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In Vitro Propagation And Conservation Strategies For A Medicinal Plant Pterocarpus Marsupium Roxb (C.G.) India

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Abstract
Pterocarpus marsupiumRoxb. is a tree species valued for its medicinal properties and high-quality timber. Despite its significance, the species faces threats of extinction due to anthropogenic activities and poor regeneration rates. In vitro multiplication techniques offer a promising approach for its conservation and sustainable use in medicine. This study compared two treatments, MS+0.1BAP and MS+0.5BAP, for their efficacy in shoot multiplication and growth parameters. The results showed that MS+0.5BAP resulted in marginally higher shoot numbers (3.60) and longer shoot lengths (6.26 cm) compared to MS+0.1BAP (3.55 shoots, 5.63 cm). This suggests the superior efficacy of higher BAP concentrations in promoting shoot elongation. Moreover, MS+0.5BAP demonstrated a higher percentage of contamination-free cultures (94.05%) and a slightly elevated survival rate (84.25%) compared to MS+0.1BAP (91.20%, 78.95%, respectively), indicating enhanced culture cleanliness and plantlet viability under higher BAP concentrations. These findings underscore the potential benefits of utilizing MS+0.5BAP for optimizing shoot multiplication and culture success in Pterocarpus marsupium tissue cultures. This study contributes to conservation efforts aimed at preserving the medicinal and ecological significance of P. marsupiumRoxb. species.
Keywords: *Propagation, in-vitro, multiplication, conservation, medicinal*

INTRODUCTION

The medicinal PlantPterocarpus marsupiumRoxb.is a, commonly known as Indian Kino or Malabar Kino has a deciduous meanshighly valued for its best timber quality and versatile leguminous tree alongside teak and rosewood native to the Deccan Peninsula, central India, and certain parts of northern India (Mohammad et al., 2022). The leaves serve multiple purposes, including use as fodder and manure. The tree also yields gum-kino, renowned for its potent astringent properties, utilized in treating various ailments such as diarrhea, dysentery, leucorrhea, hemorrhages, and toothache (Rahman et al., 2018). Aqueous infusion of the wood is traditional in managing diabetes, while water stored in vessels crafted from this wood is believed to possessantidiabetic properties (Perera, 2016). The treehas naturally propagated through seeds, but the germination rate is low (30%), and propagation through stem cutting is challenging. Due to poor propagation and overexploitation for pharmaceuticals and timber, P. marsupium has become threatened. Preserving of this species is of utmost importance, particularly when they possess significant medicinal value, faces the looming threat of extinction due to anthropogenic activities and poor regeneration rates (Thalkari et al., 2019). In vitro multiplication is essential for conserving P. marsupium, allowing controlled shoot replication and potentially increasing its population. Conservation efforts must prioritize in vitro propagation to safeguard the future of this medicinal tree species (Da Silva et al., 2018).

For decades, researchers have utilized plant tissue culture techniques to produce healthy plantlets, aiming to conserve the genetic resources of medicinal tree species and halt further declines in their populations. In vitro multiplication allows for the production of pathogen-free plantlets with consistent genetic characteristics (Ahmad et al., 2022). Moreover, in vitro multiplication techniques provide a means to propagate P. marsupium on a large scale, bypassing the need for traditional seed germination and cultivating plants in a shorter amount of time. Tree improvement via conventional breeding faces challenges due to the lengthy juvenile phase and high heterozygosity. Micropropagation has been documented in P. santalinus and P. marsupium(Pardhi et al., 2019). This method also reduces the dependency on natural habitats for collecting seeds or plant materials, thereby minimizing the impact on wild populations. Afterlooking the account of this species and their medicinal worth and timber quality, our study focusing on the propagation of in vitro multiplication techniques for the conservation of P. marsupiumspecies.

MATERIALS AND METHODS

Plant collection and surface sterilization

Thefresh, healthy and disease-free, young twigs were collected from the plants of P. marsupiumand explants were prepared by cutting them from the internodes from the sites of Barnawapara,(C.G) during year'2024.All of the explants were washed with running tap water for 5 minutes, then 70% ethanol for 2 minutes, and finally distilled water for 5 minutes. Surface sterilization of explants was performed by washing with sterile distilled water for 5 minutes, followed by different concentrations of mercuric chloride ($HgCl_2$), with leaf explants sterilized with 0.2%and stem nodes sterilized with 0.2% $HgCl_2$. Following that, two further rinses in laminar airflow with sterilized double-distilled water were performed.

Culture preparation and inoculation

All of these explants were chopped into little pieces and inoculated on the appropriate medium. All experiments in this work were performed on MS media supplemented with varied amounts of growth regulators. Murashige and Skoog (MS) medium, supplemented with appropriate plant growth regulators (PGRs) such as cytokinin and auxins, was utilized for in vitro culture. The culture media was supplemented with 40 gm sucrose and 2.5 to 3 gm clerigar for solidification, and the pH was adjusted to 5.6-5.8. The medium was steam sterilized in an autoclave set to 15 psi andtemp of 121°C. Two variations of the medium were prepared: MS medium supplemented with 0.1 mg/L benzyl aminopurine (BAP), designated as media-1, and MS medium supplemented with 0.5 mg/L BAP, designated as media-2. The sterilized explants were aseptically transferred onto the prepared MS medium in test tubes within a laminar airflow hood. Subsequently, the culture vessels were sealed with cotton plugs to maintain sterility during incubation. After the inoculation, culture bottles were shifted to a culture room with a temperature of 25±2°C and a 16-hour photoperiod provided by cool white fluorescent cool tubes.

Data Collection and Statistical analysis

After an initial 2-week incubation period, cultures were inspected for contamination, and healthy explants were transferred to fresh MS medium for shoot multiplication. Subculturing occurred every 3-4 weeks to sustain cultures and promote additional shoot proliferation. The number of initiated explants, developed plantlets, and contaminated explants were recorded for each time point and medium. Experimental data underwent statistical analysis to detect significant differences and correlations among treatments and growth parameters. Descriptive statistical approach was accomplished for the data analysis and representation.

RESULTS AND DISCUSSION

The response of P. marsupiumshoot multiplication to various in vitro culturing treatments with statistical analysis during study period was represented in Table-1. Utilizingin vitro multiplication techniques for Pterocarpus marsupiumRoxb. has pivotal for its conservation and sustainable utilization. The sustainable use of this species in medicine relies on the availability of healthy plant material, which can be reliably produced through in vitro multiplication. Thestudy provided a comprehensive examination of the observations obtained from contrasting two treatments, MS+0.1BAP and MS+0.5BAP, concerning shoot multiplication and growth parameters for P. marsupium. Analysis reveals that both treatments maintain a similar range of 3 to 4 shoots per culture vessel, with a marginally higher mean number of shoots observed in MS+0.5BAP (3.60) compared to MS+0.1BAP (3.55). Moreover, the MS+0.5BAP treatment exhibits longer shoot lengths on average (6.26 cm) than MS+0.1BAP (5.63 cm), indicating the superior efficacy of higher BAP concentrations in promoting shoot elongation. The slightly higher mean number of shoots observed in the MS+0.5BAP treatment suggests that a higher concentration of benzyl aminopurine (BAP) may indeed promote more robust shoot multiplication (Fig.1). This finding aligns with previous studies indicating the role of cytokinin, such as BAP, in stimulating shoot proliferation in various plant species (Devgun et al., 2009). Furthermore, the longer shoot lengths observed in the MS+0.5BAP treatment highlight the importance of cytokinin concentration in regulating shoot elongation, consistent with the literature on cytokinin-mediated shoot growth (McCown, 2000).

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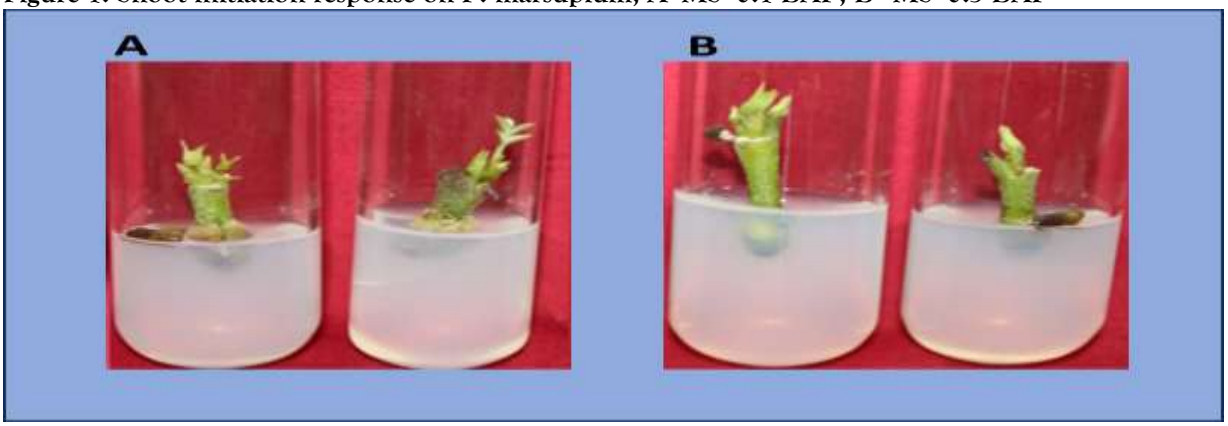
Figure 1: Shoot initiation response on P. marsupium, A- MS+0.1 BAP, B- MS+0.5 BAP



The treatment combination MS+0.5BAP demonstrates a higher percentage of contamination-free cultures (94.05%) compared to MS+0.1BAP (91.20%), suggesting enhanced culture cleanliness with increased BAP concentration. Furthermore, the survival rate is slightly elevated in MS+0.5BAP (84.25%) relative to MS+0.1BAP (78.95%), indicating improved plantlet viability under higher BAP concentrations(Fig.2).The higher percentage of contamination-free cultures in the MS+0.5BAP treatment underscores the potential antimicrobial properties of BAP or its influence on the overall health and vigor of the cultures, which could inhibit microbial contaminants.

Table 1: The response of P. marsupium to various in vitro culturing treatments with statistical analysis during study period

Observation	Treatment	Min	Max	Mean	Variance	Std. Dev.	Std. Error
Number of shoots	MS+0.1BAP	3.00	4.00	±3.55	0.26	0.51	0.11
	MS+0.5BAP	3.00	4.00	±3.60	0.25	0.50	0.11
Shoot length (cm)	MS+0.1BAP	5.30	5.90	±5.63	0.03	0.18	0.04
	MS+0.5BAP	5.80	6.60	±6.26	0.05	0.22	0.05
Contamination free (%)	MS+0.1BAP	88.00	95.00	±91.20	4.91	2.22	0.50
	MS+0.5BAP	91.00	97.00	±94.05	3.21	1.79	0.40
Survival rate (%)	MS+0.1BAP	74.00	83.00	±78.95	8.05	2.84	0.63
	MS+0.5BAP	79.00	89.00	±84.25	9.25	3.04	0.68

Figure 2. Response of P. marsupium to the number of shoots and shoot length under different treatments

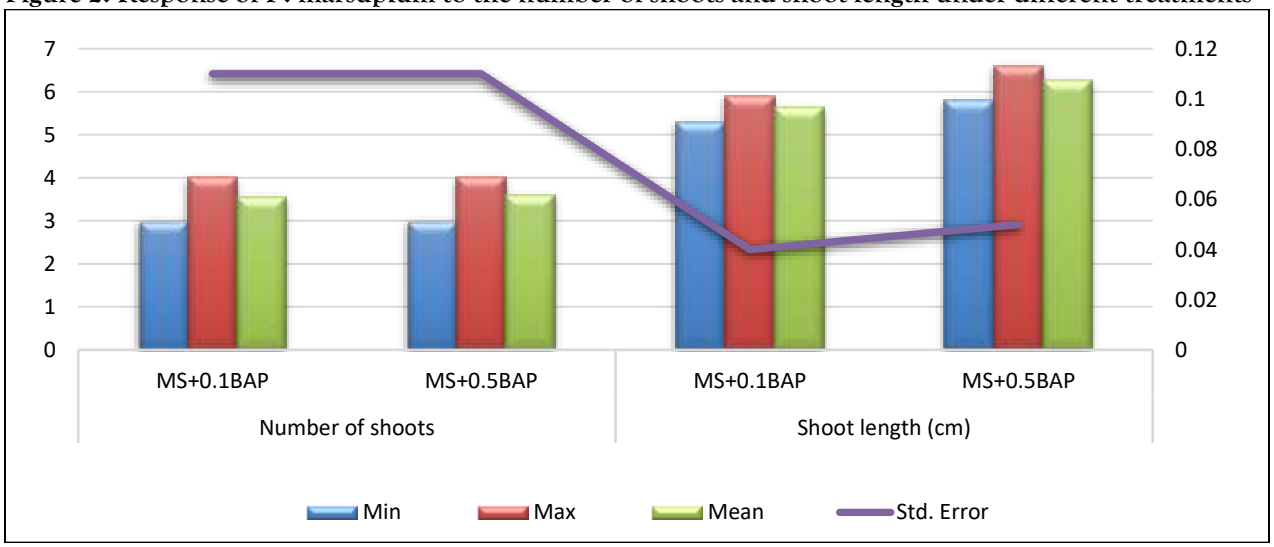
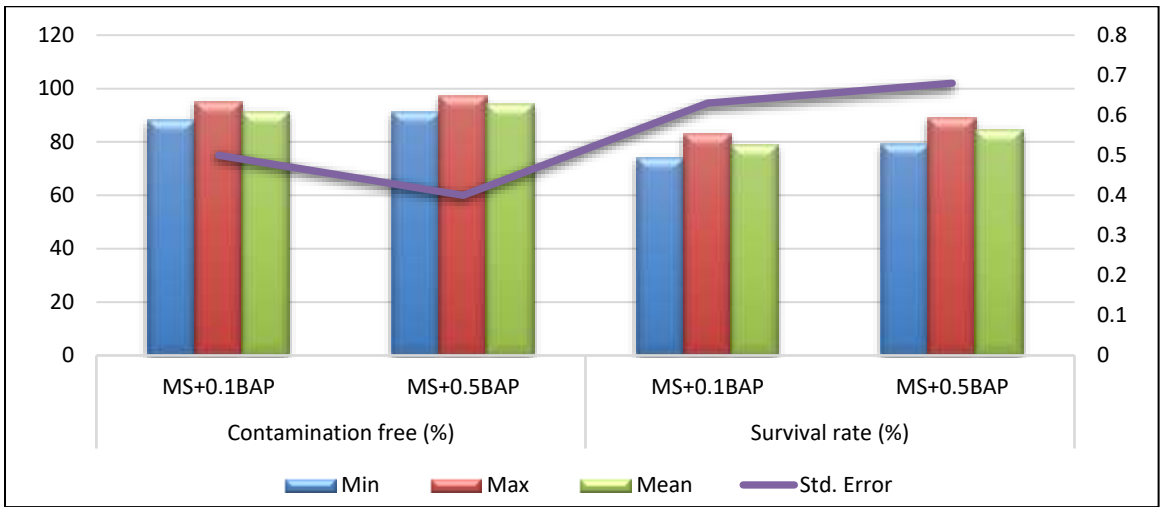


Figure 3. Response of P. marsupium to contamination-free conditions and survival rate under different treatments



This finding is supported by previous research suggesting the antimicrobial effects of certain plant growth regulators in tissue culture media (Husain et al.,2008 and Abirami et al.,2012).Additionally, the slightly elevated survival rate in the MS+0.5BAP treatment implies better plantlet viability under higher BAP concentrations, which may enhance the overall success rate of tissue culture propagation (Chand and Singh, 2004). The findings of recent studies have further elucidated the role of cytokinin in plant tissue culture and shoot proliferation. For instance, research by (Gupta et al., 2020) explored the synergistic effects of BAP and other cytokinin in enhancing shoot multiplication efficiency in medicinal plant species. Similarly, the study conducted by (Patel et al.,2019) investigated the impact of different cytokinin concentrations on shoot elongation and biomass accumulation in tissue-cultured tree species, providing valuable insights into optimizing tissue culture protocols for woody plants.

CONCLUSION

The present study underscores the importance of cytokinin concentration, particularly benzyl aminopurine (BAP), in optimizing shoot multiplication and growth parameters in P. marsupium tissue cultures. The comparison between MS+0.1BAP and MS+0.5BAP treatments reveals that higher BAP concentrations promote more robust shoot proliferation, longer shoot lengths, enhanced culture cleanliness, and improved plantlet viability. These findings highlight the critical role of cytokinin manipulation in tissue culture protocols for woody plant species like P. marsupium, offering valuable insights for optimizing propagation techniques and conserving medicinal trees.

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