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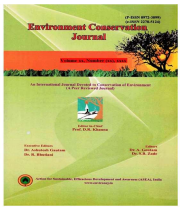

Standardization of asexual propagation techniques in rambutan (*Nephelium lappaceum* L.) for humid tropical region of India

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ABSTRACT

Rambutan (*Nephilium lappaceum* L.) is a potential exotic crop, fetching high value demand in the tropical markets of the world. In India the states like Kerala and Karnataka are pioneer in cultivation of Rambutan. It is a natural inhabitant of warm and humid climate of South East Asia gradually finding its spread and cultivation across continents of both the spheres, but in order to sustain the demand of this crop, there is an urgent need to find out the best method of propagation. In order to assess and determine a suitable vegetative propagation protocol for true to type plant multiplication in Coorg region of Karnataka, different types of budding and grafting techniques were evaluated in Rambutan. Three different methods of budding (*i.e.* patch budding, forkert budding and chip budding) were employed, among these three methods patch budding was proven to the best with a significantly higher rate of budding success (70.00 %), minimum days taken for sprouting (36.86 days) and with a lower rate of mortality (6.28 %). In a separate concurrent experiment, approach grafting method showed superior results among three different graftinf methods (*viz.* approach grafting, cleft grafting and veneer grafting) studied. It showed maximum rate of graft success (72.86 %), higher number of sprouted bud (4.29) and minimum days taken for sprouting (46.29 days) and also had a very low mortality rate (5.87 %).

Introduction

The aspect of fruit cultivation is constantly growing, promoted through the investment made by producers or by the consumers, to meet the demand of a healthy diet cart. Along with the popular fruits, obscure yet nutritious fruits are taking up their slots in supermarket shelves. This has greatly contributed to the expansion of underutilized and exotic fruits.

Among these exotic fruits, one commonly found is Rambutan (*Nephelium lappaceum* L.) which belongs to the Sapindaceae family. The name of this fruit derived from its place of origin's vernacular Malayan language word "Rambut", which translates into "Hair" in English, concerning to its soft thorn like hairy outgrowth that cover the fruit surface. Rambutan fruit varies from dark red to yellowish green in colour depending on the cultivar; ovoid in its shape with gelatinous white flesh inside adhering to its seed (Li *et al.,* 2018). Rambutan has a huge potential for industrial and neutraceutical usage in future. Edible fats extracted from seeds are found to be potential for replacing cocoa butter in confectionary industries (Solis-Fuentes *et al.,* 2011). Peel flour can be constituted as an effective ingredient in biopolymer production (Nadhirah *et al.,* 2016). Rind extractants has many effective uses such as anti-viral or anti-herpes (Nawawi *et al.,* 1999), anti-oxidant, anti-bacterial (Thitilertdecha *et al.,* 2008), anti-hyperglycemic,

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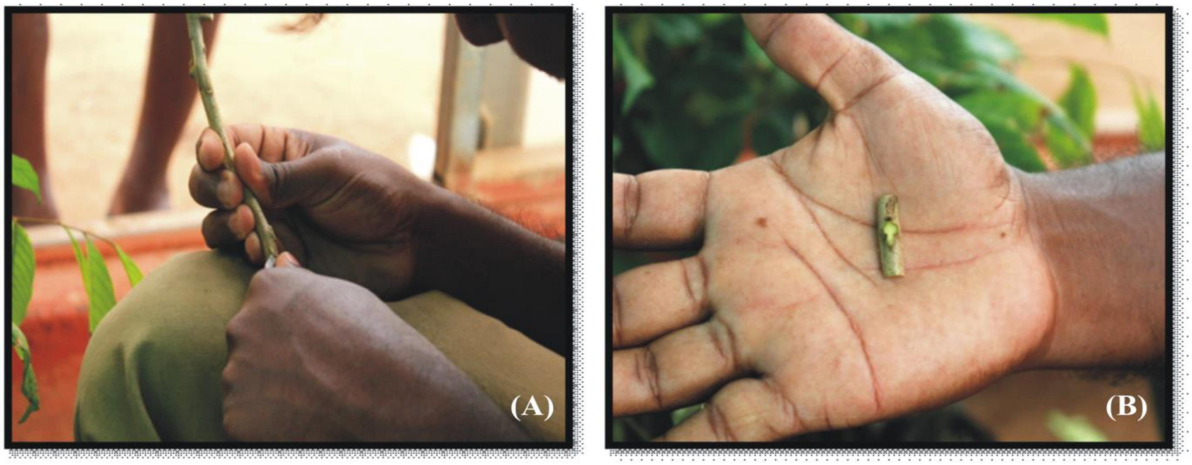
anti-inflammatory (Palanisamy *et al.,* 2011) and anti-proliferative (Kumar *et al.,* 2012). Rambutan crop has immense economic potential in market, which fetches an approximate price of ₹200/Kg of fruit for export from the states like Kerala and Karnataka. The successful commercial cultivation of this crop depends on many climatic and agronomical factors. Nevertheless a proper propagation technique is equally essential in order to supply true to type quality planting material, which eventually plays an important role in increasing the fruit yield and quality. Air layering is a common technique of propagation for Rambutan relatives such as litchi and longan. However, propagating Rambutan using air layering proved difficult (Morton, 1987). Grafting and budding success is generally determined by various factors such as grafting methods, grafting seasons, propagation environment, *etc.* (Chander *et al*., 2016). There are different grafting and budding techniques which are being practiced for commercial production of different fruit crops (Chander *et al*., 2016). As of now, there is no work done in India, for standardizing vegetative propagation methods in Rambutan. Hence, it is very much essential to arrive at a credulous method of vegetative propagation technique to maintain the genetic veracity of a variety and which can also be exploited at commercial level to increase the area of exotic crops like Rambutan. The study was undertaken to standardize suitable method for adoptable vegetative propagation technique with less rate of mortality in Rambutan under humid tropical region of Kodagu (Coorg) district Karnataka, Southern India.

Material and Methods

The experimental site of current study was located at the Central Horticultural experiment station (CHES, ICAR-IIHR), Chettalli, Kodagu, Karnataka (12°26' N latitude and 75°57' E longitude at 1050 m above mean sea level).The average annual temperature stays around 21.6 °C and the average rainfall is about 1900-1950 mm, during experimental duration average maximum temperature and average relative humidity were recorded as 29.04 °C and 97.5 % respectively. The Rambutan fruits are generally harvested during September to October month in this region. Under two separate experiments, budding and grafting operations were carried out during first week of November, 2017 and the observations were recorded upto next 120 days. One year old seedling of local variety of Rambutan, sourced from Kerala was utilized as rootstock. The seedlings of 25 to 30 cm height, thickness of a pencil grown in polybags were used for budding inside shade house. Bud woods were collected from cv. Arka Coorg Arun (CHES-27), a Rambutan selection growing in healthy manner at Rambutan orchard of CHES, Chettalli used in budding experiment. Bud woods (2.0-2.5 cm thickness) which matched with the rootstock's girth were carried to experimental site after covering in a moist sack bag to avoid desiccation. Different budding techniques were employed to find most suitable method of budding in Rambutan, Among the three different budding techniques were used the first one was patch budding (T_1), where a rectangular patch of bark (2.5 cm long and 1.0 to 1.5 cm wide) was removed from the stock and a similar patch, containing a bud was removed from the scion of Arka Coorg Arun (CHES-27). After removal, it was immediately inserted on to the stock. The patch containing bud fitted tightly at top and bottom, it was then wrapped with polythene strip (Figure 1). Under forkert budding (T_2), the root stock is prepared by giving two vertical cuts and a transverse cut above the vertical cuts to join them, along these cuts the bark was removed carefully, on which the flap of bark remains hanged down. The scion bud is prepared in same fashion as of patch budding with precise fit on root stock. The scion was then slipped into exposed portion of the stock and the flap is drawn over the inserted bud patch, followed by wrapping the region with a polythene strip and the third budding technique was chip budding (T_3), wherein a chip of bark and wood was removed from the smooth surface between the nodes of the stock. A chip of wood removed in same fashion and of similar size from the bud wood, ensuring that the bud was in middle of chip which precisely fits into stock ensuring cambium contact between chip bud and stock. It was tightly wrapped by polythene strip leaving protruding bud uncovered. After the sprout of the bud, wrapping material and above portion of stock were removed in all the treatments. In another concurrent experiment on standardization of grafting methods in rambutan, three different graftingtechniques (*viz.* G_1: Approach grafting; G_2: Cleft grafting; G_3:



A-B: Removal of patch bud from scion.

C-D: Insertion of the patch bud on rootstock.

E: Bud-stock union is wrapped with polythene

F: Sprouting of successful patch bud

Figure 1: Patch budding in Rambutan (A-F)

Side/Veneer grafting) were evaluated on same type of rootstocks used in previous budding experiment. The seedling rootstocks grown in polybags were brought in adjacent to the scion mother plant in orchard for approach grafting whereas, same types of rootstocks were placed inside shade house for cleft and side/veneer grafting. Selection of scion plant is the important factor for the propagation of fruit plants by grafting. Considering this fact, uniform bearing 10 years or older mother plant of rambutan (CHES-29) was selected as scion mother plant. The non-flowering scion shoots having dark green colored leaves, about 10-15 cm long, straight, smooth, healthy, pest and disease free were chosen from new season's growth, which were about 3-4 months old containing sufficient reserved food materials. Scion shoots were selected and marked for approach grafting on mother plant itself as it is an attached method of grafting and other selected scion shoots for detached method of grafting such as cleft and side/veneer grafting were pre-cured by defoliating them, leaving one-fourth of the petiole on the mother plants about one week before the grafting operation. After their detachment scions were carried in a poly bag to the experimental plots and kept in shady or cool place to avoid desiccation. In approach grafting technique, a thin slice of bark about 6 to 8 cm long at a height of about 20 to 25 cm above the ground level was removed from the stock. A similar reciprocate cut was made in scion with a sharp knife. Thus the cambium layer of both stock and scion were exposed. These cuts were brought together and tied tightly with the help of polythene strips (Figure 2).The rootstocks were cleft grafted after decapitating the stock 30 to 45 cm above the ground level. Beheaded stocks were split to about 5 cm depth through the centre of stem with sharp grafting knife. The scion of 15 to 20 cm size is taken from terminal shoot and wedged securely (6 to 7 cm). The cleft of the scion was slipped into the split of the stock followed by a tight wrapping of graft region with the polythene strips. In veneer grafting, the scion was inserted into the side of the rootstock, which is generally larger in diameter than the scion. First, a long sloping cut was given (2.5 to 3 cm) on the side of the rootstock at about 20 cm height. Cut was given on the scion into a wedge shape with one side slightly longer then the cut on the rootstock. Inserted the scion laid up the cambium layers. Bind the graft with wrapping material.

To evaluate the effect of treatments in these two experiments parameters like, budding success (Number of successful budding/ Total number of budding attended x 100) were recorded, successful bud showing green in colour and sprouting indicates in each treatment was counted at 20 days interval upto 60 days after budding. Similarly in grafting experiment, grafting success (%) = (Number of successful grafts/ Total number of grafted plants x 100); was recorded at 15 days interval upto 45 days after grafting. The shoot with the opened leaves from the terminal bud of scion was considered as the success of a graft. Observation regarding approach graft union was recorded by observing the greenness of the tissue. Among the other different common observations, days taken for bud sprouting, number of bud sprouts; number of leaves, number of branches, scion height, plant height, these observations were recorded on a cumulative basis 30 days interval upto 120 days after grafting. Union girth and rootstock girth were measured using vernier caliper. After 30-45 days of initial success, the grafts were observed for the next two months and in some successful budding or grafts, some scions were found to dry up due to failure of vascular connection. The number of buds or grafts mortality was recorded in percentage by using formula, Mortality (%) = (Total number of dead budded plant or grafts/ Total number of initially successful buds or grafts x 100). Design adopted for both of these experiments was randomized block design. There were three treatments with seven replications and ten numbers of grafts in each replication. The data in percentages were transformed to arc sine values for statistical analysis. The data were subjected to statistical analysis using WASP 2.0 software (ICARGOA-ICAR-Central Coastal Agricultural Research Institute). Critical difference values were tabulated at five per cent probability where "F" test was significant.

Results and Discussion

Among the different budding methods were employed to find out suitable one in Rambutan at CHES, Chettalli conditions in Karnataka, theobtained findings (Table 1) depicts that patch budding was the best among the three different

A-D: Preparing scion and rootstock for proper attachment.

E: Wrapping the graft with polythene sheet tightly.

F: Growth of rootstock and scion in successful approach graft.

G: Successfully approach grafted plants.

Figure 2: Approach grafting in Rambutan

budding treatments carried out with a significant higher rate of budding success (70.00 %), minimum days taken for sprouting (36.86 days), highest number of branches (2.86) and leaves (18.00). In addition to that, patch budding results indicating a successful bud union through significantly lowest rate of mortality (6.28 %) and maximum plant height (35.04 cm) with scion length of 17.06 cm in comparison to forkert and chip budding. Among the treatments, there was no significant variation regarding union girth (Table 1), represents the normal growth of budded plants. Normally, swelling in union region indicates lower bud growth in compared to root stock. The better performance of patch budding can be attributed to the factors like larger surface area attachment of scion bud, a precise fit on stock, less chance of injury to bud while removing the portion as patch, moreover it is easy to perform. Results from present investigation were in accordance with Morton

Table 1: Effect of different budding methods on different characters related to successful budding in Rambutan

Treatment	Budding success (%)	Days taken for bud sprouting	Number of branches	Number of leaves	Plant height (cm)	Union girth (cm)	Rootstock girth (cm)	Scion height (cm)	Mortality (%)
T_1 : Patch budding	70.00 (56.95)	36.86	2.86	18.00	35.04	0.60	0.89	17.06	6.28 (9.63)
T_2: Forkert budding	48.57 (44.16)	44.43	1.86	14.00	29.16	0.59	0.84	13.03	32.38 (34.46)
T_3 : Chip budding	24.29 (29.10)	48.86	1.57	12.71	28.86	0.69	0.80	13.53	46.43 (42.84)
Mean	47.62 (43.40)	43.38	2.10	14.90	31.02	0.62	0.84	14.54	28.36 (28.98)
S.Em±	1.95	0.75	0.23	0.71	1.10	0.04	0.04	0.43	3.35
CD (5%)	6.09	2.33	0.70	2.21	3.411	NS	NS	1.35	10.44

Note: Values in the parentheses are arc sine transformed data, NS= Non significant

Table 2: Effect of different grafting methods on different characters related to successful grafting in Rambutan

Treatment	Grafting percent success (%)	Days taken for bud sprouting	Number of bud sprouts	Number of branches	Plant height (cm)	Union girth (cm)	Rootstock girth (cm)	Scion height (cm)	Mortality (%)
G_1:Approach grafting	72.86 (58.75)	46.29	4.29	3.29	54.29	0.54	0.86	18.03	5.87 (9.30)
G_2 : Cleft grafting	32.86 (34.70)	50.57	1.57	1.86	39.20	0.49	0.83	12.93	29.48 (30.64)
G_3:Side/Veneer grafting	18.57 (25.18)	52.43	1.71	1.71	35.46	0.73	0.86	12.04	38.09 (33.52)
Mean	41.43 (39.55)	49.76	2.52	2.29	42.98	0.58	0.85	14.33	24.48 (24.49)
S.Em±	2.31	1.24	0.32	0.36	1.18	0.03	0.10	0.51	6.67
CD (5%)	7.19	3.86	1.00	1.12	3.67	0.09	NS	1.58	20.77

Note: Values in the parentheses are arc sine transformed data, NS= Non significant

(1987) and Tindall (1994) in rambutan. Obtained results were also supported by Kem and Rawat (2008) in aonla, where among the four methods of budding *i.e.*, T, Patch, Ring and Forkert budding, the maximum budding success was found in patch budding in cv. Krishna. Singh *et al*. (2003), Saroj *et al*. (2000) also observed that patch budding performed best, Prasad *et al.* (2003) also stated that patch budding gave higher success in comparison with chip budding in bael. Tripathi and Kumar (2004) and Syamal *et al*. (2013) also documented patch budding as the best budding technique. Rao *et al.* (1984) observed the maximum success under patch budding in guava than the forkert budding. Parallel reports were observed by Singh and Singh (2007) reporting that patch budding in months with high relative humidity may be adoptable for multiplication of elite tamarind genotypes. Summarized data of grafting experiments (Table 2), indicated that approach grafting was the most suitable method of grafting in rambutan under Chettalli conditions with maximum rate of graft success (72.86 %) and least day required for sprouting (46.29 days); for any successful grafting technique an early response from scion inserted is highly acceptable, It also had a higher number of sprouted bud (4.29), number of branches (3.29) and number of leaves (13.43). Approach grafting was found to be significantly, better in influencing proper scion and root stock growth. It was recorded, highest plant height (54.29 cm) and scion length of 18.03 cm along with a very low mortality rate (5.87 %). Though being considered as a cumbersome method of grafting because of essential prerequisites but in case of approach grafting, the scion used to receive continuous flow of nutrition from mother plant which act for better cambial activity led to callus development in graft

union resulting into higher success rate, a higher humidity also tends to facilitate this phenomenon. Brunner (2002) also reported, approach grafting is more dependable than cleft grafting for clonal propagation of desirable varieties in rambutan in Puerto Rico. Rajamanickam *et al*. (2002) in aonla and Bhagat *et al.* (1999) in guava also advocated the benefit of approach grafting in humid tropics. In sapota, similar results were recorded by Shirol *et al.* (2005) on grafting methods (*viz.* inarching, *in-situ* and softwood grafting) of sapota cv. Kalipatti on 18 months old khirni (*Manilkara hexendra*) rootstock, where highest graft success (85 %) was in inarch grafting. Inarching was significantly superior with 98% success rate and maximum emergence of new leaves and no. of new branches in comparison to veneer grafting, stone grafting and air layering in mango plants (Sengupta, 2005).

Conclusion

The results of current study pointed out the possibilities of exploiting patch budding and approach grafting as an answer to the quest of emerging with a suitable standard vegetative propagation approach for true to type multiplication of rambutan in Kodagu (Coorg) region of Karnataka. Patch budding can be preferred over approach grafting due its feasibility in adoption.

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Abbreviations

***cv.* :** Cultivar
CHES: Central Horticultural Experiment Station
ICAR: Indian Council of Agricultural Research
IIHR: Indian Institute of Horticultural Research
WASP: Web Agri Stat Package

Conflict of interest

The authors declare that they have no conflict of interest.

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