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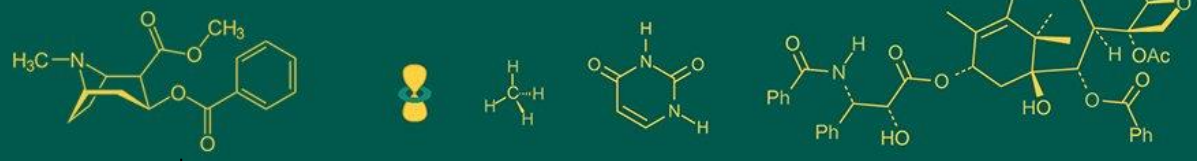

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Evaluation of different transplanting days on field survivability and growth attributes of mulberry mini clones under field conditions for *Morus indica* (V1)

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Abstract
The present scenario of the sericulture sector demands innovative propagation techniques appropriate for a range of agro-climatic circumstances. Therefore, the current study was conducted at Forest College and Research Institute in Mettupalayam in order to verify the viability of mini clonal technology for rapid plantlet regeneration. Though conventional method of stem cutting propagation was popular and widely used to generate saplings. Due to constraints like non availability of source to generate cuttings, non- uniformity in growth was overcome by mini clonal technique. Mini clones 60DAP-AC (V1) registered higher field survival percentage of 93.67 percent than stem cuttings (82.25%) under field conditions. In V1, mini clones 60DAP-AC (V1) performed better in morphological attributes like plant height (128.84 cm) and inter-nodal distance (5.72 cm) and physiological attributes like leaf area (134.73 cm^2) than plants propagated using regular stem cutting 90DAP-SC (V1) which recorded plant height (116.28cm) and inter-nodal distance (5.43 cm) and leaf area (130.60cm^2) The main objective of the study was to validate field survival and growth parameters in main field condition with respect to different days of transplantation.

Keywords: Clonal variation, mulberry, growth parameter, physiological attributes, mini clones

Introduction
The Mulberry (*Morus indica*), is a member of the Moraceae family. It is a woody, deciduous perennial tree with a deeper root system that grows quickly (Vijay and Susikaran, 2023) [17]. There are roughly 68 mulberry species in the world, with *M. indica* (Indian mulberry), *Morus alba* (White mulberry), *Morus serrata*, and *Morus laevigata* being the most widely utilized species in India (Bharathi *et al.*, 2022) [7]. With 28 chromosomes, the majority of the species in the genus *Morus* are diploid. The needed temperature range is 24°C to 28 °C, with 600–2500 mm of annual rainfall. Mulberry grows between 28°N and 55°N latitude and can withstand a variety of climates, from temperate to tropical (Magadum *et al.*, 2019) [9].

The cultivation of high-quality mulberry foliage is essential to the sustainability of sericulture since it affects not only the growth and development of silkworms but also the quality and amount of silk that is produced (Vijay *et al.*, 2023) [17]. For silkworms (*Bombyx mori* L.), mulberry leaves are the only food source. They supply almost 70% of the materials needed to biosynthesize the silk proteins, fibroin and sericin.

Various techniques of vegetative propagation are used, depending on the current environmental circumstances and soil characteristics. Mulberry plants can be propagated by a variety of techniques, including grafting, stem cuttings, and seeds (Hawramee *et al.*, 2019) [5]. Mulberries are typically propagated in India using semi-hard wood cuttings, which are then either planted directly in the main field or grown in nurseries before being moved to the field. Growing seasons and the cost of maintenance and care were the primary determinants of the rate of successful root growth of cuttings, even in nursery circumstances (Prakash *et al.*, 2017) [13]. Mulberry seeds have a 20–30% survival rate and low germination, making seed replication unfeasible (Vijayan *et al.*, 1997) [18] as a result, propagation techniques such grafting and cutting are employed Packialakshmi and Sudhagar (2019) [11].

Directly planting cuttings in the main field frequently results in unfavorable development and a low survival rate. Due to constraints like non availability of source to generate cuttings, non- uniformity in growth was overcome by mini clonal technique (Parthiban *et al.,* 2021) [10]. The utilization of new shoot tips from the plants maintained in clonal mother garden were used as a source of vegetative propagules is the primary characteristic of mini clonal technology. With two to three leaf pairs, mini-cuttings are around 12 to 15 centimeters in length (Seenivasan, 2012) [14]. Shoot apices are utilized as mini-cuttings, and they are rooted by planting them in a suitable rooting media in a glasshouse that has humidity and temperature controls (Hussein *et al.,* 2020) [6]. Because it creates a taproot-like structure, the existence of the shoot apex is crucial for guaranteeing the quality of the root system. However, the new generation of mini cuttings improves the quality of cuttings generated from rigorously maintained planting stock while quickening the pace of clonal multiplication, which reduces the length of the breeding and selection cycle (Bharathi *et al.,* 2022) [7].

Materials and Methods

The experimental location

The field experiment was conducted at Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Coimbatore district, Tamil Nadu (11˚20'N, 76˚55'E, 300m ≥ MSL with annual mean rainfall of 800mm) during academic year 2021-22. The investigation was initiated by raising stem cuttings and apical clones in clonal nursery and V1 saplings from treatment performed well in nursery were planted for further studies under main field situations following all package of practices for irrigated V1 mulberry garden. The experiment was laid out in Factorial Randomized Block Design (FRBD) with four replications and opted a spacing of 10feet ×10feet.

Rooting hormone and rooting medium for mini clonal propagation of mulberry

In plants, auxin is endogenously synthesized at growing tips from where it gets transported to other regions. It is a group of phytohormones promote plant growth along the longitudinal axis. Excised mini cuttings were subjected to exogenously synthesized auxin like Indole- 3 butyric acid which stimulate root initiation. The composition of rooting hormone includes IBA powder, fungicide, talc powder and boric acid crystal. Among different rooting hormone concentration *viz.* 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm. Plants at 3000ppm IBA treatment have performed well. Therefore, ideal rooting hormone for V1 mulberry varieties was Indole-3 butyric acid @ 3000 ppm (Kiruthika, 2020) [8]. The excised apical cuttings were dipped in appropriate rooting hormone with desired concentration and planted in root trainers. Rooting medium will significantly influence the rooting and shoot growth of the cuttings. Ideal composition of rooting media was a mix of Soil: Coir pith: FYM at 1:1:1 ratio (Kiruthika, 2020) [8]. The mini cuttings were planted without damaging the basal cambium portion and kept inside low cost poly-tunnel structure. Temperature inside poly-tunnel was maintained at 30˚C ± 3˚C and desired humidity between 80 and 85 percent. Irrigation was done weekly once.

To study the effect of different transplanting days on survival and growth parameters of V1 apical cuttings under field conditions

At nursery level, the best performed and healthy mini clones were selected and used for the study. Different days of transplanting as treatments *viz.* 50 DAP, 60 DAP, 70 DAP, 80 DAP and 90 DAP (DAP- Days After Planting in nursery) of mini clones and 90 days old mulberry sapling generated using stem cutting method of propagation were used as check to evaluate the mini clones (apical cuttings) and stem cuttings of V1 mulberry varieties. Main field was leveled and ploughed to bring soil to fine tilt. Plants were planted at wider spacing 10 feet x 10 feet to avoid interaction between plants and to evaluate the full potentiality of mini clones under field conditions. Mini clones and stem cuttings were transplanted at appropriate planting dates. Irrigation was done once in five days. Weeding was done 60 days after first treatment transplantation. The experiments were conducted in Factorial Randomized Block Design (FRBD) with four replications and ten plants per replication following regular package of practices. Observation on survival and growth parameters *viz.,* field survival (%), plant height (cm), inter-nodal distance (cm), leaf area (cm^2/plant) were taken at 30 DAT, 60 DAT, 90DAT (DAT- Days After Transplanting in main field).

Experimental details

- **Treatments:** Six.
- **Replication:** Four.
- **Crop:** Mulberry.
- **Variety:** V1.
- **Spacing:** 10 feet x 10 feet.

Treatments

- **T_1:** Variety V1 transplanting on 50^{th} day (Apical cuttings).
- **T_2:** Variety V1 transplanting on 60^{th} day (Apical cuttings).
- **T_3:** Variety V1 transplanting on 70^{th} day (Apical cuttings).
- **T_4:** Variety V1 transplanting on 80^{th} day (Apical cuttings).
- **T_5:** Variety V1 transplanting on 90^{th} day (Apical cuttings).
- **T_6:** Variety V1 transplanting on 90^{th} day (Stem cuttings) – check.

Observations recorded

The observations were recorded on survival, growth and physiological attributes of mulberry

Field survivability (%)

The percentage of mini-clones survived under field condition on 50 DAT was calculated using formula

$$\text{Mean survival per cent of explant} = \frac{\text{Total number of plants survived}}{\text{Total number of plants planted}} \times 100$$

Mulberry growth attributes

From each replication, five mulberry plants which was randomly selected under main field conditions was labelled for recording growth parameters.

Plant height (cm)
Plant height is the measure of total distance between highest point on the plant and lowest point on ground level. It was generally expressed in terms of cm.

Inter-nodal distance (cm)
Length between the nodes having fourth leaf and fifth leaf from the highest tip of the plant was recorded. The mean value were measured was generally expressed in cm.

Leaf area (cm²/ plant)
Each replication consists of ten mulberry plants from which five plants were randomly selected and fifth leaf from the tip of the plant was collected and leaf area was measured by following length and breath method recommended by Montgomery (1911) [19]. Mean value was expressed in cm²/ plant.

LA (A) = l x b x c

Where,
A- leaf area, l – leaf length, b – leaf breadth, c – constant factor (0.676)

Statistical analysis
During statistical analysis of data, the treatments which were found significant, the critical differences were calculated and analyzed at five percent level of probability. AGRES software package was used to analyze the stage wise data. The data collected from above experiments were critically analyzed by adopting Factorial Randomized Block Design (FRBD).

Results and Discussion
Effect of different transplanting days on survival and growth parameters of V1 mini clones under field conditions
Field survivability
Significant variation in field survivability of V1 mini clones was evidenced due to different days of transplantation of mini clones. The hardening period will have a direct impact on field survivability of mini clones. In V1 mulberry, at 60 DAP-AC (T_2), the field survivability registered the mean value of 93.67 percent. Whereas on 90 DAP-SC (T_6), the field survivability recorded the mean value of 82.25 percent. Higher field survivability was recorded in treatment T_5 (90 DAP-AC) with mean value of 97.86 percent followed by T_4 (80 DAP-AC) with mean 96.22 percent and poor field survival rate of 68.44 percent was witnessed in T_1 (50 DAP-AC) as presented in Table 1. From the results it was found that T_2 (93.67%), T_3 (94.23%), T_4 (96.22%), T_5 (97.86%) are statistically on par with each other.
The hardening period will have a direct impact on field survivability of mini clones. The findings are in consonance with the findings of Shekatwat *et al.* (2021) [15] who concluded properly acclimatized saplings for increase period of time have registered high survival percentage (98%) under field conditions. The result was further supported by Deb and Imchen (2010) [3] who reported the mortality of plantlets was due to poor acclimatization and transplant shock.

Table 1: Effect of different transplanting days on field survivability (%) of V1 mini clones on 50 DAT

Treatment	Field survivability (%)
V1 50 DAP-AC (T_1)	68.44 c
V1 60 DAP-AC (T_2)	93.67^a
V1 70 DAP-AC (T_3)	94.23^a
V1 80 DAP-AC (T_4)	96.22^a
V1 90 DAP-AC (T_5)	97.86^a
V1 90 DAP-SC (T_6)	82.25^b
SE(d)	2.63
CD(0.05)	5.88**

Note: AC - Apical cuttings; SC - Stem cuttings
**Highly significant, *Significant
Each value is the mean of four replications
Means followed by same alphabets are on par with each other by LSD (P=0.05)

Plant height
There was a significant difference in plant height at different growth periods *viz.*, 30 DAT, 60 DAT and 90 DAT in V1 mini clones (Figure 1). In V1, maximum plant height recorded in T_5 were 73.45 cm, 101.12 cm and 139.41 cm during 30 DAT, 60 DAT and 90 DAT. This was followed by T_4 were 70.38cm (30 DAT), 98.17 cm (60 DAT) and 135.29 cm (90 DAT) whereas T_1 showed minimum plant height of 49.67 cm, 67.89 cm and 92.53 cm during 30 DAT, 60 DAT and 90 DAT respectively. From statistical analysis, it was found that in V1 at 90 DAT, T_2 (128.84 cm) and T_3 (131.93 cm) also treatments T_4 (135.29 cm) and T_5 (139.41 cm) found to be statistically on par with each other as summarised in the Table 2.
These results are in line with observations of Bheevi (2010) [1] who noticed morphological variations in plant height of 102 cm at 60 DAT in V1 stem cuttings. The increase in plant height might be due to geotropism effect and apical dominance effect.

Table 2: Effect of different transplanting days on plant height (cm) of V1 mini clones at different time intervals

Treatment	30DAT	60DAT	90DAT
50 DAP-AC (T_1)	49.67^b	67.89 c	92.53 c
60 DAP-AC (T_2)	65.94^a	93.89^{ab}	128.84^{ab}
70 DAP-AC (T_3)	67.82^a	94.6^{ab}	131.93^{ab}
80 DAP-AC (T_4)	70.38^a	98.17^{ab}	135.29^a
90 DAP-AC (T_5)	73.45^a	101.12^a	139.41^a
90 DAP-SC (T_6)	68.14^a	91.05^b	116.28^b
SE(d)	4.54	3.38	7.10
CD(0.05)	10.12**	7.54**	15.83**

Note: AC - Apical cuttings; SC - Stem cuttings
**Highly significant, *Significant
Each value is the mean of four replications
Means followed by same alphabets are on par with each other by LSD (P=0.05)

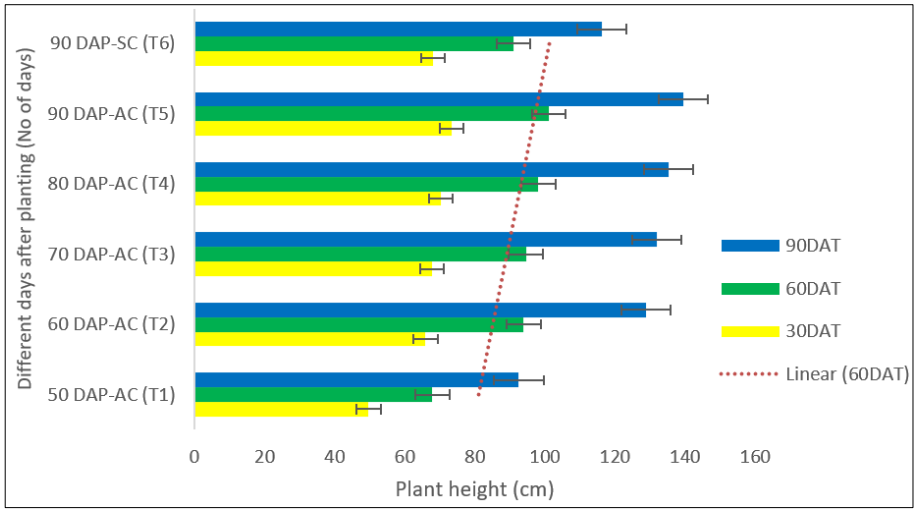

Fig 1: Effect of different transplanting days on plant height (cm) of V1 mini clones at different time intervals

Inter-nodal length

From the figure 2, it was found that inter-nodal length varied significantly due to different transplanting days. The values at 30 DAT, 60 DAT and 90 DAT in both V1 mini clones. In V1, maximum inter-nodal length registered in T_5 were 4.08cm, 4.85cm and 5.87 cm during 30 DAT, 60 DAT and 90 DAT (Table 3). This was followed by T_4 were 4.02 cm (30 DAT), 4.77 cm (60 DAT) and 5.81 cm (90 DAT) whereas T_1 recorded minimum inter-nodal length of 2.58cm, 2.96cm and 3.78cm during 30 DAT, 60 DAT and 90 DAT respectively. The values obtained were subjected to statistical analysis. In variety V1, all the treatments T_2 to T_6 except T_1 (3.78cm) was found to be on par statistically with each other at 90 DAT.

The increase in inter-nodal length was proportional to the increase in growth of the plant. These observations were further supported by Sudhakar *et al.* (2020) [16] who recorded inter-nodal distance of 5.2 cm in V1 variety at 90 DAT.

Table 3: Effect of different transplanting days on inter-nodal distance (cm) of V1 mini clones at different time intervals

Treatment	30DAT	60DAT	90DAT
50 DAP-AC (T_1)	2.58^{c}	2.96^{b}	3.78^{b}
60 DAP-AC (T_2)	3.85^{ab}	4.63^{a}	5.72^{a}
70 DAP-AC (T_3)	3.93^{a}	4.7^{a}	5.78^{a}
80 DAP-AC (T_4)	4.02^{a}	4.77^{a}	5.81^{a}
90 DAP-AC (T_5)	4.08^{a}	4.85^{a}	5.87^{a}
90 DAP-SC (T_6)	3.61^{b}	4.37^{a}	5.43^{a}
SE(d)	0.12	0.24	0.25
CD(0.05)	0.28**	0.54**	0.57**

Note: AC - Apical cuttings; SC - Stem cuttings
**Highly significant, *Significant
Each value is the mean of four replications
Means followed by same alphabets are on par with each other by LSD (P=0.05)

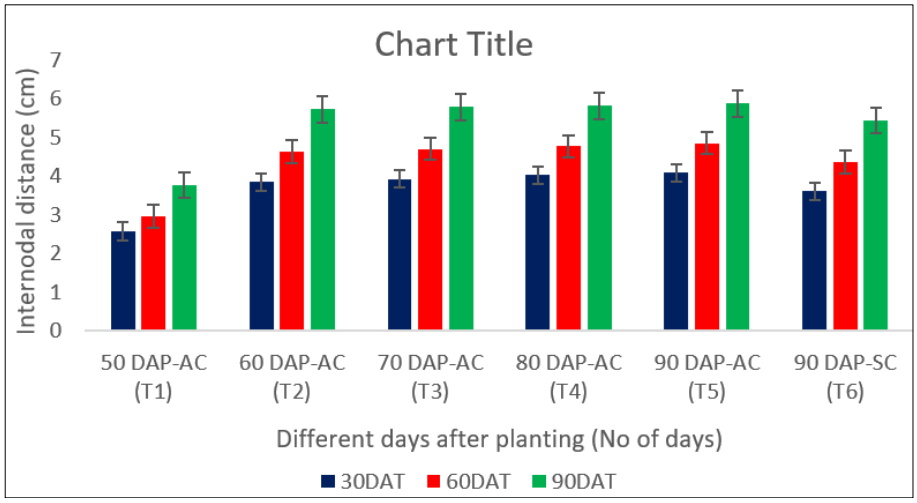

Fig 2: Effect of different transplanting days on inter-nodal distance (cm) of V1 mini clones at different time intervals

Leaf area

The leaf area values differed significantly at three intervals *i.e.* 30 DAT, 60 DAT and 90 DAT in V1 mini clones and stem cuttings was taken as check. In V1, maximum leaf area noticed in T_5 were 119.83 cm^2, 129.62 cm^2 and 157.89 cm^2 during 30 DAT, 60 DAT and 90 DAT (Figure 3). This was followed by T_4 with 116.37 cm^2 (30 DAT), 126.45 cm^2 (60 DAT) and 137.91 cm^2 (90 DAT) Whereas T_1 showed minimum leaf area of 98.72 cm^2, 109.29 cm^2 and 119.94 cm^2 during 30 DAT, 60 DAT and 90 DAT respectively (Table 4). The statistical analysis of leaf area readings revealed that in V1 at 90 DAT, T_2 (134.73 cm^2), T_3 (135.62 cm^2) and T_5 (137.91 cm^2) were found to be statistically on par with each other.

These findings are in line with Mithilasri *et al.* (2021) [10] who noticed that leaf area of 97.69 cm^2 in V1 mini clones

three months after planting and clonal variation in leaf attributes like leaf length and leaf width with respect to different accessions.

Table 4: Effect of different transplanting days on leaf area (cm^2) of V1 mini clones at different time intervals

Treatment	30DAT	60DAT	90DAT
50 DAP-AC (T_1)	98.72[b]	109.29[b]	119.94 c
60 DAP-AC (T_2)	114.1[a]	122.14[a]	134.73[b]
70 DAP-AC (T_3)	114.92[a]	123.89[a]	135.62[b]
80 DAP-AC (T_4)	116.37[a]	126.45[a]	137.91[b]
90 DAP-AC (T_5)	119.83[a]	129.62[a]	157.89[a]
90 DAP-SC (T_6)	110.08[a]	121.23[a]	130.60[cb]
SE(d)	4.91	5.04	6.42
CD(0.05)	10.94**	11.23**	14.30**

Note: AC - Apical cuttings; SC - Stem cuttings
**Highly significant, *Significant
Each value is the mean of four replications
Means followed by same alphabets are on par with each other by LSD (P=0.05)

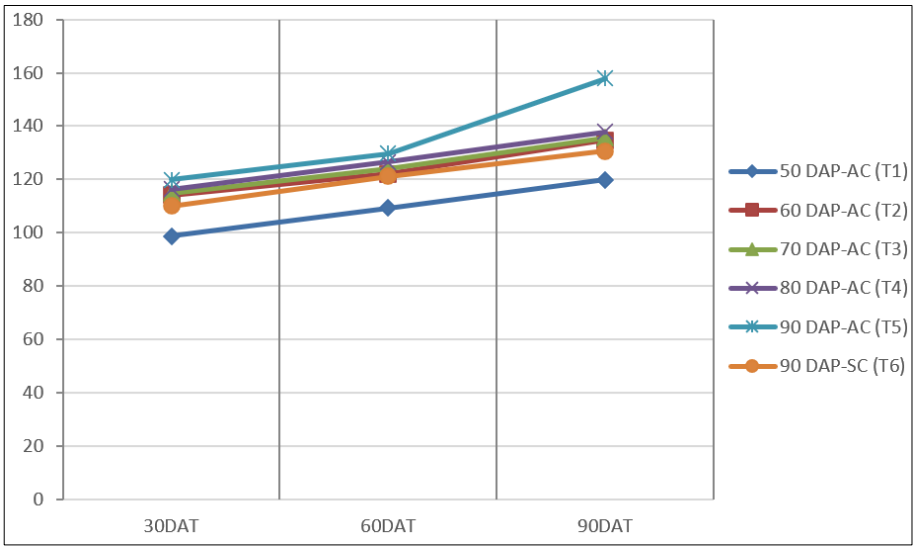

Fig 3: Effect of different transplanting days on leaf area (cm^2) of V1 mini clones at different time intervals

Conclusion

Mini clones 60 DAP-AC (V1) recorded high survival percentage of 93.67 percent compared to stem cuttings. Mini clones 60 DAP-AC (V1) registered maximum plant height of 128.84 cm. Mini-clones 60 DAP-AC (V1) showed mean nodal length of 5.72 cm. Mini clones 60 DAP-AC (V1) recorded maximum leaf area of 134.73 cm^2 when compared to check. Stem cuttings recorded field survival percentage of 82.25%, plant height of 116.28cm, intermodal distance of 5.43 cm and leaf area of 130.60cm^2. From this experimental data, we can conclude that plants regenerated using apical cuttings witnessed tremendous growth performance and field survival potential compared to plants generated using conventional stem cuttings.

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