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Standardization of propagation method of Custard Apple by Air Layering

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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 air layering. The experiment was laid out in a factorial completely randomized design with four treatments viz., pre-conditioned with 30 days of girdling (T_1), pre-conditioned with 30 days of etiolation (T_2), pre-conditioned with 30 days of girdling + etiolation (T_3) and control i.e. air layering without pre-conditioning (T_4) with three replications and all these treatments were applied at monthly interval from March to August, 2018. Among the treatments, T_2 recorded the highest percentage of success of layering (62.37%), required minimum number of days for separation of layers from mother plant (53.02 days), maximum numbers of primary roots per layer (4.76), percentage of survivability (89.05%) and number of new shoots per layer at 30,60, 90 days after planting i.e., 2.21, 2.78 and 3.09. On an average, air layering done in August (S_6) produced highest percentage of success (61.44%), minimum days for separation of layers from mother plant (51.40), numbers of primary roots per layer (5.35), numbers of new shoots per layer at 30 days (2.27), 60 days (2.75) and at 90 days (3.12) after planting in poly bags.

KEYWORDS

air layering, custard apple, etiolating, girdling, propagation

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INTRODUCTION

Custard apple belongs to the family Annonaceae and one of the finest fruits introduced in India from tropical America (Mahadevbhai and Patel, 2018). 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). Among Annonaceous fruits, custard apple is most popular in India and it is also known as shariffa and sitaphal (Thakur and Singh, 1967). Custard apple mainly propagated through seeds and therefore, it exhibits the greatest variability with regard to growth, quality, and yield attributes and have long juvenile period (Joshi *et al*, 2011).

Layering has evolved as a common means of vegetative propagation of numerous species in natural environments natural layering typically occurs when a branch touches the ground, whereupon it produces adventitious roots. At a later stage the connection with the parent plant is severed and a new plant is produced as a result. Layering is more complicated than taking cuttings, but has the advantage that the propagated portion continues to receive water and nutrients from the parent plant while it is forming roots. This is important for plants that form roots slowly, or for propagating large pieces. Layering is used quite frequently in the propagation of litchi, avocado, cherry and lemon it is also used as a technique for both creating new roots and improving existing roots.

The regeneration of adventitious roots in detached or undetached stem greatly varies with the plant species. The regeneration also affected by both external such as temperature, light, relative humidity, gravity, chemical changes, pressure and internal factors like physiological condition of the mother plant, juvenility, type of wood and presence of leaves and buds, season and presence virus etc. The morpho-physiological and biochemical characteristics together with conducive environmental conditions determine the root generating capacity of a cutting. Nutritional condition of parent plant and several pre-conditioning treatments like invigoration, girdling and etiolation, root promoting substances and season of planting are known to play an important role in rooting of cuttings, and especially on those which are difficult to root (Hess, 1967). Treatments like invigoration, girdling, etiolation etc. become more effective if these are combined with application of synthetic auxins, especially indole butyric acid (IBA) and naphthalene acetic acid (NAA). On the other hand, production of grafts and budded plants require nearly 18-24 months from rising of rootstock to make the grafts or budded plants ready for transplanting. But the requirement of time period for production of layers or cuttings is 6-8 months.

Keeping these facts in view, an investigation was carried out to standardization of the propagation method of custard apple (*Annona reticulata*) by air layering. Therefore, the present study was undertaken with the specific objective to find the effect of certain pre-conditioning treatments on rooting of air layering in custard apple and to determine the suitable time of air layering in custard apple in sub-tropical cli-

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matic condition of Assam.

MATERIALS AND METHODS

The present investigation was conducted at the Instructional cum Research Farm, Department of Horticulture, Biswanath College of Agriculture, Assam Agricultural University, Biswanath, Chariali during 2018-2019.

Plan of the experiment

Geographical location of the experimental site

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.

Climate and weather

Biswanath College of Agriculture (BNCA), located at Biswanath Chariali, falls within North Bank Plain Agro-climatic Zone (NBPZ) of Assam. The prevailing climatic condition of Biswanath Chariali is subtropical having hot and humid summer, dry and cold winter seasons. The rainy season and the summer season are overlapping and rainfall starts from March and quantum of rainfall as well as number of rainy days increases and reaches maximum in the month of August and then rainfall gradually decreases up to December. Summer is experienced from May to August and the cold winter from December to January, whereas a mild winter is experienced during September to November and February to April.

Details of the experiment

Propagation of custard apple by air layering
Design of Experiment : Factorial CRD
Treatment combinations : 24
Replication : 3
No. of layers/treatment : 30

Treatments involving conditions of air layering (Fig. 1)

T_1 : Pre-conditioned with 30 days of girdling
T_2 : Pre-conditioned with 30 days of etiolation
T_3 : Pre-conditioned with 30 days of girdling + etiolation
T_4 : Control (Air layering without pre-conditioning)

Time of air layering
S_1 : March, 2018
S_2 : April, 2018
S_3 : May, 2018
S_4 : June, 2018
S_5 : July, 2018
S_6 : August, 2018

Selection of mother plant

The age of the fruit bearing custard apple plants of ICR Farm, Department of Horticulture, B. N. College of Agriculture, AAU, Biswanath Chariali was 9-10 years old and the required mother plants for the study were selected from the plantation on the basis of plant growth, yield and quality of the fruits.

Pre-conditioning treatments for air layering

For pre-conditioning, one season mature, semi hardwood shoots having approximately 1.5-2.0 cm in diameter were selected in the mother plants. For each treatment, 30 numbers of uniform age and sizes of shoots were selected in each month of experimentation.

Girdling

Girdling was done by removing the bark of 2 cm width around the shoot at 30-35 cm from the selected shoot tip 30 days before air layering in each month of the study. After 30 days of pre-conditioning, air layering was done in the girdled portion.

Etiolation

For etiolation, 3-4 cm width around the shoot at 30-35 cm from the selected shoot tip was wrapped by black tape 30 days before air layering in each month of the study. After 30 days of pre-conditioning, the etiolated portion was girdled and air layering was done.

Girdling + Etiolation

For T_3 treatment, after girdling on the selected shoots, the girdled portion was immediately wrapped by black polythene tape for etiolation 30 days before air layering in each month of the study. After 30 days of pre-conditioning, air layering was done on the girdled and etiolated portions.

Control

In case of control treatment, immediately after removing bark of 2 cm width around the shoots at 30-35 cm from the selected shoot tip, air layering was done.

Planting of rooted layers in polybags

The air layers were detached from the mother plants when sufficient roots were visible in the rooting medium through the transparent polythene used to wrap the rooting medium. The air layers after detaching from the mother plants were planted in polybags which were earlier filled with the mixture of sandy loam soil and decomposed cow dung at equal proportion. Before planting, the wrapping material (polythene sheet) was removed without breaking the adhered rooting medium ball (Fig. 1). Then watering was done in polybags and all the polybags were kept in shade net house for further study. The layers which did not respond to the treatments and did not produce roots even after 90 days, they were discarded.

Observation recorded

Percentage of success of layering

The total number of layers produced the roots under each treatment in each replication was recorded and finally percentage of success of layering (SL) was calculated as following.

$$SL(\%) = \frac{Total\ no.\ of\ layers\ survived\ in\ each\ treatment}{Total\ no.\ of\ layers\ in\ each\ treatment} \times 100$$

Days required for separation of layers from mother plant

The duration required for layering to detach from mother plant was recorded from the day of air layering to day of detachment and expressed in days.

Number of primary roots per layer

The total numbers of primary roots in each layer were counted after cleaning the adhered medium and decayed roots from the root zone of the layers. Cleaning was done before planting in poly bags, after detachment from the mother plant.

Number of secondary roots per layer

The total number of secondary roots developed from the primary roots were counted and expressed as number of secondary roots per layer.



Fig. 1: Different treatments of air layering in custard apple

Length of longest primary root after detachment from the mother plant

The length of longest primary root of each layer was measured with a measuring scale and expressed in cm.

Percentage of survivability of layers

The survival percentage of layers in poly bags was recorded at 90 days after planting in polybags in each treatment and expressed in percentage.

Number of new shoots per layer at 30, 60 and 90 days after planting

The number of new shoots produced by each layer was counted at monthly interval from the day of planting of layers in polybags to 90 days i.e., number of shoots in each layer was recorded at 30, 60 and 90 days after planting.

RESULTS AND DISCUSSION

Percentage of success of layering

The data presented in Table 1 revealed that all the treatments under study differed significantly with respect to percentage of success of layering. The success of layering ranged from 41.18 per cent to 62.37 percent. Among the treatments, T_2 (etiolation) recorded the highest (62.37%) percentage of success while the lowest (41.18%) was observed in T_4 (control). The higher success in etiolated and girdled shoots was due to the accumulation of carbohydrates and endogenous auxins just above the etiolated and girdled area which resulted callusing and root initiation (the similar result reported by Maurya et al. (2013) Maurya *et al* (2013) in Jamaican Ackee (*Blighia sapida* L.)

Time of layering significantly influenced the percentage of success of layering. Layering done in the month of August (S_6) recorded the highest percentage of success (61.44%) which was at par with S_5 (60.82%). The least percentage (45.54%) of success was recorded in March (S_1). The interaction effect of pre conditioning treatments and time of layering had significant influence on percentage of success of layering. The highest success (71.55%) was recorded in T_2S_6 i.e. layering done in August with etiolation as pre condition treatment followed by T_2S_5 (layering done in July with pre-conditioning by etiolation). However, the least percentage (35.50%) of success was recorded in control (T_4) irrespective of time of layering.

The probable reason for maximum success during monsoon particularly during July- August could be due to congenial environment such as high humidity and temperature leading to higher cell activity and increased production of carbohydrates which caused dormant adventitious buds in girdled area to grow more rapidly into roots (Kumar, 2012). Dhillon

and Mahajan (2000) reported that August was the best time for air layering in litchi with respect to rooting success and survivability. The results are also in conformity with the Kumar (2000) in litchi, Tomar (2011) in jackfruit and Tomar (2016) in *Spondias pinnata*.

Days required for separation of layers from mother plant

Significant variation was recorded in the treatments and time of layering with respect to days required for separation of layers from mother plant as data represented on Table 1 . Of course, interaction effect between the treatments and time of layering did not show any significant variations. The shortest period (53.02 days) for separation of layers from mother plant was recorded in layers pre-conditioned with etiolation, whereas, the air layering done without pre-conditioning required longest duration of 59.47 days. In the present study, air layers were detached from the mother plants when the sufficient root formation was visible otherwise the layers attached to the mother plants until and unless root formation takes place. As the air layers pre-conditioned either by etiolating or girdling or both together produced sufficient roots earlier and therefore, these layers were detached from the mother plants within shorter period from the day of air layering.

Table 1: Percentage of success, days required for separation and number of primary shoots of air layering

Time of air layering	Success of layering (%)				Days required for separation				Number of primary roots per layer			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
S_1:March	45.67	51.33	49.67	35.50	61.58	59.33	58.67	64.50	3.43	4.64	3.67	2.67
S_2 :April	47.50	54.33	50.50	38.33	60.67	57.50	56.40	63.33	3.90	5.01	4.33	3.20
S_3 :May	48.50	61.85	53.55	41.25	55.33	53.00	54.75	58.50	4.67	5.33	4.90	3.50
S_4 :June	48.75	64.67	59.60	42.33	58.50	51.60	53.33	57.83	5.21	6.59	5.33	3.67
S_5 :July	62.45	70.50	65.66	44.67	52.25	48.10	50.35	56.67	4.85	6.67	5.67	4.15
S_6 :August	63.33	71.55	65.90	45.00	50.67	48.60	50.33	56.00	5.50	6.33	5.50	4.08
CD (P=0.05)	**T: 1.50 S: 1.84**				**T: 2.14 S: 2.62**				**T: 0.79 S: 0.97**			

Air layering done in the months of July and August showed comparatively better results; where the layers could be detached within 51.84 days and 51.40 days, respectively from the day of layering. The Shortest period for separation recorded during this period might be attributed to exclusion of light, availability of oxygen, sufficient moisture and optimum temperature and all the conditions were prevalent during June to August. Presence of high relative humidity, optimum temperature and occurrence of rains might have accelerated rooting. The results are in the line with the findings of Nautiyal (2002) where he reported that root initiation was higher in July month. Similar results were also obtained by Khan *et al* (2016) and Manga *et al* (2017).

Number of primary and secondary roots per layer

As regard to number of primary and secondary roots, significant variation was observed among the treatments and time of layering from Table 1. In the present study, etiolated layers (T_2) recorded maximum number of primary roots (5.76) and secondary roots (12.49) per layer and both primary (3.54) and secondary roots (8.32) were least in control ($T_{4)}$. Bose et al. (1986) Bose *et al* (1986) and Ezekiel *et al* (2016) remarked that rooting in air layering was encouraged by exclusion of light, availability of oxygen, sufficient moisture and optimum temperature and all the conditions were prevalent during June to August. They further remarked that internal hormonal content and co-factors could play an important role in production of roots. Baghel *et al* (2016) opined that increase in number of roots might be due to the accumulation of rooting cofactors above the etiolated and girdled portion.

Layering done during June to August recorded higher number of primary and secondary roots. On the other hand, productions of these roots were the least during March and April. The higher production of primary and secondary roots per layers produced during June to August could be attributed due to high relative humidity (91.76 – 94.22%) and temperature (32.73 – 33.0^0C) prevailed during that period. These results are in conformity with the findings of Mukherjee and Bid (1965), S Sengupta and Thakur (2001), Modi *et al* (2014), Baghel *et al* (2016) and Tomar (2016). Kumar (2012) also reported that season has pronounced effect in relation with root development in air layers.

Length of the longest primary root after detachment from the mother plant

The data on length of the longest primary root after detachment from the mother plant under study were presented in Table 2 . Significantly the highest length of primary root (6.71 cm) was recorded in T_2 (etiolation) and it was at par with T_1 (6.62 cm) and T_3 (6.58 cm) i.e. in girdling ($T_{1)}$ and girdling + etiolation (T_3), respectively. The least length (5.52 cm) of primary root was recorded in T_4 (Control). The increase in the length of primary root could be due to the accumulation of higher level of endogenous hormone and carbohydrates due

to the restriction of their downward movement caused by etiolation and girdling. The present result is in conformity with the report of Rymbai and Reddy (2010).

There was no significant difference in length of primary root with respect to time of layering. However, the longest primary root (6.57 cm) was recorded in July (S_5) and the shortest primary root (5.99 cm) was recorded in March (S_1) might be due to lower temperature and scanty rainfall recorded in that particular period of time. The similar results were obtained by Bhosale *et al* (2009), Tomar (2011), Tayade *et al* (2018) in pomegranate and Baghel *et al* (2016) in guava.primary root length varied from 5.33 cm to 6.95 cm among the treatment under study

Percentage of survivability after planting in polybags

The data presented in Table 2 revealed that all the treatments differed significantly with respect to percentage of survivability after planting in polybags. But the interaction effect between the pre conditioning treatments and time of layering had no significant effect on percentage of survivability. Among the treatments, the shoots pre-conditioned with etiolation (T_2) and air layered showed the highest percentage of survivability (89.05%) whereas, the least survival percentage (60.44%) was recorded in control (T_4) after planting in polybags. Higher survival of rooted layers in pre-conditioned with etiolation might be due to profuse rooting with longer roots with increased accumulation of dry matter leading to subsequent growth and development of layers.

Table 2: Number of secondary roots, length of longest primary root and survivability percentage of air layering

Time of air layering	Number of secondary roots per layer				Length (cm) of longest primary root				Survivability (%)			
	T_1	T_2	T_3	T_4	6.13	6.30	6.20	5.33	T_1	T_2	T_3	T_4
S_1:March	8.33	10.19	8.67	6.67	6.40	6.55	6.35	5.45	75.67	83.17	78.33	48.67
S_2 :April	8.90	11.30	9.33	8.33	6.67	6.72	6.48	5.52	78.60	85.18	80.90	56.50
S_3 :May	9.33	12.25	10.33	8.30	6.90	6.87	6.81	5.60	81.42	89.30	86.65	61.81
S_4 :June	10.50	12.90	11.71	8.67	6.83	6.95	6.85	5.66	86.66	91.67	89.00	64.67
S_5 :July	11.67	14.33	13.21	9.33	6.81	6.90	6.81	5.60	88.33	93.33	90.33	66.67
S_6 :August	12.00	14.00	13.10	8.67	6.13	6.30	6.20	5.33	85.50	91.67	87.33	64.35
CD (P=0.05)	**T: 0.80**	**S: 1.01**			**T: 0.76**	**S:NS**			**T: 1.38**	**S:1.69**		

Layering done in the month of July (S_5) showed the highest percentage (84.66 %) of survivability, which was at par with June (83.00 %) and August (82.21 %). This might be due to better growth of primary and secondary roots in the layers, production of longer primary roots which helped the layers to establish properly in the rooting medium of polybags. On the other hand, the lower survivability of layers produced in March(S_1) might be due to poor root growth and that lead to poor survival of air layers in polybags. Bose *et al* (1986) opined earlier that season is the important factor for successful layering in woody plants because of rooting on layers which is enhanced by light and presence of sufficient moisture and optimum temperature. The present findings is in conformity with the reports of Kumari and Prakash (2017) and Manga *et al* (2017) in guava who opined that increase in production of more number of roots in layers led to the higher percentage of survivability of air layers.

Number of new shoots per layer at different stages after planting

It has been observed that as the age of layers advanced, the number of new shoots per layer also increased accordingly. It is evident from the data presented on Table 3 ; the highest number of new shoots per layer was recorded at 90 days after planting. As the age of layers advanced, the number of new shoots per layer in all the stages i.e. at 30 days (2.21), 60 days (2.78) and at 90 days (3.09) after planting was maximum in layer that is preconditioned with etiolation (T_2) and it was followed by layers preconditioned with girdling + etiolation (T_3) at all the stages which recorded 2.04 at 30days, 2.49 at 60 days, and 2.85at 90 days after planting in polybags. On the other hand the layers in control condition (T_4) showed the least number of new shoots (1.40, 1.70, and 2.04) at 30 days, 60 days and 90 days respectively.

Higher production new shoot per layers gradually increased from March to August. It might be due to gradual increase in temperature and relative humidity in the later part of experimentation which ultimately favoured higher vegetative growth. Patel *et al* (2012), Bhosale *et al* (2009) and Tayade *et al* (2018) in pomegranate reported that due to absorption of more amounts of nutrients along with moisture from soil layers produces the higher number new shoots.

Table 3: Number of new shoots atdifferent days after planting of air layers

Time of air layering	Number of New shoots per layer at 30 days				Number of new shoots per layer at 60 days				Number of new shoots per layer at 90 days			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
S_1:March	1.25	1.72	1.58	1.09	1.38	1.88	2.32	1.33	1.70	2.20	2.67	1.95
S_2 :April	1.33	1.89	1.78	1.17	1.67	2.55	2.08	1.50	1.91	2.67	2.43	1.77
S_3 :May	1.53	2.08	1.99	1.33	1.85	2.87	2.33	1.67	2.06	3.17	2.77	1.89
S_4 :June	1.77	2.40	2.11	1.50	1.98	3.03	2.57	1.70	2.73	3.33	2.93	2.04
S_5 :July	2.06	2.54	2.33	1.63	2.33	3.10	2.70	1.90	2.96	3.44	3.09	2.27
S_6 :August	2.18	2.67	2.50	1.73	2.67	3.30	2.95	2.10	3.17	3.75	3.21	2.37
CD (P=0.05)	**T: 0.42 S: 0.52**				**T: 0.15 S: 0.19**				**T: 0.25 S: 0.31**			

CONCLUSION

The present investigation revealed that custard apple could be propagated by air layering. Among the different treatment combinations, etiolation of the shoot 30 days prior to air layering (T_2) $_{in}$ August (S_6) recorded significantly highest percentage of success of layering (71.55%), required shortest period for separation of layers from mother plant (48.10 days), also recorded the highest number of new shoots per layer after planting in poly bags. The shoots pre-conditioned with etiolation and air layered during June to August produced the higher number of primary roots.

From the investigation, it concluded that custard apple might be propagated by air layering during July-August with preconditioning of the shoots by girdling or etiolation 30 days prior to air layering.

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