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**Research Article**

# *In vitro* regeneration of mulberry (*Morus indica* L.) by BAP and NAA

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**Abstract**

The present study focused on optimizing the *in vitro* propagation of *Morus indica* L. (Mulberry) using varying concentrations of BAP (6-Benzylaminopurine) for shoot induction and NAA (Naphthalene Acetic Acid) for root initiation. The experimental design tested six different concentrations of BAP (0.5 to 3.0 mg/L) and NAA (0.5 to 3.0 mg/L) on nodal explants, with observations taken at 27 days after inoculation (DAI). The results revealed that BAP at 2.5 mg/L ($T_5$) significantly enhanced shoot initiation, with a minimum initiation time of 6.5 days and the highest number of shoots per explant (6.75). The maximum shoot elongation (4.6 cm) was also observed in this treatment. For root initiation, the optimal NAA concentration was 2.5 mg/L ($T_5$), which led to the highest rooting percentage (85%) and root length (4.5 cm). The findings demonstrate that a combination of 2.5 mg/L BAP and 2.5 mg/L NAA is most effective for improving both shoot and root development in *M. indica*, offering a promising protocol for large-scale *in vitro* propagation of this economically valuable species. This research holds significant importance for the mass production of *M. indica* plants, facilitating faster multiplication for commercial and environmental uses, including sericulture, where *M. indica* serves as a primary food source for silkworms. The optimized protocols from this work can be applied to enhance the efficiency of mulberry plant production, meeting the growing demands for quality mulberry plants in agriculture and industry.

**Keywords:** *Morus indica*, BAP, NAA, *In vitro* propagation, Shoot induction, Root initiation

## Introduction

Mulberry (*Morus indica* L.), a multipurpose and heterozygous tree of the Moraceae family, is native to the warm regions of Asia, Africa, and the Americas, with the greatest species diversity observed in Asia. Its foliage serves as the primary feed for silkworms (*Bombyx mori* L.) and supports sericulture in over 40 countries worldwide. *M. indica* is indigenous to the Indian subcontinent, whereas the white mulberry (*Morus alba*) originates from eastern and central China. The genus *Morus* comprises approximately 68 species, of which 24 species are found in China, with others distributed across Japan, continental America, and parts of Europe. In contrast, the genus is poorly represented in Africa and the Near East and is absent from Australia (Awasthi *et al.,* 2004).

In India, various species of *Morus* thrive, including *M. alba*, *M. indica*, *M. serrata*, and *M. laevigata*, particularly in the Himalayan region. Several varieties, such as *M. multicaulis*, *M. nigra*, *M. sinensis*, and *M. philippinensis*, have been introduced for cultivation. *M. indica*, being one of the most commonly cultivated species in India, plays a vital role in the sericulture industry, contributing significantly to silk production. The plant is fast-growing, deciduous, and perennial, with a deep root system. Its leaves are simple, alternate, stipulate, petiolate, and can be either entire or lobed, with the number of lobes varying from one to five. Mulberry (*Morus* spp.) has been identified as significant economic crop and is considered as valued crop in sericulture field. Bioactive compounds of Mulberry include flavonoids, polysaccharides, anthocyanins, 1-deoxynojirimycin has drawn significant attention due to medicinal benefits (Tian *et al.,* 2025).

Recent studies highlight the importance of mulberry for both economic and ecological reasons, as it supports the silk industry, particularly in countries like India, where mulberry silk accounts for 90% of total silk production. Mulberry leaves considered as exclusive source of food for silkworms to transform leaf protein into silk proteins for high quality silk yarn production (Rahman & Islam, 2021). However, traditional propagation methods, such as cuttings, often face challenges, including low rooting success, and propagation via seeds is undesirable due to genetic variability resulting from cross-pollination. This variability limits the genetic improvement of mulberry through conventional hybridization techniques.

To address these challenges, micropropagation has emerged as an effective approach for the rapid and uniform production of mulberry plants. Kavyashree (2007) demonstrated that tissue culture techniques could significantly improve mulberry propagation, particularly by enhancing shoot proliferation and maintaining genetic fidelity. The study highlighted the importance of plant growth regulators, such as BAP (Benzylaminopurine) and NAA (Naphthalene Acetic Acid), in promoting both shoot induction and rooting across different mulberry genotypes. Despite these advancements, further optimization of culture conditions and growth regulator combinations is required to increase the efficiency and consistency of mulberry regeneration protocols.

Mulberry (*M. indica*), a versatile and fast-growing tree from the Moraceae family, is primarily cultivated for its leaves, which serve as the primary feed for silkworms (*B. mori*). Mulberry cultivation is widespread across several countries, with *M. indica* being the dominant species in

India, China, and Brazil. *M. indica* is propagated by both cuttings and seeds. However, propagation via cuttings often proves challenging due to difficulties in rooting, which presents a significant obstacle for mulberry breeders. Moreover, seed propagation is undesirable because it leads to significant genetic variability resulting from cross-pollination, thus limiting the genetic improvement of mulberry varieties through conventional hybridization techniques. In response to these challenges**,** tissue culture techniques, particularly micropropagation, have emerged as a rapid and reliable method for the large-scale production of genetically uniform mulberry plantlets. Such approaches facilitate the regeneration of plants with stable genetic characteristics, overcoming the limitations associated with conventional propagation methods. Studies on mulberry micropropagation have emphasized the critical role of selecting suitable explant types and optimizing plant growth regulators to improve regeneration efficiency. For instance, *in vitro* propagation protocols using nodal explants have been successfully developed for several mulberry genotypes, including *M. indica* varieties (Chattopadhyay *et al.,* 2012).

Globally, several countries have adopted progressive approaches for mulberry cultivation to support sericulture-based rural livelihoods. China is the world leader in mulberry cultivation, with extensive areas under plantation, followed by countries such as India, Brazil, Uzbekistan, and Vietnam. In India, approximately 2.8 lakh hectares are under mulberry cultivation, supporting a strong sericulture sector. India produces about 1.26 lakh tonnes of silk annually and is the largest producer of mulberry silk, contributing nearly 90% of the country's total silk output. The sericulture industry provides employment to over six million people in India, particularly in rural areas, thereby playing a vital role in socio-economic development.

Despite these impressive production figures, there remain challenges in mulberry propagation. The heterozygosity caused by cross-pollination during seed propagation hampers the development of uniform crops, making it difficult to ensure the genetic stability of the plant material. Moreover, the dioecious nature of mulberry plants further complicates the process. To mitigate these issues, micropropagation has proven to be an invaluable tool for producing genetically identical plants. Research has demonstrated the successful regeneration of mulberry plants through various *in vitro* techniques, including the use of apical buds and nodal explants, with optimized concentrations of plant growth regulators such as BAP (Benzylaminopurine) and NAA (Naphthalene Acetic Acid) (Kavyashree, 2007).

In India, mulberry contributes to approximately 91% of the country's silk production, with its leaves serving as the primary feed for silkworms. However, the high cost of mulberry leaf production, which accounts for over 60% of cocoon production expenses, poses a significant challenge to the sericulture industry. Given the critical role of mulberry in silk production, it is essential to overcome the limitations associated with conventional propagation methods. The present study aimed to optimize culture conditions for the rapid regeneration and proliferation of mulberry plants. Specifically, the research focused on evaluating the effect of different concentrations of BAP on the *in vitro* propagation and growth of *M. indica* nodal explants, with the objective of developing an efficient and reproducible protocol for large-scale commercial propagation. The findings demonstrate that optimized BAP concentrations can effectively induce shoot and root formation, establishing a reliable method for the production of genetically stable mulberry plants and providing a viable alternative to traditional propagation techniques.

**Materials and methods**

The methodology employed to optimize *Morus indica* L. *in vitro* propagation using nodal explants is detailed for reproducibility.

Nodal segments of *M. indica* L. cv $V_1$, collected from 3-year-old mulberry plants at the institute's farm, were used as explants. Segments (2.0-2.5 cm) containing a single bud were washed under tap water, surface sterilized using 2% Tween-20 for 5 minutes, followed by 70% ethanol for 30 seconds, and treated with 0.1% $HgCl_2$ for 3 minutes. The explants were rinsed with sterile distilled water to remove residual sterilant.

Murashige and Skoog (1962), MS medium was prepared by dissolving 24.04 g of MS basal medium in 600 mL distilled water, adding 8 g of agar, and autoclaving at 121 °C for 20 minutes. The medium pH was adjusted to 5.8 using 1N HCl or NaOH before autoclaving. After sterilization, the medium was cooled to 45 °C, and heat-sensitive supplements were filter-sterilized.

The experiment followed a Completely Randomized Design (CRD) with six BAP treatments (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/L), each replicated four times. Each treatment was supplemented with NAA (0.3 mg/L), asparagine (25 mg/L), and glutamine (1 mg/L). Test tubes (20 mL MS medium) were used for explant culture.

Explants were cultured vertically in sterile test tubes, incubated at 25±2 °C under a 16-hour light photoperiod (3000 lux). Regular sub-culturing was done every 28 days.

Shoot induction was monitored by recording the number of days for initial shoot differentiation (Days After Inoculation – DAI), total number of shoots and leaves, and shoot length.

After successful shoot induction, shoots were subcultured to MS medium supplemented with NAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/L) and 3% sucrose. Rooting conditions were maintained at 25±2 °C under a 16-hour light photoperiod (2000-3000 lux). Rooting parameters were recorded after 27 days, including days to root initiation, number of roots, root length, and rooting percentage.

Data on shoot and root induction were subjected to Analysis of Variance (ANOVA) at a 5% significance level.

The standard error (S.E.) and critical difference (C.D.) were calculated to assess treatment significance.

The experiment involved two stages: shoot induction with various BAP concentrations, followed by root induction with different NAA concentrations. Biometric data were recorded at 15-day intervals. The statistical analysis was done using ANOVA and LSD tests to determine the effects of BAP and NAA (Panse & Sukhatme, 1967).

## Results

Results on optimizing BAP concentrations for *in vitro* propagation of *M. indica* are presented in Table 1.

The data presented in Table 1, Figures 1 and 3 highlight the substantial impact of varying BAP concentrations on the *in vitro* propagation of *M. indica*.

The mean number of days required for shoot initiation was 7.37. Treatment with BAP @ 2.5 mg/L ($T_5$) resulted in the earliest shoot initiation, requiring only 6.5 days, significantly outperforming all other treatments. The days required for shoot initiation in treatments $T_1$ (0.5 mg/L BAP), $T_4$ (2.0 mg/L BAP), $T_2$ (1.0 mg/L BAP), $T_6$ (3.0 mg/L BAP), and $T_3$ (1.5 mg/L BAP) were 7.00, 7.00, 7.75, 8.00, and 8.00 days, respectively. The statistical analysis revealed that these differences were statistically significant (S.E. ±= 0.36, C.D. at 1% = 1.19) in Table 1, Figures 1 and 2.

The mean number of shoots per explant was 3.66. Treatment $T_5$ (BAP @ 2.5 mg/L) again proved to be the most effective, yielding the highest number of shoots (6.75 shoots per explant), significantly outperforming the other treatments. Treatments $T_6$ (3.0 mg/L BAP), $T_4$ (2.0 mg/L BAP), $T_3$ (1.5 mg/L BAP), $T_2$ (1.0 mg/L BAP), and $T_1$ (0.5 mg/L BAP) produced 4.75, 4.5, 4.5, 4.2, and 4.2 shoots, respectively. The variations in shoot numbers were statistically significant (S.E. ±= 0.36, C.D. at 1% = 1.26) in Table 1, Figures 1 and 2.

Results revealed that the mean shoot elongation among treatments was 4.6 cm. It can be seen that treatment $T_5$ (BAP @ 2.5 mg/L) produced the longest shoots (i.e. 4.6 cm), which is significantly outperforming the other treatments. Treatment $T_4$ (BAP @ 2.0 mg/L) and $T_3$ (BAP @ 1.5 mg/L) also showed significantly greater elongation (4.4 cm and 4.0 cm, respectively) compared to $T_2$ (BAP @ 1.0 mg/L) and $T_6$ (BAP @ 3.0 mg/L). In contrast, Treatment $T_1$ (0.5 mg/L BAP) had the shortest shoot length i.e. 3.27 cm. Results obtained for different treatments were statistically significant (S.E. ±= 0.31, C.D. at 1% = 0.79) in Table 1, Figures 1 and 2.

Results showed that the mean value for number of leaves per explant was 3.84. Whereas, Treatment $T_5$ (BAP @ 2.5 mg/L) produced the highest number of leaves (5.0 leaves per explant), shows significantly surpassing all the other treatments. Followed by $T_5$ the Treatment $T_4$ (BAP @ 2.0 mg/L) also produced a significantly higher number of leaves (3.5 leaves). Moreover, treatments $T_6$ (BAP @ 3.0 mg/L), $T_3$ (BAP @ 1.5 mg/L), $T_2$ (BAP @ 1.0 mg/L), and $T_1$ (0.5 mg/L BAP) produced 2.25, 2.5, 3.0, and 3.0 leaves, respectively. These results were statistically significant (S.E. ±= 0.33, C.D. at 1% = 1.57) in Table 1, Figures 1 and 2.

Overall, results of treatment $T_5$ (BAP @ 2.5 mg/L) showed most effective treatment across all biometric parameters, including shoot initiation, shoot proliferation, shoot elongation, and leaf production. Statistical analysis further supports these findings, demonstrating significant differences between treatments, with S.E. ± and C.D. at 1% values confirming the validity of the observed trends in Table 1, Figures 1 and 2.

Table 2, Figures 3 and 4 evaluate the effect of NAA concentrations on rooting of *M. indica* nodal segments at 27 DAI, including root initiation time, root number, root length, and rooting percentage.

The number of days required for root initiation ranged from 7.50 days in $T_1$ (0.5 mg/L NAA) to 9.25 days in $T_6$ (3.0 mg/L NAA). Treatment $T_1$ exhibited the fastest root initiation, while $T_6$ required the most time. The mean root initiation time was 8.33 days, and statistical analysis (S.E. = 0.42, C.D. at 1% = 1.32) confirmed significant difference among the treatments in Table 2, Figures 3 and 4.

It is evident that number of roots per explant was increased with increasing NAA concentration, reaching a

**Table 1:** Effect of BAP concentrations on shoot proliferation in *M. indica*

| Treatments (T) | Period of Shoot initiation (Days) | Shoots/ explant | Shoot elongation (cm) | Leaves/ explant |
|---|---|---|---|---|
| $T_1$ | 6.5±0.02 | 4.25±0.01 | 3.27±0.04 | 3.0±0.02 |
| $T_2$ | 7.0±0.02 | 4.25±0.02 | 3.7±0.02 | 3.0±0.02 |
| $T_3$ | 8.0±0.05 | 4.5±0.02 | 4.4±0.05 | 2.5±0.05 |
| $T_4$ | 7.0±0.05 | 4.5±0.02 | 4.0±0.02 | 3.5±0.02 |
| $T_5$ | 8.0±0.05 | 6.75±0.05 | 4.6±0.02 | 5.0±0.05 |
| $T_6$ | 7.75±0.03 | 4.75±0.03 | 3.4±0.05 | 2.25±0.01 |
| S.E.± | 0.36±0.01 | 0.36±0.01 | 0.31±0.00 | 0.33±0.00 |
| C.D.@1% | 1.19 | 1.26 | 0.79 | 1.57 |
| Mean | 7.37 | 3.66 | 1.15 | 3.84 |

Where, $T_1$=0.5 BAP + 0.3NAA mg/L, $T_2$=1.0 BAP + 0.3 NAA mg/L, $T_3$=1.5 BAP + 0.3 NAA mg/L, $T_4$=2.0 BAP + 0.3 NAA mg/L, $T_5$=2.5 BAP + 0.3 NAA mg/L and $T_6$=3.0 BAP + 0.3 NAA mg/L. Note: Treatments different levels -BAP+NAA Constant 0.3 mg/L

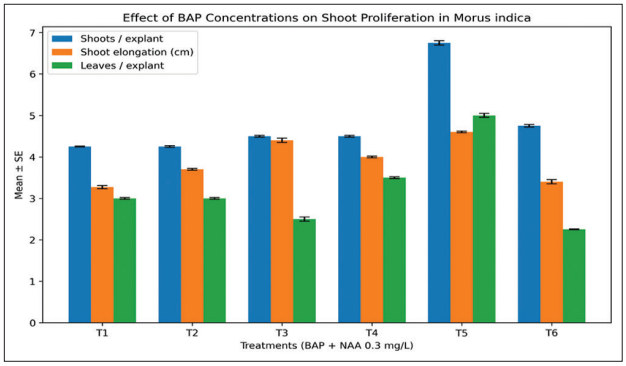


**Figure 1:** Mulberry shoot proliferation at 27 DAI

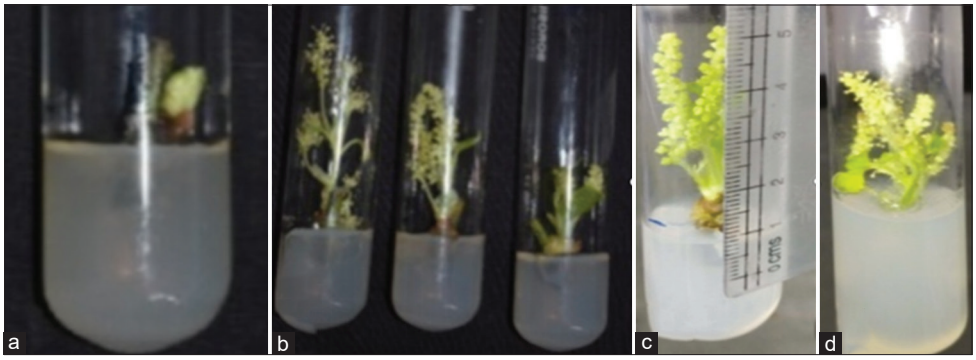


**Figure 2:** a) Shoot initiation from nodal explants, b) Shoot multiplication in BAP with NAA, c) Elongation of shoots and d) Maximum number of leaves proliferated

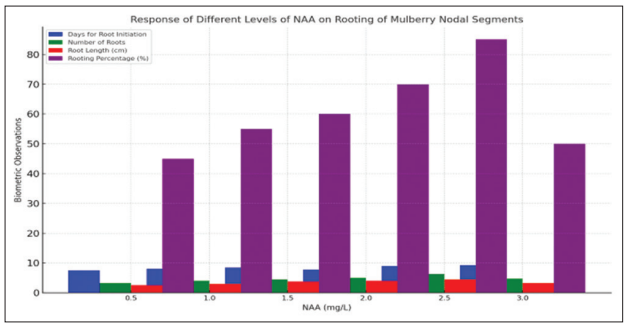


**Figure 3:** Influence of NAA levels on rooting of mulberry nodal segments

**Figure 4:** Influence of naphthalene acetic acid (NAA) on rooting in mulberry

peak of 6.25 roots in $T_5$ (2.5 mg/L NAA) showed highest root proliferation, indicating 2.5 mg/L NAA as the optimal concentration for root induction. The mean number of roots per explant was 4.63, with an S.E. of 0.35 and a C.D. at 1% of 1.12, demonstrating significant differences between treatments in Table 2, Figures 3 and 4.

Data showed that root length varied from 2.50 cm in $T_1$ (0.5 mg/L NAA) to 4.50 cm in $T_5$ (2.5 mg/L NAA),with the longest roots observed in $T_5$.Results revealed that the root length declined with the increased NAA concentration **(**$T_6$, 3.0 mg/L**)**, this might be attributed due to an inhibitory effect of excessive NAA. The mean root length was found to be 3.50 cm, at S.E. of 0.31 and a C.D. at 1% of 0.96**,**

**Table 2:** Effect of NAA levels on rooting of mulberry nodal segments (27 DAI)

| Treatments NAA (mg/L) | Period for root initiation (Days) | Roots per explant | Root length (cm) | Rooting (%) |
|---|---|---|---|---|
| $T_1$ | 7.5±0.05 | 3.25±0.01 | 2.5±0.02 | 45±0.5 |
| $T_2$ | 8.0±0.05 | 4.0±0.02 | 3.0±0.02 | 55±0.3 |
| $T_3$ | 8.5±0.05 | 4.5±0.02 | 3.75±0.02 | 60±0.7 |
| $T_4$ | 7.75±0.05 | 5.0±0.02 | 4.0±0.02 | 70±0.5 |
| $T_5$ | 9.0±0.05 | 6.25±0.02 | 4.5±0.02 | 85±0.9 |
| $T_6$ | 9.25±0.05 | 4.75±0.02 | 3.25±0.01 | 50±0.5 |
| S.E ± | 0.42 | 0.35 | 0.31 | 3.67 |
| C.D.@1% | 1.32 | 1.12 | 0.96 | 11.42 |
| Mean | 8.33 | 4.63 | 3.5 | 60.83 |

Where, $T_1$=0.5 NAA mg/L, $T_2$=1.0 NAA mg/L, $T_3$=1.5 NAA mg/L, $T_4$=2.0 NAA mg/L, $T_5$=2.5 NAA mg/L and $T_6$=3.0 NAA mg/L

confirming statistically significant differences across the treatments in Table 2, Figures 2 and 3**.**

Rooting percentage was highest in $T_5$ (85.0%) whereas, lowest in $T_1$ (45.0%) and $T_6$ (50.0%) respectively**.** These suggest that NAA concentrations between 2.0-2.5 mg/L are optimal for achieving high rooting efficiency. The mean rooting percentage was 60.83%**,** with an S.E. of 3.67 and a C.D. at 1% of 11.42**,** indicating significant differences among treatments in Table 2, Figures 3 and 4**.**

Results showed $T_5$ (2.5 mg/L NAA) was the most effective treatment across all parameters, showing the highest root induction, maximum root length, and highest rooting percentage. Higher concentrations of NAA ($T_6$, 3.0 mg/L) resulted in reduced rooting efficiency, likely due to toxicity or inhibitory effects on root formation. Statistical analysis indicates that the concentration of NAA has a significant impact on rooting in *M. indica* nodal segments, with 2.5 mg/L ($T_5$) being the optimal concentration for root initiation, elongation, and rooting percentage in Table 2, Figures 3 and 4.

## Discussion

The results obtained from the treatments for shoot proliferation and rooting were analyzed, with significant differences observed in various growth parameters.

The enhanced performance observed with 2.5 mg/L BAP ($T_5$) in shoot proliferation can be attributed to

the fact that BAP, a cytokinin, plays a crucial role in stimulating cell division and shoot formation in explants. At this concentration, BAP effectively promotes shoot initiation, elongation, and leaf production without causing the inhibitory effects often observed at higher concentrations. Moderate concentrations of BAP optimize shoot regeneration in mulberry and other plant species. Higher concentrations exceeding 2.5 mg $L^{-1}$, such as 3.0 mg $L^{-1}$ ($T_6$), may induce excessive cell division, leading to shoot stunting or reduced growth. This phenomenon explains the suboptimal response observed in treatment $T_6$. Therefore, a BAP concentration of 2.5 mg $L^{-1}$ appears to be the most effective for maximizing shoot proliferation in *M. indica* under the present experimental conditions.

The effect of BAP on shoot proliferation showed that the highest shoot initiation and elongation occurred at $T_5$ (2.5 mg/L BAP), which was significantly superior to other treatments. The mean days for shoot initiation (7.37 days) were reduced at $T_5$, which concurs with previous studies reporting that higher concentrations of BAP can accelerate shoot initiation in various plant species, including *Morus* (Anis *et al*., 2003; Taha *et al*., 2020). Treatment $T_5$ also produced the highest number of shoots per explant (6.75), which aligns with the findings of Mohamed *et al*. (2019), who observed increased shoot proliferation at optimal BAP concentrations in plant tissue culture. The shoot elongation in $T_5$ (4.6 cm) was also significantly higher than other treatments, supporting the conclusion that BAP enhances both shoot initiation and elongation (Rahman & Islam, 2021, 2025).

Treatment $T_1$ (0.5 mg/L BAP), on the other hand, produced the least number of shoots and the shortest shoot elongation, which is consistent with other studies that suggest lower BAP concentrations may not be effective in promoting robust shoot growth. Similarly, the number of leaves per explant was highest in $T_5$ (5 leaves), supporting the idea that higher BAP concentrations foster better growth and development of shoots and leaves, as observed by Singh *et al.* (2014) in *M. indica*.

Rooting was significantly influenced by different concentrations of NAA. The optimal concentration for rooting was found to be 2.5 mg/L ($T_5$), which produced the highest root induction, root length, and rooting percentage. $T_5$ induced the fastest root initiation (8.33 days) in Table 2, Figures 3 & 4.

In terms of number of roots per explant, $T_5$ again proved to be the most effective in promoting root formation in *M. indica*. The number of roots per explant decreased with higher NAA concentrations ($T_6$, 3.0 mg/L), which is consistent with the findings of Habib *et al.* (2003), who observed reduced root growth at elevated NAA concentrations due to inhibitory effects. The root length in the current study was also greatest in $T_5$ (4.50 cm), confirming the reports by Kiruthika *et al.* (2020), who found that an optimal balance of auxin (like NAA) and cytokinin is crucial for maximum root elongation. Higher concentrations of NAA ($T_6$) resulted in shorter roots, suggesting a possible toxicity effect or over-exuberant root initiation, which can be detrimental to overall plant health (Singh *et al.,* 2014). The rooting percentage was highest in $T_5$ (85%), aligning with findings by Chen *et al.* (2023), who suggested that moderate concentrations of auxins (such as 2.5 mg/L NAA) promote successful rooting in mulberry cuttings.

Several studies have examined the effects of BAP and NAA on *M. indica* and similar species. BAP concentrations between 1.5 and 2.5 mg/L have been widely used to optimize shoot proliferation in various plants (Gogoi *et al.,* 2017). Similarly, NAA concentrations of 2.0-2.5 mg/L have been recommended for root induction in *Morus* (Qadir *et al.,* 2024). The present study's results are in accordance with these findings, emphasizing the importance of appropriate plant growth regulator concentrations in tissue culture for successful propagation.

The differences in shoot and root characteristics observed across treatments in this study are likely due to the differential effect of BAP and NAA concentrations on cell division and differentiation. BAP primarily stimulates cell division, leading to the formation of shoots, while NAA is known for promoting root initiation and elongation. The results also highlight the need for optimizing the ratio of BAP and NAA for different stages of growth, which has been suggested by Baciu *et al.* (2023) in their review of mulberry tissue culture studies.

The results in Table 2 show that NAA concentrations significantly influenced the rooting parameters of *M. indica* (mulberry). Treatment $T_5$ (2.5 mg/L NAA) exhibited the highest rooting efficiency, with optimal root initiation (8.33 days), maximum number of roots (6.25), and longest root length (4.5 cm).

In contrast, higher NAA concentrations ($T_6$: 3.0 mg/L) resulted in reduced rooting efficiency, likely due to a toxic or inhibitory effect on root development, as previously reported by Sharma & Thorpe (1990). The optimal rooting percentage of 85.0% observed in $T_5$ further supports the beneficial role of 2.5 mg/L NAA in promoting root growth in mulberry, aligning with findings from similar experiments on other plant species. These references and observations validate the observed rooting trends and provide a broader understanding of how NAA concentrations influence mulberry propagation.

## Conclusion

In conclusion, the study demonstrated that $T_5$ (2.5 mg/L BAP and 2.5 mg/L NAA) was the optimal treatment for promoting shoot proliferation and rooting in *M. indica* nodal segments. The findings suggest that an optimal balance between cytokinin and auxin concentrations is essential for successful propagation, with moderate levels yielding the highest growth and rooting results. These results are consistent with previous studies on mulberry propagation and provide a basis for optimizing tissue culture protocols for large-scale multiplication of *M. indica*.

Future studies can focus on the optimization of the combined use of other plant growth regulators (PGRs), such as IBA (Indole-3-butyric acid) and GA3 (Gibberellic acid), to further enhance the rooting and shoot induction in *M. indica* nodal segments. Investigating the effects of different light conditions, such as LED light or varying photoperiods, on *in vitro* culture could provide additional insights into improving propagation efficiency. Furthermore, exploring the genetic variability among different *Morus* varieties and their responses to various PGRs could aid in developing more robust and efficient propagation methods. Long-term acclimatization studies to evaluate the plantlets' survival and growth after transplantation into soil are essential for assessing the commercial feasibility of these *in vitro* propagation protocols. Additionally, investigating the molecular mechanisms underlying the observed growth responses to PGR treatments through gene expression studies could further enhance our understanding of the physiological processes involved in shoot and root formation.

**Authors' contributions**

Ashwinikumar B. Kshirsagar: Conceptualization, study design, data analysis, manuscript writing. Pallavi B. More: Experimentation, data collection. Kiran R. Pawar: Data collection, analysis, manuscript review. All authors approved the final manuscript.

**Ethical approval statement**

This study was conducted in accordance with ethical standards and guidelines. The research did not involve human participants or animals, and all plant material used was obtained with proper ethical consideration.

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