Vegetative propagation of papaya (*Carica papaya* L.) through grafting

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In papaya orchards raised through seedlings, large variation in sex forms is a major hindrance to its cultivation on a commercial scale. Similarly, in papaya breeding, if a desirable trait is to be fixed in future generations, it is essential to multiply the plants by asexual means for evaluation on a large scale as sexual propagation has several drawbacks. Limited studies have been conducted on vegetative propagation in papaya under the tropical climate of India. Hence, the present study was initiated to standardize vegetative propagation through grafting under separate experiments. A combination of growth hormones and different aged seedlings was used to induce lateral shoots on the mother plants to utilize them as scions for grafting. Spraying BA @ 100 ppm + GA_3 @ 250 ppm on 5–6 month-old mother plants could produce more graftable size shoots. Among different methods, softwood wedge grafting recorded maximum success followed by cleft grafting. Field evaluation of grafted and seedling plants revealed that the former were dwarfs and sturdy, induced early flowering at a lower heights and came to harvest earlier than the seedlings. Though physical fruit parameters showed a significant difference between grafted and seedling plants, no difference was recorded for fruit quality parameters.

Keywords: Cytokinins, grafting, lateral shoots, papaya, vegetative propagation.

EXCEPT in South Africa, Brazil, Taiwan, etc. with a less area under grafted plants, papaya is generally propagated through seeds worldwide. Hence, commercial cultivation of papaya is hindered due to problems associated with heterozygosity, dioecy and susceptibility to major diseases. Similarly, due to variation in plants with respect to sex type, it is difficult for breeders to fix desirable characters (e.g. hermaphroditism) through sexual propagation. Several studies have been conducted to achieve success in the vegetative propagation of papaya in various countries. About 85–100% success in the rooting of 4–6 week-old cuttings has been reported when grown in perlite or manure, compared to compost made out of pine barks, where the success rate was as low as 22% (ref. 1). Commercial hybrids grafted onto *Vasconcellea cauliflora*, a wild species of papaya resistant to Papaya ringspot virus (PRSV) were found to delay the expression[2]. It is possible to develop high-yielding, disease-resistant plants with characteristics similar to the mother plants through asexual propagation[3]. Researchers have shown success in rooting in the cuttings when treated with solubor and paclobutrazol, with encouraging results[4]. Moderate-sized cuttings recorded the highest rooting compared to thicker shoots. Studies on the effect of six rootstocks on the growth and reproductive performance of papaya cv. Trang Nguyen revealed that top grafting on LD 1999 rootstock gave the highest success percentage[5]. The additional advantage of asexual propagation might be a shorter vegetative phase with dwarf stature, which can provide longer economic life[6,7]. As gynodioecious fruits have an attractive shape, size and flavour compared to fruits from female plants, asexual propagation can also maintain the hermaphrodite nature of the mother plants in addition to dwarf nature with larger trunk diameter[8,9].

Limited studies have been conducted on vegetative propagation in papaya under the tropical conditions of India. There was a requirement for standardizing vegetative propagation in papaya to facilitate speed breeding to obtain desirable traits, especially with respect to hermaphroditism and tolerance to PRSV. In this context, the present study was initiated to standardize vegetative propagation techniques in papaya through grafting, which can produce true to type in preserving the traits of interest in any crop.

Materials and methods

This study was carried out at the experimental plot of ICAR-Indian Institute of Horticultural Research, Bengaluru during 2018–2021, situated at an elevation of 890 m MSL (mean sea level), 12°68′ N lat. and 77°38′E lat. All the experiments were carried out under green shade net (50%) conditions.

Experiment 1: Standardization of chemicals to induce multiple shoots in mother plants

One-month-old seedlings were transplanted in a green shade net house at a spacing of 1.8 m × 1.8 m. When the plants were five months old, all the basal leaves were removed along with petioles by retaining 4–5 apical leaves. Care was taken not to damage the nodes at the base of the petiole. Immediately the plants were sprayed with different combinations of growth regulators, viz. hydrogen cyanamide (0.5% a.i); benzyl adenine (BA) @ 50 ppm + GA_3 @ 1000 ppm; BA

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Table 1. Weather data during the experimental period

Month (2019)	Temperature (°C) Maximum	Temperature (°C) Minimum	Relative humidity (%) 07.30 h	Relative humidity (%) 14.00 h	Rainfall (mm)
April	33.6	20.0	68.1	34.4	14.50
May	32.3	21.0	80.3	51.1	266.45
June	28.8	20.5	80.5	64.5	60.60
July	28.2	20.5	82.1	66.7	88.55
August	28.8	19.5	83.1	60.8	87.75
September	29.6	19.4	83.1	58.1	116.9
October	29.6	16.8	77.1	50.3	53.40
November	29.1	15.3	83.2	50.8	17.30
December	28.7	15.0	83.1	49.3	2.50

@ 250 ppm + GA_3 @ 500 ppm; BA @ 100 ppm + GA_3 @ 250 ppm; kintein @ 250 ppm + GA_3 @ 250 ppm and kinetin @ 100 ppm + GA_3 @ 100 ppm. After one week of spraying, the plants were decapitated. The number of lateral shoots induced was counted after 20 days. Uniformly across the treatments, 200 ppm GA_3 was sprayed to promote shoot elongation. After two months, the length (cm) of the side shoots was measured using a measuring scale. Similarly, the girth (mm) of the lateral shoots was measured using calipers. The percentage of lateral shoots with >6 mm (graftable size) was calculated accordingly.

Experiment 2: Standardization of age of mother plants to induce lateral shoots

This experiment was conducted from April 2019 to December 2019 to standardize the age of mother plants to obtain more lateral shoots which could be used as scions for grafting. Based on the results of the first experiment, the best chemical which could induce more lateral shoots (BA @ 100 ppm + GA_3 @ 250 ppm) was sprayed on four-, five-, six-, seven-, eight-, nine- and ten-month-old mother plants. After three weeks, 200 ppm GA_3 was sprayed into all the treatments to promote shoot elongation. After 30 days of treatment, the number of lateral shoots produced per plant was recorded. After two months, length of the lateral shoots and shoot diameter were recorded. The number of shoots of graftable size (>6 mm) was calculated, as explained above. Table 1 shows the weather parameters recorded during the experimental period.

Experiment 3: Standardization of grafting method for multiplication of papaya

Two-month-old, uniform-sized seedlings were used as rootstock (non-descriptive variety) for side and wedge grafting, while four-month-old rootstocks were used for cleft grafting. Two-month-old lateral shoots induced on the mother plants (mostly bisexual plants) having a diameter of >6 mm were used as scions for grafting. Different methods of grafting were employed during February 2019, as described in Harmann *et al.*[10]. After two months of grafting, the days taken for sprouting, height of plant above the graft union and success percentage were recorded.

Experiment 4: Studies on the performance of grafted and seedling plants

This experiment was conducted from June 2020 to September 2021. The grafted plants of papaya variety Arka Surya (a gynodieoecious variety having medium size, deep pink flesh with high total soluble solids) were produced by following the best treatments obtained in experiment 1 (induction of multiple shoots) and experiment 2 (method of grafting). When the grafts were two months old (August 2020), they were transplanted into the field under 50% green shade net. Similarly, two-month-old plants raised from seeds were grown to compare their performance against the grafted plants. The days taken for flowering, height at first flowering, and days taken for harvest and fruit quality parameters were recorded.

Results and discussion

Experiment 1

Among the different chemicals used for the induction of multiple shoots, a combination of BA @ 100 ppm + GA_3 @ 250 ppm could induce more shoots per plant (39.19) followed by treatment with BA @ 250 ppm + GA_3 @ 500 ppm (23.16) (Figure 1). The various treatments showed significant differences in the induction of lateral shoots. The lowest number of shoots was recorded in seedlings treated with hydrogen cyanamide, as it might be caustic resulting in scorching of the buds. BA @ 100 ppm + GA_3 @ 250 ppm could elongate the shoots significantly compared to the other treatments. The least length of lateral shoots was recorded in plants treated with kinetin @ 100 ppm + GA_3 @ 100 ppm (9.56 cm) and with H_2CN_2 (2.54 cm). Similarly, the percentage of shoots having a diameter >6 mm was significantly higher (72.56) in the treatment combination BA @ 100 ppm + GA_3 @ 250 ppm followed

Table 2. Effect of different chemicals on the induction of lateral shoots in papaya mother plants

Hormones/chemicals	Number of lateral shoots	Average length of lateral shoots (cm)	Percentage of lateral shoots with >6 mm diameter
H_2CN_2 (0.5%)	7.50^D	2.54^E	0.00^E
BA 500 ppm + GA_3 1000 ppm	21.17^{BC}	13.59^C	48.26^C
BA 250 ppm + GA_3 500 ppm	23.16^B	17.47^B	52.12^B
BA 100 ppm + GA_3 250 ppm	39.19^A	25.56^A	72.56^A
Kinetin 250 ppm + GA_3 250 ppm	19.56^C	12.86^C	47.56^C
Kinetin 100 ppm + GA_3 100 ppm	18.05^C	9.56^D	12.39^D
Mean	21.43	13.59	33.27
P-value	<0.0001	<0.0001	<0.0001

The values with different alphabets are significantly different at $P < 0.05$.

Figure 1. Induction of side shoots in mother plants sprayed with BA 100 ppm + GA_3 250 ppm.

by the treatment combination of BA @ 250 ppm + GA_3 @ 500 ppm (Table 2).

It is well established that many cytokinin compounds are known to inhibit apical dominance and thus initiate shoot proliferation[11]. In addition to the presence of cytokinins, plant hormones like GA_3 are required for shoot elongation[12]. It is not only the hormones, but their concentration also plays a major role in shoot proliferation. Sub-optimal or super-optimal concentration may have an adverse effect on shoot proliferations[13]. Multiple shoots were produced by *in vitro* callus of papaya in a medium containing 5 mg indole acetic acid (IAA) and 0.5 to 1.0 mg/l BA compared to the same concentration of IAA and 5 mg/l of kinetin[14]. The present study confirms that a combination of cytokinin and gibberellins is required for shoot proliferation and its further elongation to attain the desired length and girth. With an increase in the concentration of both cytokinin and gibberellins, there is a reduction in shoot proliferation, which clearly shows that this ideal concentration of hormones plays a major role in side shoot proliferation. Decapitation of mother trees one week after hormonal application might have also increased cytokinin concentration in the lateral nodes, as suggested by Mullar and Leyser[15]. There was an increased concentration of cytokinin in the nodal tissues and axillary buds after decapitation compared to that before decapitation[16]. Thus it was concluded that the stem is the physiologically relevant site for cytokinin synthesis in the regulation of bud activity, which subsequently translocates into the axillary buds thus bringing shoot emergence[16].

Experiment 2

Among the mother plants of different age groups, five-month-old plants when sprayed with BA @ 100 ppm + GA_3 @ 250 ppm induced more number of laterals (32.66) followed by six-month-old plants (Table 3). With an increase in the age of plants, there was a gradual reduction in the number of lateral shoots. The age of the mother plants plays a major role in obtaining healthier and larger-sized lateral shoots which can be used either as cuttings for inducing rooting or as scions for grafting. The physiological stage of the mother plants play a major role in effective shoot proliferation. Plants that have just completed their juvenile phase and making a transition towards the reproductive phase might be ideal for the induction of more laterals as evinced in the present study[17]. Usually, seedling papaya plants enter the reproductive phase and start producing flowers from the seventh to eighth month onwards. In papaya plants, well-developed pith is conspicuous during the early stages of development and with progress in age of the plants, the stems become hollow due to the breakdown of pith at the internodes as they mature and the fibres also become thicker and harder[18]. Due to the lignification of nodal regions in mature plants, axillary buds may have reduced capacity to sprout from such nodes. However, during the early stages of development, due to the presence of pith and soft stem the axillary buds in the nodal region may be physiologically active and start sprouting after the application of cytokinin compounds. Hence, in the present study

Table 3. Effect of age of rootstock on the induction of multiple shoots in papaya plants

Treatment				
Age of mother plants (months)	Month (2009)	No. of lateral buds	Average length of lateral shoots (cm)	Lateral shoots of >6 mm thick (%)
4	May	22.72^{B}	15.76^{D}	45.56^{C}
5	June	32.66^{A}	35.65^{A}	72.26^{A}
6	July	30.89^{A}	32.56^{A}	69.26^{A}
7	August	29.56^{A}	22.26^{B}	51.23^{B}
8	September	28.06^{AB}	19.56^{BC}	49.26^{BC}
9	October	15.43^{C}	21.56^{B}	48.86^{C}
10	November	11.60^{D}	24.56^{B}	54.56^{B}
General mean		22.10	24.81	56.12
P-value		<0.0001	<0.0001	<0.0001

The values with different alphabets are significantly different at $P < 0.05$.

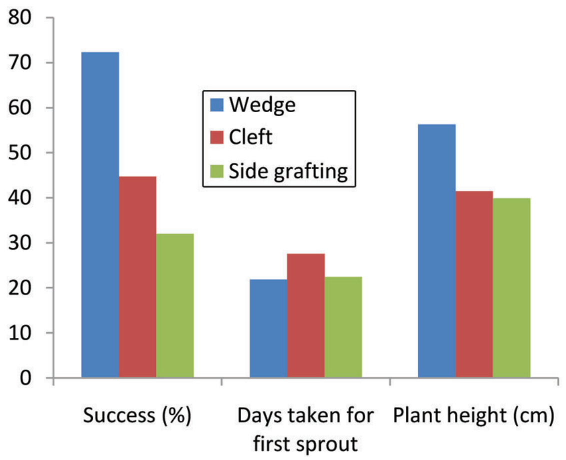

Figure 2. Effect of different methods on the success of grafting in papaya.

more lateral shoots could be seen in the mother plants till the age of six months, after which there was a reduction in the emergence of lateral shoots.

In addition, the prevailing climate may also play a major role in lateral shoot induction. In the present study, the seedlings which attained 5–6 months of age (coinciding with June–August) had a favourable climate with respect to temperature and humidity (Table 1). The reduced number of shoots in the fourth month may be due to both immature stages of mother plants, and prevailing high temperatures and low humidity (April–May). Similarly, low temperatures coupled with more humidity and frequent showers might have reduced the lateral shoot induction in mother plants aged 7–10 months (September–November). Environmental variables such as light, temperature, ambient humidity, wind speed, edaphic characteristics and biotic factors such as mycorrhizal fungi and genotype, significantly affect the physiology of growth and development in papaya[19,20]. It has also been demonstrated that orchards located in regions with a mean temperature of 25°C promote excellent vegetative growth, fruit quality and high sugar compared to regions with a mean temperature below 20°C and above 30°C (ref. 21).

Experiment 3

Among different methods of grafting, wedge grafting recorded 72% success followed by a cleft (46%) and side grafting (32%), while days taken for the first sprout was early in wedge and side grafting (22 days) than in cleft grafting (30 days). The length of grafted plants after two months was highest in wedge-grafted plants (56 cm) compared to 46 cm and 42 cm in cleft and side-grafted plants respectively (Figure 2).

Several studies are being conducted to identify the best grafting methods and the results vary according to local climate, variety and rootstocks. Some factors that directly influence graft success are the method of grafting, compatibility, vigour and health of the plants and aftercare of the grafted plants[22]. Field grafting of female branches on male branches of the same plant has been reported[23]. As a result, the papaya grafting success was confirmed. However, under South Indian conditions, field grafting has also been reported in CO-1 and Coorg Honey Dew cultivars[24]. About 80% success in tongue grafting was reported using tape as the fastening device compared to cleft grafting[25]. With further modifications in tongue grafting and seedlings grown in polythene bags, graft success up to 92.5% could be obtained compared to other methods. Softwood wedge grafting is a simple technique, and both rootstocks and scions are in the active growing stage with soft stem; callus formation might be quick followed by better dedifferentiation to connect the vascular tissues of stock and scion[26]. In citrus, wedge grafting was identified as the most suitable method followed by cleft grafting[27]. The disadvantage of cleft grafting is that temperature and humidity need to be rigorously controlled, which is not the case in softwood grafting[28]. Another disadvantage of cleft grafting is that the stock diameter should be considerably large, and the diameter of stock and scion should exactly match for maximum success[29]. However, in the present study, there was slight mismatch in the diameter of stock and scion as scions were retained on mother plants for more days causing lignification of scions thus resulting in least graft success in cleft grafting. The least success percentage recorded in side grafting after two months might be due to slow graft union formation. Similar results were reported where 80% graft success was recorded after 15 weeks in side grafting compared to the first two methods of grafting[30].

Experiment 4

Significant differences in various vegetative and reproductive parameters were recorded among seedling and grafted plants (Table 4). Height at first flowering was 55 cm above ground level after 155 days of planting in grafts, while it was 120 cm above ground level after 198 days of planting in seedlings. The first harvest took 276 days for grafted plants and 362 days for seedling plants. Significant difference was observed for average fruit weight, fruit length, fruit width and fruit volume with highest values for grafted plants compared to seedlings. However, no difference was recorded for fruit quality parameters. It has been well established that grafting induces dwarfing effect on plants compared to seedlings. Plants produced from clonal propagation tend to bear fruits earlier and at very lower trunk height[31–33]. In a study to compare grafted and seedling plants in papaya, grafted plants were dwarfs and sturdy, with lower bearing position, faster development of leaves and higher leaf chlorophyll content compared to seedling plants[34]. The possible reason for dwarfing in grafted plants is due to the rate at which vegetative shoots grow and the time over which they grow[35]. In the present study also we recorded reduced internodal length on grafted plants compared to seedling plants. The reduced internodal distance might also contribute to reduced plant height in grafted plants. The slow growth of the grafted plants resulted in increased stem girth which might also be one of the reasons for dwarfing in the grafted plants[36]. The other possible reason for dwarfing may be due to reduced leaf area in the grafted plants which are produced with fewer internodes and closer orientation to intercept less amount of sunlight that might reduce the growth rate of the grafted plants[37]. In addition to reducing plant height, the rootstocks are known to induce precocious flowering[38]. Grafting is a strategy for inducing flowering in many fruit crops as some studies have suggested transmission of floral stimulus from rootstock to scion, in addition to the transport of mobile elements throughout the plants[39]. In the present study also significant difference was observed for time to first flowering, wherein most of the photosynthates produced might have been diverted for reproductive growth than vegetative growth which may induce early flower buds. In a study on the behaviour of three papaya types propagated by grafting, the grafted plants of Taunung 01 variety recorded shorter height and large trunk diameter at 90 days after planting. First flowering was recorded 35 days after planting at 65 cm height compared to seedling plants which took 70 days to flower at the height of 106.8 cm (ref. 38). The grafted plants also produced more fruits than seedling plants. Grafted Intenzza papaya variety recorded dwarfing effect with early precocity and harvest compared to seedlings[39]. No difference was recorded for fruit quality parameters. Similar studies on the non-significant effect of grafting on fruit quality parameters were conducted earlier, though there was a significant effect on fruit yield[33,40–42]. In addition to grafting, it was reported that yield in ratoon crops could be increased by heading back (pollarding) the tree after first harvest at the height of 60 cm compared to heading back at less than 60 cm height or more[43].

Table 4. Growth, flowering habit and fruit quality parameters in grafted and seedling papaya plants

Parameters	Grafted plants	Seedling plants	Significance
Stem girth at first flowering node (cm)	19	13	*
Number of nodes at first flowering	37	46	*
Height at first flowering (cm)	55	120	*
Days for first flowering	155	198	*
Days for first harvest	276	362	*
Average fruit weight (g)	743.7	478.35	*
Fruit volume (cm^3)	508.7	379.6	*
Fruit length (cm)	17.82	14.86	*
Fruit width (cm)	10.27	6.36	*
Pulp thickness (cm)	2.41	2.31	NS
Pulp colour (cm)	Orange	Orange	NS
TSS (°B)	11.73	11.98	NS
Cavity index	21.28	22.73	NS

*Significantly different at $P < 0.05$; NS, Not significant.

Conclusion

Vegetative propagation of papaya through grafting plays a major role in facilitating breeders to fix the desired characteristics in the progeny and its further evaluation. Standardization of the age of seedlings and chemicals for induction of lateral shoots to use them as scions is the first step in successful grafting experiments. In the present study, 5–6-month-old mother plants sprayed with BA @ 100 ppm + GA_3 @ 250 ppm could induce more lateral shoots which could be used as scions for grafting. Softwood wedge grafting recorded maximum success compared to side and cleft grafting. The grafted plants were dwarfs, could produce flowers and fruits at a lower height and came to harvest early compared to seedling plants.

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