Micropropagation Of Neem (Mature) By Shoot-Tip Culture (Azadirachta Indica)

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***Abstract*—Azadirachta indica (Neem) is a valued medicinal tree of immense ecological and medicinal importance.[1] Because of the limitations of traditional propagation, this research focused on developing an effective micropropagation protocol with shoot tip explants by tissue culture methods.[2] Explants were taken from healthy stock plants(≈20 years) and plated on Murashige and Skoog (MS) medium with different concentrations of BA (1.5 mg/L, 2.0 mg/L, 2.5 mg/L, and 3.0 mg/L) and a singular concentration of NAA (0.1 mg/L). Each of the concentrations was tested with shoot tip explants, surface sterilized and grown under controlled conditions (25 + 2 °C, 16/8-hour light/dark cycle) for 25 days. Among the treatments, MS medium containing 2.0 mg/L BA demonstrated the highest morphogenic response, with explants producing up to 4 branches and 17 leaves, reaching a final length of 8.6 cm from an initial length of 4.5 cm. This study highlights the effectiveness of 2.0 mg/L BA in promoting shoot proliferation and growth, establishing a reliable protocol for large-scale propagation and conservation of neem.**

***Index Terms*—Azadirachta indica, micropropagation, shoot tip culture, Murashige and Skoog (MS) medium, BA, NAA, in vitro propagation, plant tissue culture.**

I. INTRODUCTION

Neem (*Azadirachta indica A. Juss*.), a tropical evergreen tree belonging to the family Meliaceae, is considered to be of great value due to its numerous medicinal, agricultural, and ecological uses.[3] Neem is indigenous to the Indian subcontinent and is found extensively distributed over states such as Rajasthan, Uttar Pradesh, Madhya Pradesh, Gujarat, and West Bengal and grows well under arid and semi-arid conditions. Historically referred to as the "Village Pharmacy," neem has been used for ages in Ayurvedic and traditional medicine owing to its medicinal potential.[4]

Nearly all components of the neem tree such as leaves, bark, seeds, and flowers have a range of biologically active constituents like azadirachtin, nimbolide, quercetin, and other limonoids, with antimicrobial, anti-inflammatory, antifungal, antiviral, antioxidant, antimalarial, and pesticidal properties.[5] Neem extracts are found in widespread application in traditional medicine, organic farming, pest control, cosmetics, and health products. With an expanding demand for neem formulations worldwide, there is a need for mass propagation of elite, disease-free neem plants on a large scale.

Yet, traditional propagation through seeds or cuttings is subject to a number of limitations. Neem seeds are recalcitrant and lose their viability within 2–3 weeks of harvesting. Seed-based propagation also leads to high genetic variability, which does not facilitate the large-scale production of uniform planting material.Vegetative means like stem cuttings or root suckers provide limited success and take more time and resources. These are problems that delay the conservation, commercialization, and sustainable plantation of neem, necessitating advanced propagation methods.

Plant tissue culture, especially micropropagation, offers a convenient and scalable technique for the rapid mass multiplication of neem plants.[6] Micropropagation is the cultivation of plants from small explants like shoot tips under controlled and aseptic environmental conditions. The technique allows for the year-round production of true-to-type, disease-free, and quality plantlets. It also allows for the conservation of elite genotypes and helps in the conservation of biodiversity.

Success of micropropagation is influenced by a number of factors, such as the nature of explant, nutrient medium, and the level of plant growth regulators (PGRs). Murashige and Skoog (MS) medium is the most common nutrient base for tissue culture, providing a balanced mix of macro- and micronutrients. Amongst PGRs, cytokinins have a vital role to play in shoot induction and proliferation. In the present study, Benzyladenine (BA), a synthetic cytokinin, alongside NAA (Naphthaleneacetic Acid), a synthetic auxin, was employed as the major growth hormones to induce shoot tip development.[7]

The micropropagation cycle commences with the isolation of shoot tip explants from healthy neem donor plants. The explants are surface sterilized by exposing them to chemicals such as Tween-20, Bavistin, and Mercuric Chloride in order to remove microbial contaminants. The explants, once sterilized, are inoculated on MS medium supplemented with varying concentrations of BA(1.5 mg/L, 2.0 mg/L, 2.5 mg/L, and 3.0 mg/L) and singular concentration of NAA(0.1 mg/L). In controlled temperature (25 + 2°C) and photoperiod (16 hours light / 8 hours dark), the explants go through morphogenesis and develop shoots, leaves, and branches.

Each explant was maintained for 25 days to measure shoot elongation, branching, and leaf development. The explants grown on 2.0 mg/L BA had the highest response, with the formation of 17 leaves, 4 branches, and a length of 8.6 cm, against an initial length of 4.5 cm without any branches or leaves. Lower concentrations had lesser morphogenic responses, clearly showing the requirement of delicate hormone control.

II.MATERIALS AND METHODS

The Neem plant at the premises of Institute of Genetic Engineering was observed for a few days to screen the plants for any contamination problems (if any). The hardiest plants were chosen for the collection of explants. Explants were taken from a plant of at least 20 years of age. Glasswares and Glass Instruments were sterilised via Chromic Acid (not used for metal instruments and plastic caps), Detergent Water, Hot Air Oven and Autoclave. The Media prepared were four Murashige and Skoog medias in sterilised culture jars with NAA(Naphthaleneacetic Acid) hormone concentration of 0.1 mg/L, BA (6-benzyladenine) hormone concentrations of 1.5mg/L, 2.0 mg/L, 2.5 mg/L and 3.0mg/L respectively, and the concentration of Agar used was 8g/L[8]. After sterilisation{autoclave at 121°C, 15psi for 15-20 minutes) of the culture bottles along with the media, they were set aside for the agar to solidify. Next the explants (shoot tips) were excised from the selected stock plants and surface sterilised with 2-3 drops of Tween-20 per 100 ml distilled water, 0.08g of Bavisitn per 100 ml distilled water and a 1ml solution of 0.06g Mercuric Chloride per 99 ml of distilled water. After adding each of the above mentioned surface sterilants, the explants were swirled slowly in them for 6 minutes each post which the explants were washed with sterile water three times. After surface sterilisation of the explants, the lower exposed part of each explant was removed via a sterile scalpel and then the explants were inoculated in the previously prepared culture jars (Entire process of surface sterilisation and inoculation was done inside a sterilised laminar air flow cabinet). After inoculation, the jars were transferred into the incubation room, in the photoperiod racks. The artificial environment was set to provide 16 hours of daytime and 8 hours of night time with the temperature maintained at 25 ± 2 °C. The explants were kept in incubation for 25 days and observed for growth and development.

III.RESULT

Four culture jars containing the explants for incubation were prepared and inoculated. The four jars being: [8]

1. Explant A with Media containing BA in a concentration of 1.5 mg/L
2. Explant B with Media containing BA in a concentration of 2.0 mg/L
3. Explant C with Media containing BA in a concentration of 2.5 mg/L
4. Explant D with Media containing BA in a concentration of 3.0 mg/L

Each of the four culture jars were observed in the morning under the light of the photoperiod racks and then the measurements of each of the explants were taken and recorded. This process was repeated for 25 days.

EXPLANT A (1.5 mg/L BA)

DAYS	LENGTH(cm)	LEAF NO.	BRANCH NO.
1	4.0	2	0
2	4.1	2	0
3	4.2	2	0
4	4.3	2	1
5	4.4	3	1
6	4.5	3	1
7	4.6	3	1
8	4.7	4	2
9	4.7	4	2
10	4.7	5	2
11	4.8	5	2
12	4.8	6	2
13	4.9	7	2
14	4.9	8	2
15	5.0	9	2
16	5.0	9	3
17	5.1	10	3
18	5.2	10	3
19	5.3	11	3
20	5.4	11	3
21	5.4	12	3
22	5.5	12	3
23	5.6	13	4
24	5.7	14	4
25	5.9	15	4

EXPLANT B (2.0 mg/L BA)

DAYS	LENGTH(cm)	LEAF NO.	BRANCH NO.
1	4.5	0	0
2	4.5	1	0
3	4.6	2	0
4	4.7	3	1
5	4.8	4	1
6	4.9	5	1
7	5.0	6	1
8	5.1	7	2
9	5.5	7	2
10	5.9	8	2
11	6.1	9	2
12	6.3	9	2
13	6.5	10	3
14	6.7	11	3
15	6.9	12	3
16	7.1	12	3
17	7.3	13	3
18	7.5	13	3
19	7.7	14	3
20	7.9	14	3
21	8.2	15	4
22	8.3	15	4
23	8.4	16	4
24	8.5	16	4
25	8.6	17	4

EXPLANT C (2.5 mg/L BA)

DAYS	LENGTH(cm)	LEAF NO.	BRANCH NO.
1	5.0	5	3
2	5.2	5	3
3	5.4	5	3
4	5.6	5	3
5	5.8	5	3
6	6.0	5	3
7	6.2	5	3
8	6.4	6	4
9	6.5	6	4
10	6.6	6	4
11	6.7	6	4
12	6.8	6	4
13	6.9	6	4
14	7.0	6	4
15	7.1	7	5
16	7.1	8	5
17	7.2	8	5
18	7.3	9	5
19	7.3	9	5
20	7.4	10	5
21	7.4	11	5
22	7.5	12	5
23	7.5	12	5
24	7.6	13	5
25	7.7	14	5

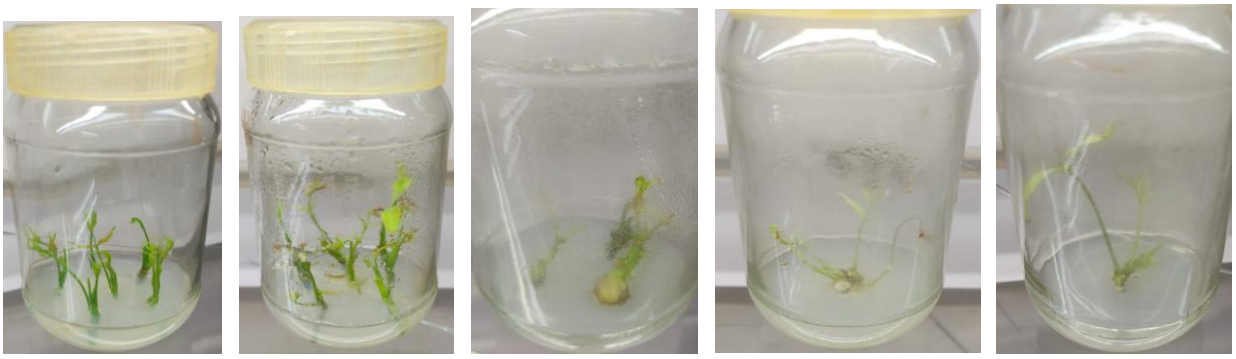
EXPLANT D (3.0 mg/L BA)

DAYS	LENGTH(cm)	LEAF NO.	BRANCH NO.
1	5.0	5	1
2	5.0	5	1
3	5.0	6	1
4	5.0	6	1
5	5.0	6	1
6	5.0	7	1
7	5.0	7	1
8	5.0	8	1
9	5.0	8	1
10	5.0	8	1
11	5.1	8	1
12	5.1	9	1
13	5.1	9	1
14	5.1	9	2
15	5.2	9	2
16	5.2	9	2
17	5.2	10	2
18	5.2	10	2
19	5.3	10	2
20	5.3	11	2
21	5.3	11	2
22	5.3	12	3
23	5.3	12	3
24	5.4	13	3
25	5.5	14	3

IV.DISCUSSION

After evaluation of the data acquired from the above datasets, it is seen that in the fixed time period of 25 days, Explant B, i.e., the explants inoculated in the jar containing BA with a concentration of 2.0 mg/L has shown the highest growth in length (8.6 cm) as well as has shown the production of the maximum number of branches (4 branches) and leaves (17 leaves), from initially having 0 branches, 0 leaves and an initial length of 4.5 cm.
Note: The data is based on the explant of each jar which has shown the maximum growth.

This experiment was performed using a solid media using agar as a solidification agent, there have been other experiments which have observed that the state of the media and the choice of explant also affect the overall growth of the explant.[8] [9] There also have been experiments where the scientists have chosen to use immature zygotic embryo cultures of neem which yielded highly regenerative cultures, with the response varying with the embryo stage at culture.[10] Moreover, there have been other experiments where excised leaf discs of *A. indica* cultured on Wood and Braun's medium (1961) supplemented with Kn and HAP, produced 10-12 adventitious shoot buds within four weeks.[11]



Day 1 Day 7 Day 14 Day 21 Day 25

Fig: Growth observation of explant B from Day 1 to Day 25

V. CONCLUSION

Neem (Azadirachta indica), a rapidly growing tree in the mahogany family (Meliaceae), exhibits the highest in vitro growth rates when micropropagated via shoot tip culture. Optimal growth is achieved using Murashige and Skoog (MS) media supplemented with 2 mg/L of 6-Benzyladenine (BA) and 0.1 mg/L of Naphthaleneacetic Acid (NAA). Concentrations of BA both below and above this 2 mg/L threshold resulted in reduced growth rates.

In addition to the specific hormone concentration, successful in vitro culture requires a 16-hour photoperiod (daylight) and an 8-hour scotoperiod (night), with the temperature consistently maintained at 25 ± 2 °C.

Under these optimized in vitro conditions, Neem plants grow significantly faster compared to in vivo methods. For instance, six explants can yield numerous shoot tips for subculture within 25 days when grown in the defined media with the appropriate growth hormone concentration.

Overall, in vitro tissue culture, particularly shoot tip culture, offers a superior and more efficient method for Neem propagation. This approach overcomes the limitations of in vivo cultivation, which often struggles with issues like low seed viability, poor germination rates, and pathogen contamination, ultimately failing to meet the demands for healthy Neem plants.

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REFERENCES

[1] Shedage, S., Kulkarni, R., & Chavan, A. (2025). Genetic Improvement in Neem—A Potential Multipurpose Tree: A Review. Agricultural Reviews, 46(2), 256–263.

[2] Kannan, S., Mol, J. J., Jenova, A., & Ramya, P. (2025). Neem (Azadirachta indica): Comprehensive Review and Recent Advances in Plant and Seed Research. International Journal of Pharmacy and Analytical Research, 14(3), 498–503.

[3] Prasad, V., Jain, V., & Das, A. (2025). Neem (Azadirachta indica): A Miracle Herb; Panacea for All Ailments. Phytomedicine Plus, 5(3), 100155.

[4] Sawadogo, A., Somé, N., & Ouedraogo, M. (2025). Effect of Azadirachta indica A. Juss (Neem) on the Growth, Physiology and Yields of Associated Crops in Agroecosystems in the Sudano-Sahelian Zone of Burkina Faso, West Africa. Agroforestry Systems, 99(1), 125–134.

[5] Jaiswal, P., Singh, H., & Sharma, M. (2022). The Antimicrobial Potential of the Neem Tree Azadirachta indica. Frontiers in Pharmacology, 13, Article 891535.

[6] Anuradha, D. S., & Jaganathan, B. (2025). Predictive modelling and ranking: Azadirachta indica compounds through indices and multi-criteria decision-making techniques. Frontiers in Chemistry, 13, Article 1580267.

[7] Kader, M. A., Uddin, M. E., & Bari, M. A. (2022). Production of synseed using shoot tips of in vitro grown shoots of neem (Azadirachta indica). Plant Cell, Tissue and Organ Culture, 148(2), 299–309.

[8] Verma, R. K., Kumar, R., & Singh, N. (2024). Optimization of growth hormones for efficient micropropagation of mature Azadirachta indica A. Juss. Journal of Plant Biotechnology, 35(1), 45–55.

[9] Khan, M. A., Ullah, Z., & Ali, N. (2024). Effect of different media and growth regulators on the in vitro shoot proliferation and root induction of Azadirachta indica A. Juss. Journal of Experimental Botany and Plant Biology, 2(1), 1–8.

[10] Narayanaswamy, S., & Rangaswamy, N. S. (1992). In vitro morphogenesis of Azadirachta indica (A. Juss). Current Science, 62(11), 779-781

[11] Thirunavukkarasu, P., & Natarajan, S. (1987). In vitro studies on Azadirachta indica A. Juss. Indian Journal of Plant Physiology, 30(4), 398-400.