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An Analysis of Pollen Parameters, Fruit Set and Fruit Development During Attempts on Intergeneric Hybridization of Papaya (*Carica papaya* L.)

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Authors' contributions

This work was done in collaboration among all the six authors. Authors KS and AJJ designed the study. Author RMA performed the experiment, statistical analysis and wrote the first draft of the manuscript. Authors KS and AJJ supervised the study and analysed the data. Authors PP, PM and CNC corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Research was conducted at the University Orchard, Horticultural College and Research Institute, to investigate the extent of pollen production, pollen fertility, fruit set, fruit growth and seed set as well as seedling vigour when two wild relatives of papaya (*Vasconcellea cauliflora* and *Vasconcellea candamarcensis*) are involved in the hybridization programme as male parents with three papaya cultivars (CO 7, TNAU Papaya CO 8 and Pusa Nanha) as female parents. Among the two wild

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relatives, *V. candamarcensis* was more polleniferous but with significantly lesser pollen viability and germination than *V. cauliflora*. Among the six intergeneric crosses, although the fruit set ranged from 60.00% to 93.33% the fruit retention till harvest was only 30.77 per cent to 35.71 per cent. The study also revealed metaxenic influence of wild parent on the growth of the fruits derived after crossing. Seed set was observed only with CO 7 as female parent but the seed size and weight, germination and seedling vigour recorded by intergeneric crosses of CO 7 with *Vasconcellea* were found to be low. The implications of the observations are discussed in relevance to employing the wild relatives for improvement of papaya.

Keywords: Papaya; Vasconcellea; PRSV; intergeneric hybrid; pollen fertility, fruit and seed set.

1. INTRODUCTION

Papaya (*Carica papaya* L., *Caricaceae*) is an economically important fruit crop being grown in the tropics and subtropics for its nutritionally rich fruits and high income returns. It is also commercially exploited for the 'papain' a proteolytic enzyme tapped from the fruits and used for pharmaceutical, textile and beverage industries [1]. The devastating incidences of the Papaya Ring Spot disease caused by Papaya Ring Spot Virus (PRSV) across the different growing regions in the world including India is considered as being most challenging problem to be effectively addressed [2-3]. PRSV is easily transmitted by aphids (majorly, *Myzus persicae*) in a non-persistent manner. Efforts to develop PRSV resistant varieties, so far has not been very successful by conventional hybridization. However, the genetic engineering approach has resulted in development of PRSV resistant varieties such as 'Sun Up' and 'Rainbow' [4,2]. Nevertheless the public sentiments and government policies are not yet in favour of adoption of Genetically Engineered (GE) varieties of food crops so far in India. Hence, conventional hybridization involving wild relatives of papaya having resistance to PRSV disease is the only available alternate approach for developing resistant varieties.

The *Vasconcellea* genera was earlier grouped with *Carica* genera only, but based on the chloroplast DNA characteristics, almost all the *Carica* species except *C.papaya* are now grouped under *Vasconcellea* [5]. Two wild relatives of papaya belonging now to *Vasconcellea* genera (*V. cauliflora and V.candamrcensis*) are known to possess resistance to PRSV by earlier workers [6-8]. The *V. candamarcensis* is also a possible genetic resource for improving cold tolerance in the cultivated varieties [9,6,10,11]. Hence, attempts were made in the present study to involve them for hybridization programme to generate PRSV resistant hybrids. The problem often encountered with conventional wide hybridization is that the extent of fruit and seed set is very low or almost nil. Before taking up large scale hybridization program using wild relatives, it may be essential to have preliminary studies on the extent of fruit set or seed set that one may achieve when such attempts are made. This will help to strategically plan hybridization program as well as identify means and ways of improving the seed set. Hence in the study, investigations was embarked on to determine the extent of pollen production, pollen fertility, fruit set, fruit growth and seed set as well as seedling vigour when these two wild relatives of papaya; *V. cauliflora* and *V. candamarcensis* are involved in the hybridization programme as male parents.

2. MATERIALS AND METHODS

The hybridization attempts were made at the University Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, using a gynodioecious variety, CO 7 and two dioecious varieties *viz*., TNAU papaya CO 8 and Pusa Nanha as female parents and the PRSV resistant crop wild relatives *viz*., *Vasconcellea cauliflora* and *V. candamarcensis* as male parents. Since, *V. candamarcensis* is a highland papaya, it is maintained at Horticultural Research Station, TNAU, Ooty, at the Nilgiris District of Tamil Nadu, having mild temperate conditions and located at an altitude of 2240 m above mean sea level at a distance of about 86 km from Coimbatore. The cultivated papaya varieties and *V. cauliflora* are maintained at the germplasm bank at the Coimbatore (411 m above MSL) having tropical climatic conditions and moderately cool weather during winter. The salient features of the parents used in the study were as follows.

CO 7

A gynodioecious variety developed through multiple crosses between CP 75 (Pusa Delicious x CO 3) and Coorg Honey Dew. The fruits are oblong in shape with attractive red colour pulp. It produces an average of 110 fruits per tree with an estimated yield of 180 t/ha.

TNAU Papaya CO 8

A red pulped dioecious variety suitable for dessert and papain purpose. It is derived through improvement of CO 2 with the high yielding potential of 220-230 t/ha with an average of 80 fruits per tree.

Pusa Nanha

A very dwarf dioecious variety highly suitable for high density planting and kitchen gardening. It is developed through mutation breeding. The average plant height is 120 cm and the fruiting starts at a height of 30-40 cm. The fruits are small to medium in size with yellow coloured pulp. The yield per plant is 25 to 30 kg and the estimated yield is about 60-65 t/ha.

Vasconcellea cauliflora

A wild species, semi-dwarf plant (0.89 m); dioecious in nature; bearing non-edible small sized fruits with an average fruit length and width was 6.8 cm and 3.7 cm, respectively. The fruits are oblong in shape with ridges on it [12]. It was reported to be a resistant source for PRSV [6,7,8].

Vasconcellea candamarcensis (Syn: Vasconcellea pubescens)

This wild relative is tall statured, polygamous in nature and bears small sized fruits with ridges on it; adapted to cool hill regions of Nilgiris in Tamil Nadu; reported to be a resistant to PRSV and tolerant to cold [9,6,10,11].

2.1 Pollen Parameters

The study on pollen grains includes the pollen output per anther, pollen viability and pollen germination. The unopened flowers (which are in the process of flowering the next day) of the parents involved in this study were collected at early morning between 06.30 and 07.30 am and the flowers were then dissected in laboratory for pollen grain collection. In case of *Vasconcellea candamarcensis*, the unopened male flowers which possessed natural viable pollen grains were collected from the bisexual plant at 05.00 to 06.00 pm at HRS, Ooty and brought immediately after packing in small poly bags with pinholes for aeration and placed in cool thermocol boxes maintained at the temperature of 6-8ºC using ice packs surrounding the thermacol layers , to HC & RI, Coimbatore and kept overnight at room temperature for collection of pollen grains in the next day by 7.00 am. The pollen output per anther was quantified by using the haemocytometer [13]. The pollen viability was estimated by staining the pollen grains using one per cent acetocarmine staining solution. The number of viable, red stained pollen grains per microscopic field were counted and expressed in per cent. Pollen germination was measured by incubating the pollen grains in a germination medium containing 0.15 M sucrose, 2.43 mM boric acid and 2.12 M calcium nitrate at 28°C for 24 hours [14]. The pollen grain with a pollen tube length greater than its diameter was considered to be germinated pollen and the germination was expressed in per cent.

2.2 Hybridization

In dioecious varieties *viz*., TNAU Papaya CO 8 and Pusa Nanha, the unopened and fully developed female flowers were bagged before its attainment of stigma receptivity using the butter paper bags. The bag was removed and the petals were forcibly opened. To improve fruit and seed set, sucrose solution (5%) was smeared using a fine tipped camel-hair brush gently over the receptive stigmatic surface [15] and the collected viable pollen grains of *Vasconcellea* were dusted on it at 06.30 to 07.30 am. The artificially pollinated flowers were then labelled for periodical observations and again covered using the butter paper bag. The artificially pollinated flowers were then allowed to grow until the fruits get harvested. In gynodioecious variety CO 7, the unopened and fully developed hermaphrodite flower was selected during a day prior to the anthesis. The petals were forcibly opened and emasculated carefully without damaging the ovary or style using the fine tipped forceps. After the completion of emasculation, the flower was bagged using the butter paper bag and tied using a string over the petiole of leaf and made sure that no space was provided in the bag to prevent the entry of any foreign pollen. The collection of viable pollen grains, application of sucrose solution and dusting of pollens over the receptive stigma was done by following the same procedure as in the case of dioecious plants.

2.3 Observations Recorded

The observations recorded include, fruit set percentage, fruit retention per cent till harvest, fruit length, fruit circumference, fruit weight, number of seeds, seed length, seed diameter, dry seed weight, seed germination percentage and seedling vigour.

The per cent fruit set was calculated by dividing the number of fruits set to the number of flowers crossed and multiplied with 100. The fruit retention per cent till harvest was calculated by dividing the number of fruits harvested to the number of fruits set and multiplied with 100. The fruit growth pattern of the crossed fruits was studied by measuring the fruit length and fruit circumference at monthly interval *viz*., 1, 2, 3 and 4 months after pollination (MAP). Fruit length was measured from the pedicel end to the stylar end of the fruit by using a measuring tape and expressed in centimetres. Fruit circumference was measured at midpoint of the fruit length by using a measuring tape and expressed in centimetres. Fruit weight was observed by weighing the fruit in an electronic weighing balance and the average of four fruits were calculated and expressed in kilograms. Number of seeds was counted manually from each harvested fruit. The seed length and seed diameter were observed in at least 12-20 twenty seeds depending upon the seed recovery and the average was calculated. The length and diameter of the seed was measured using a digital Vernier calliper and expressed in millimetres. Seed weight was calculated by weighing the seeds in an electronic weighing balance and expressed as 100 seed weight in grams. After the removal of mucilagenous sarcotesta from the collected seeds, they were shade dried and used for germination in nursery in standard pot mixture. The extent of germination and seedling vigour was also observed. The seed germination per cent was calculated by manually counting the number of plumules emerged at thirty days after sowing and expressed in per cent. The seedling vigour was calculated by the product of seed germination percentage and seedling length and expressed without units [16].

2.4 Statistical Analysis

The experiment was conducted in a randomized complete block design. The pollen parameters, fruit and seed parameters were statistically analysed using ANOVA with significance at probability level (P) of 0.05 by SPSS 16.0 version.

3. RESULTS AND DISCUSSION

3.1 Pollen Parameters

The pollen parameters *viz*., pollen output per anther, pollen viability and pollen germination were estimated in the parents involved in the intergeneric hybridization study Table 1.

3.1.1 Pollen output per anther

The pollen output per anther ranged from 14,125 to 20,125 among the parents. Significantly higher amount of pollen grains per anther was recorded in the polygamous type *V. candamarcensis* (20,125/anther) while significantly lower amount of pollen grains per anther was recorded in the gynodioecious type CO 7 (14,125/anther). The pollen output per anther of dioecious types ranged between 16,125 to 17,375. Higher pollen production is an attribute of out crossing species [17] and in gynodioecious varieties like CO 7, the necessity of such out crossing for successful fruit set is not as much as that required in dioecious varieties. Perhaps, this may be one reason for reduced pollen production in CO 7 papaya as compared to the dioecious varieties. As against earlier published model pointing that dioecy in flowering plants have evolved independently in a number of groups from hermaphrodite as monoecious ancestry [18], in papaya the origin of both hermaphrodite or gynodioecious forms are considered to have occurred from the wild populations which are mostly dioecious [19]. The necessity for production of more pollen in the wild population seems to have become persistent in the present day cultivated dioecious genotypes too.

3.1.2 Pollen viability

The pollen viability ranged from 68.45% to 92.31% among the parents. The varieties 'TNAU Papaya CO 8' and 'Pusa Nanha' were more or less similar with 87.19% and 86.44% pollen viability respectively (Table 1). Pollen viability was significantly higher in 'CO 7' (92.31%) wheras in *V. candamarcensis,* it was significantly lower (68.45%). Varying Influence of geographical location on pollen production of genotypes have been reported [14,20,21]. Higher pollen viability is advantageous in the hybridization programme and low pollen viability observed in *V. cauliflora* needs attention and it may be necessary to collect more pollen in the wild relatives of papaya for hybridization programme.

3.1.3 Pollen germination

The pollen viability as determined by acetocarmine staining is generally considered satisfactory from the breeder's point of perception. However, the pollen growth is additionally required to be observed to understand whether the pollens are capable of germinating on the stigmatic surface and result in successful fertilization, especially in hybridization attempts involving wild relatives.

The pollen germination ranged from 40.42% to 62.54% among the genotypes in the present study. A similar trend was observed that all the three papaya cultivars registering higher pollen germination per cent than the wild relatives. Although, the gynodioecious 'CO 7' was less polleniferous, it registered higher viability (stainability) and germination and thus possibly compensating for male reproductive efficiency. *V.candamarcensis* registered higher pollen germination than *V. cauliflora* in the present study. Higher fruit set and seed set is expected to result with higher pollen viability and germination provided there are no post pollination or post zygotic difficulties. Considering the extent of pollen viability and germination, it is suggested to collect pollen grains from more number of *V. candamarcensis* flowers than *V. cauliflora* for the hybridization program to incorporate resistance genes.

3.2 Fruit Set and Fruit Development

Although, the pollen stainability and pollen germination were relatively moderate in the wild relatives, it may be necessary to study the fruit set as well as seed set in such distant hybridization programmes. In many such attempts, embryo abortion usually results leading to absence of fruit set or seed set. Incompatibility problems and zygotic abortion post fertilization have been also reported earlier in papaya [22-24]. To overcome poor seed set, Dinesh *et al*. [15] suggested the use of sucrose solution to facilitate pollen germination and pollen tube growth whenever *V. cauliflora* was used as a male parent in the intergeneric hybridization. The role of sucrose serves as the best carbohydrate source for pollen germination in vivo, which helps in maintaining osmotic pressure of the medium and act as a nutritive substrate for pollen metabolism [25]. A similar attempt was followed in present study to overcome the limitations in fruit set.

Among the six intergeneric crosses, the fruit set ranged from 60.00% to 93.33% Table 2. The cross combination of 'TNAU Papaya CO 8' x *V. candamarcensis* recorded the highest extent of fruit set (93.33%) followed by the cross 'TNAU Papaya CO 8' x *V. cauliflora* (86.67%). However, the fruit retention was only 30.77 per cent only in CO.8 x *V. cauliflora* and 35.71 per cent in CO.8 x *V. candamarcensis* during the crossing attempts (Table 2) The cross combination of 'CO 7' x *V. cauliflora* recorded the highest fruit retention (80.00%) followed by the cross 'CO 7' x *V. candamarcensis* (66.67%). Based on these observations, the excessive fruit drop in the intergeneric crosses involving TNAU Papaya CO 8 may be associated with the lower concentration of endogenous auxin production. Hence, it is suggested to attempt exogenous auxin application such as NAA to overcome the fruit drop problem. Since, CO 7 is a gynodioecious variety, it may be possible to develop gynodioecious PRSV resistant hybrid from crosses involving CO 7. Similar attempts of distant hybridization with gynodioecious varieties have been carried out at IIHR, Bangalore [15,26].

3.3 Fruit Growth Pattern

The fruit growth pattern of four intergeneric crosses were also compared in the present study (Fig. 1). Among the crossed and set fruits, the fruit length ranged from 7.50 to 10.85 cm at 1MAP, 9.40 to 15.45 cm at 2 MAP, 13.85 to 21.60 cm at 3 MAP and 16.75 to 27.25 cm at 4 MAP (Table 3). Fruit length was significantly higher in the intervarietal cross of 'CO 7' x 'TNAU Papaya CO 8' Table 3. It was followed by the intergeneric cross 'CO 7' x *V. cauliflora* in general except at 3MAP. The intergeneric crosses of 'CO 7' x *V. candamarcensis* and 'TNAU Papaya CO 8' x *V. candamarcensis* recorded a significantly lower fruit length.

The fruit circumference in the four crosses ranged from 10.25 to 15.50 cm at 1 MAP to 23.50 to 34.20 cm at 4 MAP. Fruit circumference was significantly higher in the intervarietal cross 'CO 7' x 'TNAU Papaya Co 8' and was followed by the intergeneric cross 'CO 7' x *V. cauliflora* (Table 3). The intergeneric cross of 'CO 7' x *V. candamarcensis* recorded a significantly lower fruit circumference. While comparing the fruit size among the intergeneric crosses also, significantly bigger sized fruits were observed in the crosses involving *V. cauliflora* than with *V. candamarcensis*. It is normally expected that the fruit size to be similar to the other fruits of the female parent pollinated with its own pollen or under natural pollination during development. But the small size the fruits developed from crossed ovary as a result of fertilization by wild pollen in the present study reveals metaxenic influence of *Vasconcellea* sp on papaya varieties.

In the present study, periodical destructive sampling of fruit weight could not be carried out as the objective was to get fully developed fruits after hybridization to generate intergeneric hybrids. Hence, fruit weight was recorded during the harvest time. The fruit weight of the crosses ranged from 0.674 to 1.186 kg. The fruit weight was significantly higher in the intervarietal cross 'CO 7' x 'TNAU Papaya Co 8' and was followed by the intergeneric cross 'CO 7' x *V. cauliflora* (Table 3). The intergeneric cross of 'CO 7' x *V. candamarcensis* recorded a significantly lower fruit weight. Since, the fruit size is positively correlated with the fruit weight; the results of fruit weight also registered a similar pattern of higher fruit weight in the crosses involving *V. cauliflora* than with *V. candamarcensis*. The results of fruit weight also shows the possibilty of metaxenic influence of *Vasconcellea* sp on papaya varieties.

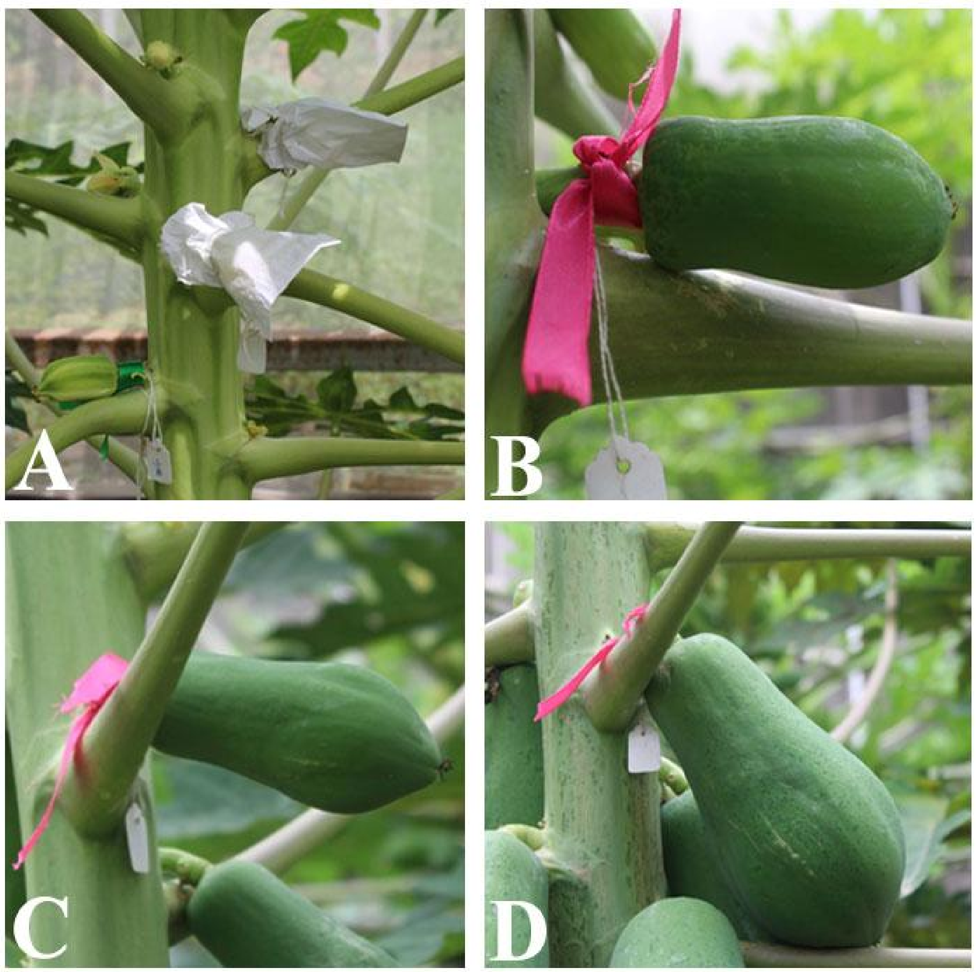

Fig. 1. Fruit growth of Co 7 x *V.cauliflora* A) Flowers of Co 7 pollinated with *V.cauliflora*, B) Fruit growth at 1 MAP, C) Fruit growth at 2 MAP and D) Fruit growth at 4 MAP

Table 1. Pollen parameters of the parents involved in the intergeneric hybridization attempts

Parent	Sex form	Pollen output per anther (numbers)	Pollen viability (%)	Pollen germinability (%)
		Mean ± SE		
Co 7	Gynodioecious	14,125 ± 1124[c]	92.31 ± 0.71[a]	57.12 ± 2.07[ab]
TNAU Papaya Co 8	Dioecious	16,375 ± 865[bc]	87.19 ± 1.34[b]	61.49 ± 2.58[a]
Pusa Nanha	Dioecious	17,375 ± 934[ab]	86.44 ± 1.56[b]	62.54 ± 2.72[a]
Vasconcellea cauliflora	Dioecious	16,125 ± 926[bc]	80.27 ± 1.00[c]	50.37 ± 2.67[b]
Vasconcellea candamarcensis	Polygamous	20,125 ± 1007[a]	68.45 ± 1.02[d]	40.42 ± 3.43[c]
SEd	-	1,474.49	1.75	4.12
CD (P = 0.05)	-	2993.43	3.53	8.37

Mean values with the same alphabetic letter do not differ significantly

Table 2. Per cent of fruit set, fruit developed and number of seeds from the intergeneric crosses

Cross*	No. of crosses made	Per cent fruit set	Per cent fruit retention	No. of seeds recovered
1 x 4	15	66.67	80.00	626
2 x 4	15	86.67	30.77	Nil
3 x 4	15	73.33	27.27	Nil
1 x 5	15	80.00	66.67	12
2 x 5	15	93.33	35.71	Nil
3 x 5	15	60.00	33.33	Nil

**The number indicates its corresponding parent*

1 : Co 7

2 : TNAU Papaya Co 8

3 : Pusa Nanha

4 : Vasconcellea cauliflora

5 : Vasconcellea candamarcensis

Table 3. Fruit growth pattern of the intergeneric crosses

Combination*	Fruit length (cm)				Fruit circumference (cm)				Fruit weight (kg)
	1 MAP	2 MAP	3 MAP	4 MAP	1 MAP	2 MAP	3 MAP	4 MAP	
1 x 4	9.70 ± 0.25^{b}	14.45 ± 0.60^{a}	19.35 ± 0.35^{b}	23.10 ± 0.36^{b}	12.70 ± 0.25^{b}	20.70 ± 0.48^{a}	24.75 ± 0.57^{b}	29.13 ± 0.41^{b}	0.886 ± 0.021^{b}
2 x 4	8.70 ± 0.29^{c}	12.58 ± 0.74^{b}	16.43 ± 0.53^{c}	20.18 ± 0.54^{c}	11.40 ± 0.47^{bc}	15.25 ± 0.54^{b}	20.30 ± 0.38^{c}	24.93 ± 0.51^{c}	0.798 ± 0.021^{c}
1 x 5	7.50 ± 0.35^{d}	10.50 ± 0.35^{c}	13.85 ± 0.25^{d}	16.75 ± 0.18^{e}	10.25 ± 0.18^{c}	13.50 ± 0.35^{c}	17.00 ± 0.35^{d}	23.50 ± 0.35^{c}	0.674 ± 0.015^{d}
2 x 5	8.05 ± 0.04^{d}	9.40 ± 0.14^{c}	14.20 ± 0.14^{d}	18.10 ± 0.07^{d}	11.80 ± 0.85^{bc}	14.20 ± 0.57^{bc}	19.95 ± 0.32^{c}	24.00 ± 0.35^{c}	0.681 ± 0.032^{d}
1 x 2	10.85 ± 0.25^{a}	15.45 ± 0.67^{a}	21.60 ± 0.57^{a}	27.25 ± 0.53^{a}	15.50 ±0.71^{a}	21.05 ± 0.39^{a}	27.10 ± 0.78^{a}	34.20 ± 1.56^{a}	1.186 ± 0.026^{a}
SEd	0.30	0.64	0.39	0.43	0.74	0.62	0.57	0.96	0.03
CD (P = 0.05)	0.65	1.40	0.84	0.95	1.60	1.35	1.24	2.10	0.06

**The number indicates its corresponding parent. Mean values with the same alphabetic letter do not differ significantly.*

1 : Co 7

2 : TNAU Papaya Co 8

3 : Pusa Nanha

4 : Vasconcellea cauliflora

5 : Vasconcellea candamarcensis

MAP : Months after pollination

In the present study, the monthly incremental rate of change in fruit length and fruit circumference over a four month period were higher in the intervarietal crosses followed by the crosses of *V. cauliflora* and *V. candamarcensis* (Fig. 2a and b). In general, in the crossed fruits, the growth was rapid till 30 days after pollination followed by a slight fall in rate of growth till two months and again the rate of growth rate tends to be in an upward pattern till two and a half months after pollination (Fig. 2a). The rate of fruit growth was slow during the period between one to two months after pollination. It may be possible that resource allocation may be distributed for leaf expansion or new flowers and fruits that are freshly set or for seed and pulp development during the slow fruit growth phase.

In many fleshy fruits it was generally observed that during the early phase of the fruit growth, rapid cell division and enlargement leads to increases in pulp volume while the embryo volume remains small during the intermediate phase seed development followed by rapid radial longitudinal and radial cell enlargement leading to increase in fruit size [27]. Varying rates of fruit growth and variation in fruit size after hybridization attempts with cultivated varieties or wild relatives of papaya clearly depicts that pollen source could influence the fruit growth rate after fruit set. The functional genes that may involve in the process either that was stimulated following hybridization or gene regulation events post pollination after fruit set or zygote formation need to be studied further.

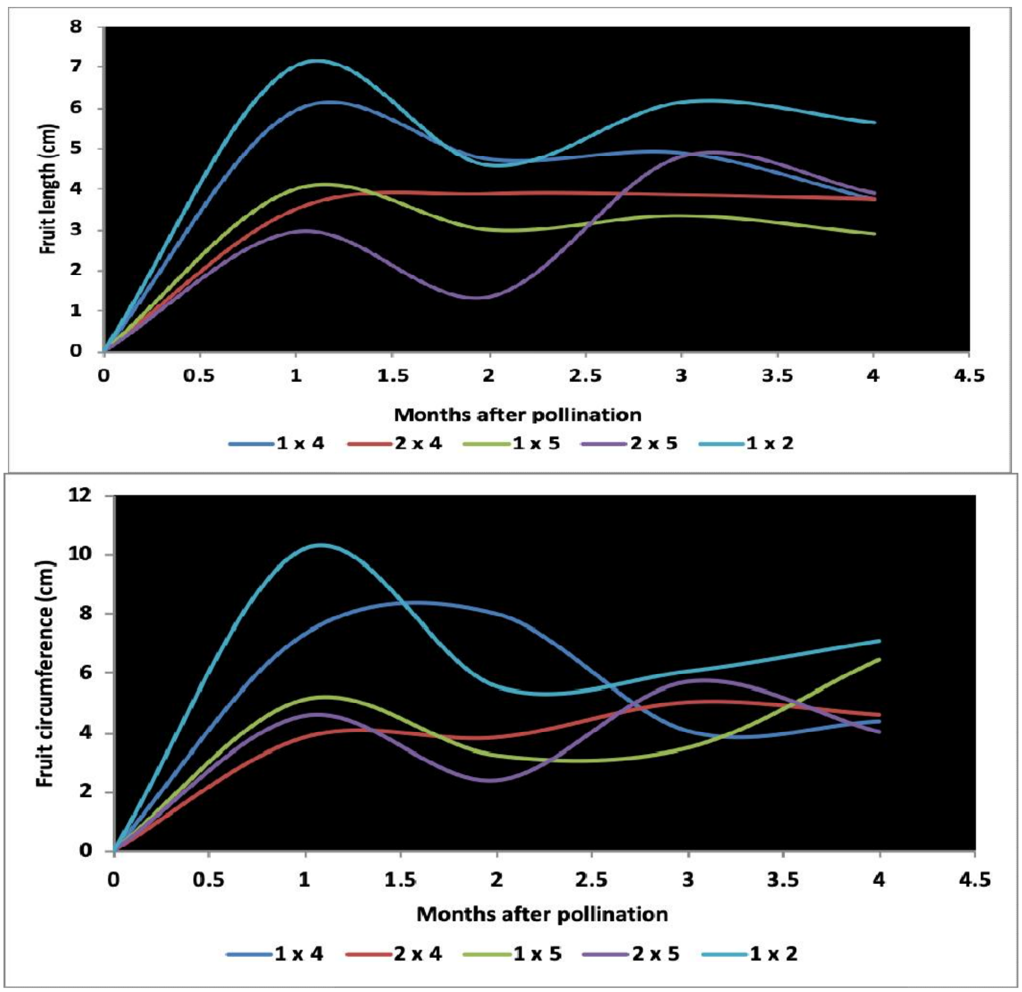

Fig. 2. Growth of fruits of intergeneric and interspecific crosses a) growth pattern with respect to fruit length and b) Growth pattern with respect to fruit circumference. 1 (CO 7), 2 (TNAU Papaya CO 8), 4 (*V. cauliflora*) and 5 (*V. candamarcensis*)

3.4 Seed Parameters

Among the six combinations of intergeneric crosses, only in two crosses *viz*., 'Co 7' x *V. cauliflora* and 'Co 7' x *V. candamarcensis* was found to set seeds in the hybridized fruits (Fig. 3). The other four combinations yielded only seedless fruits even though 5% of sucrose solution was used during hybridization process to aid in pollen germination over the stigma and aiding its growth through the style [15,28]. In the present study, seed less ness was observed in the cross combination involving the dioecious parents TNAU papaya CO.8 and as well in Pusa Nanha when crossed with *Vasconcellea*. This is in contrast to earlier results obtained in TNAU where in seed set was observed in CO 8 and Pusa Nanha in similar attempts (28). Hence, the influence of season of pollination or post zygotic abortion of ovules could not be ruled out as fruit development was nearly normal in both these combinations after crossing and fruit set.

In the present study, 626 seeds were recovered from the cross CO 7 x *V. cauliflora* and 12 seeds from the cross CO 7 x *V. candamarcensis* (Table 4). Higher seed recovery in *V. cauliflora* crosses can be attributed to higher pollen germination capacity than *V. candamarcensis*. Deepa *et al.* [26] also reported that the better seeds recovery in crosses involving *V. cauliflora* than *V. candamarcensis* and *V. parviflora* when involved as male parent.

In the present study, the seed length of the intergeneric crosses and its parents ranged from 4.22 mm to 8.43 mm (Table 4). In the parents, the seed length was significantly higher in *V. cauliflora* (8.43 mm) followed by *V. candamarcensis* (7.92 mm) and 'CO 7' (6.45 mm). The intergeneric cross 'CO 7' x *V. candamarcensis* recorded a significantly lower seed length of 4.22 mm. The seed diameter of the intergeneric crosses and its parents ranged from 2.59 mm to 7.37 mm (Table 4). The seed diameter was significantly higher in *V. cauliflora* (7.37 mm) and in *V. candamarcensis* (7.19 mm). The intergeneric cross 'CO 7' x *V. candamarcensis* recorded a significantly lower seed diameter and was found to be on par with the 'CO 7' x *V. cauliflora*.

The intergeneric crosses 'CO 7' x *V. candamarcensis* and 'CO 7' x *V. cauliflora* registered significantly less seed weight than the parents (Table 4). In the cross 'CO 7' x *V. cauliflora* the observed 100 seed weight was only 0.58 g while in the pollen parent *V. cauliflora*, the observed 100 seed weight was 3.77 g. Such reduction in seed size has been earlier observed in wide hybridization attempts in other crops too [29].

In case of the parents, the seed germination was 88.89 % in 'Co 7 as against the low germination of 26.67 % in *V. cauliflora* and 23.08 % in *V. candamarcensis.* However in the cross 'Co 7' x *V. cauliflora*, there is a drastic fall in seed germination to a 12.46 % whereas in the cross 'Co 7' x *V. candamarcensis*, there was no seed germination from the twelve seeds sown (Table 4). Based on a systemic study, Jimenez and Horovitz [30] described the failure in fertilization or seed development in crosses involving *C. papaya* and five *Vasconcellea* species as a consequence of gene pool interactions. According to them, *C. papaya* represented in the third gene pool that never produced viable seeds when crossed with the members of first genepool (*V. cauliflora, V. candicans, V microcarpa.* and *V. monoica*) or second gene pool (*V. goudotiana*) and embryo rescue and culture was suggested

Table 4. Seed length, diameter, 100 seed weight, seed germination and seedling vigour index of the intergeneric hybrids

Parent and Cross	Seed length (mm)	Seed diameter (mm)	100 seed weight (g)	Seed germin-ability (%)	Seedling vigour index
Co 7 x *V. cauliflora*	4.75 ± 0.07[d]	2.78 ± 0.05[c]	0.58	12.46	204.34[c]
Co 7 x *V. candamarcensis*	4.22 ± 0.07[e]	2.59 ± 0.05[c]	0.41*	Nil	-
Co 7	6.45 ± 0.13[c]	4.67 ± 0.07[b]	1.79	88.89	2192.62[a]
V. cauliflora	8.43 ± 0.13[a]	7.37 ± 0.14[a]	3.77	26.67	372.49[b]
V. candamarcensis	7.92 ± 0.09[b]	7.19 ± 0.12[a]	3.11	23.08	-
SEd	0.16	0.15	-	-	71.94
CD (P = 0.05)	0.32	0.29	-	-	145.21

**Estimated data from mean seed weight. Mean values with the same alphabetic letter do not differ significantly* to recover hybrids [31,24]. According to Manshardt and Wenslaff [22] also the major barriers in interspecific hybridization of papaya include post zygotic barriers such as ovule and embryo abortion, as well as a lack of endosperm development. This may be the reason behind the low or failure of seed germination in intergeneric crosses in the present study with CO2 or TNAU Papaya CO.8. In the present study, it could be however observed that *V. cauliflora* as a pollen parent was able to fertilize and result in viable seeds in cultivar CO 7. It may be possible that environmental factors may lead to such viable seed production apart from the inherent genetic constitution of the female parent. Successful seed set and recovery with dioecious cultivars, CP 50 and Pusa Nanha was earlier achieved even under Coimbatore conditions itself when crossed with *Vasconcellea cauliflora* [28]. Further studies on the influence of environmental factors contributing to better seed set in such intergeneric hybridisation attempts could help to draw inferences on such processes that may overcome interspecific barrier in hybridization.

Seedling vigour index is an important parameter to assess the healthiness of the seedling to withstand and establish in the field condition. In the current study, the seedling vigour index ranged from 204.34 to 2192.62 (Table 4). The seedling vigour index was significantly higher in ‘Co 7’ (2192.62) followed by *V. cauliflora* (372.49). The intergeneric cross ‘Co 7’ x *V. cauliflora* recorded a significantly lower seedling vigour index of 204.34. This implies that immense attention of the breeder is required to optimize the nutrient requirements and further care to stabilize the growth of seedlings of intergeneric hybrids before transplanting for evaluation.

Fig. 3. Cross section of the intergeneric hybridized fruit A) Co 7 x *V. candamarcensis*, B) TNAU Papaya Co 8 x *V. candamarcensis*, C) Pusa Nanha x *V. candamarcensis*, D) Co 7 x *V. cauliflora*, E) TNAU Papaya Co 8 x *V. cauliflora* and F) Pusa Nanha x *V. cauliflora*

4. CONCLUSION

The analysis of the pollen parameters, fruit set and development pattern, seed set aspects on development of intergeneric hybrids of papaya revealed that among the two wild relatives, *V. candamarcensis* was more polleniferous than *V. cauliflora* but the pollen viability and germination was better in *V. cauliflora*. Hence more crossing attempts are required to develop intergeneric hybrids when these two wild species are used as parents in conventional hybridization. Moreover, further research is required to unravel the reason behind the poor fruit retention in intergeneric crosses involving TNAU Papaya Co 8. A conspicuous metaxenic effect was observed when pollen of wild relatives were employed. Though many attempts were made to effect distant crosses between the *V. cauliflora* and *C. papaya*, only some viable seeds were developed by employing 5% of sucrose solution especially in the cross CO 7 x *V. cauliflora*. Hence, attempts can be further made to generate more such crosses to further identify a gynodioecious segregant with resistance attributes at later generations. The reduction in seed size and weight, germination and seedling vigour observed in intergeneric crosses of CO 7 with *Vasconcellea* implied possible post zygotic genetic abnormalities and complications. The possibility for embryo rescue from such distant crosses needs to be studied further to recover more progenies from the intergeneric crosses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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