P-ISSN: 2618-0723
E-ISSN: 2618-0731

NAAS Rating: 5.04
www.extensionjournal.com

International Journal of Agriculture Extension and Social Development

Volume 7; SP-Issue 8; August 2024; Page No. 204-207

Received: 13-05-2024
Accepted: 20-06-2024

Indexed Journal
Peer Reviewed Journal

Dardizing cutting size of mulberry (*Morus sinensis*) for mini clonal technology

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DOI: https://doi.org/10.33545/26180723.2024.v7.i8Sd.980

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Abstract
An attempt was made to standardize a sound protocol for mini clonal propagation of mulberry (*M. sinensis*). Different sized apical shoot cuttings *viz*., 2 cm, 4 cm, 6 cm, 8 cm, 10 cm and 15 cm were harvested from mother plants of *M. sinensis.* Such cuttings were treated with different growth regulators *viz*., IBA and NAA @ 5000 ppm and raised in mistless polytunnel. The growth parameters *viz*., shoot length, rooting percentage, root length and root shoot ratio were taken. Among the sizes, cutting size of 15 cm treated with IBA @ 5000 ppm recorded highest survival percentage of (43.00%) followed by NAA (35.00%).

Keywords: Cutting size, IBA, NAA, growth, *Morus sinensis*

Introduction

Mulberry (*Morus sp*., Moraceae) is a fast growing, deciduous woody perennial tree with a deep rooting system with its leaves being simple, alternate, stipulate, petiolate, entire or lobed, lobes may vary from 1-5. Inflorescence is catkin type with a pendent or drooping peduncle bearing unisexual flowers. India, there are many species of *Morus* are present, of which *M. alba, M. indica. M. serrata* and *M. laevigata* grow wild in the Himalayas. Several varieties belonging to *M. multicaulis, M. nigra, M. sinensis* and *M. philippinensis* have been introduced. Most of the Indian varieties of mulberry belong to *M. indica* (Sanjappa, 1989) [1]. Mulberry is one of the most economically important tree crops in Asia as the leaves of mulberry is the sole food for silkworm Bombyx mori L. Nearly 70 per cent of silk produced by silkworm is directly derived from the protein of mulberry leaves as the quality and quantity of mulberry leaves have a direct impact on cocoon harvest (Datta, 2000) [2]. Increased carbohydrate and protein contents of leaves are favorable for healthy growth and development of silkworm and mulberry plants are used as fodder for livestock (Vijayan *et al*., 1998) [3].

Mulberry is amenable for sexual and asexual modes of reproduction. Owing to heterozygosity of parents, propagation through seeds is not commercially viable as seed grown plants show high degree of variability and poor survival percentage 20-30 per cent (Vijayan, 1997) [4]. Therefore, propagation of mulberry for large scale production is done using stem cuttings. Though propagation through stem cutting is easy, it has some restriction *viz*., low rooting potential in (MR2 and matigara black), less number of harvests per plant and long juvenile period. Additional problem involved in developing saplings in nursery is the maintenance and management cost for 3-6 months. So, to overcome these limitations, it was planned to establish an alternate method with which mulberry can be propagated rapidly in a cost-effective way.

Mini clonal propagation provides such an alternative tool for rapid and cost-effective multiplication for mulberry as large number of clones could be produced in short time and space. Mini clonal technology has been developed for Casuariana and Melia and successfully implemented in Tamil Nadu for commercial multiplication (Parthiban, 2016) [5]. In contrast when compared to stem cutting, mini cutting system showed improved rooting potential, rooting speed, and rooting quality as well as reduced cost. Additionally, this system offer propagules with increased uniformity and greatly reduces topophysis effect (Francisco *et al*., 2004) [6]. Against this backdrop it was planned to standardize the protocol for mini clonal propagation for mulberry.

Materials and Methods

The present studies were carried out in the Department of Sericulture, Forest College and Research Institute, Mettupalayam, Tamil Nadu, India to standardize the sound protocol for mini clonal propagation of mulberry variety MR2 of (*M. sinensis*) cuttings of different sizes were collected from donor nursery plants. Experimental site is situated at 300m above mean sea level and lies between 11º 19' N latitude and 76º 56' E longitude. The climate is sub-tropical to tropical and rains are concentrated during July-August.

Preparation mini clonal cuttings

Apical shoot cuttings were excised from mother garden. The cuttings were further trimmed to different length of (2, 4, 6,

8, 10 and 15 cm). All the leaves of the shoot except two leaves near the apical point were removed and pre-treated with 0.2% Bavistin to prevent the cuttings from fungal infection before using for different hormonal treatments. A slanting cut was given at the basal part of all the cuttings and subjected to different root inducing hormones *viz*., Indole Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) at 5000 ppm on quick dip basis. Such treated cuttings were planted in poly bags of 10 x 15 cm size and kept inside the low-cost poly tunnels. Watering was done once in a week in order to maintain desired humidity between 80 and 90 per cent.

The temperature in the poly tunnel was maintained at 33 ± 1 °C. The studies were conducted in a Completely Randomized Block Design (CRD) with four replications, with 25 cuttings per replication. Most of the cuttings started rooting in 25th day. Observations on shoot length (cm), rooting percentage, root length (cm) and root shoot ratio (%) were recorded at three growth periods *viz*., 30 DAP, 60 DAP and 90 DAP.

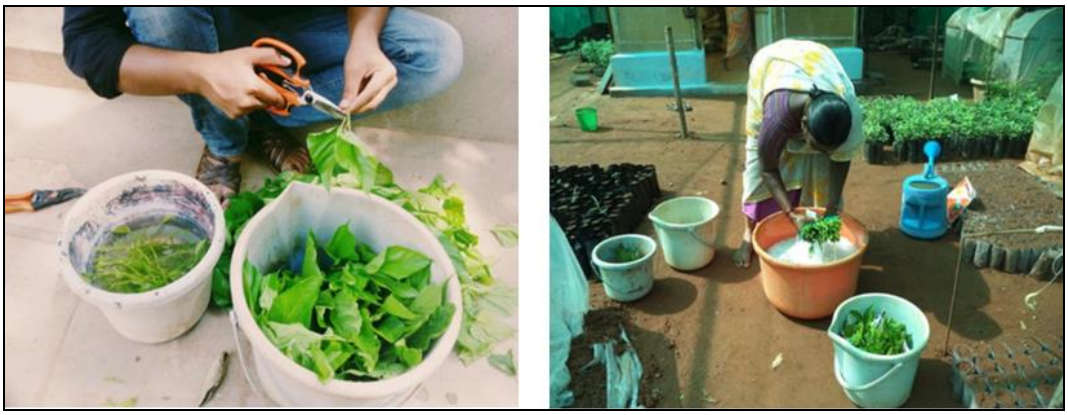

Fig 1a: Clonal ramets Trimming process of *Morus sinensis* and pre-treating the clonal ramet with 0.2% Bavistin

Fig 1b: Planting of Clonal ramets of *Morus sinensis* in poly bags and covering the planted cutting using transparent polythene sheet to maintain humidity and temperature

Fig 1c: Root initiation from planted Clonal ramets of *Morus sinensis* at 25th day

Results

In the present study on the "Dardizing cutting size of Mulberry (*Morus sinensis*) for Mini Clonal Technology, mini cuttings of various sizes of *viz.*, (2, 4, 6, 8, 10 and 15 cm) were subjected to two different growth regulators *viz*., NAA an IBA at different concentrations to standardize ideal cutting size for mini clonal propagation of *M. sinensis*. Growth parameters *viz*., shoot length, root length, number of roots, number of leaves and Root shoot ratio were recorded. Among the different sizes of cuttings investigated apical shoot cuttings of 15 cm recorded superiority in majority of growth parameters at three growth periods *viz*., 30 DAP, 60 DAP and 90 DAP. Hence cutting size of 15 cm was found to be optimum for clonal multiplication of *M. sinensis.*

Among the different concentration of growth regulators used, cuttings treated with IBA 5000 ppm (Table 1.) registered superiority in overall performance *viz*., Shoot length, root length, number of roots, number of leaves and Root shoot ratio, followed by NAA 5000 ppm (Table 2). Maximum shoot length recorded at 90 DAP (22.05 cm), root length (26.40 cm), Number of leaves per plant (11.50), Number of roots per plant (15.25) and Root shoot ratio (1.26).

Discussion

It was found that apical shoot bud cutting of 15 cm size was better in overall performance. This might be attributed due to optimum cutting size, temperature, light, aeration and moisture supply to the cuttings. Similar studies were conducted in *Morus alba* towards standardizing different sized cuttings and found that cuttings of 6 inches length proved to be superior in terms of sprouting percentage, plant height and number of leaves. However this study was associated with hardwood cuttings against the apical shoot bud cuttings diploid in the study.

Out of two growth regulators (IBA and NAA) used, IBA reigned supreme in terms of morphometric traits *viz*., (sprouting percentage, rooting percentage, average number of roots, average root length per cuttings, average shoot length, average root length and root shoot ratio). Auxins were known to trigger the rooting of cuttings of many species (Sardoei *et al*., 2013; Sevik and Kerim Guney 2013; Surendran *et al.*, 2000) [7, 9, 10].

Among auxins, the superiority of IBA to that of other auxins had been reported earlier by many investigators in Eucalyptus (Teotonio Francisco de Assis *et al*., 2004) [6], Lawsonia inermis (Hasan Sarhadi *et al*., 2013) [7], *Azadirachta indica, Casuarina equisetifolia, Ceiba pentandra, Gmelia arborea* and *Thespesia populnea* (Parthiban *et al*., 1999) [10], Citrus medica (Sulaiman *et al*., 2015) [11] and *Cordyline terminalis*.

In this study two growth regulators *viz*., NAA, IBA were employed. IBA 5000 ppm reigned supreme in terms of survival parentage, sprouting percentage, rooting percentage, shoot length, root length, average number of roots and average root length in three growth periods *viz*., 30DAP, 60DAP and 90DAP. The dose of hormone is an important factor in the rooting of cuttings. Similar results were found in *Morus nigra* where in the hardwood cutting treated with IBA at 5000 ppm performed superior in terms of callusing percentage, rooting percentage, number of roots and root length (Fatma Koyuncu and Eylem 2003) [13]. High concentration of IBA resulted in high percentage of rooting of cuttings in *Jatropha curcas*, *Simarouba glauca* (Kala *et al*., 2017) also attests the superiority of IBA in rooting of cuttings reported in the present study.

In a holistic perspective, the current study recommends that propagation of *Morus sinensis* using standard cutting size of 15 cm on treatment with IBA at 5000 ppm is suitable for mass multiplication of mini clones of mulberry.

Table 1: Effect of IBA on growth attributes of *M. sinensis*

Treatments	15 cm cuttings														
	Shoot length (cm)			Root length (cm)			Root/shoot			No. of roots/cutting			No. of leaves/cutting		
	30DAP	60DAP	90DAP	30DAP	60DAP	90DAP	30DAP	60DAp	90DAP	30DAP	60DAP	90DAP	30DAP	60DAP	90DAP
T1-1000ppm	2.13*	7.07*	12.37*	4.15*	8.92*	15.92*	1.96*	1.28*	1.26 *	3.25*	5.50*	8.75*	2.25*	3.50*	5.00*
T2-2000ppm	3.60*	9.15*	14.05*	5.83*	11.42*	17.75*	1.68*	1.26*	1.24*	4.00*	6.25*	10.25*	2.50*	4.75*	6.50*
T3-3000ppm	4.50*	11.5*	16.12*	6.75*	14.10*	20.07*	1.52*	1.24*	1.22*	5.25*	7.00*	11.75*	4.75*	5.5*	7.50*
T4-4000ppm	5.80*	13.3*	19.02*	8.80*	16.07*	23.10*	1.52*	1.21*	1.20*	6.25*	8.75*	12.50*	5.25*	6.75*	9.00*
T5-5000ppm	7.38*	16.22*	22.05*	11.30*	19.10*	26.40*	1.55*	1.19*	1.17*	8.00*	11.00*	15.25*	7.00*	8.00*	11.50*
Control	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mean	4.07	9.70	14.10	6.30	11.77	17.37	1.54	1.18	1.20	4.63	6.58	9.92	3.67	4.92	6.75
S.Ed	0.43	0.39	0.50	0.69	0.48	0.59	0.12	0.01	0.02	0.58	0.58	0.70	0.65	0.65	0.55
CD (.05%)	0.90	0.83	1.06	1.46	1.01	1.25	0.25	0.03	0.06	1.22	1.23	1.48	1.37	1.37	1.16

*Significant at 5% Level

Table 2: Effect of NAA on growth attributes of *M. sinensis*

Treatments	15 cm cuttings														
	Shoot length (cm)			Root length (cm)			Root/shoot			No. of roots/cutting			No. of leaves/cutting		
	30DAP	60DAP	90DAP	30DAP	60DAP	90DAP	30DAP	60DAp	90DAP	30DAP	60DAP	90DAP	30DAP	60DAP	90DAP
T1-1000ppm	2.40*	6.05*	11.25*	4.35*	7.65*	14.45*	1.83*	1.26*	1.28*	3.25*	5.00*	8.75*	2.50*	4.00*	5.75*
T2-2000ppm	4.43*	7.40*	13.00*	6.43*	9.17*	16.35*	1.59*	1.24*	1.25*	3.00*	6.50*	10.25*	2.75*	4.75*	7.00*
T3-3000ppm	5.20*	9.22*	16.05*	7.40*	11.32*	19.17*	1.42*	1.22*	1.19*	3.75*	7.25*	11.50*	3.25*	5.25*	7.75*
T4-4000ppm	7.40*	10.12*	18.40*	9.23*	12.20*	21.32*	1.25*	1.20*	1.15*	4.75*	8.00*	12.25*	4.00*	7.00*	8.25*
T5-5000ppm	8.40*	12.17*	19.80*	10.70*	14.57*	22.30*	1.27*	1.19*	1.15*	6.25*	9.75*	14.75*	5.50*	7.50*	10.25*
Control	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mean	4.80	7.66	13.38	6.52	9.32	15.76	1.39	1.18	1.17	3.67	6.25	9.75	3.17	4.91	6.66
S.Ed	0.26	0.24	0.4679	0.49	0.27	0.32	0.07	0.01	0.03	0.86	0.67	0.66	0.65	0.71	0.79
CD (.05%)	0.56	0.51	0.9829	1.04	0.57	0.67	0.16	0.03	0.08	1.81	1.42	1.40	1.37	1.50	1.60

*Significant at 5% Level

Conclusion

In conclusion, this study effectively demonstrates that mini clonal propagation is a viable and efficient method for mulberry (*Morus sinensis*) multiplication. Among the various cutting sizes tested, 15 cm apical shoot cuttings showed superior growth metrics, including shoot length, root length, and root-shoot ratio. Additionally, treatment with Indole Butyric Acid (IBA) at 5000 ppm yielded the best results across all evaluated parameters, outperforming Naphthalene Acetic Acid (NAA). This method offers a promising alternative to traditional propagation techniques, reducing costs and improving rooting success, which is crucial for large-scale mulberry cultivation and silkworm productivity.

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