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Exploring the compatibility of interspecific grafting and budding methods in propagation of breadfruit (*Artocarpus altilis*)

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ABSTRACT

An experiment was conducted during the rainy (*kharif*) and winter (*rabi*) seasons of 2023–24 at College of Agriculture, Kerala Agricultural University, Vellanikkara, Kerala to identify suitable vegetative propagation methods and compatible rootstocks for breadfruit [*Artocarpus altilis* (Parkinson) Fosberg.]. The experiment was laid out in factorial randomized complete block design (F-RCBD) with two factors, namely methods of propagation and types of rootstocks. Patch budding and softwood grafting were tried using one-year-old rootstocks of three different species, such as *A. heterophyllus* (Jackfruit), *A. camansi* (Breadnut) and *A. hirsutus* (Wild jack/Anjili). Success percentage, growth parameters and histological characteristics of budded and grafted plants were evaluated. Results revealed that patch budding was the most suitable propagation method with a success rate of 33.3% in rainy season compared to softwood grafting (3.33%) and *A. camansi* is more compatible with *A. altilis* (success rate 38.33%) compared to other two species. Patch budding on *A. camansi* in rainy season was identified as the superior with a success rate of 66.67% followed by patch budding on *A. hirsutus* (33.33%) and softwood grafting on *A. camansi* (10%). In contrast, *A. heterophyllus* exhibited poor compatibility with *A. altilis* and failed to produce viable grafted or budded plants, suggesting its incompatibility with breadfruit.

Keywords: *Artocarpus* rootstocks, Histology, Interspecific compatibility, Propagation

Breadfruit [*Artocarpus altilis* (Parkinson) Fosberg.] is a tropical staple fruit crop that remains underutilised and neglected despite having high nutritional value and potential for achieving food security. Fruits are rich in carbohydrates, minerals and vitamins and have low fat content (Parrotta 1994). They can be eaten as baked, boiled, roasted, fried or made into flour. It requires less maintenance and is resilient to climate changes. Breadfruit is commonly propagated by clonal propagation methods such as suckers, root cuttings or air layering (Roberts-Nkrumah 2012). Vegetative propagation is required for both seedless and seeded varieties of breadfruit due to their recalcitrant seeds (Ragone 2007). Its commercial cultivation is limited due to the unavailability of quality planting materials. In conventional methods of propagation using root cuttings and suckers, continuous mass production cannot be achieved as the number of planting materials produced is less and such trees are susceptible to wind damage due to the lack of taproot system (Zhou *et al.* 2014).

Grafting or budding offers a wide range of benefits such as wider adaptability, true-to-type nature, low juvenile phase, dwarfism, etc. Interspecific grafting and budding using different species as rootstocks improve disease resistance and stress tolerance and also provide better tap root system. Several researchers have reported interspecific grafting of breadfruit with other related species such as *Artocarpus mariannensis* (Nandwani and Kuniyuki 2005), *A. camansi* (Medagoda and Chandrarathna 2007, Solomon *et al.* 2012), *A. sericicarpus* (Zhou and Underhill 2018), *A. odoratissimus* (Zhou and Underhill 2019) and *A. lakoocha* (Zhou and Underhill 2022). *A. heterophyllus, A. camansi* and *A. hirsutus* are locally available closely related species and adapted to the climatic conditions of Kerala. If they are compatible with breadfruit, it will be a breakthrough in its commercial cultivation. Hence this study was undertaken to identify the compatible rootstocks and to standardise the propagation methods in breadfruit.

MATERIALS AND METHODS

An experiment was conducted during the rainy (*kharif*) and winter (*rabi*) seasons of 2023–24 at College

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of Agriculture, Kerala Agricultural University, Vellanikkara (10°54'N, 76°28'E; at an elevation of 22.25 m amsl), Kerala. The experimental site had lateritic soil with acidic *p*H. The area receives most of its rainfall from the south-west monsoon, between June and September. The experiment was laid out in factorial randomized complete block design (F-RCBD) with two factors (method of propagation and types of rootstocks) having a total of six treatments replicated three times each with 10 plants. Patch budding (P_1) and softwood grafting (P_2) were employed with rootstocks R_1, *A. hirsutus*; R_2, *A. camansi* and R_3, *A. heterophyllus*. The treatment combinations were P_1R_1; P_1R_2; P_1R_3; P_2R_1; P_2R_2; and P_2R_3.

Seeds of the rootstocks were collected from the homesteads in Thrissur district and were sown in polythene bags of size 5" × 7" filled with potting mixture comprising of soil, vermicompost and coir pith compost in 4:1:1 proportion. One-year-old plants were used as rootstock. Tender green terminal scion shoots of 15 cm length were collected in the morning on the day of grafting from the college orchard. The scions were carefully cut from the selected mother tree using sharp secateurs and immediately wrapped in a moist cloth to prevent the buds from drying out. The wrapped scions were then transported in a polythene cover to the site for budding and grafting, which was performed on the same day of scion collection. The rootstock was decapitated at 15 cm height, followed by a longitudinal downward cut of 2.5 cm at the pruned end for softwood grafting. The basal end of the scion was shaped into a wedge by making two slanting cuts on opposite sides, inserted into the cleft of the rootstock, and then secured with a 150 gauge transparent polythene strip (1.5 cm wide, 30–45 cm long). After moistening, the graft was covered with a small 100 gauge tubular polythene bag to maintain humidity. Grafted plants were kept in shade and watered regularly. Once new growth appeared, the polythene cap was removed. For patch budding, budwoods were collected from the mother plants maintained in the college orchard in the early morning. Branches with dormant plump buds were carefully collected and kept moistened. The budwood was prepared by removing the leaves, leaving the petiole intact to serve as a handle during the insertion of the bud into the rootstock. A patch of bark measuring 2.0–2.5 cm in length and 0.8–1.0 cm in width was removed from the rootstock at a height of 15 cm above ground level. A similar-sized piece of bark containing an active scion bud was taken from the scion bud wood and placed onto the exposed portion of the rootstock. It was then securely tied with a polythene strip to ensure proper union. About a month after budding, the polythene strip was removed. If the buds were found green, the top growth of the rootstock was decapitated above one node, which facilitated the bud to sprout and put forth new flushes.

The different growth parameters, such as days to sprout, number of leaves, number of branches and shoot length were recorded at 15 days interval. Success percentage, number of roots and average length of roots were noted 120 days after grafting/budding (DAG/DAB). The success percentage was calculated by using the formula:

$$\text{Success percentage} = \frac{\text{Number of successful grafted or budded plants}}{\text{Total number of grafted or budded plants}} \times 100$$

The anatomical study of bud and graft union was carried out at 120 DAG/DAB. For anatomical studies, thin sections of the stem were taken from the region of graft or bud union using a Leica sliding microtome. Suitable sections were selected for anatomical studies and the remaining were preserved for future reference. Sections were stained using Safranin dye for 2–5 min followed by a dehydration process involving a graded series of alcohol solutions (30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% and 100%) and an alcohol-xylene mixture (50:50) followed by treatment with xylene (100%) each for about 2–5 min. The sections were then kept in xylene till they became clear. Later, it was mounted on the slide using a DPX mountant covered with a coverslip and observed under the MoticTM Trinocular Microscope at 4x magnification. Images were taken using Image analyser software Digi Pro.7. The data were analysed using factorial CRBD, following the methodology outlined by Panse and Sukhatme (1985) and analysed through ANOVA, with critical difference values calculated at a 5% level of significance, using KAU GRAPES software (Gopinath *et al.* 2020).

RESULTS AND DISCUSSION

All parameters showed significant variation across propagation methods, rootstock types and their interactions. The success rate also varied by season, with a notably higher percentage of success observed during the rainy season compared to winter. Among propagation methods, patch budding (P_1) achieved the highest success rate, with 33.33% in the rainy season and 22.22% in winter, in contrast to softwood grafting (P_2), which maintained a lower success rate of 3.33% in both seasons. Among the rootstocks tested, *A. camansi* (R_2) exhibited the highest success rate in both seasons, reaching a peak of 38.33% during the rainy season. Out of the three rootstocks evaluated, *A. heterophyllus* (jackfruit) (R_3) consistently showed negative results, indicating its incompatibility with *A. altilis* despite repeated trials across different seasons and rootstock ages. This indicates that several physiological, biochemical and molecular mechanisms are involved in scion rootstock interaction (Habibi *et al.* 2022). In examining the combined effects of propagation methods and rootstock types, patch budding on *A. camansi* (P_1R_2) yielded the highest success rate (66.67%), followed by patch budding on *A. hirsutus* (P_1R_1) at 33.33% and softwood grafting on *A. camansi* (P_2R_2) at 10%. Notably, softwood grafting on *A. camansi* (P_2R_2) showed a consistent success rate across both seasons (10%), whereas patch budding in *A. camansi* and patch budding in *A. hirsutus* displayed higher values during the rainy season (Fig. 1). This may be attributed to the high humidity which reduces water loss and provides conditions

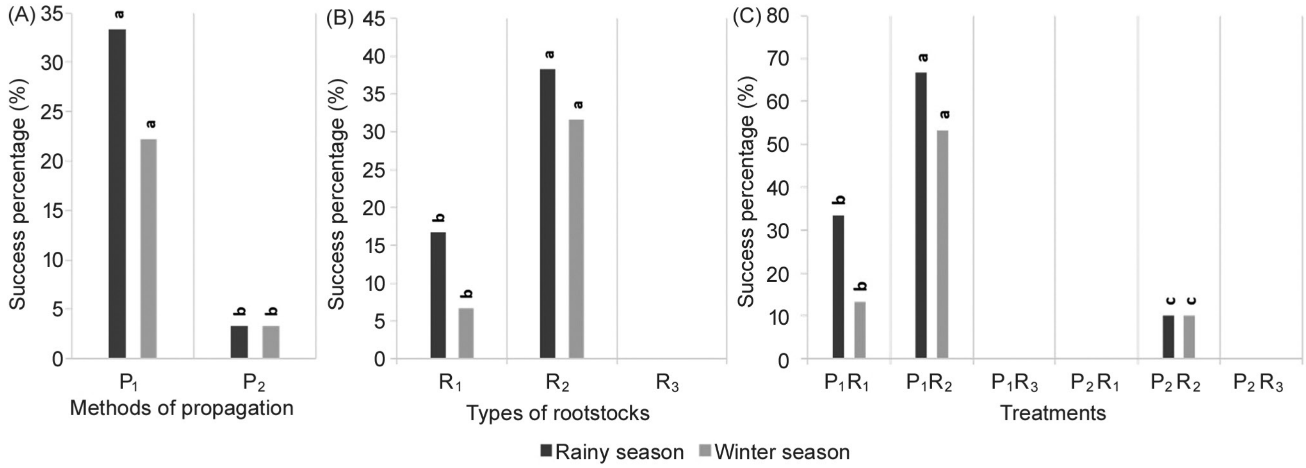

Fig. 1 Effect of propagation methods (A), rootstock (B) and interaction between propagation methods and rootstock (C) on success percentage of graftage under different seasons.
Values that share same letter are not significantly different from each other while values with different letters are significanly different at p=0.05. Treatment details are given under Materials and Methods.

beneficial for successful union between the rootstock and scion.

Among the two propagation methods, early sprouting was observed with softwood grafting (10.11 days) compared to patch budding (26.67 days) (Table 1). However, patch budding outperformed softwood grafting in all other parameters, including maximum number of leaves, greater shoot and root length and higher number of roots. The early sprouting is greatly influenced by the scion. The reserved food materials available in scion helps to produce early sprouting in grafted plants which is absent in patch budding and also the polythene cover over the graft improves the microclimate around the graft union by maintaining optimal humidity and preventing desiccation of scion tissues, thereby enhancing early sprouting (Visen *et al.* 2010). Bharad *et al.* (2006) and Jevoor *et al.* (2024) in Jamun and Baskaran *et al.* (2008) in jackfruit reported similar findings, as they observed that softwood grafting led to earlier sprouting compared to patch budding. The successfully grafted and budded plants remained single-stemmed throughout the study period, producing no branches. This growth pattern aligns with the typical development of breadfruit, which generally reaches a height of 4 m (13 ft) or more before branching (Ragone 2006). Maximum number of leaves was recorded for the patch budding during the period from 45–120 DAB (0.80 and 4.24, respectively) compared to softwood grafting (0.33 and 1.33, respectively) (Fig. 2). This is in agreement with Joshi *et al.* (2011) in custard apple where the scion length and leaf count were found superior for patch budding compared to softwood grafting. For patch budding, shoot length was 10.25–18.47 cm during the study period compared to softwood grafting (8.26–9.83cm) (Table 2). The greater vegetative growth observed in patch budding may be attributed to the fact that patch bud has high meristematic potential which makes early connections with the cambium of rootstock and utilises the food and nutrients for its vegetative growth while in grafting food and nutrients are initially utilised for the survival of the scion, leaving less nutrients available for vegetative growth, which leads to reduced vegetative development compared to patch budding. Root parameters such as the number of roots and the average length of roots were found highest for patch budding (35.92 cm and 20.93 cm, respectively) compared to softwood grafting (14.12 cm and 10.39 cm, respectively). Better performance of patch bud compared to softwood grafting may be due to strong stock-scion interaction and more number of leaves which leads to the production of more photosynthates, and better nutrient and water transfer thus promoting more vigorous growth (Koepke and Dhingra 2013).

Interspecific grafting is generally effective and compatible with many fruit plants (Ogden and Campbell 1980, Hosomi 2015, Al-Aizari 2024), though some species exhibited incompatibility issues (Gainza *et al.* 2015, Reig *et al.* 2018). Successful grafting between different species, particularly within the same genus, has been documented in *Prunus* spp. (Gainza *et al.* 2015), *Solanum* spp. (Kawaguchi *et al.* 2008, Bahadur *et al.* 2024), *Cucurbita* spp. (Lee and Oda 2003), *Passiflora* spp. (Silva *et al.* 2018), *Citrus* spp. (Muralidhara and Gowda 2019), etc. In this study, the rootstocks *A. camansi* (breadnut) (R_2) and *A. hirsutus* (anjili/wild jack) (R_1) produced successful union with breadfruit. Among these two, R_2 outperformed R_1 in morphological parameters like the number of leaves (1.20–5.50 from 45–120 DAG/DAB), shoot length (20.21–30 cm from 45–120 DAG/DAB), number of roots (51.82) and the average length of roots (31.40 cm). Early sprouting was observed for R_1 (20.23 days) compared to R_2 (34.95 days). Heterograft compatibility in plants is influenced by the genetic distance between the scion and rootstock. Greater the genetic distance, higher will be the graft incompatibility (Schoening and Kollmann 1997). Breadnut is considered as wild, seeded, ancestral form of breadfruit (Ragone 2006). This might be the reason for the highest success rate of breadnut as rootstock for breadfruit. Medagoda and Chandrarathna (2007) and Solomon *et al.* (2012) have also reported the suitability of *A. camansi* as a rootstock for breadfruit. *A. heterophyllus* failed to perform graft/bud union with *A. altilis* showing incompatibility between two different species.

Interaction effect between the two factors also revealed a significant difference in the time taken for sprouting. Softwood grafting in *A. camansi* showed the shortest sprouting time (30.33 days), followed by patch budding in *A. camansi* (39.56 days), which was at par with patch budding in *A. hirsutus* (40.47 days) (Table 1). As mentioned before, the available food material in the scion resulted in early sprouting in softwood grafting. Highest leaf number (1.4–7.0) was observed in patch budding in *A. camansi* from 45–120 DAG/DAB (Fig. 2). Maximum shoot length was observed during 45–90 DAG/DAB for softwood grafting in *A. camansi* (24.77–27.4 cm) followed by patch budding in *A. camansi* (15.65–25.34 cm), while in the later stages at 105 and 120 DAG/DAB, highest shoot length was recorded in patch budding in *A. camansi* (29.03 cm and 30.5 cm, respectively) succeeded by softwood grafting in *A. camansi* (28.4 cm and 29.5 cm, respectively). During the entire study period, the lowest shoot length was observed for patch budding in *A. hirsutus* (Table 2). Patch budding in *A. camansi* recorded more roots (61.27) followed by patch budding in *A. hirsutus* (46.5) and softwood grafting in *A. camansi* (42.37). Patch budding in *A. camansi* exhibited highest root length (31.63 cm) which was at par with softwood grafting in *A. camansi* and patch budding in *A. hirsutus* with a root length of 31.17 cm (Table 1). *A. heterophyllus* (R_3) in both methods and *A. hirsutus* in softwood grafting failed to produce successful union with breadfruit. Genetic, enzymatic, physiological and anatomical compatibility resulted in early and well-established connection between scion and rootstock in patch budding on *A. camansi*. This was justified in histological analysis also.

Anatomical evaluation of graft sections provides initial insights into the compatibility or incompatibility of graft unions (Kankaya *et al.* 1999). According to Dolgun *et al.* (2008), three key stages in graft formation were identified- callus formation, cambial differentiation and continuity and the development of vascular tissues. In this study also, similar stages were observed at the stock-scion union. Anatomical studies conducted at 120 DAG/DAB revealed a well-developed connection between the rootstock and scion in patch budding on *A. camansi* (Fig. 3A). The adhesion line, formed by the thickened cell wall of rootstock and scion, typically visible in the early stages of graft union (Frey *et al.* 2020), was absent in sections, indicating compatibility between breadfruit and breadnut. Xylem differentiation was observed in some regions, while the presence of necrotic areas suggests pathological infections, highlighting the need for improved hygienic practices to enhance the success rate. At the same time, in

Table 1 Effect of methods of propagation and types of rootstocks on establishment of grafted and budded plants of breadfruit (pooled data)

Treatments	Days to sprout	Average length of roots (cm)	Number of roots
P_1	26.67[a]	20.93[a]	35.92[a]
P_2	10.11[b]	10.39[b]	14.12[b]
CD	1.44	0.91	1.75
CV	7.60	5.64	6.81
SEM±	0.47	0.30	0.57
R_1	20.23[b]	15.58[b]	23.25[b]
R_2	34.95[a]	31.40[a]	51.82[a]
R_3	-	-	-
CD	1.76	1.11	2.14
CV	7.60	5.64	6.81
SEM±	0.57	0.36	0.70
P_1R_1	40.47[a]	31.17[a]	46.50[b]
P_1R_2	39.56[a]	31.63[a]	61.27[a]
P_1R_3	-	-	-
P_2R_1	-	-	-
P_2R_2	30.33[b]	31.17[a]	42.37[c]
P_2R_3	-	-	-
CD	2.49	1.57	3.03
CV	7.60	5.64	6.81
SEM±	0.81	0.51	0.98

Values that share same letter are not significantly different from each other while values with different letters are significanly different at p=0.05. Treatment details are given under Materials and Methods.

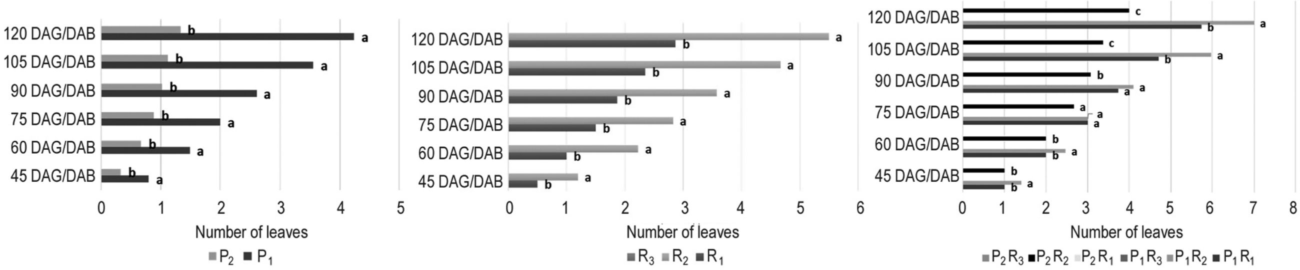

Fig. 2 (A) Effect of method of propagation (A), rootstock (B) and interaction between propagation methods and rootstock (C) on leaf number in breadfruit.
DAG/DAB, Days after grafting/budding. Values that share same letter are not significantly different from each other while values with different letters are significanly different at p=0.05. Treatment details are given under Materials and Methods.

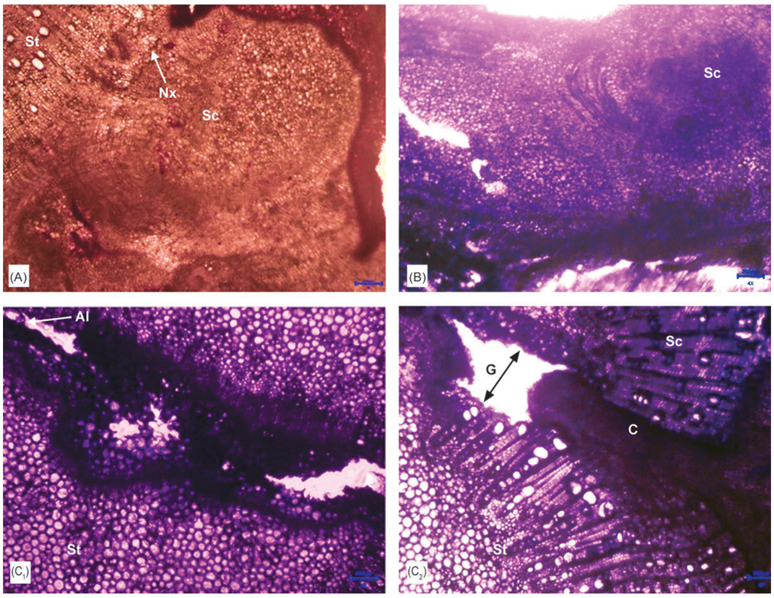

Fig. 3 Microscopic image of the section from patch budding of breadfruit on (A), *A. camansi*; (B), *A. hirsutus*; (C), Softwood grafting on *A. camansi*. C_1, Necrotic line present in between rootstock and scion; C_2, Callus connection between rootstock and scion. Scale bar- 200 µm. Sc, Scion; St, Rootstock; C, Callus; N_l, Necrotic line; A_l, Adhesion line; G, Gap; Nx, New xylem.

Table 2 Effect of methods of propagation and types of rootstocks on shoot length of breadfruit

Treatments	Shoot length (cm)					
	45 DAG/ DAB	60 DAG/ DAB	75 DAG/ DAB	90 DAG/ DAB	105 DAG/ DAB	120 DAG/ DAB
P_1	10.25^{a}	12.00^{a}	13.65^{a}	14.85^{a}	17.20^{a}	18.47^{a}
P_2	8.26^{b}	8.46^{b}	8.69^{b}	9.13^{b}	9.47^{b}	9.83^{b}
CD	0.22	0.38	0.36	0.48	0.33	0.47
CV	2.37	3.67	3.18	3.86	2.38	3.25
SEM±	0.07	0.12	0.12	0.15	0.11	0.15
R_1	7.50^{b}	8.15^{b}	9.20^{b}	9.60^{b}	11.28^{b}	12.45^{b}
R_2	20.21^{a}	22.54^{a}	24.30^{a}	26.37^{a}	28.72^{a}	30.00^{a}
R_3	-	-	-	-	-	-
CD	0.28	0.47	0.45	0.58	0.4	0.58
CV	2.37	3.67	3.18	3.85	2.38	3.25
SEM±	0.09	0.15	0.14	0.19	0.13	0.19
P_1R_1	15.00^{c}	16.30^{c}	18.40^{c}	19.20^{c}	22.57^{c}	24.9^{c}
P_1R_2	15.65^{b}	19.71^{b}	22.54^{b}	25.34^{b}	29.03^{a}	30.5^{a}
P_1R_3	-	-	-	-	-	-
P_2R_1	-	-	-	-	-	-
P_2R_2	24.77^{a}	25.37^{a}	26.07^{a}	27.40^{a}	28.40^{b}	29.5^{b}
P_2R_3	-	-	-	-	-	-
CD	0.39	0.67	0.63	0.82	0.57	0.82
CV	2.37	3.67	3.18	3.85	2.38	3.25
SEM±	0.13	0.22	0.21	0.27	0.18	0.27

DAG/DAB, Days after grafting/budding. Values that share same letter are not significantly different from each other while values with different letters are significanly different at p=0.05. Treatment details are given under Materials and Methods.

softwood grafting on *A. camansi*, the adhesion line remained visible, with a gap observed between the rootstock and scion. Grafting shock was evident from the presence of a pronounced necrotic line (Fig. 3C_1). Due to uneven thickness between the rootstock and scion, the cambial connection was only established on one side, where it began producing new cells towards the gap, which may be bridged in the later stages of graft union development (Fig. 3C_2). Patch budding on *A. hirsutus* sections also displayed the connection between scion and stock indicating the compatibility between breadfruit and *A.hirsutus* (Fig. 3B).

Different methods of propagation and types of rootstocks had a significant impact on the success percentage as well as the growth parameters of breadfruit. The study revealed that patch budding is the best propagation method compared to softwood grafting and *A. camansi* and *A. hirsutus* can be used as rootstocks for breadfruit. Patch budding on *A. camansi* showed a higher success per cent followed by patch budding on *A. hirsutus.* These promising rootstocks can be utilised commercially after field evaluation. *A. camansi* can be recommended for farmers to be used as a rootstock against *A. altilis* for mass multiplication and patch budding to be followed for production of quality planting materials. It can be practiced at multilocation to assess its performance in different regions.

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