Plant Archives Vol. 24, No. 2, 2024 pp. 577-582 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.081

STANDARDIZATION OF SOFT WOOD GRAFTING FOR CUSTARD APPLE CULTIVARS UNDER VARIOUS ENVIRONMENTAL CONDITION

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(Date of Receiving-01-02-2024; Date of Acceptance-11-04-2024)

ABSTRACT

Custard apple plants traditionally propagated by seeds result in significant fruit variability. Vegetative propagation provides true-to-type crops with shorter juvenile phases. This study aimed to standardize softwood grafting techniques for three custard apple cultivars, namely Arka Sahan, Island Gemand Balangar, under three different propagation environmental conditions: open field, shade house, and mist house. The experiment was conducted following a factorial completely randomized design with nine treatments replicated three times. Results indicated that cv. Arka Sahan exhibited the shortest duration for initial sprouting (8.53 days) and produced the highest number of sprouts per graft (3.83), girth at graft union (11.86 mm), length of sprouted shoot (29.50 cm), internodal length of sprouted shoot (3.13 cm), number of leaves per graft (28.00), graft success (97.77%), survivability (92.55%), total chlorophyll content (61.66 SCMR Values), stomatal conductance (350.66 mmol $m^{-2}s^{-1}$) and leaf area per graft (1134.52 cm^2), when placed under mist house conditions. This was followed by cv. Island Gem under mist house conditions.

Key words : *Annona*, Propagation, Cultivars, Environmental condition.

Introduction

Custard apple (*Annona squamosa* L.) holds significant importance as a dry land fruit in India and belongs to the Annonaceae family. It is among the finest fruits introduced to India. The *Annona* genus comprises over 100 species, with five producing edible fruits, including *Annona squamosa* L. (Custard apple), *Annona reticulata* (Bullock's heart) and *Annona cherimoya* (Hanumanphal), which hold commercial importance. In India, it is also referred to as sitaphal, sugar apple and sweet soap (Popenoe, 1974).

Custard apple plants have traditionally been propagated using seeds since long time. However, this method often leads to significant variability in fruit size, shape and quality. Vegetative propagation offers a solution by producing crops that are true-to-type and have a shorter juvenile phase. Softwood grafting emerges as a practical and efficient alternative, enabling the production of grafts within a year and thereby reducing the cost associated with raising them. Softwood grafting yields high success rates and increased survival percentage of quality grafts, minimizing the risk of mortality. This process contributes to the establishment of orchards that are more uniform and of higher quality (Ram and Pathak, 2006).

The presence of shade during and after grafting has been observed to positively influence the success of grafting. Light serves as a crucial stimulus for initiating photosynthetic activity, thus enhancing the nourishment of grafts. The rate of photosynthetic activity, however, varies depending on the degree of shading (Swamy, 1993). Given the rising demand for custard apple planting material, there is a pressing need to ensure year-round production of grafts by creating optimal environmental conditions. This can be achieved through the use of

protected structures such as mist chambers and shade houses, which can significantly increase the success rate by providing favourable conditions. Therefore, in consideration of current needs, growing conditions, and future requirements, it is imperative to standardize the appropriate propagation environment for softwood grafting in custard apple.

Materials and Methods

The study was conducted at the Main Horticulture Research and Extension Centre (MHREC), University of Horticultural Science, Bagalkot, during the period of 2020-21. This location falls within the northern dry zone of Karnataka (zone -3), positioned at $16^0 10'$ north latitude and $17^0 42'$ east longitude, with an elevation of 542 meters above mean sea level. Scion material was obtained from elite custard apple varieties, namely Arka Sahan, Balanagar, and Island Gem, sourced from the mother block of the University of Horticultural Sciences, Bagalkot.

Ten days before grafting, the selected scion shoots underwent curing, where leaves were removed using secateurs while leaving the bud intact. Rootstock comprised one-year-old local cultivar *Annona* seedlings of optimum size. Grafting was conducted on February 15, 2021. Following grafting, all three cultivars were transferred to mist house, shade house and open field conditions, with 30 plants assigned to each condition, to evaluate their performance under different growth environments.

Various parameters were recorded, including the number of days taken for sprouting, number of sprouts per graft, girth at graft union, length of sprouted shoot, girth of sprouted shoot, internodal length of sprouted shoot, number of leaves per graft, graft success, graft survivability, leaf area, total chlorophyll content and stomatal conductance. After grafting, observations were made regularly to monitor graft greening until sprouting or drying occurred, with means computed thereafter. Ninety days post-grafting, parameters such as number of leaves developed per graft, length of sprouted shoot (cm), girth of sprouted shoot (mm), internodal length of sprouted shoot (cm) and leaf area (cm^2) were recorded. Girth at graft union (mm) of five randomly selected observational plants was measured separately in each treatment using digital vernier callipers. Grafts were continuously monitored up to 90 days post-grafting and final survival rates were recorded. Total chlorophyll content was assessed using a chlorophyll meter (SPAD-502), while stomatal conductance was measured using a porometer at the 90-day mark post-grafting. Data were analysed according to Panse and Sukhatme (1985).

Results and Discussion

Number of days taken for first sprouting

The data in Table 1 revealed an early onset of sprouting in the mist house condition, occurring at 10.12 days. Specifically, among the cultivars, cv. Arka Sahan exhibited significantly faster initial sprouting at 9.79 days. Additionally, the interaction effect between the propagation environment and custard apple cultivars on the number of days taken for first sprouting was notably minimized in cv. Arka Sahan grafted plants under mist house conditions, emerging at 8.53 days. This variation could be attributed to the optimal temperature and higher relative humidity prevalent in the mist house environment. The warmer and humid atmosphere within the mist house likely contributed to soil warming and facilitated early contact between the cambium layers of the stock and scion, leading to early callus formation and subsequent bud sprouting. Similar findings were reported by Singh and Singh (2007) in Jamun and Syamal *et al.* (2013) in Bael.

Number of sprouts per graft

The data in Table 1 indicated that the highest number of sprouts per graft was observed among plants kept in the mist house (3.51) across different propagation environments and Annona cultivars. Specifically, cv. Arka Sahan recorded the highest number of sprouts per graft (3.16) among cultivars. Furthermore, the interaction effect between propagation environment and Annona cultivars showed that cv. Arka Sahan grafts maintained in the mist house condition exhibited the maximum number of sprouts per graft (3.83). This could be attributed to the genetic performance of the scion and the favourable microclimate conditions inside the mist house, which promoted early and maximum sprouting of the scion. This finding aligns with the results reported by Bajpai *et al.* (1989).

Girth at graft union

In Table 1, it was observed that grafts maintained under mist house conditions exhibited the highest girth of graft union (10.64 mm) among different propagation environments. Similarly, among the cultivars, cv. Arka Sahan displayed the maximum girth of graft union (9.96 mm). Furthermore, considering the interaction effect between growing conditions and Annona cultivars, the maximum girth of graft union was observed in cv. Arka Sahan grafts under mist house conditions (11.86 mm). This variation may be attributed to the availability of favourable environmental conditions, facilitating early healing at the graft union. This, in turn, leads to accelerated

physiological tissue development characterized by higher meristematic activities, cell division and cell elongation at the union, promoting plant growth and the accumulation of stored food materials or carbohydrates at the union. These findings are consistent with those reported by Gurudutta *et al*. (2004); Mulla *et al*. (2011) in Jamun and Jalal *et al*. (2018) in Aonla.

Length of sprouted shoot (cm)

The data presented in Table 1 showed that among various propagation environments, the mist house condition yielded the longest sprouted shoot (25.88 cm), while Arka Sahan exhibited the longest sprouted shoot (25.96 cm) among Annona cultivars. Specifically, when considering the interaction between Annona cultivars and propagation environment, cv. Arka Sahan maintained in the mist house condition displayed the longest sprouted shoot (29.50 cm). The increase in sprout length is likely attributed to the warmer and more humid environment inside the mist house, which promotes soil warming. Consequently, the warmer conditions inside the mist house positively influence sprout length. These findings parallel those of Pandey *et al*. (2004).

Internodal length of sprouted shoot (cm)

The data in Table 2 revealed that grafts maintained under mist house conditions exhibited significantly longer internodal lengths in sprouted shoots (2.83 cm). Among different Annona cultivars, Island Gem displayed significantly longer internodal lengths in sprouted shoots (2.81 cm). In terms of interaction effect, cv. Arka Sahan under mist house conditions recorded significantly longer internodal lengths in sprouted shoots (3.13 cm). This variation is likely due to the physiological condition of the scion and favourable environmental conditions in the mist house, promoting higher physiological activity and carbohydrate production, thereby stimulating increased vegetative growth and internodal length. Similar findings

Table 1 : Influence of propagation environment and *Annona* cultivars on different growth parameters.

Propagation environment	Number of days taken for first sprouting				Number of sprouts per graft				Girth at graft union (mm)				Length of sprouted shoot (cm)			
	V_1	V_2	V_3	Mean	V_1	V_2	V_3	Mean	V_1	V_2	V_3	Mean	V_1	V_2	V_3	Mean
C_1	8.53	11.99	9.85	**10.12**	3.83	3.03	3.66	**3.51**	11.86	9.04	11.02	**10.64**	29.50	19.16	29.00	**25.88**
C_2	9.63	12.96	10.52	**11.03**	3.13	2.53	3.06	**2.91**	9.46	8.51	10.10	**9.36**	26.30	13.06	27.00	**22.12**
C_3	11.22	14.66	12.22	**12.70**	2.51	2.10	2.43	**2.35**	8.56	8.04	8.36	**8.32**	22.10	10.83	20.66	**17.86**
Mean	**9.79**	**13.20**	**10.86**		**3.16**	**2.55**	**3.05**		**9.96**	**8.53**	**9.81**		**25.96**	**14.35**	**25.55**	
	S. Em ±		**CD at 5%**		**S. Em ±**		**CD at 5%**		**S. Em ±**		**CD at 5%**		**S.Em ±**		**CD at 5%**	
C	0.24		0.72		0.03		0.10		0.17		0.53		0.34		1.03	
V	0.24		0.72		0.03		0.10		0.17		0.53		0.34		1.03	
C×V	0.41		1.25		0.05		0.17		0.30		0.92		0.59		1.76	

C_1- Mist house, **C_2**- Shade net, **C_3**- Open field, **V_1**– ArkaSahan, **V_2** - Balanagar, **V_3** -Island Gem, **DAG -** Days after grafting.

Table 2 : Influence of propagation environment and *Annona* cultivars on different growth parameters.

Propagation environment	Internodal length of sprouted shoot (cm)				Number of leaves per graft				Leaf area per graft (cm^2)			
	V_1	V_2	V_3	Mean	V_1	V_2	V_3	Mean	V_1	V_2	V_3	Mean
C_1	3.13	2.34	3.03	**2.83**	28.00	22.99	25.81	**25.60**	1134.52	803.54	1007.85	**981.97**
C_2	2.70	2.10	2.90	**2.56**	24.37	21.33	23.50	**23.06**	926.31	660.54	886.69	**824.51**
C_3	2.30	1.89	2.50	**2.23**	20.77	17.93	20.04	**19.58**	755.47	519.69	696.86	**657.34**
Mean	**2.71**	**2.11**	**2.81**		**24.38**	**20.45**	**23.11**		**938.77**	**661.26**	**863.80**	
	S.Em±		**CD at 5%**		**S.Em±**		**CD at 5%**		**S.Em±**		**CD at 5%**	
C	0.03		0.10		0.20		0.60		8.50		25.49	
V	0.03		0.10		0.20		0.60		8.50		25.49	
C × V	0.06		0.17		0.34		1.04		14.72		44.15	

C_1- Mist house, **C_2**- Shade net, **C_3**- Open field, **V_1**- ArkaSahan, **V_2** - Balanagar, **V_3** -Island Gem, **DAG -** Days after grafting.

Table 3 : Influence of propagation environment and *Annona* cultivars on different growth parameters.

Propagation environment	Chlorophyll content (SCMR)				Stomatal conductance (mmol $m^{-2}s^{-1}$)			
	V_1	V_2	V_3	Mean	V_1	V_2	V_3	Mean
C_1	54.80	46.79	54.66	**52.08**	283.33	253.33	282.66	**273.10**
C_2	57.58	51.66	56.10	**55.12**	319.00	271.66	310.00	**300.22**
C_3	61.66	53.66	61.66	**58.99**	350.66	324.66	342.66	**339.32**
Mean	**58.01**	**50.70**	**57.47**		**317.66**	**283.32**	**311.77**	
	S.Em ±		**CD at 5%**		**S.Em ±**		**CD at 5%**	
C	0.33		1.00		1.94		5.83	
V	0.33		1.00		1.94		5.83	
C × V	0.58		1.74		3.37		10.10	

C_1- Mist house, C_2- Shade net, C_3- Open field, V_1- ArkaSahan, V_2 - Balanagar, V_3 -Island Gem, **DAG** - Days after grafting.

have been reported by Ram *et al*. (2012) in mango; Shinde *et al*. (2015) in custard apple and Kholia *et al*. (2017) in guava.

Number of leaves per graft

The number of leaves per graft was detailed in Table 2. Notably, grafts maintained under mist house conditions exhibited the highest number of leaves, significantly reaching 25.60. Among the various custard apple cultivars, cv. Arka Sahan showed the maximum number of leaves at 24.38. A significant interaction effect between different propagation environments and cultivars was observed regarding the number of leaves per graft, with the highest number recorded in cv. Arka Sahan under mist house conditions, totalling 28.00 leaves per graft. The early sprouting, coupled with optimal temperature and relative humidity, likely contributed to the production of the maximum number of leaves, possibly due to increased synthesis of photosynthates. Additionally, the development of more sprouts, heightened meristematic activity, and improved graft healing could also be responsible. These findings align with those of Pujari and Magdum (1991) in Sapota.

Leaf area per graft (cm^2)

The data presented in Table 2 indicates that among different propagation environments, the mist house condition exhibited significantly maximum leaf area (981.97 cm^2). In terms of cultivars, cv. Arka Sahan displayed significantly maximum leaf area per graft (938.77 cm^2). However, considering the interactions, cv. Arka Sahan grafts under mist house conditions recorded the highest leaf area per graft (1134.52 cm^2). The increased leaf area may be attributed to the favourable weather conditions prevailing in the mist house, which likely contribute to enhanced leaf growth. Similar results have been reported by Sivudu *et al.* (2014) in jamun; Mulla *et al.* (2011) in jamun; Rani *et al.* (2015) in guava and Chander *et al.* (2016) in Jamun.

Chlorophyll content (SCMR)

Chlorophyll content was mentioned in Table 3. Grafts maintained under open condition recorded significantly maximum amount of chlorophyll content (58.99) among the different custard apple cultivars, significantly maximum chlorophyll content was noticed in cv. Arka Sahan (58.01). Among the different treatment interactions also significant variations were noticed with respect to chlorophyll content. Significantly maximum amount of chlorophyll content was noticed in cv. Arka Sahan and Island Gem kept under open condition (61.66).

Stomatal conductance (gs)

Data presented in table 3 shows that grafts placed under open field condition recorded significantly maximum stomatal conductance (339.32 mmol m^2s^{-2}). Regarding cultivars, the data revealed that the ArkaSahan recorded significantly maximum stomatal conductance (317.66 mmol m^2s^{-2}). Data regarding interaction effect between propagation environment and *Annona* cultivars was also found significant. ArkaSahan kept under open condition had significantly maximum stomatal conductance (350.66 mmol m^2s^{-2}). This might be due to better leaf surface available in cultivar providing more number of stomata and optimum temperature and relative humidity maintained under open condition that helps in getting maximum stomatal activity compared to the other condition because stomata opening and closing is completely depends on environmental conditions *viz*., temperature, relative humidity and light and Minimum stomatal conductance was noticed in mist house because of higher relative humidity in mist house compare to open field and shade net condition and these findings were in accordance with Sharma *et al.* (2016) in custard apple.

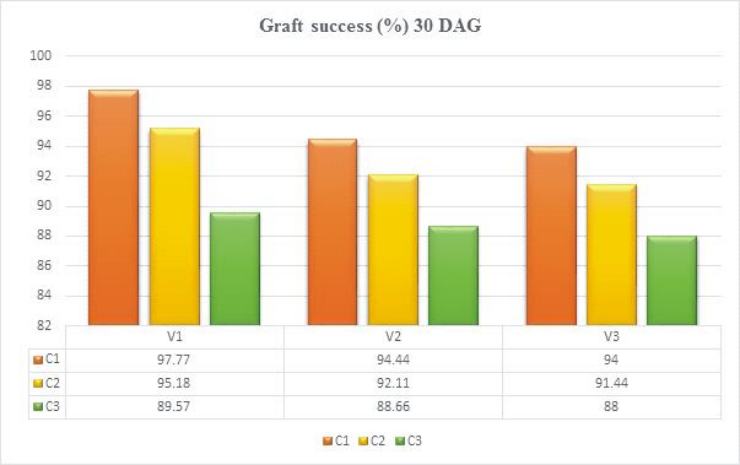

Fig. 1 : Influence of propagation environment and Annona cultivars on graft success (%)30 DAG. **C_1**- Mist house, **C_2**- Shade net, **C_3**- Open field, **V_1**- ArkaSahan, **V_2** –Balanagar, **V_3** -Island Gem, **DAG** - Days after grafting.

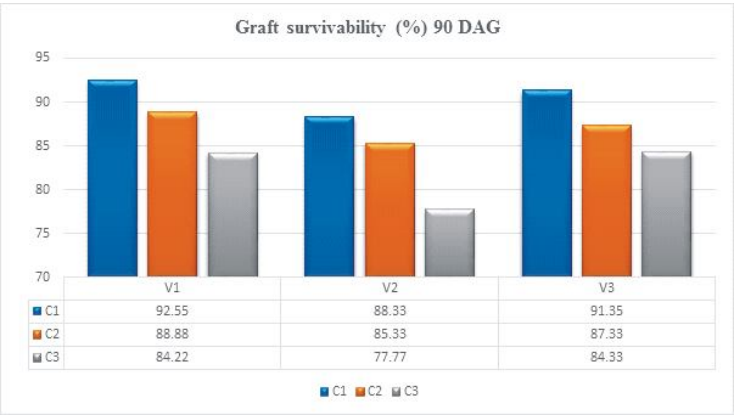

Fig. 2 : Influence of propagation environment and *Annona* cultivars on graft survivability (%) 90 DAG. **C_1**- Mist house, **C_2**- Shade net, **C_3**- Open field, **V_1**–ArkaSahan, **V_2** - Balanagar, **V_3** - Island Gem, **DAG** - Days after grafting.

Graft success (%)

Graft success percentage at 30Days after grafting revealed that the mist house condition exhibited significantly higher graft success at 95.40 per cent. Among the different cultivars, cv. Arka Sahan achieved the highest graft success rate at 94.17 per cent. A significant interaction effect between the propagation environment and Annona cultivars on graft success was observed, with cv. Arka Sahan under mist house conditions showing the highest graft success rate at 97.77 per cent (Fig. 1). This could be attributed to the favourable humidity and temperature levels maintained in the mist house. The advantages of the mist house environment may be attributed to prolonged high humidity levels, which prevent tissue desiccation at the scion and stock interface, there by promoting rapid development of callus tissues and facilitating better graft union. Similar results have been reported by Jose and Valsalakumari (1991), Dhanraj (1996) in jackfruit and Jholgiker (2001) in custard apple.

Graft survivability (%)

The choice of propagation environment demonstrated significant importance regarding graft survivability at 90 days after grafting (DAG). Notably, the mist house environment exhibited superior results, achieving maximum graft survivability at 90.74 per cent. Among the cultivars, Arka Sahan showed the highest graft survival rate at 90 DAG, reaching 88.55 per cent. There was an observed interaction between the propagation environment and Annona cultivars at 90 DAG. Specifically, when Arka Sahan cultivar was placed under mist house conditions, it demonstrated the highest graft success rate at 92.55 per cent (Fig. 2). The increased survivability of Arka Sahan grafts in the mist house environment could be attributed to optimal temperature and physiological conditions of the scion, which favor the proliferation of new parenchymatous callus between the rootstock and scion, along with favorable callus formation due to higher humidity (Hartmann *et al*., 1997). Similar outcomes were reported by Desai and Patil (1984); Sivudu *et al*. (2014) in mango, as well as by Shinde *et al.* (2010) in jamun.

Conclusion

In the present investigation both the growth of the graft and survivability was found to be highest when the grafts of cv. ArkaSahankept under mist house condition followed by cv. Island Gemkept under mist house. The congenial temperature and relative humidity in the mist house at the initial stage might have helped in better graft success and survivability. It can be concluded that grafts maintained under mist house condition during the initial days of grafting may be helpful in large scale and off-season production of grafts in *Annona*to get vigorous and good quality seedlings in shorter period of time.

Acknowledgement

We extend our heartfelt appreciation to the Main Horticulture Research and Extension Centre (MHREC) at the University of Horticultural Science, Bagalkot, for their consistent support and generous provision of resources during the duration of this study.

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