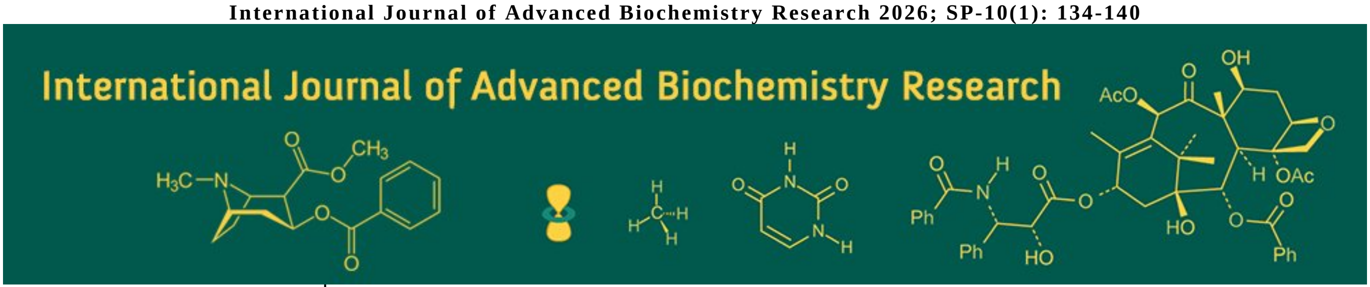

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Performance of different *Annona* species on the grafting success and growth of Soursop (*Annona smuricata* L.)

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Abstract
Soursop (*Annona muricata* L.) is traditionally propagated from seeds, but this results in considerable variability among seedlings due to cross-pollination, leading to inconsistent orchard performance. Vegetative propagation through grafting offers a dependable alternative, provided there is compatibility between the scion and rootstock. Hence, the present study designed to know the grafting effectiveness of six *Annona* species i.e. *A. muricata, A. squamosa, A. glabra, A. reticulata, A. montana, and A. mucosa* as potential rootstocks for soursop in controlled nursery conditions. A Completely Randomized Design (CRD) including six treatments with four replications was employed for softwood grafting. In documented data about graft success, plant survival, vegetative growth, root development, specific leaf weight (SLW), and chlorophyll content at 30-, 60-, and 90-days post-grafting (DAG). The results revealed that *A. montana* and *A. reticulata* exhibited the highest compatibility with soursop, with graft success rates of 80.00% and 62.50%, and survival rates of 67.50% and 55.00%, respectively. These rootstocks facilitated early sprouting, promoted higher shoots, increased leaf production, enhanced graft unions, and developed more robust root systems. Conversely, grafts on *A. squamosa* and *A. glabra* were entirely unsuccessful by 90 days after grafting, indicating that the two species were incompatible either physically or physiologically. Grafts on *A. montana* and *A. reticulata* exhibited significantly enhanced physiological characteristics, including specific leaf weight (SLW) and chlorophyll content. This indicates that the leaves exhibited more strength and photosynthesis functioned more efficiently. The research indicates that *A. montana* and *A. reticulata* serve as effective rootstocks for enhancing graft success and initial growth in soursop.

Keywords: Grafting, annona, graft compatibility, rootstock, scion

Introduction
Soursop (*Annona muricata* L.) is a tropical fruit tree extensively cultivated in tropical regions due to its aromatic fruits, health benefits, and emerging use in medicine. Despite the significance of soursop, large-scale cultivation is typically challenging because of its susceptibility to soil-borne diseases, inconsistent yields, and sensitivity to environmental fluctuations.

Soursop (*Annona muricata* L.) is commonly propagated from seeds. Propagation by seeds will produce variation among plants, leading to inconsistent orchard performance. To overcome this problem, propagation through vegetative must be done i.e., by grafting. Vegetative propagation by grafting requires the availability of rootstocks and scions [1].

Annonaceous fruits are often propagated via seeds, resulting in significant variety in growth, productivity, and fruit quality among the trees in the orchard. Due to the high variability and heterozygosity among the seedlings, asexual techniques are the easy ways to overcome the problems of seed propagation. The research work on the development of superior-performing as well as known varieties are meager. However, quite often we do stumble upon trees exhibiting prolific yield with superior quality. If such promising trees are further perpetuated through vegetative means, the desirable types can be multiplied and establish the orchard with uniform fruit quality [2].

Among different methods of propagating Annona vegetatively, layering and cuttings are not typically employed for commercial multiplication; nonetheless, budding and grafting are considered advantageous. Rootstocks significantly contribute to the propagation of plants. The compatibility of rootstock and scion is crucial for budding and grafting. Furthermore, the cultivation environment significantly influences the establishment of favorable conditions for optimal growth and development of propagated plants [3]. Annona has shown a higher percentage of success when propagated by grafting in comparison to other methods [10]. Availability of high-quality planting material would augment the cultivation of *Annona muricata*. This can be achieved by selecting suitable rootstock species to increase the efficiency of its propagation through grafting.

Differential anatomical compatibility, cambial alignment, sap flow, and physiological responses post-grafting significantly influence graft survival and growth rate. Identifying the optimal rootstock that synergizes effectively with various plants and exhibits rapid growth can mitigate common challenges in horticulture, such as poor seed germination, uneven root development, and premature seedling mortality [4, 5].

This study seeks to evaluate the influence of particular *Annona* rootstock species on the grafting compatibility and initial growth performance of soursop. The study aims to identify rootstocks that enhance the survival, vigor, and long-term performance of soursop plants by assessing graft union success, scion establishment, and vegetative development. Results will provide valuable insights for farmers, nursery managers, and researchers.

2. Materials and Methods

The experiment was conducted in the Fruit Genetic Diversity Block of the College of Horticulture, Department of Fruit Science in Bengaluru, Karnataka. The location is situated at 12° 58' N latitude and 77° 35' E longitude, with an elevation of 930 meters above sea level. The region possesses a temperate tropical climate conducive to fruit cultivation.

2.1. Rootstock prepration and nursery management

Rootstocks of six *Annona* species *A. muricata*, *A. squamosa*, *A. glabra*, *A. reticulata*, *A. montana*, and *A. mucosa* were raised in 200-gauge black polybags measuring 8" × 6". Rootstock of six-month-old seedlings that were healthy, rapidly growing, and uniform in height and girth. During growth of the rootstock, standard nursery practices such as irrigation, weed management, and pest control were employed.

2.2 Scion selection and curing

Scion shoots were obtained from healthy, disease-free, and pest-free mature soursop (*Annona muricata*) trees, which were situated in the Fruit Genetic Diversity Park at the College of Horticulture in Bengaluru. The scions were pre-cured to enhance the likelihood of successful grafting, selected mature branches from the current season. The pre-cured scions were collected from 8:00 to 9:00 AM on the grafting day. Upon severance from the mother plant, the scions were enveloped in damp fabric to prevent desiccation and used it for grafting.

Experimental set up and treatment

The experiment employed a Completely Randomized Design (CRD) with six treatments representing different *Annona* rootstock species. Four replicas of each treatment were produced, and 10 grafts were implemented for each treatment to ensure an adequate sample size and statistically reliable results. Softwood grafting was employed to disseminate all treatments, ensuring uniformity in the grafting procedure across all experimental units.

Sl. No.	Treatment	Rootstock species
1	T_1	*Annona muricata*
2	T_2	*Annona squamosa*
3	T_3	*Annona glabra*
4	T_4	*Annona reticulata*
5	T_5	*Annona montana*
6	T_6	*Annona mucosa*

Data Collection and Observation

Observations about the growth and development of the graft were recorded at 30-, 60-, and 90-days post-grafting (DAG). Root characteristics were assessed destructively at 90 days after grafting (DAG). Three grafts were randomly selected for each replication, and the identical plants were employed across all observation periods to maintain consistency. The duration from grafting to the emergence of the initial sprout on the scion was documented. At 30 days after grafting (DAG), the success of the graft was assessed by calculating the proportion of grafts that had developed, utilizing the formula:

At 30, 60, and 90 days after grafting (DAG), we enumerated the leaves on each graft and measured the height from the graft union to the terminal shoot. A digital Vernier caliper was employed to measure the graft girth at the graft union. The girth measurements were subsequently computed utilizing the formula:

$$\text{Graft success (\%)} = \frac{\text{Number of grafts sprouted}}{\text{Total number of grafts prepared}} \times 100$$

Every 30 days, the quantity of sprouts per graft was documented, and a digital Vernier caliper was employed to measure the diameter at the graft union. At 90 days after grafting, the grafts were carefully extracted to assess the root parameters. The taproot length was assessed from the collar region to the root apex, and the quantity of primary roots emerging from the main taproot was enumerated. Weigh the freshly uprooted roots using an electronic balance, and subsequently weigh the dried roots after subjecting them to an oven at 70°C for 48 hours.

The equation for determining graft survival at 90 days after grafting (DAG) was:

$$\text{Graft survivability (\%)} = \frac{\text{Number of grafts survived}}{\text{Total number of grafts prepared}} \times 100$$

The mortality rate was calculated by subtracting the survival rate from 100%. We determined the specific leaf weight (SLW) by

$$\text{SL W} = \frac{\text{Leaf dry weight (g)}}{\text{Total leaf area (cm}^2\text{)}}$$

The DMSO extraction method to quantify the chlorophyll content in the sample. Measured absorbance at 645 nm and 663 nm. The subsequent equations was employed to get the chlorophyll fractions:

Chlorophyll “a” = {12.7 (A 663) - 2.69 (A 645)} × V/(1000 × W×a)

Chlorophyll "b" = {22.9 (A 645) - 4.68 (A 663)} × V/(1000 × W×a)

Total chlorophyll = Chlorophyll "a" + Chlorophyll "b"

Where,
A= Absorbance at specific wavelengths (OD values)
V= Volume of chlorophyll extracted (ml)
W= Fresh weight of the sample (g)
a= Path of the light (1cm)

The total chlorophyll content was obtained by summing chlorophyll a and chlorophyll b. These metrics provided extensive insights into the physiological and morphological responses of soursop grafts to several Annona rootstocks.

Statistical Analysis
The obtained data was analyzed using Analysis of Variance (ANOVA) with the Web Agri Statistical Package (WASP 2) developed by the ICAR Research Complex, Goa. Prior to analysis, percentage statistics such as graft success and survivorship were modified by arcsine square-root transformation. A significance level of 1% (p = 0.01) was employed for testing. Critical Difference (CD) values were computed wherever the F-test indicated significance, following the statistical procedures of [6].

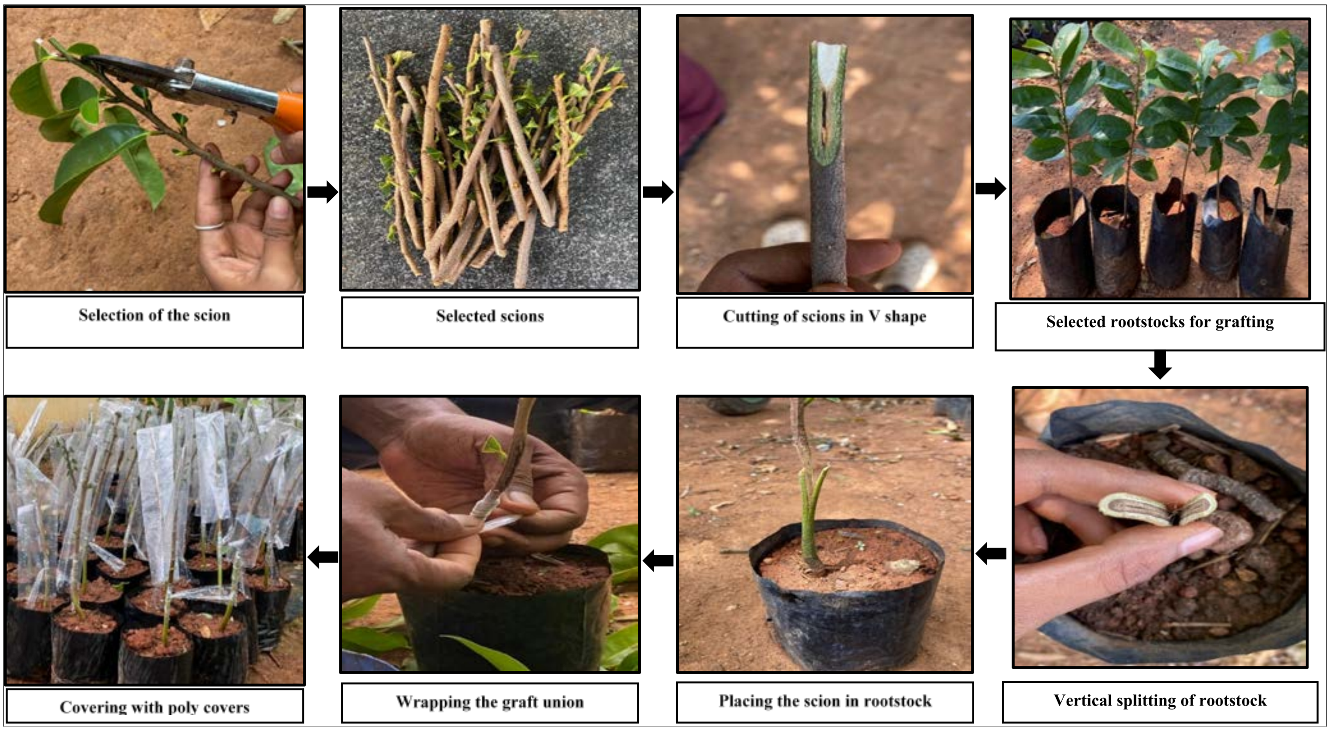


Fig 1: Sequential steps in soft wood grafting technique

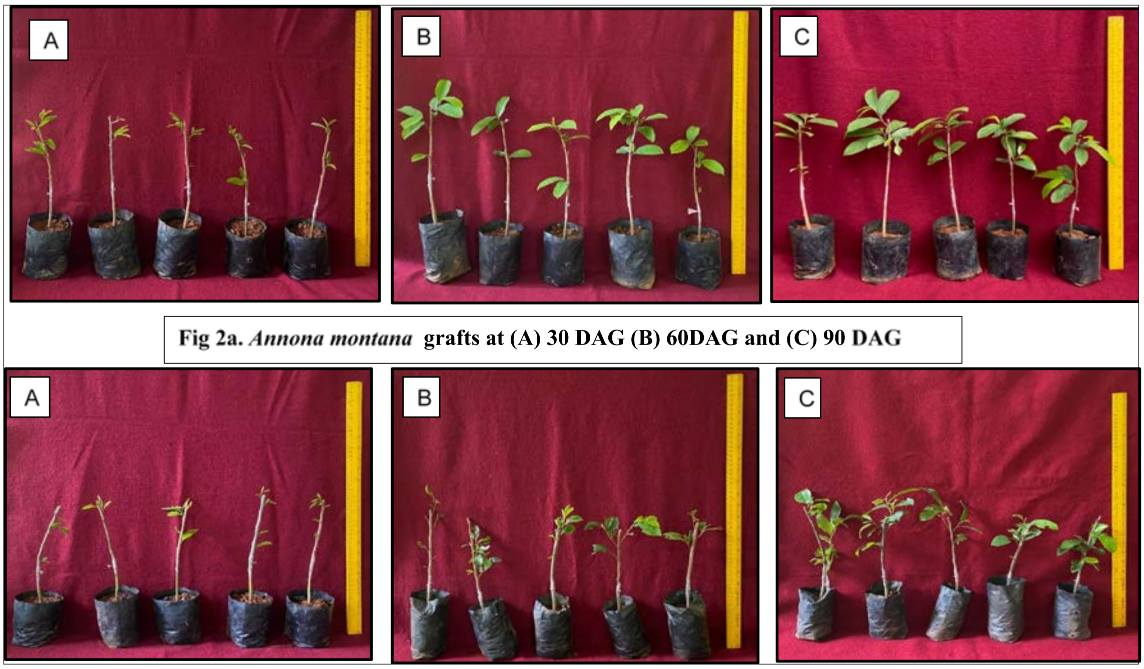


Fig 2b. *Annona reticulata* grafts at (A) 30 DAG (B) 60DAG and (C) 90 DAG

Fig 2: Best root stock combination

3. Results and Discussion

3.1 Graft Success (%)

The percentage of graft success at 30 days after grafting (DAG) differed significantly depending on the rootstock species. Treatment T_5 (*Annona montana*) achieved the highest success rate 80.00%, which was significantly higher than all other treatments. T_4 (*A. reticulata*) achieved 62.50%, while T_1 (*A. muricata*), T_3 (*A. glabra*) and T_6 (*A. mucosa*) produced success rates around 52.50-55.00%. The lowest success (42.50%) was recorded for T_2 (*A. squamosa*) (Table 1).

These findings align with previous reports indicating that *A. montana* can be a suitable rootstock for soursop grafting. Grafting of *A. muricata* scions onto *A. montana* rootstocks yielded graft-success rates comparable to use of *A. muricata* rootstocks [7]. The superior performance of *A. montana* in the present study suggests better anatomical or physiological compatibility with the scion than some of the other rootstocks tested.

Table 1: Effect of different *Annona* rootstocks on the graft success, graft survival, and mortality percentage of soursop grafts at 30 and 90 days after grafting

Treatments	Graft success (%)	Graft Survivability (%)	Graft Mortality (%)
	30 DAG*	90 DAG	
T1 *(Annona muricata)*	55.00^{bc} (47.87)	37.50^{b} (37.76)	62.50^{b} (52.24)
T2 *(Annona squamosa)*	42.50^{c} (40.69)	0.00^{c} (0.00)	100.00^{a} (90.00)
T3 *(Annona glabra)*	55.00^{bc} (47.87)	0.00^{c} (0.00)	100.00^{a} (90.00)
T4 *(Annona reticulata)*	62.50^{bc} (52.24)	55.00^{a} (47.87)	45.00^{c} (42.13)
T5 *(Annona montana)*	80.00^{a} (63.43)	67.50^{a} (55.24)	32.50^{c} (34.76)
T6 *(Annona mucosa)*	52.50^{bc} (46.43)	12.50^{c} (20.70)	87.50^{a} (69.30)
S.Em.±	5.30	4.60	4.60
C.D. @ 5 %	15.76	13.67	13.67

*Values in parenthesis are arc sin transformation data
*DAG - Days after grafting

3.2 Graft Survivability (%) and Graft Mortality (%)

At 90 DAG, survivability also varied markedly across treatments (S.Em ±4.60, $CD_{0.05}$ =13.67). T_5 again led with 67.50% survivors, followed by T_4 with 55.00%. T_1 achieved 37.50%, much lower than the top two. Importantly, T_2 (*A. squamosa*) and T_3 (*A. glabra*) recorded zero survival, and T_6 achieved only 12.50% survival (Table 1).

This pattern suggests that although initial graft union may form, long-term compatibility and physiological continuity vary strongly by rootstock. The complete failure of *A. squamosa* and *A. glabra* as rootstocks in this experiment indicates strong incompatibility under these conditions. Literature on *Annona* grafting supports the notion that rootstock selection is critical: while *A. glabra* is tolerant to flooding and used for rootstock under specific conditions, compatibility issues have been reported [8]. The marked difference in survivability between *A. montana* and the poorest-performing rootstocks underscores the importance of assessing both short-term graft take and longer-term survival.

Graft mortality presented an inverse trend to survivability. T_2 and T_3 had 100% mortality, T_6 had 87.50%, T_1 had 62.50%, T_4 had 45.00%, and T_5 had the lowest mortality at 32.50% (Table 1). The fact that *A. montana* (T_5) showed both the highest success and lowest mortality suggests superior compatibility and vigor of the resulting graft union.

3.3 Number of Days to First Sprouting

Variations in the number of days to first sprouting among treatments were significant (Table 2). T_5 sprouted earliest at 12.33 days, followed by T_4 at 15.75 days, and T_1 at 18.00 days. Treatments T_2 and T_3 took much longer (23.92 and 24.83 days respectively). Early sprouting may indicate good physiological connectivity and vitality of the graft union; therefore, the superior performance of *A. montana* and *A. reticulata* affirm their suitability as rootstocks.

Table 2: Effect of different rootstocks of *Annona* on number of leaves and number of sprouts per grafts of Sour sop at 30, 60 and 90 days after grafting

Treatments	Number of days taken for first sprouting	Number of sprouts per graft			Number of leaves per graft		
		30 DAG*	60 DAG	90 DAG	30 DAG	60 DAG	90 DAG
T1 *(Annona muricata)*	18.00^{b} (25.10)	5.75 (13.87)	7.75^{a} (16.16)	7.25^{b} (15.62)	5.16^{ab} (13.13)	10.41^{a} (18.82)	14.66^{a} (22.51)
T2 *(Annona squamosa)*	23.92^{a} (29.28)	5.91 (14.07)	0.00^{c} (0.00)	0.00^{d} (0.00)	2.58^{c} (9.24)	0.00^{b} (0.00)	0.00^{c} (0.00)
T3 *(Annona glabra)*	24.83^{a} (29.89)	4.91 (12.80)	0.00^{c} (0.00)	0.00^{d} (0.00)	4.08^{bc} (11.65)	0.00^{b} (0.00)	0.00^{c} (0.00)
T4 *(Annona reticulata)*	15.75^{c} (23.38)	6.25 (14.48)	7.91^{a} (16.33)	11.58^{a} (19.89)	6.91^{a} (15.24)	10.25^{a} (18.67)	16.91^{a} (24.28)
T5 *(Annona montana)*	12.33^{d} (20.56)	5.33 (13.350	5.83^{a} (13.97)	7.41^{b} (15.80)	6.25^{ab} (14.48)	12.91^{a} (21.06)	18.66^{a} (25.59)
T6 *(Annona mucosa)*	18.75^{b} (25.66)	6.66 (14.96)	3.16^{b} (10.24)	2.83^{c} (9.68)	5.66^{ab} (13.76)	2.75^{b} (9.55)	5.66^{b} (13.76)
S.Em.±	0.58	0.60	0.72	0.84	0.70	1.08	1.31
C.D. @ 5 %	1.73	NS	2.15	2.50	2.10	3.22	3.90

*Values in parenthesis are arc sin transformation data
*DAG - Days after grafting

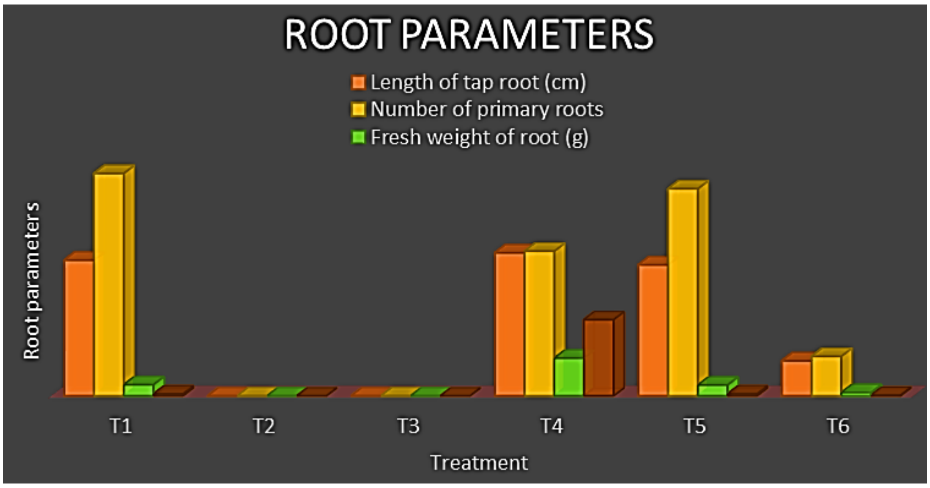
3.4 Vegetative Growth, Root Traits and Physiological Parameters

The number of sprouts per graft, number of leaves, graft height and graft girth, diameter at graft union, and root traits (tap-root length, primary root number, fresh & dry root weights) all followed patterns consistent with the earlier results (Table 3; Fig 3): rootstocks T_5 and T_4 generally outperformed the rest. For example, at 90 DAG, T_4 had the highest number of sprouts (11.58) followed by T_5 (7.41) and T_1 (7.25), while T_2 and T_3 produced none. Leaf numbers at

90 DAG were highest in T_5 (18.66 leaves) followed by T_4 (16.91) and T_1 (14.66). In root parameters, T_1, T_4 and T_5 produced measurable tap-root lengths (23-24 cm) whereas T_2 and T_3 recorded none.

Specific Leaf Weight (SLW, Fig 4) and chlorophyll content further supported these growth differences. T_4 and T_5 registered the highest SLW (0.03 g/cm^2), and for chlorophyll, T_5 recorded the highest total chlorophyll (3.42 mg/g FW), followed by T_4 (2.50) and T_1 (2.38). Treatments T_2 and T_3 recorded essentially zero values (Fig 5).

These results reaffirm that rootstock choice not only influences graft take and survival, but has downstream effects on vegetative development, root system establishment, leaf physiological properties and likely the future fruiting capacity. Previous studies have noted similar trends: in sugar-apple (Annonaceae) grafted combinations, root systems with better development translated to higher leaf chlorophyll content and improved growth [9].



T_1: *Annona muricata*, T_2: *Annona squamosa*, T_3: *Annona glabra*, T_4: *Annona reticulata*, T_5: *Annona montana*, T_6: *Annona mucosa*

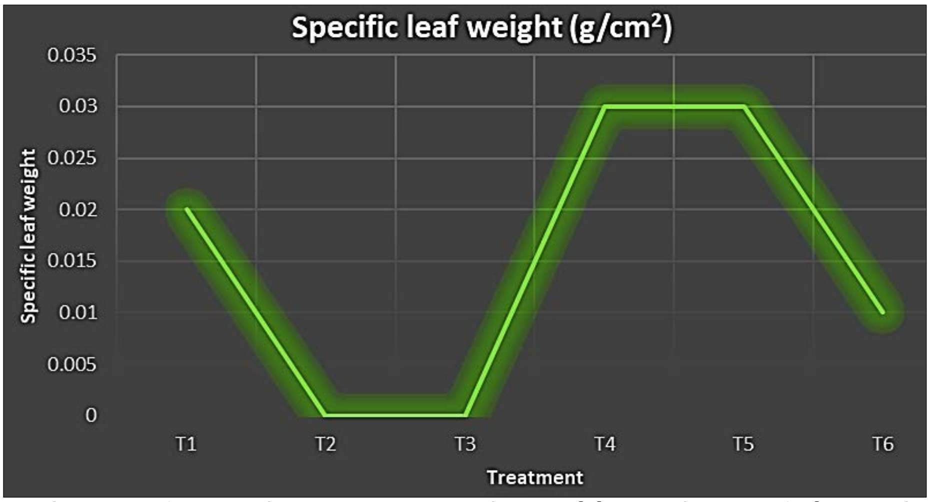
Fig 3: Effect of different rootstocks of *Annona* on root parameters of Sour sop grafts at 90 days after grafting (DAG)

The overall pattern from these results clearly highlights that among the six *Annona* species, *Annona montana* (T_5) and *Annona reticulata* (T_4) exhibited superior compatibility with soursop scion in the experimental conditions. They both delivered high graft success, higher survival, early sprouting, vigorous vegetative growth and strong root development. In contrast, *A. squamosa* and *A. glabra* performed poorly, indicating likely graft incompatibility or sub-optimal physiological interactions.

The compatibility between scion and rootstock is a critical determinant of graft success and subsequent growth. Successful grafting depends on anatomical alignment, vascular connectivity, and physiological integration including nutrient and water translocation [14, 10]. The findings here suggest that *A. montana* and *A. reticulata* rootstocks may facilitate more effective graft-union integration for soursop scions.

However, the complete failure of *A. squamosa* and *A. glabra* under these conditions raises questions about their viability as rootstocks for soursop under similar nursery regimes. Previous literature indicates that although *A. glabra* can perform as a rootstock under certain stress conditions (e.g., flooding tolerance), it may be incompatible with some scions [11-13]. Incompatibility may manifest as poor graft union formation, weak vascular connectivity, or physiological stress leading to death over time.

The physiological measurements (SLW and chlorophyll content) further reflect the nutritional and functional status of the grafted plants. Higher chlorophyll content in grafts on *A. montana* and *A. reticulata* indicates better leaf health and photosynthetic potential, likely translating into improved growth and future yield.



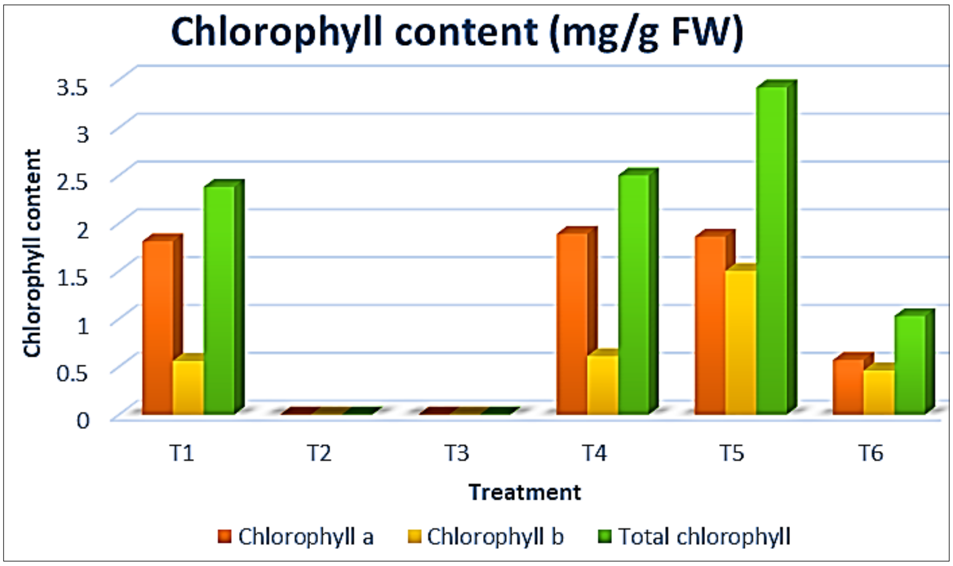
T_1: *Annona muricata*, T_2: *Annona squamosa*, T_3: *Annona glabra*, T_4: *Annona reticulata*, T_5: *Annona montana*, T_6: *Annona mucosa*

Fig 4: Effect of different rootstocks of *Annona* on specific leaf weight

Table 3: Effect of different rootstocks of *Annona* on graft height, girth and graft union girth of Sour sop grafts at 30, 60 and 90 days after grafting

Treatments	Graft height (cm)			Graft girth (mm)			Diameter at graft union (mm)		
	30 DAG*	60 DAG	90 DAG	30 DAG	60 DAG	90 DAG	30 DAG	60 DAG	90 DAG
T_1 (*Annona muricata*)	16.40^{ab} (23.89)	19.66^{a} (26.32)	22.47^{a} (28.30)	16.64^{a} (24.07)	18.96^{a} (25.81)	23.58^{a} (29.05)	7.32^{c} (15.70)	9.11^{ab} (17.57)	9.48^{ab} (17.93)
T_2 (*Annona squamosa*)	15.16^{bc} (22.91)	0.00^{c} (0.00)	0.00^{c} (0.00)	15.85^{bc} (23.46)	0.00^{c} (0.00)	0.00^{c} (0.00)	8.51^{ab} (16.96)	0.00^{d} (0.00)	0.00^{d} (0.00)
T_3 (*Annona glabra*)	15.00^{c} (22.79)	0.00^{c} (0.00)	0.00^{c} (0.00)	14.82^{c} (22.64)	0.00^{c} (0.00)	0.00^{c} (0.00)	7.96^{bc} (16.39)	0.00^{d} (0.00)	0.00^{d} (0.00)
T_4 (*Annona reticulata*)	16.70^{a} (24.12)	19.30^{a} (26.06)	25.01^{a} (30.01)	17.55^{a} (24.77)	20.47^{a} (26.90)	20.47^{a} (26.90)	9.22^{a} (17.68)	9.52^{a} (17.97)	10.07^{a} (18.50)
T_5 (*Annona montana*)	15.20^{bc} (22.95)	18.02^{a} (25.120	23.34^{a} (28.89)	17.23^{a} (13.55)	21.79^{a} (27.83)	23.14^{a} (28.7)	7.18^{c} (15.54)	7.37^{b} (15.75)	8.03^{b} (16.46)
T_6 (*Annona mucosa*)	14.70^{c} (22.54)	8.13^{b} (16.57)	7.85^{b} (16.27)	16.51^{a} (13.26)	9.29^{b} (16.80)	8.35^{a} (9.39)	6.93^{c} (15.26)	3.40^{c} (10.63)	3.20^{c} (10.30)
S.Em.±	0.41	1.32	1.43	0.12	1.73	0.86	0.33	0.61	0.64
C.D. @ 5 %	1.21	3.94	4.26	0.38	5.21	2.60	1.00	1.82	1.92

*Values in parenthesis are arc sin transformation data
*DAG - Days after grafting



T_1: *Annona muricata*, T_2: *Annona squamosa*, T_3: *Annona glabra*, T_4: *Annona reticulata*, T_5: *Annona montana*, T_6: *Annona mucosa*

Fig 5: Effect of different rootstocks of *Annona* on chlorophyll content of Sour sop grafts at 90 days after grafting

4. Conclusion

This study showed that the choice of rootstock significantly influences the grafting success and growth rate of soursop (*Annona muricata* L.). *A. montana* and *A. reticulata* were the most suitable matches among the six species examined. They exhibited the highest rates of graft success and survivorship, accelerated sprouting, enhanced vegetative growth, superior root system development, and increased chlorophyll content. The results indicate that the soursop scion and the other plants exhibited effective physiological compatibility. *A. squamosa* and *A. glabra* exhibited complete graft failure, whereas *A. mucosa* displayed very restricted compatibility. *A. montana* and *A. reticulata* are the optimal rootstocks for enhancing the growth of soursop.

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