and colour of emerging leaf of different polyembryonic mango genotypes.

Genotypes	Days to germination	Germination (%)	Colour of emerging leaf	Range	
				No. of segments/kernel	No. of seedlings emerged/stone
Vellaikolumban	34.51	57.89	Greyed orange Grp 177 C	2-10	1-4
Bappakai	32.78	84.00	Greyed Purple Grp 187 A	3-9	1-3
Moreh	37.45	60.00	Greyed Purple Grp 187 A	3-12	1-6
All Season	29.41	94.29	Greyed Yellow Grp 160 C	2-6	1-7
Kensington	43.32	60.00	Greyed orange Grp 177 B	3-12	1-5
Manipur	40.15	80.00	Greyed Yellow Grp 162 A	2-8	1-5
Moovandan	30.25	88.89	Greyed Yellow Grp 160 A	3-7	1-5
Carabao	45.12	15.00	Greyed Purple Grp 187 A	3-8	2-3
Chandrakaran	40.42	33.33	Greyed Purple Grp 187 B	2-7	1-5
Olour	33.52	58.62	Greyed Orange Grp 170 A	3-15	1-6
Peach	36.85	20.00	Grey Brown Grp 199 A	2-9	1-5
Starch	38.45	10.00	Greyed orange Grp 166 A	3-10	1-3
Prior	35.56	10.00	Greyed orange Grp 165 A	2-11	1-3
Vattam	39.24	40.63	Greyed Yellow Grp 160 C	3-9	1-2
Turpentine	31.52	50.00	Greyed Purple Grp 187 A	2-11	1-5
Thali	34.51	20.00	Greyed orange Grp 166 A	3-7	1-2
Chengavarikai,	33.12	50.00	Greyed orange Grp 174 A	5-13	1-8
IRS	36.45	50.00	Greyed orange Grp 166 A	3-9	1-4
Kitchener	34.52	30.00	Greyed orange Grp 166 A	2	1-3
Kurukkan	41.52	60.00	Greyed orange Grp 165 A	3-8	1-3
Gomavu	35.74	57.14	Greyed orange Grp 165 B	3-7	1-2
M. Odorata	31.14	27.27	Greyed Purple Grp 187 C	2-8	1-2
M. zeylanica	37.41	6.90	Greyed orange Grp 177 A	3-10	1-7

kernels and number of seedlings per stone among the 21 polyembryonic genotypes of *Mangifera indica* and its two related species namely *M. zeylanica* and *M. odorata.* Similar variations observed based on number of segments per kernels, Manila and Ataulfo cultivars of mango having an average 3.4 and 3.2 embryos per seed respectively with more than 80% seeds having 2 to 4 embryos per seed was represented by Ochoa *et al.* (14). Our study recorded most of seedlings emerged per stone in Moreh. Sane *et al.* (18) observed maximum number of seedlings per stone in Moreh and Nekkare

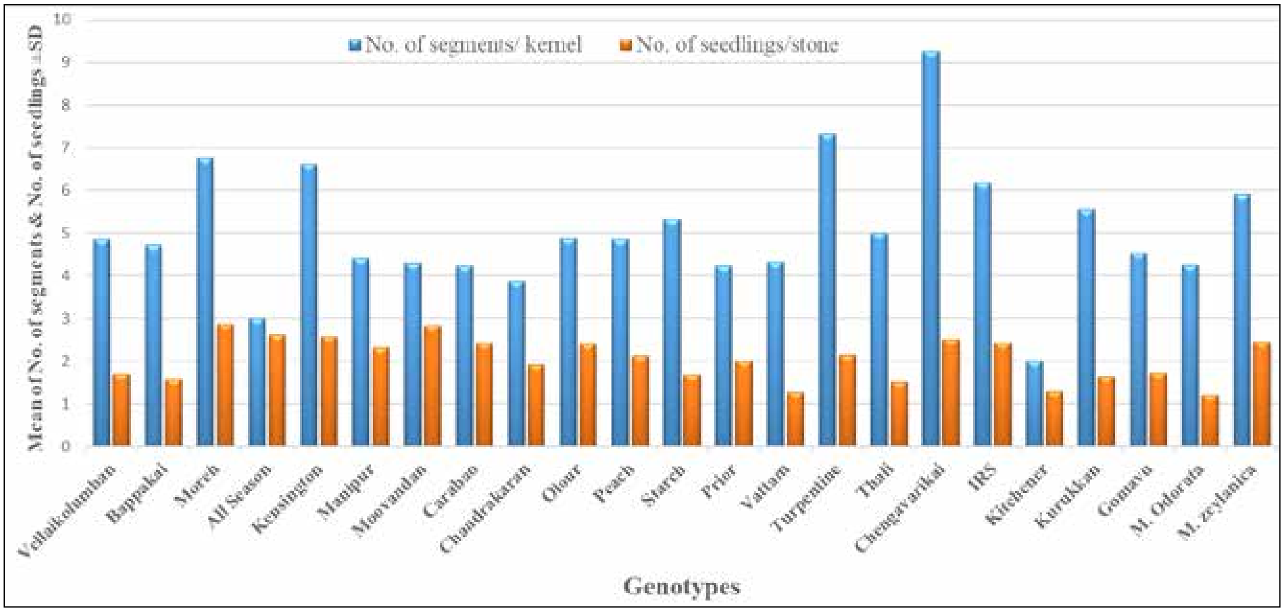

Fig. 2. Number of segments/kernel and number of seedlings/stone.

among seven open pollinated polyembryonic genotypes (Nekkare, Moreh, Olour, Vellaikolumban, Kurukkan, Peach and Bappakai). Variation in extent of polyembryony were reported by Abirami *et al.* (1), Shaban (19) and Kumar *et al.* (14). However, Rao and Reddy (15) reported that genotype Peach recorded the maximum polyembryony while least was with Kurukkan among 14 genotypes viz Starch, Kitchener, Prior, Chandrakaran, Nekkare, EC-95862, Olour, Moovandan, Bappakai, Peach, Kensington, Mylepelian, Kurukkan and Vellaikolumban. The number of seedlings per seed varied with the cultivar and environmental conditions (Andrade-Rodríguez *et al.,* 2). Sturrock (20) observed that in polyembryonic stone, variation in sprouts was due to aberration of embryo, which is mediated through other extraneous factors. The number and type of embryo produced varied from tree to tree and also at different positions on a single tree. The variation has been suggested to be controlled by minor genes, pollen sources and environmental conditions (Khan and Roose, 5). Our present study, more comprehensive than the earlier reported ones involving the number of embryos present as well as the number of seedlings emerged per kernel in several polyembryonic genotypes at a time, established that not all embryos present in a stone of polyembryonic genotype succeed to germinate and grow to seedlings and that the variation in expression of polyembryony was not due to xenia.

The data on number of days to germination, germination percentage, colour of emerging leaf, and number of seedlings per stone in different polyembryonic genotype are presented in Table 2. All season took least days (29.41) for germination followed by Moovandan (30.25) with most of the genotypes taking 30 to 40 days, but Carabao needing the maximum (45.12). Maximum germination percentage was also in All Season (94.29 %) followed by Moovandan (88.89%) while minimum was in *M. zeylanica* (6.90%) followed by Starch and Prior (10.00). Colour of emerging leaf were in different groups like greyed orange, purple and yellow in all the genotypes except for Peach in Greyed brown group. Each genotype had the same leaf colour among its multiple seedlings; hence, colour of emerging leaf cannot distinguish the nucellar from zygotic seedlings, or alternatively all emerged seedlings could be nucellar. The similar findings on germination aspects were also reported by Sane *et al*. (18). The cultivars with less stone weight exhibited slow germination due to less endosperm content in the stone, which might have supplied less nutrient and food material for germination (Rao and Reddy, 16). The differing results to our study on germination were reported by Kumar *et al.* (9) noticing highest germination in Kurukkan followed by Olour and Shaban (19) recording maximum in Sabre followed by 13-1 and minimum in Sukkary.

Among the polyembryonic genotypes, Bappakai (34.0 cm) showed maximum plant height followed by Gomavu (32.9cm) and minimum was in Prior (12.2cm) followed by *M. zeylanica* (12.4cm), Olour (13.1cm) and Carabao (13.2cm) at 30 DAT. At 180

DAT, the plants were tallest in Moovandan (54.1cm) followed by Bappakai (53.5cm), All Season (52.7cm) and Gomavu (51.5cm), the shortest being in Peach (28.2cm) followed by Prior (28.4cm). The rate of leaf emergence varied with genotype, Bappakai (11.0) recording the highest and it was lowest in Chandrakaran (2.6) followed by Vellaikolumban (3.2) at 30 DAT. At 180 DAT, *M. zeylanica* (21.9) followed by IRS (21.0) recorded the higher rate of leaf emergence than Vellaikolumban (11.6) and Starch (12.0). At 30 DAT, Bappakai (4.8mm) recorded higher plant girth followed by Thali (4.3mm), while it was lower in Prior (1.4mm) followed by Carabao (1.6mm). Bappakai (8.1mm) and Chengavarikai (7.6mm) had higher plant girth, while it was lowest in Carabao (4.2mm) followed by Starch (4.7mm) at 180 DAT. Significant differences were observed for vigour index-I among different genotypes at 180 DAT, being highest in All Season (4978.00) followed by Moovandan (4816.62) and Bappakai (4496.77) and the lowest was registered in *M. zeylanica* (256.00) (Table 3). Different plants high or low growth potential seems to be due to the stone characteristics in terms of size and weight as well as faster germination. The present findings on growth parameters conform with that of Sane *et al*. (18). The findings by Rao and Reddy (16) revealed that maximum seedling height and number of leaves were recorded in Mylepelian

Table 3. Growth parameters of different polyembryonic mango genotypes recorded after transplanting.

Genotypes	Plant Height (cm)		Rate of leaf emergence		Plant Girth (mm)		Vigour Index-I
	30 Days	180 Days	30 Days	180 Days	30 Days	180 Days	180 Days
Vellaikolumban	22.6^{bcd}	44.6^{cd}	3.2^{jk}	11.6^{h}	3.2^{efgh}	6.2^{efg}	2582.11
Bappakai	34.0^{a}	53.5^{a}	11.0^{a}	19.1^{abcde}	4.8^{a}	8.1^{a}	4496.77
Moreh	15.5^{fghij}	36.4^{fgh}	5.2^{efghi}	18.8^{abcde}	2.3^{ij}	6.2^{efg}	2184.60
All season	24.3^{bc}	52.7^{ab}	4.2^{hijk}	18.5^{abcdef}	3.0^{fghi}	6.6^{cdef}	4978.00
Kensington	22.0^{bcd}	36.5^{fgh}	4.8^{fghij}	15.8^{efgh}	3.7^{bcde}	5.9^{fgh}	2191.98
Manipur	22.6^{bcd}	38.2^{defg}	4.7^{fghij}	15.6^{efgh}	3.0^{efghi}	5.5^{ghi}	3056.00
Moovandan	26.7^{b}	54.1^{a}	6.0^{cdefg}	20.8^{abc}	3.4^{defg}	7.0^{bcd}	4816.62
Carabao	13.2^{hij}	31.6^{hi}	4.8^{fghij}	14.8^{fgh}	1.6^{kl}	4.2^{j}	475.20
Chandrakaran	18.4^{defgh}	37.2^{fgh}	2.6^{k}	12.2^{h}	2.7^{hij}	5.3^{hi}	1243.10
Olour	13.1^{ij}	38.6^{defg}	3.8^{ijk}	19.6^{abcde}	2.9^{fghi}	6.1^{fg}	2262.76
Peach	14.7^{ghij}	28.2^{i}	4.8^{fghij}	16.8^{bcdefg}	2.8^{ghij}	5.6^{gh}	565.40
Starch	16.4^{efghij}	32.4^{ghi}	5.6^{defgh}	12.0^{h}	3.4^{defg}	4.7^{ij}	324.10
Prior	12.2^{j}	28.4^{i}	4.6^{ghij}	15.4^{efgh}	1.4^{l}	5.2^{hi}	284.17
Vattam	20.3^{cdef}	46.4^{bc}	6.4^{bcdef}	17.1^{bcdefg}	3.7^{bcde}	6.5^{cdef}	1885.93
Turpentine	16.4^{efghij}	34.6^{fghi}	6.2^{cdefg}	16.5^{defg}	3.4^{defgh}	6.7^{cdef}	1734.35
Thali	19.9^{cdefg}	35.6^{fgh}	7.5^{bc}	16.8^{cdefg}	4.3^{ab}	6.5^{def}	712.00
Chengavarikai	21.0^{cde}	44.3^{cde}	6.8^{bcde}	18.8^{abcdef}	4.0^{bcd}	7.6^{ab}	2215.65
IRS	19.7^{cdefg}	38.0^{efgh}	6.4^{bcdef}	21.0^{ab}	3.5^{cdef}	7.0^{bcde}	1903.15
Kitchener	17.9^{defghi}	33.4^{ghi}	5.7^{defgh}	18.2^{abcdef}	3.9^{bcd}	7.0^{bcde}	1003.11
Kurukkan	15.9^{efghij}	35.8^{fgh}	7.0^{bcd}	16.8^{bcdefg}	3.4^{defgh}	7.3^{abc}	2149.20
Gomavu	32.9^{a}	51.5^{ab}	7.9^{b}	20.3^{abcd}	4.2^{abc}	7.0^{bcde}	2947.83
M. Odorata	19.8^{cdefg}	41.0^{cdef}	5.6^{defgh}	13.6^{gh}	3.5^{cdef}	6.5^{def}	1120.01
M. zeylanica	12.4^{j}	37.1^{fgh}	5.3^{efghi}	21.9^{a}	2.1^{jk}	5.6^{gh}	256.00
C.D.	5.3	6.5	1.7	4.1	0.7	0.7	
SE(m)	1.8	2.2	0.6	1.4	0.2	0.2	
SE(d)	2.6	3.2	0.8	2.0	0.3	0.3	
C.V.	16.4	9.9	18.4	14.6	12.9	7.4	

Note: Each value represents the mean value of ten plants. NS indicates non-significant differences among the genotypes at p= 0.05. Values represented with at least one common letter as superscript are not statistically different at $P \leq 0.05$ using DMRT.

Table 4. Number of segments/kernel and number of seedlings/stone, for different cross combinations.

Cross Combination	No. of segments/ Kernels	No. of seedlings/ Stone	Cross Combination	No. of segments/ Kernels	No. of seedlings/ Stone	Cross Combination	No. of segments/ Kernels	No. of seedlings/ Stone
	Mean ± SD	Mean± SD		Mean± SD	Mean± SD		Mean±SD	Mean±SD
Vellaikolumban x Alphonso	5.75±2.03	2.70±1.24	Olour x Alphonso	6.00±1.41	4.00±0.00	Totapuri x Olour	1±0	1±0
Vellaikolumban x Dashehari	4.20±1.58	2.29±0.99	Olour x Dashehari	4.20±1.58	1.67±0.58	Totapuri x Vellaikolumban	1±0	1±0
Vellaikolumban x Totapuri	5.26±2.07	2.61±1.09	Olour x Totapuri	4.85±1.53	2.61±1.09			
Vellaikolumban x Banganapalli	4.85±1.53	2.08±0.95	Olour x Banganapalli	5.00±0.71	1.50±0.50			
Vellaikolumban x Neelum	5.40±2.30	1.67±0.58	Olour x Neelum	5.14±2.13	2.33±1.15			
Vellaikolumban x Rumani	4.71±2.27	2.25±1.26	Olour x Rumani	5.75±2.03	3.00±0.00			
Vellaikolumban x Amrapali	6.00±0.82	3.00±0.00	Olour x Amrapali	6.25±0.71	1.50±1.00			

and minimum in Starch while the vigour index-I was highest in Vellaikolumban and lowest in Kitchener among the 14 polyembryonic genotypes. Abirami *et al.* (1) observed maximum seedling height, girth and number of leaves in Nekkare and concluded that Starch, Peach and Kurukkan were the less vigorous genotypes. Khobragade *et al*. (6) noted Peach as dwarf genotype.

The number of segments per kernel and number of seedlings emerged per stone recorded in seeds obtained by crossing Vellaikolumban and Olour with seven male parents, indicated little influence of pollen parent on expression of polyembryony. In the crosses involving monoembryonic Totapuri as female with polyembryonic Vellaikolumban or Olour as the male, there was no influence of pollen parent on expression on polyembryony. From these results, it can be concluded that there is no xenic effect in mango for the expression of polyembryony (Table 4).

The differential expression of extent of polyembryony in twenty one polyembryonic genotypes of *Mangifera indica* and 2 related species *viz*; *M. zeylanica* and *M. odorata* revealed that Moreh, Moovandan, Chengavarikai, All season and Turpentine genotypes showed high extent of polyembryony and also early germination and germination percentage. It was not possible to distinguish the nucellar and zygotic seedlings with the colour of emerging leaf. The genotype Moovandan, Bappakai, All season and Gomavu were highly vigorous whereas the Peach, Prior, Corabao, M. *zeylanica*, and Vellaikolumban were less vigorous genotypes. Further, this study has also proved that there was no xenic effect on expression of polyembryony in mango and that not all embryos succeed to germinate and grow to seedlings; hence polyembryony need to be assessed by the number of segments present in each kernel rather than the number of seedlings emerging out of it.

AUTHORS' CONTRIBUTIONS

Material preparation, implementation of research work, data collection and analysis were (NMK, RMK, MS); Draft of the manuscript (NMK, RMK, MS) and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

DECLARATION

The authors declare no conflict of interest

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