REVIEW ARTICLE

Plant tissue culture techniques for *in vitro* mass multiplication of *Prosopis cineraria* (L.) in Thar Desert of Rajasthan

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Kumar R, Bhandari S, Sarita. Plant tissue culture techniques for *in vitro* mass multiplication of *Prosopis cineraria* (L.) in Thar Desert of Rajasthan. AGBIR.2024;40(4):1185-1187.

Prosopis cineraria (L.) is commonly known as Khejri or Shami. It is a multipurpose tree species found in arid and semi-arid regions of the Indian subcontinent. Its ecological significance includes soil stabilization and nitrogen fixation, while economically, it provides timber, fuelwood and fodder. Medicinally, various parts of the tree are used in traditional remedies for conditions like diabetes and respiratory ailments. Conventional propagation methods for *Prosopis cineraria* are often inadequate for its large-scale production. *In vitro* tissue culture techniques offer a viable alternative for mass multiplication and conservation of this valuable species. This review provides an in-depth analysis of various plant tissue culture methods employed for the *in vitro* propagation of *Prosopis cineraria*. The review also discusses the challenges, optimization strategies and potential applications of these techniques.

Key Words: *Prosopis cineraria; Tissue culture; Cytokinin; Soil*

INTRODUCTION

Prosopis cineraria (L.), commonly known as Khejri or ghaf, plays a significant role in combating desertification in the Thar Desert of Rajasthan [1]. It is a member of the family Fabaceae. It is widely recognized for its adaptability to harsh environmental conditions, making it a keystone species in the Thar Desert and other arid regions. The Khejri tree is unique in that it thrives in all desert climates. It is known as Kalptaru, which means all parts of the tree are useful. It is also referred to as the "wonder tree" and consequently, the "king of desert" [2-4]. Regular surveys conducted in 2010 in Jodhpur, Nagpur, Sikar, Churu and Jhunjhunu by the Arid Forest Research Institute, Jodhpur, revealed that the percentage mortality of Khejri trees ranged from 18.08% to 22.67%, with an average mortality of 20.93% in these districts [5,6]. This resilient tree species is well-adapted to arid and semi-arid conditions, making it an invaluable asset in the efforts to mitigate the adverse effects of desertification. It reduces desertification through soil stabilization, soil fertility improvement, water conservation, support for biodiversity, provision of economic benefits, carbon sequestration and enhancement of ecosystem resilience. Its multifaceted role underscores the importance of conserving and promoting this tree species in arid regions like Rajasthan. The tree is valued for its nutritional, medicinal and ecological benefits. Despite its importance, natural regeneration is slow and overexploitation has led to its decline. Micropropagation is an effective technique for producing disease-free plants quickly, rare species quickly multiplying, genetically modifying plants and producing bioactive substances produced from plants [7,8]. Tissue culture techniques offer a potential solution for the rapid propagation and conservation of *Prosopis cineraria* [9-11].

LITERATURE REVIEW

Tissue culture techniques

Plant tissue culture involves the *in vitro* cultivation of plant cells, tissues or organs under controlled environmental conditions on a nutrient medium. This method allows for the rapid multiplication of plants and is particularly beneficial for the propagation of species that are difficult to propagate by conventional methods.

Explants selection and preparation: For the micropropagation of *Prosopis cineraria*, the selection of suitable explants is critical. Commonly used explants include nodal segments, shoot tips and cotyledonary nodes. Explants must be sterilized to prevent microbial contamination, typically using a combination of alcohol and sodium hypochlorite [12].

Culture initiation and shoot multiplication: Explants are placed on a nutrient medium supplemented with essential macro and micronutrients, vitamins and Plant Growth Regulators (PGRs) such as auxins and cytokinins. Murashige and Skoog (MS) medium is frequently used for initiating cultures due to its comprehensive nutrient composition [13]. The induction of multiple shoots from the explants is achieved by optimizing the concentration and combination of cytokinins (e.g. Benzylaminopurine) and auxins (e.g. Indole-3-acetic acid). A high cytokinin to auxin ratio typically promotes shoot proliferation. Subculturing the explants onto fresh media every 3-4 weeks ensures continuous shoot multiplication. After pruning thorny mature trees of *P. cineraria*, the nodal shoot section was used to generate the highest number of 10-12 shoots on Murashige and Skoog's (MS) medium, which contained 0.1 mg/l Indole-3-Acetic Acid (IAA)+2.5 mg/l Benzylaminopurine (BAP)+additives [14]. For the purpose of standardizing shoot formation in nodal segment of *Prosopis cineraria*, a variety of auxins, including Naphthalene Acetic Acid (NAA), IAA, IBA and 2,4-Dichlorophenoxyacetic acid (2,4-D) and cytokinins, such as kinetin and BAP, were added to Murashige and Skoog's (MS) medium. It was discovered that 3-Indoleacetic Acid (IAA: 3.0 mg/l) combined with kinetin (0.05 mg/l) was the most effective combination for multiple shoot differentiation [9].

Callus induction, somatic embryogenesis and organogenesis: Callus induction is the initial step in many plant tissue culture processes. It involves the formation of an undifferentiated mass of cells (callus) from explants under controlled conditions. Somatic embryogenesis involves the development of embryos from somatic or non-reproductive cells, which can then develop into complete plants. Organogenesis is the process by which explants form organs like shoots and roots. Direct and indirect organogenesis can be induced by culturing explants or calli on media with specific PGR combinations [15].

Root induction, acclimatization and transfer to soil: Once adequate shoot multiplication is achieved, shoots are transferred to a rooting medium. This medium is generally fortified with auxins such as Indole-3-Butyric Acid (IBA) to induce root formation. Rooting efficiency can be enhanced by adjusting the concentration of IBA and other supplements like activated charcoal to reduce phenolic exudation. For the purpose of standardizing root production, a variety of auxins, including NAA, IAA, IBA and 2,4-D and cytokinins, including kinetin and BAP, were added to Murashige and Skoog's (MS) medium. After differentiation, shoot segments were obtained and multiplied by subculturing on 1/2 MS that was devoid of auxins and cytokinins. In white's base medium containing 0.8% agar, 3.0 mg/l Indole-3-Butyric Acid (IBA) and 0.05 mg/l kinetin, shoot segments spontaneously

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Received: 26-Jun-2024, Manuscript No. AGBIR-24-139942; **Editor assigned:** 28-Jun-2024, Pre QC No. AGBIR-24-139942 (PQ); **Reviewed:** 12-Jul-2024, QC No. AGBIR-24-139942; **Revised:** 19-Jul-2024, Manuscript No. AGBIR-24-139942 (R); **Published:** 26-Jul-2024, DOI:10.35248/0970-1907.24.40.1185-1187

produced tap roots within 15-20 days. With 60% of the rooted plants, transplantation to pots with a soil-vermiculite mixture (3:1) was completed effectively in *Prosopis cineraria* [9]. On MS medium with 0.1 mgl^{-1} NAA+1.0 mgl^{-1} BAP+additives, differentiated shoots multiplied most effectively. After extracting the differentiated shoots, the original explant of *Prosopis cineraria* was transplanted six times on new medium in order to produce multiple shoots. After pulsing 100 mgl^{-1} IBA for 4 hours, shoots were rooted by culture on hormone-free half-strength MS medium [14]. The acclimatization stage is significant for the survival of *in vitro* cultured plants. Plantlets with well-developed roots are gradually acclimatized to *ex vitro* conditions by transferring them to soil or a soil-vermiculite mixture under high humidity and controlled temperature conditions. This step involves gradually reducing humidity to adapt the plantlets to ambient conditions.

DISCUSSION

Applications and benefits in the Thar Desert

Ecological restoration: *Prosopis cineraria* is pivotal in combating desertification and restoring degraded lands in the Thar Desert. Its deep root system stabilizes the soil, reduces erosion and improves water retention.

Livelihood support and food for animals: The tree provides various products such as fodder, fuelwood and medicinal compounds, supporting local livelihoods. Its leaves and pods are highly nutritious for livestock, especially during the dry season when other forage resources are scarce.

Soil fertility enhancement: *Prosopis cineraria* enriches soil fertility through nitrogen fixation and leaf litter decomposition, which adds organic matter to the soil. This improves the soil's physical and chemical properties, promoting the growth of other vegetation (Figure 1).

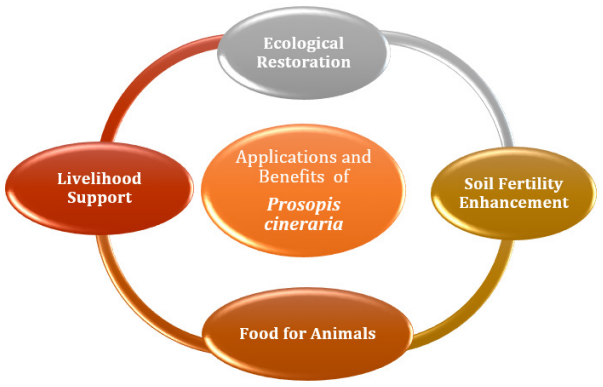

Figure 1) *Applications and benefits of Prosopis cineraria*

Challenges and optimization strategies

Contamination: Contamination is a critical issue in tissue culture, often caused by bacteria, fungi or yeast, which can compromise the culture's success. To address this, surface sterilization of plant material is essential. Mercuric Chloride ($HgCl_2$), though highly effective, is less commonly used due to its toxicity and environmental concerns. Sodium Hypochlorite (NaOCl) is a safer, more widely used alternative, typically applied at 1%-5% concentrations. Both agents work by eliminating surface microorganisms, but proper handling and timing are significant to avoid damaging the plant tissues. Consistent use of these sterilization protocols is key to maintaining aseptic conditions and ensuring culture success.

Somaclonal variation: Somaclonal variation refers to genetic changes that can arise during tissue culture, potentially altering the traits of regenerated plants. This variation may result from stress or genetic instability in the cultured cells. To ensure that tissue-cultured plants maintain the desired genetic characteristics of the parent, regular monitoring is significant. Employing molecular markers such as Polymerase Chain Reaction (PCR) and Simple Sequence Repeats (SSR) allows for precise detection of genetic deviations. These markers help in assessing clonal fidelity, ensuring that the propagated plants are true to type and maintain consistent quality, which is essential for breeding programs and commercial production.

Media optimization: Media optimization is vital for tissue culture success, including macronutrients, micronutrients and Plant Growth Regulators (PGRs), is significant for successful tissue culture. Adjustments must be made continuously based on the type of explant and its developmental stage to promote optimal growth and development.

Acclimatization: Acclimatization involves gradually transitioning *in vitro* plants to *ex vitro* conditions. This process starts with high humidity and reduced light intensity to ease the plants adaptation to external environments. Gradual exposure to these changing conditions helps improve survival rates and ensures a smoother adaptation to natural growth conditions (Figure 2).

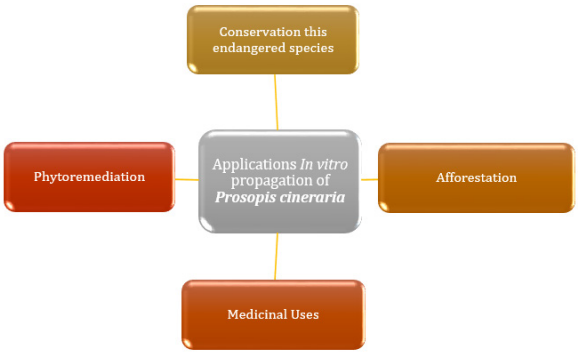

Figure 2) *Applications in vitro propagation of Prosopis cineraria*

Applications

In vitro propagation of *P. cineraria* has numerous applications as follows:

Conservation: *Prosopis cineraria* is a significant species for arid and semi-arid ecosystems, but it is endangered due to habitat loss and other environmental pressures. *In vitro* propagation, which involves growing plants in a controlled, sterile environment, is essential for its conservation. This technique enables the mass multiplication of the species, thus creating large numbers of genetically identical plants that can be reintroduced into their natural habitats. This approach helps in preserving the genetic diversity and ensuring the survival of the species in the wild.

Afforestation: Rapid propagation through tissue culture supports large-scale afforestation projects, especially in arid and semi-arid regions where *Prosopis cineraria* is naturally adapted. By generating a high volume of seedlings quickly and efficiently, *in vitro* propagation facilitates the restoration of degraded lands and contributes to the greening of these challenging environments. This not only helps in combating desertification but also in enhancing local biodiversity and improving soil quality.

Phytoremediation: *Prosopis cineraria* is well-suited for phytoremediation, a process where plants are used to clean up contaminated soils or water. The species' ability to thrive in degraded soils and tolerate harsh conditions makes it a valuable candidate for such projects. *In vitro* propagation ensures a consistent supply of healthy plants that can be used in these remediation efforts, helping to restore soil fertility and remove pollutants from the environment.

Medicinal uses: *Prosopis cineraria* has a history of use in traditional medicine and *in vitro* propagation supports the consistent supply of plant material for the extraction of bioactive compounds. These compounds are used in various medicinal preparations, including treatments for ailments such as inflammation, diabetes and gastrointestinal issues. By providing a steady source of high-quality plant material, tissue culture helps ensure that traditional medicinal practices can continue effectively and sustainably.

CONCLUSION

Prosopis cineraria or Khejri, is a vital species for arid and semi-arid regions due to its ecological, economic and medicinal benefits. Its role in soil stabilization, nitrogen fixation and provision of essential resources underscores its importance in combating desertification and supporting local livelihoods.

However, traditional propagation methods are often inadequate for its large-scale production. *In vitro* tissue culture techniques offer an effective solution for the rapid and disease-free multiplication of *P. cineraria*. These methods, while potential, face challenges such as contamination and somaclonal variation, which require ongoing optimization. Successful implementation of tissue culture can significantly enhance conservation efforts, support afforestation projects and provide a consistent supply of plant material for medicinal uses, thereby ensuring the sustainability and continued utility of this significant species.

Tissue culture techniques offer a sustainable and efficient method for the mass multiplication of *Prosopis cineraria*. Advances in callus induction, somatic embryogenesis, organogenesis and micropropagation have significantly improved propagation efficiency. Despite challenges such as contamination and somaclonal variation, ongoing research and optimization continue to enhance the effectiveness of these techniques. The application of *in vitro* propagation methods can play a pivotal role in the conservation, afforestation and utilization of *Prosopis cineraria*.

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