Investigations into vegetative propagation of papaya (*Carica papaya* L.) through grafting

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Abstract

In papaya orchards raised through seedlings, large variation in sex forms is a major hindrance in its cultivation on commercial scale. Similarly, in papaya breeding, if a desirable trait is to be fixed in future generations, it's essential to multiply the plants by asexual means to evaluate it on large scale as sexual propagation has several drawbacks. Very limited studies are being attempted on vegetative propagation in papaya under tropical climate of India. Hence, this work was initiated to standardize vegetative propagation through grafting under separate experiments. Combination of growth hormones and different aged seedlings were used to induce lateral shoots on mother plants to use them as scions for grafting. Spraying BA@100 ppm + GA_3@250 ppm on five to six month old mother plants could produce more number of graftable size shoots. Among different methods of grafting, softwood wedge grafting recorded maximum success followed by cleft grafting. Field evaluation of grafted and seedling plants revealed that grafted plants were more dwarf and sturdy, induced early flowering at lower height and came to harvest early than seedlings. Though fruit physical parameters showed significant difference between grafted and seedling plants, no difference was recorded for fruit quality parameters.

Keywords: Papaya, vegetative propagation, grafting, lateral shoots, cytokinins

Introduction

Throughout the world papaya is propagated through seeds except in South Africa, Brazil, Taiwan etc. where very little area is under grafted plants. Hence, commercial cultivation of papaya is hindered due to problems associated with heterozygocity, dioeciousness and susceptibility to major diseases. Similarly, due to variation in plants with respect to sex type, it is very difficult for breeders to fix desirable characters (eg. Hermophroditeness) through sexual propagation. Several studies were attempted to achieve success in vegetative propagation of papaya in different countries. About 85-100 % success in rooting of four to six week old cuttings have been reported when grown in perlite or manure, compost made out of pine barks although the rooting success was as low as 22%[1]. Commercial hybrids grafted onto *Vesconcelea cauliflora*, a wild spp of papaya resistant to Papaya Ring Spot virus (PRSV) was found to delay the expression[2]. It's possible to develop high yielding disease resistant plants with similar characteristics of mother plants through asexual propagation[3]. Some researchers have shown success of rooting in cutting when treated with solubor, paclobutrazol with encouraging results[4]. Moderate sized cuttings recorded highest rooting compared to thicker shoots. Some studies on effect of six rootstocks on growth and reproductive performance of papaya cv. Trang Nguyen revealed top grafting on LD 1999 rootstock gave the highest success percentage[5]. The additional advantage of asexual propagation might be shorter vegetative phase with dwarf stature which can provide longer economic life[6,7]. As gynodioecious fruits are having attractive shape, size and flavor compared to fruits from female plants, asexual propagation can also maintain hermaphrodite nature of mother plants in addition to dwarf nature with larger trunk diameter[8, 9].

Very limited work is being done with vegetative propagation studies in papaya under tropical conditions of India. There was a requirement for standardizing vegetative propagation in papaya to facilitate speed up of breeding work to get desirable traits especially with respect to hermaphrodite and tolerance to PRSV. In this context, this work 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

The present investigations were carried out at experimental plot of ICAR-Indian Institute of Horticultural Research, Bengaluru during 2018 to 2021. It is situated at an elevation of 890 meters above sea, $12^{0}68^{'}$ North latitude and $77^{0}38^{'}$ East latitude. All the experiments were carried out under green shade net (50%) conditions.

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

Seedlings of one month old were transplanted in green shade net house at spacing of 1.8 ×1.8 m. When the plants were 5 month old, all the basal leaves were removed along with petioles by retaining apical 4-5 leaves. Care was taken not to damage the nodes present at the base of petiole. Immediately the plants were sprayed with different combinations of growth regulators viz., Hydrogen Cyanamide (0.5 % a.i); BA@50 ppm + $GA_3$1000 ppm; BA@250 ppm + $GA_{3@}$500 ppm; BA@100 ppm + GA_3@250 ppm; Kintein@250ppm + GA_3@250ppm and Kinetin@100ppm + GA_3@100ppm. After 1 week of spraying, the plants were decapitated. Number of lateral shoots induced was counted after 20 days. Uniformly across the treatments, 200ppm GA_3 was sprayed to promote shoot elongation. After 2 months, length of side shoots was measured using measuring scale. Similarly girth of lateral shoots was measured using calipers and expressed in millimeter. The percent of lateral shoots with >6 mm (graftable size) were calculated accordingly.

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

This experiment was conducted during April 2019 to December 2019 to standardize age of mother plants to obtain more number of lateral shoots which can be used as scions for grafting. Based on the results of first experiment, the best chemical which could induce more lateral shoots **(**BA@100 ppm + GA_3@250 ppm) was sprayed on 4th, 5^{th}, 6^{th}, 7^{th}, 8^{th}, 9^{th} and 10^{th} month old mother plants. After 3 weeks,, 200ppm GA_3 was sprayed to all the treatments to promote shoot elongation. After 30 days of treatment, number of lateral shoots produced per plant was recorded. After 2 months,, the length of lateral shoots and shoot diameter were recorded. The number of shoots having graftable size (>6mm) were calculated as explained above. The weather parameters recorded during experimental period is shown in table 1.

Experiment 3: Standardization of grafting method for multiplication of papaya

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

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

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

Results and Discussion

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

Among different chemicals used for induction of multiple shoots, combination of BA@100ppm + GA_3@250 ppm could induce more number of shoots (39.019) per plant (Fig 1) followed by plants treated with BA@250ppm + GA_3@500ppm (23.16). The different treatments showed significant differences in induction of lateral shoots. Lowest number of shoots was recorded in seedlings treated with hydrogen cyanamide as it might be caustic resulting in scorching of buds. Among different chemicals, BA@100ppm + 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 Kinetin100@ppm + GA_3@100 ppm (9.56 cm) and with H_2CN_2 (2.54 cm). Similarly the percent of shoots having shoot diameters of > 6mm was significantly higher (72.56 %) in the treatment combination BA@100ppm + GA_3@250 ppm followed 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 presence of cytokinins, some other plant hormones like GA_3 is required for shoot elongation[12]. It is not only the hormones, but its concentration plays major role in shoot proliferation. Either suboptimal or super optimal concentrations may have adverse effect on shoot proliferations[13]. Many multiple shoots were produced by *in vitro* callus of papaya in media containing 5 mg IAA and 0.5 to 1.0 mg BA/L compared to same concentration of IAA along with 5 mg/L of kinetin[14]. In present study it is confirmed that combination of both cytokinin and gibberellins is required to bring about shoot proliferation and its further elongation to attain desirable size in terms of length and girth. With increase in concentration of both cytokinin and gibberellins there was reduction in shoot proliferation which clearly shows right concentration of hormones play a major role in side shoot proliferation. Decapitation of mother trees one week after hormonal application might have also increased cytokinin concentrations in lateral nodes as suggested[15]. There was increased concentration of cytokinin in nodal tissues and axillary buds after decapitation compared to before decapitation and thus they concluded nodal stem as the physiologically relevant site for cytokinin synthesis in the regulation of bud activity which subsequently transports into axillary buds thus bringing about shoot emergence[16].

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

Among mother plants of different ages tried, five month old plants when sprayed with BA@100ppm + GA_3@250 ppm induced more number of laterals (32.66) followed by 6 month old plants. With increase in age of plants, there was gradual reduction in the number of lateral shoots. Age of the mother plants play major role in getting healthier and better 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 plays a major role for effective shoot proliferations. Plants which have just completed their juvenile phase and the transition towards reproductive phase might be ideal for induction of more lateral as evinced in current study[17]. Usually seedling papaya plants enter reproductive phase and start producing flowers from 7th to 8th month onwards. In papaya plants well developed pith is very conspicuous during early stages of development and with progress in age of the plant, the stems become progressively hollow due to breakdown of pith at the internodes as they mature and fibers also become thicker and harder[18]. Because of the lignifications of nodal regions in matured plants, there may be reduced capacity for axillary buds to sprout from such nodes. However, during early stages of development, due to presence of pith and soft stem the axiallary buds in nodal region may be physiologically active and start sprouting after application of cytokinin compounds. Hence, in the present study more number of lateral shoots could be seen in mother plants till the age of 6 months and later there was reduction in emergence of lateral shoots.

In addition, the prevailing climate may also play a major role in lateral shoot induction. In current study, the seedlings which attained 5 to 6 month age (coincided with June-August) had favorable climate with respect to temperature and humidity (Table 1). The reduced number of shoots in 4th month may be due to both immature stages of mother plants and prevailing high temperature and low humidity (April – May). Similarly, less temperature coupled with more humidity and frequent showers might have reduced the lateral shoot induction in mother plants of age from 7 to 10 months (September to November). Environmental variables such as light, temperature, ambient humidity, wind speed, edaphic characteristics, and biotic factors such as mycorrhizal fungi and genotype, significantly affects the physiology of growth and development in papaya[19, 20]. It was also demonstrated that orchards located in regions with mean temperature of 25°C promotes excellent vegetative growth, fruit quality and high sugar compared to regions having mean temperature below 20°C and above 30°C [21].

Experiment 3: Standardization of grafting method for multiplication of papaya

Among different methods of grafting, wedge grafting recorded 72% success followed by cleft (46%) and side grafting (32%) while days taken for 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 (Fig 2).

Several studies are being done to find the best grafting methods and the results varied according to local climate, variety and rootstocks. Some of the factors which directly influence graft success are method of grafting, compatibility, vigour and health of the plants and aftercare of grafted plants[22]. Field grafting of female branches on the male branches of the same plant was reported for the first time [23], and confirmed the grafting success in papaya. However, under southern Indian conditions, field grafting has also been reported[24] in CO-1 and Coorg Honey Dew cultivars. About 80% success in tongue grafting was reported using tape as fastening device compared to cleft grafting[25]. With further modification in the tongue grafting and seedlings grown in polythene bags, they could obtain graft success upto 92.5% compared to other methods. Softwood wedge grafting is a simple technique and both the rootstock and scions are in active growing stage with soft stem, the callus formation might be quick followed by better dedifferentiation to connect the vascular tissues of stock and scion [26]. In grafting studies of citrus, it was opined that wedge grafting as the most suitable method followed by cleft grafting[28]. The disadvantage of cleft grafting is that temperature and humidity needs to be rigorously controlled which is not the case in softwood grafting[28]. Another disadvantage of cleft grafting is the stock diameter should be considerably greater and the diameter of stock and scion should exactly match to get maximum success [29]. However in present study there was slight mismatch in diameter of stock and scion as we kept scion for more number of days on mother plants causing lignification and thus not suitable for use as scion. The least success percent recorded in side grafting after two months might also 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 first two months[30].

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

Significant differences for 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 at 120 cm above ground level after 198 days of planting in seedlings. Grafted plants took 276 days for first harvest while it was 362 days in seedling plants. Significant difference was observed for average fruit weight, fruit length, fruit width and fruit volume with highest values for grafted plants than seedlings. However, no difference was recorded for fruit quality parameters. Its well established that grafting induces dwarfing effect on any 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 dwarf and sturdy, lowered 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 rate at which vegetative shoots grow and the time period over which they grow [35]. In our study also we recorded reduced internodal length on grafted plants compared to seedling plants. The reduced internodal distance might also contribute for 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 grafted plants [36]. The other possible reason for dwarfing may be due to reduced leaf area in grafted plants which are produced with less internodes with closer orientation to intercept lesser sunlight which might reduce the growth rate of grafted plants [37]. In addition to reducing plant height the rootstocks are known to induce precocious flowering [9]. Grafting is a strategy for inducing flowering in many fruit crops as some studies have suggested transmission of floral stimulus from rootstock to scions in addition to transport of mobile elements throughout the plants[39]. In present study also significant difference was observed for time to first flowering wherein the most of the photosynthates produced might have diverted for reproductive growth than vegetative growth which may induce early flower buds. In a study to see the behavior of three papaya types propagated by grafting, grafted plants of Taunung 01 variety recorded shorter height, large trunk diameter at 90 days after planting. First flowering was recorded on 35 days after planting at 65 cm height compared to seedling plants which took 70 days to flower at the height of 106.8cm [38]. 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 non significant effect of grafting on fruit quality parameters were reported by many previous workers though there was significant effect on fruit yield [33, 40, 41, 42.] In addition to grafting, it was reported that yield in ratoon crop can be increased by heading back (pollarding) the tree after first harvest at the height of 60 cm compared to heading back at less than 60cm height or more than it [43].

Conclusion:

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

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Table 1. Weather data during experimental period

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

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

Hormones/chemicals	No of lateral shoots	Average length of lateral shoots (cm)	% of lateral shoots with > 6mm dia
H_2CN_2 (0.5%)	7.50^{D}	2.54^{E}	0.00^{E}
BA 500ppm+GA_3 1000ppm	21.17^{BC}	13.59^{C}	48.26^{C}
BA 250ppm+GA_3 500ppm	23.16^{B}	17.47^{B}	52.12^{B}
BA 100ppm+GA_3 250ppm	39.19^{A}	25.56^{A}	72.56^{A}
Kinetin 250ppm+GA_3 250ppm	19.56^{C}	12.86^{C}	47.56^{C}
Kinetin 100 ppm+GA_3 100ppm	18.05^{C}	9.56^{D}	12.39^{D}
Mean	21.43	13.59	33.27
P value	<0.0001	<0.0001	<0.0001

Table3. Effect of age of rootstocks on induction of multiple shoots in papaya plants

Treatment				
Age of mother plants	**Time of the year**	**No. of lateral buds**	**Average Length of lateral shoots (cm)**	**Lateral shoots of >6 mm thickness (%)**
4 months	May	22.72^{B}	15.76^{D}	45.56^{C}
5 months	June	32.66^{A}	35.65^{A}	72.26^{A}
6 months	July	30.89^{A}	32.56^{A}	69.26^{A}
7 months	August	29.56^{A}	22.26^{B}	51.23^{B}
8 months	September	28.06^{AB}	19.56^{BC}	49.26^{BC}
9 months	October	15.43^{C}	21.56^{B}	48.86^{C}
10 months	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**

Table4. 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 color (cm)	Orange	Orange	NS
TSS (°B)	11.73	11.98	NS
Cavity index	21.28	22.73	NS

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

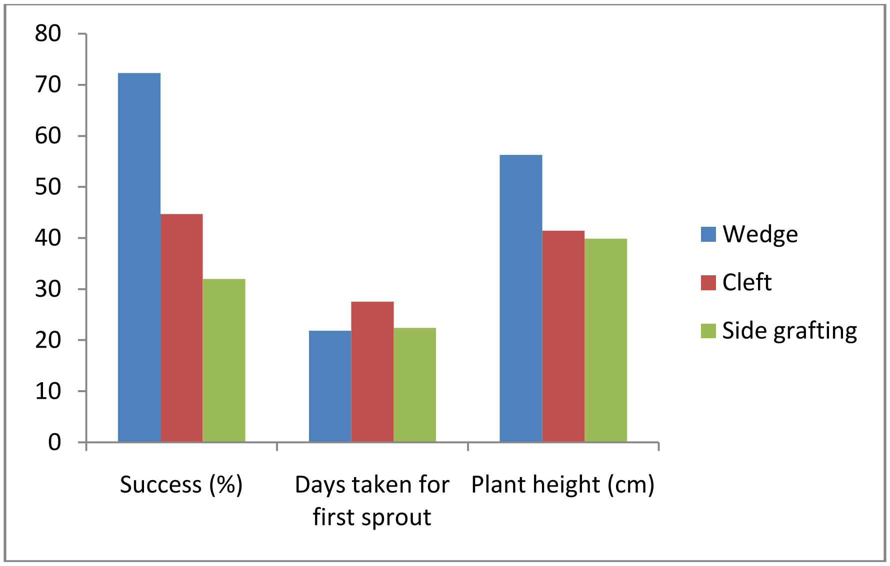

Figure 2. Effect of different methods of grafting on success of grafting in papaya