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Standardization of propagation method of Custard Apple (*Annona reticulata*) by stem cuttings

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Abstract
An experiment was carried out at instructional cum research farm, Department of Horticulture, BNCA, AAU, Biswanath Chariali during 2018-2019 to standardize the propagation method of custard apple (*Annona reticulata*) by stem cuttings. For carrying out the propagation by cuttings, three types of cuttings *viz.,* Softwood cuttings (T_1), Semi hardwood cuttings (T_2) and Hardwood cuttings (T_3) were prepared and planted in poly bags at monthly interval from February to August, 2018 *i.e.* S_1 (February), S_2 (March), S_3 (April), S_4 (May), S_5 (June), S_6 (July) and S_7 (August). The experiment was laid out in factorial CRD with three replications. Among the treatments, T_2 (Semi hardwood) recorded the highest percentage of survivability (44.57%) at 60 days after planting of cuttings, number of primary roots (3.72) of cuttings, longest primary roots at 90 days after planting of cuttings (8.46 cm) and number of primary shoot (5.15) per cuttings at 90 days after planting of cuttings. Cuttings planted in July (S_6) recorded the highest numbers of primary roots (3.74) per cuttings. The percentage of survivability was highest (29.97%) in April (S_3) recorded at 60 days after planting of cuttings. Among all the treatment combinations, T_2S_7 (semi hardwood cuttings done in the month of August) recorded maximum length (9.20 cm) of primary roots at 90 days after planting of cuttings and number of primary shoots (6.67) per cuttings at 90 day after planting of cuttings.

Keywords: Stem cutting, custard apple, propagation, survivability

Introduction
Custard apple belongs to the family Annonaceae and one of the finest fruits introduced in India from tropical America (Mahadevbhai and Patel, 2018) [8]. *Annona* fruits are one of the world's best testing fruits due to the sweet, creamy, flesh and fragrant flavor when fully ripe (Sasidharan and Jayadev, 2017) [19]. It comes under family Annonacea and native of the West Indies but it was cultivated since early times throughout Central America to Southern Mexico (Kopade and Jadav, 2013).

Both sexual and asexual methods of propagation of the more important, commercially grown Annona species are reviewed (George and Nissan, 1987) [4]. Propagation of most Annona species by seed is not recommended as the seedlings are genetically diverse and most are characterized by a long juvenile period, irregular bearing and poor fruit quality (Leslie, 1922; Campbell and Phillips, 1983a) [7, 1]. Although some species of Annona, such as soursop (*A. muricata* L.), can be easily propagated by cuttings (Morton, 1967) [10], most are difficult to strike. The sugar apple has been successfully propagated in Florida by stem cuttings using healthy branches of mature wood taken during the dormant period (Noonan, 1953; Ochse *et al.,* 1961) [13, 14]. Shoots that are about 0.5-1 cm in diameter are cut into 13-15-cm lengths. They are set in sand to a depth of 4/5 of their length with at least 1 bud exposed above the surface. The cuttings produce roots in about 25-35 days. In India and the Philippines, however, the propagation of sugar apple using stem cuttings has not been successful (Miraflores, 1915; Venkataratanam and Satyanaranaswamy, 1956) [9, 24].

Keeping these facts in view, an investigation was carried out to standardization of the propagation method of custard apple (*Annona reticulata*) by stem cuttings. Therefore, the present study was undertaken with the specific objective to determine the suitable time and type of stem cuttings in propagation of custard apple.

Materials and Methods
The present investigation entitled "Standardization of propagation method of custard apple (*Annona reticulata*) by stem cutting was conducted in the Instructional cum Research Farm,

Department of Horticulture, Biswanath College of Agriculture, Assam Agricultural University, Biswanath, Chariali during 2018-2019. The experimental site is situated at $26^0 43' 32''$ N latitude and $93^0 08' 01''$ E longitudes having an elevation of 86.70 m above mean sea level. The topography of the land is uniformly plain. The experiment involved three treatments viz., Softwood cuttings (T_1), Semi hardwood cuttings (T_2) and Hardwood cuttings (T_3) was laid out in factorial Completely Randomized Design with three replications. The cuttings was planted in poly bags at monthly interval from February to August, 2018 *i.e.* S_1 (February), S_2 (March), S_3 (April), S_4 (May), S_5 (June), S_6 (July) and S_7 (August). Statistical observations recorded during field experiment were subjected to the statistical analysis of variance (ANOVA) by factorial Completely Randomized Block Design (CBD). Significance and non-significance of the variance due to different treatments and different period were determined by calculating the respective 'F' value as the method described by Panse and Sukhatme (1985) [16].

Preparation of stem cuttings

Uniform and healthy shoots of desirable size (1.5-2.0 cm in diameter) were collected from custard apple plants of Instructional cum Research farm of Biswanath College of Agriculture, From the collected shoots, softwood, semi hardwood and hardwood stem cuttings of 15-20 cm lengths were prepared for planting in polybags filled with sandy loam soil and decomposed cow dung at equal proportion. As per the technical programme, three different types *i.e.* softwood, semi hardwood and hardwood stem cuttings were prepared and planted at monthly interval from February, 2018 to August, 2018. A slanting cut was given at the basal end of each cutting with care to avoid the splitting of the cut end or detachment of bark from the wood portion.

Method of planting and after care of cuttings

In the pre-filled polybags (size: 8" x 10") with growing medium, a hole was made up to a depth of 5-6 cm with a bamboo stick and each of the prepared cuttings were placed in the holes of each polybags. The base of the each cutting was pressed firmly and the polybags were watered thoroughly to settle down the medium.

The cuttings in the polybags were kept in shade net house for further study. Watering was done regularly so as to keep the medium with sufficient moisture for easy rooting. As and when weeds were observed, these were pulled out carefully without disturbing the cuttings whenever necessary.

Observation recorded

Percentage of survivability of cuttings at 60 days after planting: The total number of stem cuttings survived in each treatment was recorded at 60 days after planting of cuttings and final survival percentage was calculated as following.

$$\text{Success of cuttings (\%)} = \frac{\text{Total no. of cuttings survived in each treatment}}{\text{Total number of cuttings of each treatment}} \times 100$$

Number of primary roots per cuttings at 90 days after planting of cuttings

The cuttings were uprooted carefully from the polybags to record the observation on roots at 90 days after planting of cuttings in polybags. The number of primary roots of cuttings of each type of stem cuttings was counted and recorded.

Length (cm) of longest primary roots at 90 days after of planting of cuttings

Among the primary roots, the longest root of each rooted cuttings was measured in 'cm' by measuring scale from the base to the tip of root at 90 days after planting and the mean length of three replications was recorded and expressed in cm.

Days taken for shoot emergence from planting of cuttings

Period required for each cutting to produce new shoot was calculated out by counting the number of days from the date of planting of cuttings to emergence in each treatment. The duration was expressed in days.

Number of primary shoots per cuttings at 90 days after planting of cuttings

The number of primary shoots produced by each cutting was counted at 90 days after planting of cuttings in polybags and the average of three replications was recorded

Result and Discussion

Percentage of survivability at 60 days after planting of cuttings: The differences in percentage of survivability of cuttings at 60 days due to treatments were highly significant as presented in Table 1. The highest percentage of survivability (44.57%) was observed in treatment T_2 (semi hardwood) followed by treatment T_3 (25.75%). However, very low percentage of survivability was observed in treatment T_1 (7.68%) at 60 days after planting of cuttings. Survivability of cuttings depend upon number of factors namely, type of plant species, age of the shoot taken for cuttings, rooting medium, environment and age of the mother plants. It might be due to the result of reserved sufficient plant metabolites and auxins in semi hardwood cuttings. The present result is in support of work of Chadha (2001) [2] who opined that the shoot younger than 6 months and older than 18 months are not suitable for stem cuttings.

The percentage of survivability differed significantly at 60 days after plantings in respect to time of cuttings. Cuttings done in the month of April (S_3) showed highest percentage (29.97%) of survivability followed by May (28.01%). On the other hand, cuttings done in the month of August (S_7) recorded lowest percentage of survivability (20.48%). The higher percentage of survivability of rooted cuttings might be attributed to higher number of primary roots, branches and leaves which was always found to be beneficial for profuse growth which helped to more uptakes of water and nutrients leading to more survivability of cuttings.

Interaction effect between the treatment and time of cuttings were found significant. The treatment combination T_2S_3 *i.e.* semi hardwood cuttings done in the month of April (S_3) showed highest percentage of survivability (49.75%) followed by T_2S_4 (47.50%) *i.e.* semi hardwood cuttings done in the month of May. However, the treatment combination of T_1S_7 (softwood cuttings done in the month of August) showed lowest percentage (6.50%) of survivability which was at par with softwood cuttings done in the month of February (T_1S_1), March (T_1S_2), May (T_1S_4), June (T_1S_5) and July (T_1S_6) and in August (T_1S_7). Singh *et al.* (2015) showed that the maximum survival percentage (77.37%) was observed under the rainy season planting time. The present finding also gets support from the work of Ozcan *et al.* (1993) [15] who reported that stem cuttings planted during July-August produced higher number of roots leading to higher survivability of cuttings.

Table 1: Percentage of survivability at 60 days after planting of cuttings

Time of cuttings	T_1 (Softwood)	T_2 (Semi hardwood)	T_3 (Hardwood)	Mean
S_1(February)	8.00	43.50	26.33	25.94
S_2(March)	8.50	44.67	28.50	27.22
S_3 (April)	9.17	49.75	31.00	29.97
S_4 (May)	7.67	47.50	28.87	28.01
S_5 (June)	7.07	46.67	25.37	26.37
S_6 (July)	6.90	43.33	21.87	24.03
S_7 (August)	6.50	36.60	18.35	20.48
Mean	7.68	44.57	25.75	

S. Ed ± T: 0.41 S: 0.63 T vs S: 1.10
CD (P=0.05) T: 0.84 S: 1.29 T vs S: 2.23
NS: Non- significant

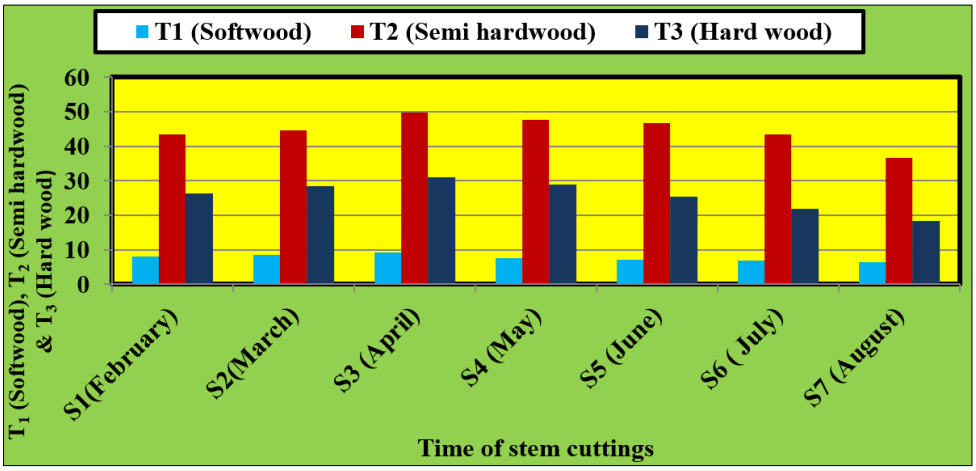

Fig 1: Percentage of survivability at 60 days after planting of cuttings

Number of primary roots per cuttings at 90 days after planting of cuttings

There were significant differences among the treatments observed in number of primary roots per cuttings. The data on number of primary roots per cuttings were presented in Table 2. Maximum numbers of primary roots (3.72) was recorded in T_2 (semi hardwood) followed by T_3 (3.07). Development of higher number of primary roots in semi hardwood cuttings might be due to the presence of sufficient carbohydrates and natural auxins that encouraged the formation of callus leading to initiation of roots. This is in accordance with the findings of Parmar *et al.* (2018) [17].

The number of primary roots per cuttings differed significantly in respect to time of cuttings. Cuttings done in the month of July (S_6) showed maximum number (3.74) of primary roots per cuttings followed by August (3.62) and they are at par with S_3 (3.21), S_4 (3.41) and S_5 (3.58). On the other hand, minimum number of primary roots (1.79) per cuttings was recorded in S_1 which showed no significant variation with S_2 (2.22). The maximum number of primary root might be attributed to the fact that presence of higher number of rapidly dividing cells leads to early callusing and ultimately cause rooting.

The interaction effect between the treatment and time of cuttings with respect to number of primary roots per cuttings was found non-significant. However, maximum numbers of primary roots (4.50) per cuttings was observed in combination with T_2S_6 *i.e.* semi hard wood cuttings done in the month of July. Samanci (1990) [20] emphasised that semi hardwood cuttings taken in July and August yielded average rooting of 70-90 per cent.

Table 2: Numbers of primary roots per cuttings at 90 days after planting of cuttings

Time of cuttings	T_1 (Softwood)	T_2 (Semi hardwood)	T_3 (Hardwood)	Mean
S_1 (February)	1.25	2.13	2.00	1.79
S_2 (March)	1.67	2.67	2.33	2.22
S_3 (April)	2.58	3.90	3.17	3.21
S_4 (May)	2.67	4.17	3.40	3.41
S_5 (June)	2.90	4.33	3.53	3.58
S_6 (July)	3.05	4.50	3.67	3.74
S_7 (August)	3.01	4.40	3.45	3.62
Mean	2.44	3.72	3.07	

S. Ed ± T: 0.18 S: 0.28 T vs S: 0.49
CD (P=0.05) T: 0.37 S: 0.57 T vs S: NS
NS: Non- significant

Length (cm) of longest primary roots at 90 days after planting of cuttings

The data presented in Table 3 revealed that all the treatments under the study differed significantly in respect to longest primary roots at 90 days after cuttings. The length of primary roots varied from 5.09 cm and 8.46 cm. Among the treatments, maximum length (8.46 cm) was recorded in T_2 (semi hardwood) followed by T_3 (6.96 cm) and minimum length (5.09 cm) of primary roots was recorded in T_1 (softwood). The longest primary roots produced by semi hardwood cuttings might be due to the well developed adventitious roots as observed in the present study which resulted better growth of the primary roots. Singh (1963) justified that longer primary root in semi hardwood cuttings might be due to the presence of higher amount of food

reserves within the cuttings and greater accumulation of sugars in the cuttings.

The effect of time of cuttings had no significant influence on length of primary roots at 90 days after planting of cuttings. Maximum primary root length (7.53 cm) was recorded in S_6 (July) followed by S_5 (7.17 cm). It was observed that the interaction effect between the treatments and time of cuttings did not have significant influence on length of longest primary roots at 90 days after planting of cuttings. There were no significant variations in length of primary roots of the cuttings due to planting of cuttings at different months. These results are confirmatory with earlier findings of Muhammad-Luqman *et al.* (2004) in guava and Narula (2016) [12] in plum.

Table 3: Length (cm) of longest primary roots at 90 days after planting of cuttings

Time of cuttings	T_1 (Softwood)	T_2 (Semi hardwood)	T_3 (Hardwood)	Mean
S_1 (February)	4.50	7.33	6.17	6.00
S_2 (March)	4.67	8.10	6.80	6.52
S_3 (April)	4.90	8.31	7.15	6.78
S_4 (May)	5.08	8.50	7.33	6.97
S_5 (June)	5.24	8.67	7.60	7.17
S_6 (July)	5.55	9.17	7.87	7.53
S_7 (August)	5.70	9.20	5.81	6.90
Mean	5.09	8.46	6.96	
S. Ed ±	T: 0.34	S: 0.51	T vs S: 0.89	
CD (P=0.05)	T: 0.68	S: NS	T vs S: NS	

NS: Non- significant

Days taken for shoot emergence from planting of cuttings

There were significant treatment differences observed in days taken for shoot emergence from plantings of cuttings. Data presented on Table 4 revealed that minimum numbers of days (15.11) taken for shoot emergence was recorded in T_2 (semi hardwood). However, maximum days (17.52) were recorded in T_1 (softwood) which was at par with T_3 (16.81 days).

Semi hardwood cuttings produced new shoots within shortest period and it might be justified that due to the presence of sufficient plant metabolise, semi hardwood cuttings established properly and developed more number of roots which led to early emergence of new shoot in semi hardwood cuttings.

The days taken for shoot emergence were significantly influenced by the time of cuttings. Maximum numbers of days (19.39) for shoot emergence was recorded in S_1 (February) followed by S_2 (17.40 days). On the other hand, minimum numbers of days was recorded in S_6 which was at par with S_4 and S_5. Time of planting of cuttings also influenced the duration required to emerge the shoots in cuttings. The early shoot emergence might be due to the presence of higher amount of reserved food materials in the cuttings and favourable environmental conditions during June-July (Vyas *et al.*, 2017) [25]. Kumar *et al.* (1995) [6] also found the similar results and showed that cuttings taken in July required fewer days for sprouting and showed better shoot and root growth than those taken in February.

The interaction effect of treatments and time of cuttings was found non- significant in respect to days taken for shoot emergence from planting of cuttings. However, combination of semi hardwood cuttings (T_2) which was done in the month of July (S_6) took minimum days (13.33) for shoot emergence.

Table 4: Days taken for shoot emergence from planting of cuttings

Time of cuttings	T_1 (Softwood)	T_2 (Semi hardwood)	T_3 (Hardwood)	Mean
S_1 (February)	21.33	17.67	19.17	19.39
S_2 (March)	18.15	16.17	17.90	17.40
S_3 (April)	17.00	15.33	16.50	16.27
S_4 (May)	16.67	14.17	15.67	15.50
S_5 (June)	15.67	13.97	15.17	14.93
S_6 (July)	16.17	13.33	14.77	14.75
S_7 (August)	17.67	15.17	18.50	17.11
Mean	17.52	15.11	16.81	
S. Ed ±	T: 0.40	S: 0.62	T vs S: 1.07	
CD (P=0.05)	T: 0.82	S: 1.25	T vs S: NS	

NS: Non- significant

Number of primary shoot per cuttings at 90 days after planting of cuttings

The data on number of primary shoot per cuttings at 90 days after planting of varied significantly in the present study. Table 5 revealed that, highest number (5.15) of primary shoots was recorded in T_2 (semi hardwood) and lowest number (2.91) was recorded in T_1 (softwood) which was at par with T_3 (3.40). Accelerated number of primary shoot development in semi hardwood cuttings was the result of development of higher number of primary roots which enhanced the uptake of nutrients and water from soil. Similar results also found by Rajan and Markose (2007) [18] who reported that semi hardwood cuttings of pomegranate resulted higher number of new shoots.

Number of primary shoot per cuttings at 90 days after planting significantly influenced with respect to time of cuttings. Cuttings done in the month of August (S_7) showed highest number (5.15) of primary shoot at 90 days after planting which was at par with S_6 (4.97) *i.e.* cuttings done in the month of July. However, lowest numbers of primary shoots per cuttings was recorded in S_1 which had no significant difference with S_2. Higher number of primary shoots in cuttings was recorded in cuttings planted during June to August. There was high rainfall, relative humidity and higher temperature during this period and these environmental factors might have become congenial in development of more number of shoots in cuttings. As reported by Chang and Yih (1996) [3], increase in branch number in Nagatenga (*Rhus semialata*) might be due to increase in root system which absorbed more nutrients for its growth. Similar findings were also reported by Ullah-Tahseen *et al.* (2005) [23] in guava.

Interaction effect between the treatment and time of cuttings did not influenced significantly with respect to number of primary shoot per cuttings at 90 days after planting of cuttings. The numbers of primary shoots per cuttings varied from 1.17 to 6.67 numbers among all the treatment combinations.

Table 5: Number of primary shoot per cuttings at 90 days after planting of cuttings

Time of cuttings	T_1 (Softwood)	T_2 (Semi hardwood)	T_3 (Hardwood)	Mean
S_1 (February)	1.17	2.90	2.17	2.08
S_2 (March)	1.90	3.67	2.75	2.77
S_3 (April)	2.67	5.17	3.10	3.64
S_4 (May)	2.90	5.50	3.17	3.85
S_5 (June)	3.33	5.67	3.80	4.26
S_6 (July)	4.10	6.50	4.33	4.97
S_7 (August)	4.30	6.67	4.50	5.15
Mean	2.91	5.15	3.40	

S. Ed ± T: 0.25 S: 0.39 T vs S: 0.27
CD (P=0.05) T: 0.52 S: 0.79 T vs S: NS
NS: Non- significant

Conclusion

On the basis of this experiment, it may be concluded that propagation of custard apple by stem cuttings might be possible if semi hardwood cuttings (T_2) are planted during July-August. However, further study on the use of rooting hormone would definitely increase the percentage of success in air layering and stem cuttings.

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