**COMPARISON ON PHYSIOLOGICAL TRIATS OF MINI CLONAL LEAVES OF MULBERRY TREATED WITH DIFFERENT GROWTH HORMONES**

**ABSTRACT**

In this study leaves of mini clonal cutting of mulberry treated with different growth regulating hormones *viz*., (IBA and NAA) were analysed for their physiological traits *viz.*, (chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll content, chlorophyll a/b ratio and soluble protein content). Their result shows that IBA reigned supreme in terms of physiological traits when compared with NAA and this explains furtherance of IBA for propagation of mini clonal cuttings of mulberry.

**Key words**: NAA, IBA, Mini clonal leaves

**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. 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).

 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). Therefore, propagation of mulberry for large scale production is done using stem cuttings by planting the cutting directly in the field or raising saplings in nursery and then transplanting to main field. Though propagation through stem cutting is easy, it has some restriction *viz.,* low rooting potential in (MR2 variety), 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 (Kapur *et al.,* 2001).

 Owing to these limitations, it was planned to developed an alternative method for propagation of mulberry. Mini clonal technology is well known propagation techniques successfully developed for forest trees with promising results. Since mulberry is perennial tree, this provides 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). The cuttings have to be treated with plant growth regulators to promote growth. Plant growth regulators are synthetic substances which produce identical results like natural plant hormones which are synthesized in the plants in a very small amount. They are also known as **Phytohormones**. Plant Growth Regulators are used in agricultural and horticultural technology to stimulate the developmental processes of the plants. The most common plant growth regulator or auxin used is Indole-3-butyric acid (IBA) or Naphthalene acetic acid (NAA) to stimulate rooting in cuttings. There are three types of cuttings that are used for propagation– Softwood cutting, Semi-hardwood cutting and Hardwood cutting. (Rahman et al., 2004).

This study was undertaken to access the leaf quality of mini clonal cuttings treated with different growth regulating hormones *viz*., of IBA and NAA. Leaves are excised from mini clonal cutting treated with IBA and NAA @ 1000, 2000, 3000, 4000, 5000 ppm and analysed for physiological traits *viz*., (chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll content, chlorophyll a/b ratio and soluble protein content).

**MATERIALS AND METHODS**

The experimental material comprised of semi-hard wood cuttings of MR2 variety (*Morus sinensis).* Nursery experiments were carried out at Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam(11o19’N, 76o56’E, 300 meters MSL, 800 mm, pH 7.1during 2016-2017. The propagules required for standardizing cutting size for mini clonal propagation were collected from juvenile stem cuttings of MR2.

**Species under study**

**i) Species description**

Species : *Morus sinensis*

Tamil name : KalpaVruksha

Common name : Indian Mulberry

**ii) Climate and soil**

Mulberry thrives under various climatic conditions ranging from temperate to tropical, located north of equator between 28˚N and 55˚N latitude. The ideal range of temperature is from 24˚ to 28˚C. Mulberry grows well in places with an annual rainfall ranging from 600 to 2500 mm and flourishes well in soil that are flat, deep, loamy to clayey and porous with good moisture holding capacity with ideal pH ranging from 6.2 to 6.8.

**Optimization of rooting hormone for Mini clonal propagation of mulberry**

 Different concentrations of rooting hormones *viz*., 1000, 2000, 3000, 4000 and 5000 ppm were used. Instead of stock solution, talc based formulation of rooting hormone prepared by adding known quantity of rooting hormone *viz*. (100mg, 200mg, 300mg, 400mg, and 500mg/100g of talc respectively). Selected Mini-cuttings were dipped in the rooting hormone and planted in polybags and raised in polytunnel. Leaves from the cuttings are excised after 40 and 60 DAP Parthiban *et.al*., (2016)

**Estimation of chlorophyll**

The concentrations of chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll were estimated at 40 days and 60 days old cuttings by adopting the method of Yoshida *et al.,* (1976) and expressed as mg per gram of fresh weight.

Matured young fresh leaf samples of 250 mg were collected, washed in distilled water and then ground with 10 ml of 80 per cent acetone using pestle and mortar. The homogenate solution was centrifuged at 500 rpm for 10 minutes. The supernatant was collected and the volume was made upto 25 ml using 80 per cent acetone. The optical density of the content was measured at 663 and 645nm (Tanee and Albert, 2013). Then chlorophyll ‘a’, chlorophyll ‘b’ total chlorophyll and soluble protein content were calculated using the following formulae:

**Total chlorophyll** (mg g-1) = (8.02 x OD at 663) + (20.2 x OD at 645) X dilution factor

Where,

 V = Volume made (25ml)

 W = Weight of fresh sample taken (0.250g)

**Estimation of soluble protein**

This was estimated using Lowry’s method (Lowry *et al*., 1951) and expressed in mg/g.

**Statistical design**

The data obtained were subjected to statistical analyses to evaluate the possible relationship between different parameters and analysis of variance employed in nursery by adopting Completely Randomized Design described by Panse *et al*. (1985). The stagewise data were analysed separately using AGRES software.

**RESULT AND DISCUSSION**

Different concentrationsoftwo growth regulators *viz*., IBA and NAA were used for propagation of mini clonal cuttings harvested from clonal hedge mulberry garden. Such treated cuttings were analyzed for both morphological and physiological traits. This study focusses on physiological traits *viz*., (chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll content, chlorophyll a/b ratio and Soluble protein content). Result shows that IBA gains the positive trends over NAA.

**Chlorophyll and Soluble protein Content of leaves excised from IBA treated leaves of min clonal Cuttings**

**Chlorophyll ‘a’ (mg g-1)**

 Significant variation in chlorophyll ‘a’ due to IBA treatments was evidenced and it ranged from 3.25 mg g-1 (T5) to 2.58 mg g-1 (T1). The average chlorophyll ‘a’ registered was 2.87. Compare to control (3.14 mg g-1), only one treatment (T5) registered significantly higher chlorophyll ‘a’ and the least value for chlorophyll ‘a’ was recorded by T1 (2.58 mg g-1) (Table 1).

**Chlorophyll ‘b’ (mg g-1)**

Chlorophyll ‘b’ differs significantly due to treatments. It varied between 1.47 mg g-1 (T1) and 2.93 mg g-1 (control). All treatments of IBA registered significantly lower chlorophyll ‘b’. However compare to general mean 2.22 mg g-1 three treatments *viz*., T3 (2.36 mg g-1), T4 (2.65 mg g-1) and T5 (2.93 mg g-1) registered significantly higher chlorophyll ‘b’ (Table 1).

**Total chlorophyll (mg g-1)**

There was a significant variation in the total chlorophyll content among treatments. It ranged from 2.90 mg g-1 (T5) to 2.32 mg g-1 (control). The general mean recorded for total chlorophyll is 2.70 mg g-1. All the treatments exhibited significantly higher total chlorophyll content than control. Among them, treatment (T5) recorded superiority for this parameter (Table 1).

**Chlorophyll a/b**

Variation in chlorophyll a/b ratio was significantly influenced by IBA treatments. The deviation for chlorophyll a/b ratio varied from 1.06 (control) to 1.56 (T1). Compared to control, all treatments registered higher chlorophyll a/b ratio. Among the treatments, significantly higher chlorophyll a/b was registered by T1 (1.56) and lowest value of 1.11was recorded by T5 (Table 1).

**Soluble protein (mg g-1)**

Significant differences in soluble protein were observed among different concentrations of IBA. The variation ranged from 23.58 mg g-1 (T1) to 40.62 (T5). The general mean for soluble protein recorded was 34.57 mg g-1. Among the treatments T5 recorded highest soluble protein content of 40.62 mg g-1. Four treatments *viz*. T2 (25.31 mg g-1), T3 (32.10 mg g-1), T4 (38.78 mg g-1) and T5 (40.62 mg g-1) expressed superiority over control. (Table 1)

**Chlorophyll and Soluble protein Content of leaves excised from NAA treated leaves of min clonal Cuttings**

**Chlorophyll ‘a’ (mg g-1)**

Chlorophyll ‘a’ differed significantly due to NAA treatments. It ranged from 3.14 mg g-1 (control) to 1.88 mg g-1 (T1). Compare to control all the treatments recorded significantly lower chlorophyll ‘a’ (Table 2).

**Chlorophyll ‘b’ (mg g-1)**

Different concentration of NAA showed significant variation for Chlorophyll ‘b’. The highest chlorophyll ‘b’ was recorded in control (3.08 mg g-1). Compare to control all treatments exhibited significantly lower chlorophyll ‘b’. Two treatments *viz*., T5 (2.74 mg g-1) and T4 (2.50 mg g-1) recorded significantly higher chlorophyll ‘b’ than general mean 2.43 mg g-1 (Table 2).

**Total chlorophyll (mg g-1)**

There was a significant variation in the total chlorophyll content among treatments studied. It ranged from 2.32 mg g-1 (control) to 1.11 mg g-1 in (T1). The general mean recorded for total chlorophyll content was 1.67 mg g-1. All treatments registered significantly lower total chlorophyll than control (Table 2).

**Chlorophyll a/b**

A significant difference was observed for chlorophyll a/b ratio. Compare to control only one treatment T1 registered significantly higher chlorophyll a/b (1.09). And least value for chlorophyll a/b was recorded by (T5) 0.88 (Table 2).

**Soluble protein (mg g-1)**

Significant difference in soluble protein is due to different treatments of NAA and it varied between 12.18 mg g-1 in control and 17.62 mg g-1 in T5 (NAA 5000 ppm). Compare to control four treatments recorded higher protein content and lowest was observed in T1.

Out of two growth regulators used IBA registered superiority in terms of physiological traits. This explains IBA improves the root growth and root hair formation of cuttings which in turn influences nutrient uptake and leaf quality. Mini cuttings of *Ligusturum japonicum* treated with IBA @ 2000 ppm improves chlorophyll, sprouting percentage Celik, H *et.al*., (2019). Similar result was observed in pomegranate, maximum number of leaves/cuttings, leaf area and chlorophyll content recorded in cutting treated with IBA @ 5000 ppm G Rao *et al*., (2022) Successive root growth was noticed in bay leaf layering treated with IBA @ 4000 ppm during May to July reported by Mozunder, SN, *et al*., (2021).

Morphological characters *viz*., Survival percentage, shoot length, root length of mogra founded to higher when treated with IBA @ 1500 ppm over control Makwana *et al*., (2024). Nath *et al*.,(2025) also reported similar trend NAA with orgafol in combination with Azospirillum and Phosphobacteria showed higher chlorophyll content in mulberry leaf.

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), and *Cordyline terminalis* (Parvaneh Rahdari *et al*., 2014).

**Conclusion**

Holistically, by considering all the parameters investigated *viz*., chlorophyll of IBA and NAA treated mini clonal cuttings. IBA @ 5000 ppm treated cutting are found to be superior in all physiological traits to NAA @ 5000 ppm treated cuttings hence IBA 5000 ppm is fixed as an optimum rooting concentration for clonal propagation of *Morus sinensis.*

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, manuscript.

**REFERENCE**

1. Celik, H., Cakir, S., Celik, D and Altun, B (2019). Effect of leaf size and IBA on rooting and root quality of Japanese privet, rock cotoneaster and ornamental pomegranate mini-cutting. International Society for Horticultural Science, 1263, 253-260.
2. Francisco de Assis, A.G.Fett and A.C.Alfenas, (2004). Current techniques and prospects for the clonal propagation of hardwoods with emphasis on *Eucalyptus*. **Plantation forestry Biotechnology for the 21st  century**: 303-333.
3. Husen, A., M. Iqbal, S.N. Siddique and S.S. Sohrab. (2015). Effect of Indole-3-Butyric Acid on clonal propagation of mulberry (*Morus alba* L.) stem cuttings: Rooting and associated biochemical changes. ***Natl. Acad. Sci***, 7: 1-16.
4. Makwana, R.J, Rathva, V.D and Panchal (2024). B.H. Effect of IBA concentration (Indole Butaric Acid) and Growing condition on performance of cuttings in mogra (*Jasminum sambac*). Journal of Experimental Agriculture International, 46(10):744-750.
5. Mozunder, SN, Kamal, MM, Haque, MI, and Shahiduzzaman, (2021). M Effect of time and IBA concentration on the performance of bay leaf layering. International Journal of Research - GRANTHAALAYAH, 9(7):15.
6. Noor Rahman, Tehsimullah, Ghulam Nabi and Taslim Jan (2004) Effect of different growth-regulators and types of cuttings on rooting of guava. Quarterly Science Vision Vol.9 : 1-5.
7. Parthiban, K.T. and R. Seenivasan. (2016). Forestry Tecnologies A Complete value chain approach. **Scientific publisher**, ISBN: 978-93-86102-60-7
8. Parvanesh Rahdari, Mojgan Khosroabadi and Katayum Delfani. (2014). Effect of different concentration of plant hormones (iba and naa) on rooting and growth factors in root and stem cuttings of *Cordyline Terminalis.* ***Journal of Medical and Bioengineering***, 3(3): 190-194.
9. Siva Koteshwara Rao G, Irfan Ahmad Bisati, Umar Iqbal, Amit kumar and Sajad Ahamad Bhat (**2022)**. Effect of IBA concentration on vegetative parameters of pomegranate cuttings under temperate conditions of Kashmir. ***The Pharma Innovation Journal,* 11(6): 177-182.**
10. Vijayan, K., M.K. Raghunath, K.K. Das, A. Tikader, S.P. Chakraborthi, B.N. Roy and S.M.H. Qadri. (1997), Studies on leaf moisture of mulberry germplasm varieties. ***Indian J. Seric***, 36(2): 155-157.
11. Geetha, T., & Murugan, N. (2017). Plant Growth Regulators in Mulberry. Annual Research & Review in Biology, 13(3), 1–11
12. Nath, I., Rajagopal, S., Dutta, P. L., Ahmed, M. H., & Saikia, K. (2025). Impact of Liquid Bioformulations on Mulberry Leaf Quality and Physiological Traits. International Journal of Plant & Soil Science, 37(7), 512–521.

**Table 1. Effect of IBA on chlorophyll content of *M. sinensis***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatments** | **Chlorophyll a** | **Chlorophyll b** | **Total****chlorophyll** | **Chlorophyll a/b** | **Protein content mg/g** |
| T1 – 1000 ppm | 2.58 | 1.47 | 2.62\* | 1.76\* | 23.58\* |
| T2 - 2000 ppm | 2.61 | 1.67 | 2.74\* | 1.56\* | 25.31\* |
| T3 - 3000 ppm | 2.89 | 2.36 | 2.79\* | 1.22\* | 32.10\* |
| T4 - 4000 ppm | 3.05 | 2.65 | 2.84\* | 1.15\* | 38.78\* |
| T5 - 5000 ppm | 3.25\* | 2.93 | 2.90\* | 1.11\* | 40.62\* |
| Control (V1) | 3.14 | 2.96\* | 2.32 | 1.06 | 20.04 |
| **Mean** | **2.87** | **2.22** | **2.70** | **1.31** | **34.57** |
| **SEd** | **0.03** | **0.02** | **0.03** | **0.02** | **0.058** |
| **CD (.05)** | **0.06** | **0.05** | **0.06** | **0.04** | **0.12** |

\*Significant at 5% level

**Table 2. Effect of NAA on chlorophyll content of *M. sinensis***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatments** | **Chlorophyll a** | **Chlorophyll b** | **Total****chlorophyll** | **Chlorophyll a/b** | **Protein content mg/g** |
| T1 – 1000 ppm | 1.88 | 1.73 | 1.11 | 1.09\* | 10.43 |
| T2 - 2000 ppm | 2.08 | 2.13 | 1.64 | 0.97 | 12.80\* |
| T3 - 3000 ppm | 2.17 | 2.38 | 1.58 | 0.91 | 15.27\* |
| T4 - 4000 ppm | 2.24 | 2.50 | 1.59 | 0.90 | 16.11\* |
| T5 - 5000 ppm | 2.41 | 2.74 | 1.75 | 0.88 | 17.62\* |
| Control (V1) | 3.14\* | 3.08\* | 2.32\* | 1.02 | **12.18** |
| **Mean** | **2.32** | **2.43** | **1.67** | **0.96** | **14.07** |
| **SEd** | **0.02** | **0.03** | **0.03** | **0.01** | **0.14** |
| **CD (.05)** | **0.05** | **0.06** | **0.07** | **0.04** | **0.29** |

\*Significant at 5% level