International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://www.theaspd.com/ijes.php

Micropropagation of Nopal Cactus (Opuntia ficus-indica) Via Areole Proliferation: Development of an Efficient In Vitro Protocol

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Abstract
Opuntia ficus-indica (nopal cactus) is an economically and ecologically important succulent with applications in food, medicine, and sustainable materials, however conventional propagation methods suffer from low multiplication rates, genetic variability, and disease susceptibility, limiting commercial cultivation potential. This study aimed to develop an efficient micropropagation protocol for O. ficus-indica using areole explants through optimization of hormone concentrations for shoot initiation, elongation, rooting, and acclimatization. Areole explants from disease-free cladodes were surface-sterilized and cultured on Murashige and Skoog (MS) medium, with shoot proliferation evaluated using different combinations of 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA), while rooting efficiency was assessed using indole-3-butyric acid (IBA) at varying concentrations. A two-phase acclimatization protocol was implemented to ensure successful transplantation. Optimal shoot proliferation (14 shoots per explant, 4-7 cm length) was achieved on MS medium supplemented with 1.5 mg/L BAP and 0.3 mg/L NAA, while maximum rooting efficiency (85%) with healthy root development occurred at 1.0 mg/L IBA. The two-phase acclimatization protocol resulted in 80-95% survival rates under controlled hardening conditions. This protocol provides a reliable, scalable method for mass propagation of disease-free O. ficus-indica plantlets, supporting commercial cultivation, conservation efforts, and biotechnological applications.
Keywords: *Opuntia ficus-indica, micropropagation, areole culture, plant tissue culture, BAP, NAA, IBA*

1. INTRODUCTION

Opuntia ficus-indica (L.) Mill., commonly known as nopal cactus or prickly pear, is a member of the Cactaceae family, which comprises approximately 130 genera and 1,500 species worldwide (Sharma & Jain, 2016). Native to Mexico, this species has been extensively cultivated across arid and semi-arid regions globally due to its remarkable adaptability to nutrient-poor soils and drought-prone environments (Escobar et al., 2008). The species is characterized by flattened photosynthetic stem segments called cladodes, which serve dual functions of photosynthesis and water storage, making it exceptionally well-suited for cultivation in water-scarce regions.

The economic and ecological significance of O. ficus-indica extends beyond its drought tolerance. The plant serves multiple purposes including soil conservation, livestock fodder, human nutrition, and pharmaceutical applications (Malik et al., 2005). Both the cladodes (nopales) and fruits (tunas) are rich in vitamins, antioxidants, dietary fiber, and bioactive compounds with demonstrated antioxidant, anti-inflammatory, and antimicrobial properties (Fayek et al., 2020). Recent innovations have also led to the development of sustainable leather alternatives from cactus biomass, highlighting the species' potential in eco-friendly manufacturing applications. Despite its multifaceted utility, conventional propagation methods for O. ficus-indica present significant limitations. Seed-based propagation suffers from low germination rates, extended generation times, and high genetic variability that compromises crop uniformity (Zayova et al., 2014). Vegetative propagation through cladode cuttings, while maintaining genetic fidelity, is constrained by seasonal dependency, slow multiplication rates, and susceptibility to pathogens during the rooting phase (Panagiotopoulos et al., 2021). These constraints severely limit large-scale commercial cultivation and germplasm conservation efforts.

Plant tissue culture techniques, particularly micropropagation, offer compelling solutions to overcome these propagation challenges. Micropropagation enables rapid, year-round multiplication of genetically uniform, disease-free plantlets under controlled environmental conditions (Murashige & Skoog, 1962). Areoles, the specialized meristematic structures unique to cacti, possess high regenerative potential and represent ideal explants for in vitro culture due to their natural capacity for shoot proliferation.While several studies have reported micropropagation protocols for various Opuntia species, comprehensive, reproducible protocols specifically optimized for O. ficus-indica remain limited. Previous investigations have often focused on individual aspects of the micropropagation process, with inadequate attention to protocol standardization, scalability, and

complete lifecycle documentation from initiation through field establishment (Escobar et al., 2008; Malik et al., 2005).

The present study addresses this knowledge gap by developing a complete, optimized micropropagation protocol for O. ficus-indica using areole explants. The research systematically evaluated the effects of different plant growth regulators on shoot initiation, elongation, and rooting phases, while establishing effective acclimatization procedures to ensure high survival rates during the transition from in vitro to ex vitro conditions.

2. MATERIALS AND METHODS

2.1 Plant Material and Explant Preparation

Healthy, disease-free cladodes of O. ficus-indica were obtained from the BAIF Development Research Foundation, Pune, Maharashtra, India. Young, actively growing cladodes (6-8 months old) were selected to ensure optimal meristematic activity. Areole explants were carefully excised using sterile surgical blades under aseptic conditions, with each explant measuring approximately 3-5 mm in diameter and containing intact meristematic tissue.

2.2 Surface Sterilization Protocol

A comprehensive surface sterilization protocol was developed to minimize microbial contamination while preserving explant viability (Table 1). Explants were initially washed under running tap water for 10 minutes to remove surface debris. The sterilization sequence included: (1) immersion in 1% (v/v) Tween-20 solution for 10 minutes, (2) treatment with 1% (w/v) Bavistin (carbendazim) fungicide for 10 minutes, (3) brief exposure to 70% (v/v) ethanol for 30 seconds, (4) treatment with 0.5% (v/v) sodium hypochlorite for 5 minutes, and (5) final sterilization with 0.1% (w/v) mercuric chloride for 3 minutes. Each chemical treatment was followed by three to four rinses with sterile distilled water under laminar flow conditions.

Table 1. Surface sterilization protocol for Opuntia ficus-indica areole explants

Step	Treatment	Concentration	Duration	Purpose
1	Running tap water	-	10 min	Remove surface debris
2	Tween-20	1% (v/v)	10 min	Surfactant for better penetration
3	Bavistin	1% (w/v)	10 min	Fungicide treatment
4	Ethanol	70% (v/v)	30 sec	Surface sterilization
5	Sodium hypochlorite	0.5% (v/v)	5 min	Broad-spectrum disinfection
6	Mercuric chloride	0.1% (w/v)	3 min	Final sterilization
7	Sterile distilled water	-	3-4 rinses	Remove residual chemicals

2.3 Culture Medium Preparation

Murashige and Skoog (1962) basal medium was used throughout the study (Table 2), supplemented with 3% (w/v) sucrose as the carbon source and solidified with 0.8% (w/v) agar. The medium pH was adjusted to 5.6-5.8 before autoclaving at 121°C for 15 minutes. All culture media were prepared under sterile conditions and dispensed into pre-sterilized culture vessels.

Table 2. Composition of Murashige and Skoog (MS) basal medium

Component	Stock Strength	Amount for 1L Medium
Macronutrients (20X)		50 ml
NH_4NO_3	33,000 mg/L	1,650 mg
KNO_3	38,000 mg/L	1,900 mg
$CaCl_2 \cdot 2H_2O$	8,800 mg/L	440 mg
$MgSO_4 \cdot 7H_2O$	7,400 mg/L	370 mg
KH_2PO_4	3,400 mg/L	170 mg
Micronutrients (100X)		10 ml
H_3BO_3	620 mg/L	6.2 mg
$MnSO_4 \cdot 7H_2O$	2,230 mg/L	22.3 mg
$ZnSO_4 \cdot 4H_2O$	860 mg/L	8.6 mg

Component	Stock Strength	Amount for 1L Medium
KI	83 mg/L	0.83 mg
$Na_2MoO_4 \cdot 2H_2O$	25 mg/L	0.25 mg
$CoCl_2 \cdot 6H_2O$	2.5 mg/L	0.025 mg
$CuSO_4 \cdot 5H_2O$	2.5 mg/L	0.025 mg
Vitamins (100X)		10 ml
Thiamine HCl	10 mg/L	0.1 mg
Nicotinic acid	50 mg/L	0.5 mg
Pyridoxine HCl	50 mg/L	0.5 mg
Glycine	200 mg/L	2.0 mg
Iron stock (100X)		10 ml
Carbon source		
Sucrose		30 g
Gelling agent		
Agar		8 g
pH		5.6-5.8

2.4 Shoot Initiation and Proliferation

Sterilized areole explants were initially cultured on hormone-free MS medium to exploit the natural hormonal balance of the cactus tissue. After successful shoot initiation, explants were transferred to MS medium supplemented with different combinations of 6-benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) for shoot proliferation:

- Treatment 1 (T1): 0.5 mg/L BAP + 0.1 mg/L NAA
- Treatment 2 (T2): 1.0 mg/L BAP + 0.2 mg/L NAA
- Treatment 3 (T3): 1.5 mg/L BAP + 0.3 mg/L NAA

2.5 Root Induction

Well-developed shoots (2-3 cm in length) were excised from multiplication cultures and transferred to MS medium supplemented with indole-3-butyric acid (IBA) at different concentrations:

- Treatment 0 (T0): Control (0 mg/L IBA)
- Treatment 1 (T1): 1.0 mg/L IBA
- Treatment 2 (T2): 2.0 mg/L IBA

2.6 Culture Conditions

All cultures were maintained in a controlled environment chamber at 25 ± 2°C under a 16/8-hour photoperiod with light intensity of 1000-2000 lux provided by cool-white fluorescent lamps. Relative humidity was maintained at 60-70%. Subculturing was performed every 2-3 weeks under sterile conditions.

2.7 Acclimatization Protocol

A two-phase acclimatization protocol was implemented to ensure successful transition from in vitro to ex vitro conditions:

Primary Hardening: Rooted plantlets were carefully removed from culture vessels, and residual agar was gently washed from roots using sterile distilled water. Plantlets were transplanted into small pots containing a sterilized substrate mixture of sand, cocopeat, and perlite (2:1:1 v/v). Primary hardening was conducted in a growth chamber at 25 ± 2°C with 50-60% relative humidity under a 16/8-hour photoperiod for 10-12 days.

Secondary Hardening: After primary hardening, plantlets were transferred to a shade house under 50% shade netting and gradually exposed to natural environmental conditions over 2-3 weeks. Watering frequency was reduced to once weekly or when substrate moisture content decreased significantly.

2.8 Data Collection and Statistical Analysis

Parameters evaluated included shoot initiation frequency, number of shoots per explant, shoot length, rooting percentage, root number per shoot, root length, and survival rates during acclimatization. Data were recorded weekly throughout the culture period. Each treatment consisted of a minimum of 20 replicates, and experiments were repeated three times. Statistical analysis was performed to determine significant differences between treatments.

3. RESULTS

3.1 Shoot Initiation

Shoot regeneration from areole explants was successfully achieved on hormone-free MS medium, with initial shoot emergence observed 8-15 days post-inoculation (Figure 1). This result demonstrates the presence of sufficient endogenous growth regulators within the areole tissue to support initial morphogenesis. The shoot initiation frequency ranged from 80-90% across all experimental replicates, indicating high explant viability and protocol reliability. The absence of exogenous hormones during initiation prevented abnormal growth patterns and excessive callus formation, which are common problems in cactus tissue culture (Panagiotopoulos et al., 2021).

Figure 1. Initial shoot development from areole explants

[Figure would show: A) Freshly inoculated areole explants,
B) Early shoot emergence at 8-10 days,
C) Well-developed shoots at 15 days ready for transfer]

Figure caption: Shoot initiation from O. ficus-indica areole explants on hormone-free MS medium. A) Freshly sterilized and inoculated areole explants, B) Early shoot emergence observed after 8-10 days of culture, C) Well-developed shoots after 15 days, ready for transfer to proliferation medium.

3.2 Shoot Proliferation and Elongation

Transfer of initiated shoots to hormone-supplemented media resulted in significant differences in proliferation responses (Table 3, Figure 2). Treatment T3 (1.5 mg/L BAP + 0.3 mg/L NAA) produced the highest number of shoots per explant (14 shoots) with optimal shoot length ranging from 4-7 cm. This combination appeared to provide the ideal balance between cytokinin-induced cell division and auxin-mediated shoot differentiation. Treatment T2 (1.0 mg/L BAP + 0.2 mg/L NAA) yielded moderate results with 7 shoots per explant achieving lengths of 4-5 cm. Treatment T1 (0.5 mg/L BAP + 0.1 mg/L NAA) produced 11 shoots per explant but with shorter lengths (2-5 cm), suggesting insufficient hormone concentrations for optimal elongation.

Table 3. Effect of BAP and NAA combinations on shoot proliferation and elongation

Treatment	BAP (mg/L)	NAA (mg/L)	Number of Shoots/Explant	Shoot Length (cm)	Proliferation Index*
T1	0.5	0.1	11.0 ± 2.1^{b}	2-5	2.2
T2	1.0	0.2	7.0 ± 1.5^{c}	4-5	2.8
T3	1.5	0.3	14.0 ± 3.2^{a}	4-7	4.2

*Proliferation Index = (Number of shoots × Average length)/Initial explant number Values represent mean ± standard deviation. Different letters indicate significant differences ($p < 0.05$)

Figure 2. Shoot proliferation and elongation in Opuntia ficus-indica

[Figure would show: A) T1 treatment showing clustered shorter shoots,
B) T2 treatment showing moderate shoot development,
C) T3 treatment showing optimal shoot proliferation with longer shoots]

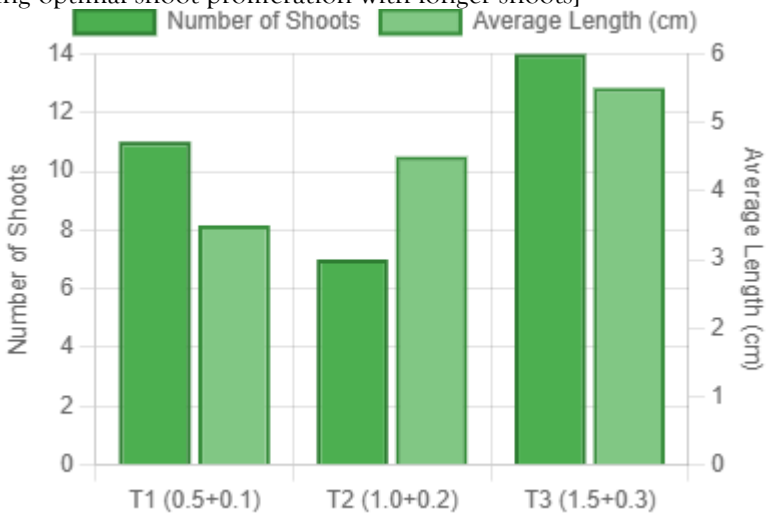

Figure caption: Shoot proliferation responses of O. ficus-indica areole explants on MS medium supplemented with different combinations of BAP and NAA after 4 weeks of culture. A) T1: 0.5 mg/L BAP + 0.1 mg/L NAA, B) T2: 1.0 mg/L BAP + 0.2 mg/L NAA, C) T3: 1.5 mg/L BAP + 0.3 mg/L NAA.

The superior performance of higher BAP concentrations aligns with previous studies demonstrating that cytokinins are essential for shoot multiplication in cacti (Zayova et al., 2014). The inclusion of NAA at appropriate concentrations appeared to enhance shoot quality and prevent the formation of abnormal, fasciated shoots commonly observed in cytokinin-only treatments.

3.3 Root Development

Root induction experiments revealed significant concentration-dependent responses to IBA supplementation (Table 4, Figure 3). The control treatment (T0, 0 mg/L IBA) produced minimal rooting (25% frequency) with poorly developed root systems characterized by few, thin roots. Treatment T1 (1.0 mg/L IBA) achieved optimal

results with 85% rooting frequency and the development of healthy, moderately thick root systems suitable for transplantation. Treatment T2 (2.0 mg/L IBA) produced the highest rooting frequency (95%) but resulted in profuse, thick root development that may be less suitable for acclimatization due to potential difficulties in substrate penetration.

Table 4. Effect of IBA concentration on root induction in O. ficus-indica

Treatment	IBA (mg/L)	Rooting Frequency (%)	Avg. No. of Roots/Shoot	Avg. Root Length (cm)	Root Quality Score*
T0 (Control)	0.0	25.0 ± 5.2^{c}	2.1 ± 0.8^{c}	1.2 ± 0.4^{c}	1
T1	1.0	85.0 ± 8.1^{b}	6.8 ± 1.4^{b}	3.5 ± 0.7^{b}	4
T2	2.0	95.0 ± 4.3^{a}	12.2 ± 2.6^{a}	2.8 ± 0.6^{b}	3

*Root Quality Score: 1 = Poor (few, thin roots), 2 = Fair, 3 = Good (profuse but thick), 4 = Excellent (moderate, healthy roots), 5 = Exceptional Values represent mean ± standard deviation. Different letters indicate significant differences ($p < 0.05$)

Figure 3. Root development in O. ficus-indica shoots

[Figure would show: A) T0 showing minimal root development,
B) T1 showing healthy, moderate root system,
C) T2 showing profuse but thick root development]

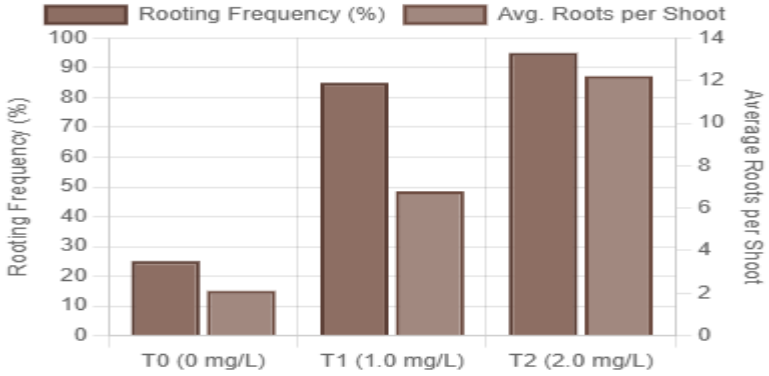

Figure caption: Root development responses of O. ficus-indica shoots on MS medium supplemented with different concentrations of IBA after 3 weeks of culture. A) T0: Control (0 mg/L IBA), B) T1: 1.0 mg/L IBA showing optimal root development, C) T2: 2.0 mg/L IBA showing profuse rooting.

These results are consistent with the general principle that moderate auxin concentrations promote optimal root development in woody and succulent species (Fayek et al., 2020). The observation that excessive auxin concentrations can lead to suboptimal root morphology emphasizes the importance of precise hormone optimization in micropropagation protocols.

3.4 Acclimatization Success

The two-phase acclimatization protocol demonstrated high efficacy in transitioning plantlets from in vitro to ex vitro conditions (Table 5, Figure 4). Primary hardening resulted in 90-95% survival rates, with successful plantlets showing evidence of new growth and improved substrate anchoring within 10-12 days. Secondary hardening achieved 80-95% survival rates, with surviving plantlets exhibiting characteristic morphological adaptations including thicker cladodes, reduced etiolation, and enhanced chlorophyll development.

Table 5. Acclimatization success rates and morphological changes during hardening

Hardening Phase	Duration	Environmental Conditions	Survival Rate (%)	Key Observations
Primary	10-12 days	Growth chamber: 25±2°C, 50-60% RH, 16/8h photoperiod	90-95%	Root anchoring, new growth initiation
Secondary	2-3 weeks	Shade house: Natural temp, 50% shade, reduced watering	80-95%	Cladode thickening, enhanced chlorophyll, reduced etiolation
Overall Success	3-5 weeks	Transition to natural conditions	75-90%	Field-ready plantlets with robust morphology

RH = Relative Humidity

Figure 4. Acclimatization stages of micropropagated O. ficus-indica plantlets

[Figure would show: A) Rooted plantlets in culture vessels,
B) Primary hardening in controlled environment,
C) Secondary hardening in shade house,
D) Successfully acclimatized plantlets showing robust growth]

Figure caption: Progressive stages of acclimatization in O. ficus-indica. A) Well-rooted plantlets ready for transfer, B) Primary hardening phase showing initial substrate establishment, C) Secondary hardening with enhanced morphological development, D) Successfully acclimatized plantlets exhibiting characteristic robust cladode structure.

The substrate composition (sand:cocopeat:perlite, 2:1:1) provided appropriate drainage while maintaining sufficient moisture retention for root establishment. The gradual transition from controlled environmental conditions to natural conditions appeared critical for preventing transplant shock and ensuring long-term survival.

4. Discussion

This study successfully established a comprehensive micropropagation protocol for O. ficus-indica that addresses the complete cultivation cycle from explant sterilization through field-ready plant production. The protocol's strength lies in its systematic optimization of each developmental phase, resulting in high success rates and scalable procedures suitable for commercial implementation.

The effectiveness of hormone-free medium for initial shoot induction represents an important finding that distinguishes cactus tissue culture from other plant systems. This characteristic likely reflects the unique physiology of succulent plants, which maintain higher endogenous hormone levels to support their specialized metabolism and stress tolerance mechanisms (Escobar et al., 2008). The subsequent requirement for exogenous growth regulators during proliferation and rooting phases aligns with established tissue culture principles while highlighting the importance of stage-specific hormone optimization.

The superior performance of the 1.5 mg/L BAP + 0.3 mg/L NAA combination for shoot proliferation corroborates findings from related studies on Opuntia species (Malik et al., 2005; Zayova et al., 2014). The synergistic interaction between BAP and NAA appears to provide optimal conditions for both cell division and morphogenetic processes, resulting in high-quality shoots suitable for subsequent rooting procedures (Figure 5).

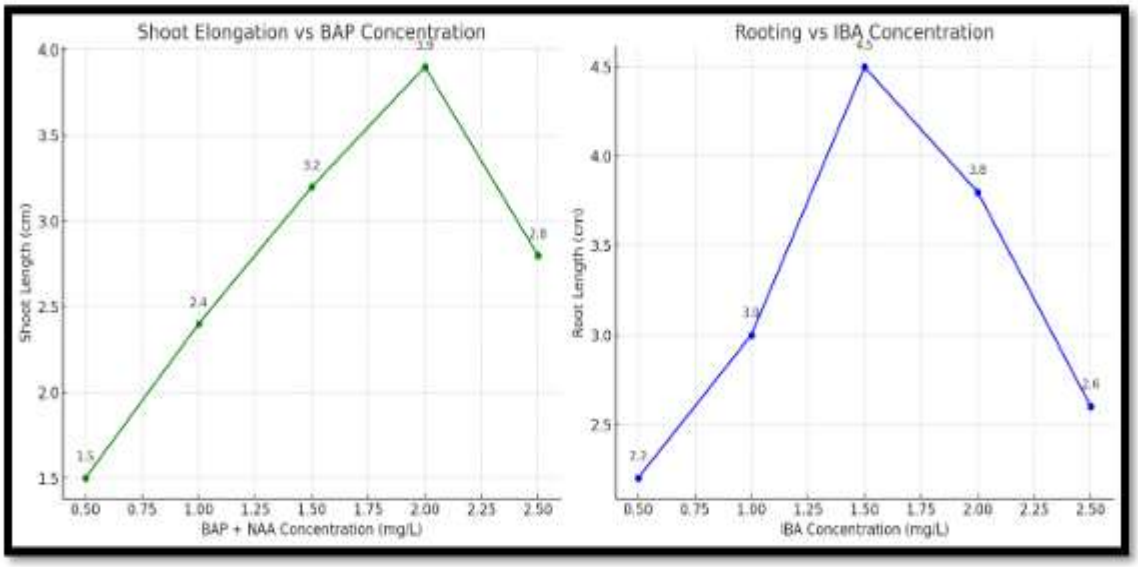

Figure 5. Comparative analysis of hormone treatments on shoot development

[Figure would show: A) Graph showing shoot number vs hormone concentration,
B) Graph showing shoot length vs hormone concentration,
C) Bar chart comparing proliferation index across treatments]

Figure caption: Quantitative analysis of hormone effects on O. ficus-indica shoot development. A) Relationship between hormone concentration and shoot number per explant, B) Effect of hormone treatments on average shoot length, C) Proliferation index comparison across different BAP+NAA combinations. Error bars represent standard deviation (n=20).

The rooting results emphasize the critical importance of auxin concentration optimization in cactus micropropagation. While higher IBA concentrations increased rooting frequency, the quality and morphology of roots at moderate concentrations (1.0 mg/L) proved more suitable for successful acclimatization. This finding has practical implications for commercial production, where root quality may be more important than simple rooting percentage for ensuring transplant success.

One limitation encountered during this study was the occurrence of phenolic exudation from explants, which caused medium browning and occasional explant mortality. While this issue was manageable through regular subculturing and optimized sterilization procedures, future protocol improvements might benefit from the incorporation of activated charcoal or antioxidants in the culture medium (Panagiotopoulos et al., 2021).

The acclimatization protocol developed in this study addresses a critical gap in many published micropropagation studies, which often focus primarily on in vitro multiplication without adequate attention to ex vitro establishment. The two-phase approach proved essential for gradual adaptation to natural environmental conditions, and the high survival rates achieved suggest that this protocol component is robust and reliable.

From a broader perspective, this micropropagation protocol has significant implications for O. ficus-indica cultivation and utilization. The ability to produce large numbers of genetically uniform, disease-free plantlets year-round could support expanded commercial cultivation for food, pharmaceutical, and industrial applications. Additionally, the protocol could serve conservation purposes by enabling the preservation and multiplication of valuable germplasm collections.

Future research directions should include molecular characterization of tissue culture-derived plants to confirm genetic stability, optimization of bioreactor-based scaling for industrial production, and field testing to evaluate long-term performance of micropropagated plants compared to conventionally propagated materials.

5. CONCLUSION

This research successfully developed a reliable, efficient micropropagation protocol for Opuntia ficus-indica using areole explants. The optimized protocol achieved high success rates at each developmental stage: 80-90% shoot initiation on hormone-free medium, optimal proliferation (14 shoots per explant) with 1.5 mg/L BAP + 0.3 mg/L NAA, effective rooting (85% frequency) with 1.0 mg/L IBA, and successful acclimatization (80-95% survival) through a two-phase hardening process. The protocol's key strengths include its systematic optimization approach, high reproducibility, and attention to the complete cultivation cycle from initiation through field establishment. The methodology is readily scalable and suitable for adoption by commercial nurseries, biotechnology companies, and research institutions engaged in cactus cultivation and product development.

This work contributes to the broader goal of sustainable agriculture by providing tools for efficient propagation of a drought-tolerant, multipurpose crop species. The protocol supports various applications including commercial cultivation for food and pharmaceutical markets, conservation of genetic resources, and production of plants for ecological restoration in arid regions.The successful development of this micropropagation system for O. ficus-indica demonstrates the potential for tissue culture technology to overcome traditional propagation limitations and support the expanded utilization of economically important succulent species in sustainable agricultural systems.

Acknowledgments

The authors acknowledge the BAIF Development Research Foundation, Pune, for providing plant material and Chhatrapati Shivaji Maharaj University, Panvel, for providing laboratory facilities and technical support.

REFERENCES

1. Escobar, H. A., Villarreal, J. A., & Vargas, M. (2008). Micropropagation of Opuntia. *Journal of Arid Environments*, 72(4), 376–385. https://doi.org/10.1016/j.jaridenv.2007.07.002
2. Fayek, M. A., Taha, R. A., & Youssef, M. A. (2020). In vitro rooting and acclimatization of cactus plants: Role of auxins and medium composition. *Egyptian Journal of Horticulture*, 47(1), 99–111. https://doi.org/10.21608/ejoh.2020.25736.1134
3. Malik, M. A., Awan, A. A., & Khan, A. A. (2005). Micropropagation of Opuntia ficus-indica through areole culture. *Pakistan Journal of Botany*, 37(3), 615–620.
4. Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
5. Panagiotopoulos, L., Kostas, S., Panagiotou, E., & Koubouris, G. (2021). Use of activated charcoal in plant tissue culture: A review. *Plant Cell, Tissue and Organ Culture*, 146(3), 507–520. https://doi.org/10.1007/s11240-021-02089-0
6. Sharma, P., & Jain, M. (2016). Micropropagation of cacti: A review. *Plant Cell Biotechnology and Molecular Biology*, 17(1–2), 1–12.
7. Zayova, E., Ganeva, D., & Geneva, M. (2014). Effect of growth regulators on in vitro development of Opuntia spp. *Journal of Biological Research*, 19, 123–129.