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BIOLIFE RESEARCH ARTICLE

IN VITRO PROPAGATION OF *CEROPEGIA BULBOSA* (LUSHI)

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ABSTRACT

Medicinal plants are coming forward as reliable lifesaving medicines now days. Hence the world Health Organization (WHO) has an inventory of medicinal plants listing over 20,000 species. As a part of the strategy to reduce burden on developing countries, WHO currently encourages, recommends and promotes the inclusion of herbal drugs in national health-care programmes. Today the global market of herbal products is estimated to be around US $40 billion and growing at a rate of 15-20% annually (pate, 1985). Medicinal plants have curating properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more parts of the plants. These plant metabolites, according to their composition, are grouped as alkaloids, glycosides, according to their composition, are grouped as alkaloids, glycoside, corticosteroids, essential oils, etc. However, it should be stated in all fairness that our knowledge of the genetic and physiological make-up of most of the medicinal plants is poor and we know still less about biosynthetic pathways leading to the formation of active constituents for which these plants are valued.

Keywords: *Ceropegia bulbosa*, WHO, Medicinal Plants, alkaloids, glycosides.

INTRODUCTION

Medicinal plants have served as an indispensable source of therapeutic agents for centuries and continue to play a significant role in modern healthcare systems. The World Health Organization (WHO) recognizes the importance of medicinal plant resources and has documented more than 20,000 species with established or potential medicinal value. The increasing demand for herbal medicines is attributed to their therapeutic efficacy, relatively low incidence of adverse effects in India. Consequently, herbal products have emerged as an important component of global healthcare, with a rapidly expanding international market estimated to grow at 15–20% annually (Pate, 1985). The pharmacological potential of medicinal plants arises primarily from diverse secondary metabolites, including alkaloids, glycosides, terpenoids, corticosteroids, essential oils, flavonoids, and phenolic compounds, which exhibit antimicrobial, antioxidant, anti-inflammatory, anticancer, and immunomodulatory activities. Despite their immense medicinal significance, the biosynthetic pathways, genetic diversity, and physiological mechanisms regulating the production of these bioactive constituents remain inadequately understood in many species. This knowledge gap has stimulated increasing interest in the conservation, propagation, and biotechnological improvement of medicinal plants to ensure their sustainable utilization and continuous availability for pharmaceutical and therapeutic applications.

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Traditional medicinal systems such as Ayurveda, Siddha, Unani, and Tibetan medicine have relied extensively on plant-based remedies for the prevention and treatment of numerous human ailments. India possesses one of the richest traditions of ethnomedicine, supported by thousands of licensed practitioners and centuries of documented indigenous knowledge. Ancient texts, including those on Dravyaguna Shastra, Vriksha Ayurveda, and Krishi Shastra, describe the medicinal properties of numerous plant species as well as their applications in agriculture and veterinary medicine. More than 8,500 flowering plant species are reported to be utilized by over 4,600 ethnic communities throughout the country for human and animal healthcare, while nearly 1,800 plant species have been comprehensively documented in Ayurvedic literature for their biological activities and therapeutic formulations (Bhagyalakshmi and Singh, 1988). However, increasing anthropogenic pressure, habitat degradation, overexploitation, and unsustainable harvesting practices have resulted in a rapid decline in several medicinal plant populations, including threatened taxa such as *Ceropegia bulbosa* var. *lushi*. These challenges highlight the urgent need for effective conservation strategies capable of preserving valuable germplasm while ensuring a sustainable supply of planting material for pharmaceutical research and commercial cultivation.

Plant tissue culture has emerged as one of the most effective biotechnological approaches for the rapid propagation, conservation, and genetic improvement of valuable medicinal plants. Since the development of the Murashige and Skoog (MS) culture medium, in vitro culture techniques have revolutionized plant biotechnology by enabling the regeneration of complete plants from isolated cells, tissues, organs, and protoplasts under controlled environmental conditions (Murashige and Skoog, 1962). Subsequent advances have significantly improved regeneration protocols for numerous plant species previously regarded as recalcitrant, thereby expanding opportunities for genetic transformation, somatic embryogenesis, organogenesis, germplasm conservation, and large-scale clonal multiplication (Jdeda and Tanabe, 1989). Continued research has also enhanced our understanding of cellular totipotency, embryogenic competence, and the optimization of culture media and growth regulators required for efficient in vitro regeneration (Naidu and Emehute, 1997). Beyond propagation, tissue culture provides an effective platform for the production of valuable phytochemicals, disease-free planting material, and elite genotypes while minimizing dependence on natural populations. Consequently, it has become an indispensable tool in medicinal plant biotechnology and biodiversity conservation (Dohroo, 1988).

Among the threatened medicinal species, *Ceropegia bulbosa* var. *lushi* possesses considerable medicinal and conservation importance but exhibits limited natural regeneration owing to habitat destruction, poor seed viability, and excessive collection from wild populations. Conventional propagation methods are inadequate for meeting conservation and commercial requirements, necessitating the development of reliable in vitro propagation protocols. Micropropagation through nodal explants offers an efficient approach for the rapid multiplication of genetically uniform, disease-free plantlets while preserving elite germplasm for future utilization. Optimization of culture media supplemented with suitable concentrations of cytokinins such as 6-benzylaminopurine (BAP) and kinetin, together with auxins including naphthalene acetic acid (NAA), indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA), plays a crucial role in regulating shoot induction, multiplication, rooting, and acclimatization. Therefore, the present investigation was undertaken to establish an efficient and reproducible protocol for the in vitro propagation of *Ceropegia bulbosa* var. *lushi*, thereby contributing to its conservation, sustainable utilization, and future exploitation as an important medicinal resource.

MATERIALS AND METHODS

Plant Material

The present study was undertaken to develop an efficient in vitro propagation protocol for *Ceropegia bulbosa* var. *lushi*, a threatened medicinal plant belonging to the family Asclepiadaceae. The genus *Ceropegia* comprises approximately 200 species distributed throughout tropical and subtropical regions of Asia, Africa, Australia, Malaysia, and the Canary and Pacific Islands (Anonymous, 1992; Bruyns, 2003). India harbors 44 species of *Ceropegia*, of which 27 are endemic to Peninsular India, particularly the Western Ghats, and many are categorized as endangered because of habitat destruction and overexploitation (Ahmedullah and Nayar, 1986; Nayar and Sastry, 1983). The species exhibits poor natural regeneration due to low seed germination, cross-pollination, and limited vegetative propagation through tubers and stem cuttings. The tuberous roots possess considerable medicinal importance and contain starch, sugars, gums, albuminoids, fats, crude fiber, and the alkaloid ceropegine, which is traditionally used in the treatment of diarrhoea and dysentery (Kirtikar and Basu, 1935; Nadkarni, 1976). Considering its medicinal significance and threatened status, micropropagation using meristematic tissues provides an effective strategy for rapid clonal multiplication and long-term conservation.

Explant Collection and Surface Sterilization

Healthy tubers of *Ceropegia bulbosa* var. *lushi* were collected from the Gogababa Hills and roadside areas of the university campus, Aurangabad, Maharashtra, India. The tubers were established under greenhouse conditions in the Botanical Garden of the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar. Apical shoot tips, axillary buds, nodal segments, and meristematic tissues were excised from two-month-old healthy donor plants and used as explants for in vitro culture. The explants were thoroughly washed under running tap water for 10 min, followed by rinsing with distilled water for 5 min to remove adhering contaminants. Surface sterilization was carried out by immersing the explants in 70% ethanol followed by treatment with 0.3% (w/v) mercuric chloride ($HgCl_2$) for 5 min under aseptic conditions. Subsequently, the explants were rinsed three times with sterile double-distilled water inside a laminar airflow cabinet to remove traces of the sterilizing agent. Sterilized explants were trimmed into appropriate sizes to maximize the exposed surface area before inoculation onto the culture medium.

Culture Medium and Culture Conditions

Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) was employed for the initiation and multiplication of shoots from apical shoot tips, axillary buds, and nodal explants. The medium was supplemented with different concentrations of the cytokinins 6-benzylaminopurine (BAP) and kinetin (Kn) to evaluate their effects on shoot induction and proliferation. For in vitro rooting, regenerated shoots were transferred to half-strength MS medium supplemented with varying concentrations of the auxins indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and α-naphthalene acetic acid (NAA). All media contained 3% (w/v) sucrose as the carbon source and were solidified with 3 g L^{-1} Clerigel. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C and 15 psi for 20 min. Cultures were incubated in a growth room maintained at 25 ± 2°C under a 16-h photoperiod provided by cool white fluorescent lamps. A minimum of ten culture vessels were maintained for each treatment throughout the experiment.

Experimental Design and Data Recording

The experiments were conducted using a completely randomized design with five independent replicates per treatment. Observations on shoot induction frequency, number of shoots per explant, and shoot length were recorded after 25 days of culture. Mean values (μ) and standard errors (SE) were calculated for each treatment to evaluate the effects of different plant growth regulator combinations on in vitro shoot multiplication and regeneration efficiency.

RESULTS AND DISCUSSION

Shoot Induction and Multiple Shoot Formation

Apical shoot tips, axillary buds, and nodal explants of *Ceropegia bulbosa* var. *lushi* cultured on hormone-free Murashige and Skoog (MS) medium did not exhibit shoot induction or multiple shoot formation, indicating that exogenous plant growth regulators are essential for in vitro morphogenesis. Multiple shoot induction was achieved only on MS medium supplemented with different concentrations of 6-benzylaminopurine (BAP) either alone or in combination with indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA). Among the treatments tested, MS medium containing 1.6 mg L^{-1} BAP combined with 0.2 mg L^{-1} IBA produced the highest percentage of shoot multiplication and the greatest shoot length. Both apical shoot tips and axillary buds responded positively to cytokinin supplementation; however, nodal explants exhibited the highest regenerative potential. These observations demonstrate that the presence of actively dividing meristematic tissues, together with an optimum cytokinin-to-auxin ratio, plays a crucial role in initiating and sustaining multiple shoot formation in *C. bulbosa* var. *lushi*.

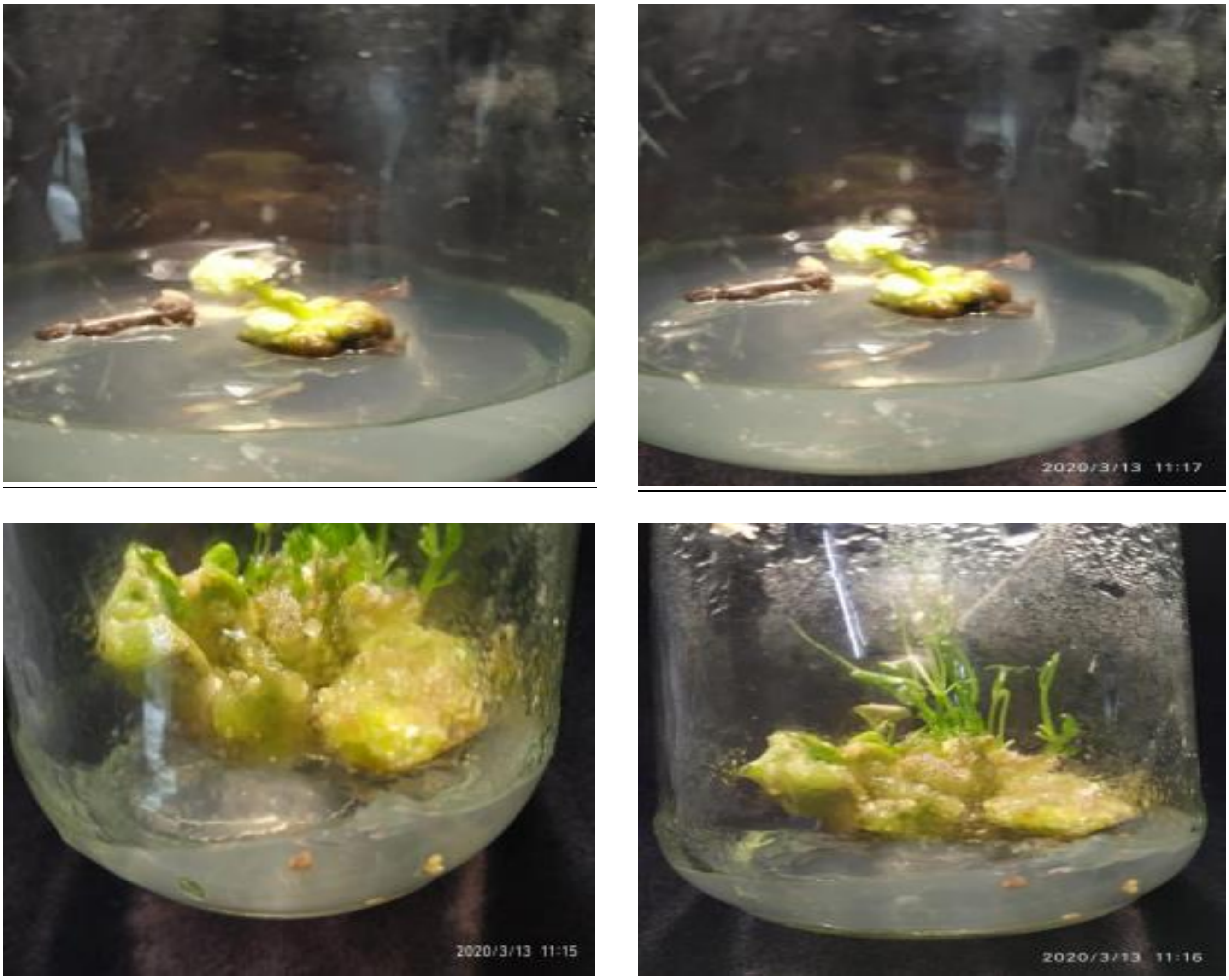

Figure-1. *In vitro* shoot induction of *Ceropegia bulbosa* var. lushi on Murashige and Skoog (MS) medium supplemented with different concentrations of BAP and kinetin (KIN). Initiation of multiple shoots within 10 days of inoculation.

Callus Induction

Callus induction was evaluated on three basal media, namely MS, White's, and Gamborg's media, supplemented with different combinations of BAP, kinetin (KIN), indole-3-acetic acid (IAA), and IBA. Callus initiation was observed within 10–15 days of inoculation, although considerable variation in callus induction frequency and fresh biomass was recorded among different media and growth regulator combinations. The highest callus induction and fresh weight were obtained on MS medium supplemented with 0.5 mg L^{-1} BAP and 1.0 mg L^{-1} IAA, whereas Gamborg's medium containing 1.0 mg L^{-1} IBA and White's medium exhibited comparatively lower callus induction frequencies. The superior response of MS medium may be attributed to its balanced nutrient composition and its interaction with suitable concentrations of auxins and cytokinins. Similar observations have been reported in other medicinal plants where MS medium proved to be more effective for callus induction and biomass production. Comparable antioxidant characteristics of callus cultures have also been reported in *Desmodium gangeticum*, where significant superoxide scavenging activity was observed in callus tissues (Narendra et al., 2014).

Shoot Multiplication

Multiple shoot induction was initiated within 10 days of culture on MS medium supplemented with various concentrations of BAP and KIN (Fig. 1). Shoot induction frequency ranged from 80–100% in

Table 1. Effect of different concentration of growth regulators on MS media for the adventitious shoot regeneration from the nodal callus of *Desmodium gangeticum.*

Explant	Auxin	Cytokinins	Colour of callus	Callus / Shoot Induction Frequency (%)
	IAA	BAP		
LEAF	0.5	0.5	Swelling	--
		1.0	Greenish	80.00
		1.5	Greenish	60.00
		2.0	Shoot	60.00
		2.5	Shoot	80.00
		3.0	Shoot	60.00
STEM	0.5	0.5	Swelling	--
		1.0	Brownish	40.00
		1.5	Whitish	60.00
		2.0	Whitish	60.00
		2.5	Shoot	60.00
		3.0	Shoot	80.00

• **a- Callus**

Mean values within columns followed by the same letter are not significantly different at 5% level.

BAP-containing medium and 90–100% in KIN-containing medium (Table 1). Substantial shoot proliferation was achieved within 30–45 days of culture. The number of shoots per explant varied from 40 to 46 on BAP-supplemented medium, with the maximum response observed at 15 µM BAP. In contrast, KIN produced 43–49 shoots per explant, with the highest multiplication recorded at 10–15 µM KIN. Shoot length ranged between 7.0 and 9.5 cm under both cytokinin treatments. The maximum shoot length (9.46 cm) was obtained on MS medium containing 15 µM KIN, while shoots cultured on 15 µM BAP attained an average length of 9.24 cm. These findings indicate that although both cytokinins effectively promoted shoot initiation and proliferation, KIN was comparatively more efficient than BAP in enhancing shoot multiplication from nodal explants. Similar genotype-dependent responses to cytokinins have been reported in several medicinal plants (Patnaik and Chand, 1996; Mohamed et al., 1999; Kumar et al., 1998; Sahoo and Chand, 1998; Velayutham, 2003; Baskaran and Jayabalan, 2005; Bhat et al., 2010). While BAP has been reported to induce superior shoot proliferation in many species (Kadota and Niimi, 2003; Martinussen et al., 2004; Vasudevan et al., 2004), the present investigation demonstrated that KIN produced a comparatively higher multiplication rate in *C. bulbosa* var. *lushi*, suggesting species-specific hormonal requirements.

Root Induction

Regenerated shoots were transferred to MS medium supplemented with different concentrations (5–25 µM) of IBA and 2,4-dichlorophenoxyacetic acid (2,4-D) for root induction. Root initiation was observed within 10 days of culture in all treatments (Fig. 2). However, the quality and quantity of roots varied depending on the auxin concentration.

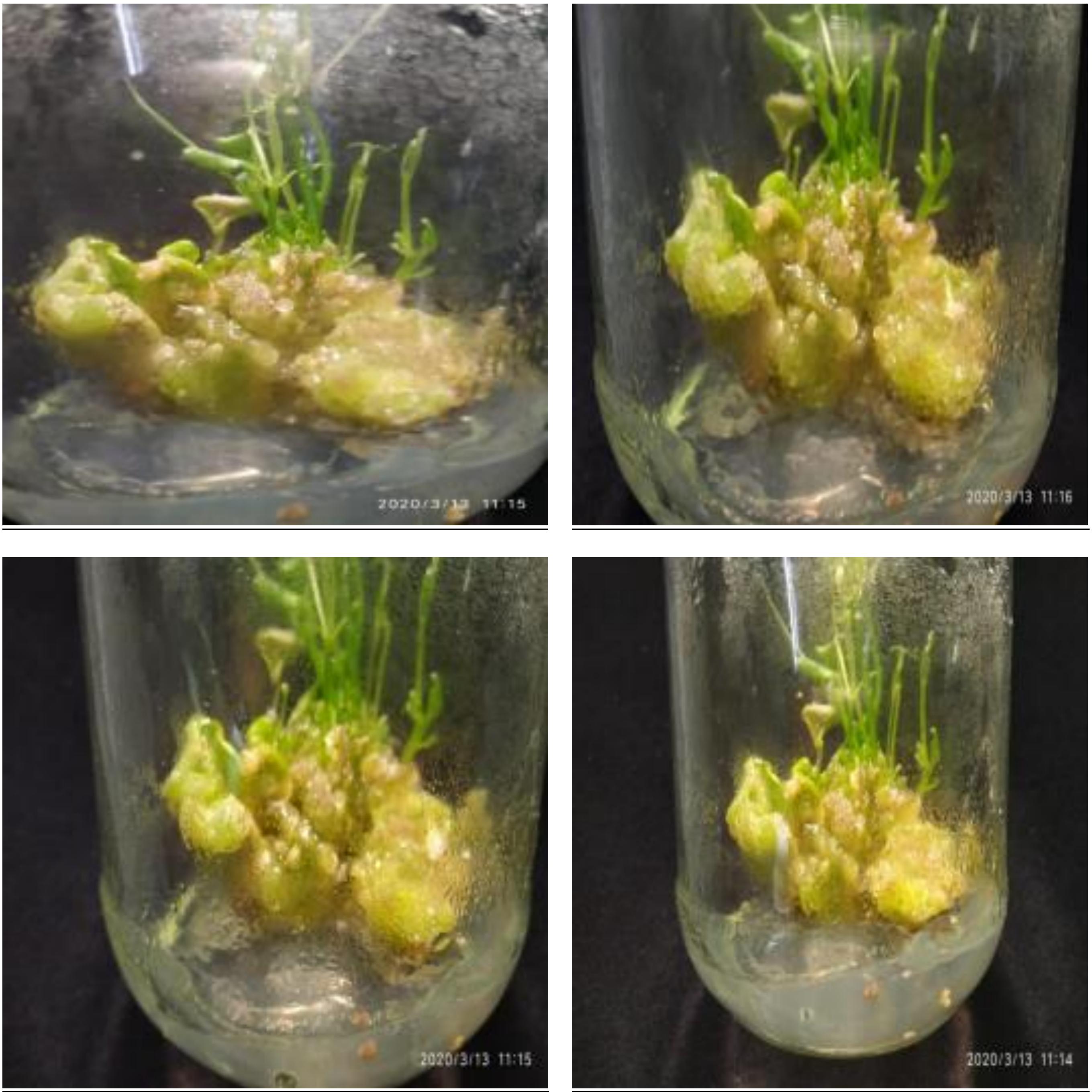

Figure-2. *In vitro* multiple shoot proliferation of *Ceropegia bulbosa* var. lushi on Murashige and Skoog (MS) medium supplemented with different concentrations of BAP and kinetin (KIN). Shoot multiplication on BAP-supplemented MS medium; shoot multiplication on KIN-supplemented MS medium.

The highest rooting response was obtained on medium containing 10–15 µM IBA, which produced a maximum of 47 roots per shoot with an average root length of 5.5 cm within 30 days. In comparison, 2,4-D induced fewer and less vigorous roots. Similar observations have been reported in *Aristolochia indica* (Manjula et al., 1997), *Gymnema sylvestre* (Komalavalli and Rao, 2000), *Avicennia marina* (Al-Bahrany and Al-Khayri, 2003), and *Eclipta alba* (Baskaran and Jayabalan, 2005), where IBA proved superior for rhizogenesis. Conversely, IAA and NAA have been reported to be more effective in species such as *Syzygium cumini*, *Cichorium intybus*, *Rubus chamaemorus*, *Plumbago zeylanica*, *Aerva lanata*, and *Solanum nigrum* (Bajaj and Gill, 1986; Jain and Babbar, 2003; Velayutham and Ranjithakumari, 2003; Martinussen et al., 2004; Suganya et al., 2005; Jabeen et al., 2005; Lunavath et al., 2013). The present findings indicate that IBA is the most suitable auxin for in vitro rooting of *C. bulbosa* var. *lushi*.

Hardening and Acclimatization

Well-rooted plantlets were successfully transferred to paper cups containing a sterilized potting mixture of red soil, farmyard manure, and sand in the ratio of 2:1:1 (Fig. 2). Following gradual acclimatization under greenhouse conditions, approximately 80% of the regenerated plantlets survived and established successfully under field conditions. During acclimatization, the regenerated plants developed healthy light-green tubers, which subsequently produced new shoots upon subculture, demonstrating the regenerative potential of the propagated plants. Similar observations on successful shoot proliferation and regeneration have been reported in *Tylophora indica* (Shah and Kapoor, 1976; Gupta et al., 2010; Swapna et al., 2024). Repeated subculturing of proliferating shoot cultures on hormone-free medium effectively maintained the juvenile nature of the explants and enhanced multiplication efficiency, corroborating earlier findings by Chaudhari and Jha (2004). The highest shoot multiplication was recorded during the third subculture cycle, indicating enhanced regenerative competence following repeated culture. Comparable regenerative responses from nodal explants have also been documented in *Plumbago indica* (Das and Rout, 2002; Namthabad et al., 2014). The optimized protocol established in the present study provides a rapid and reliable method for large-scale propagation and conservation of the threatened medicinal species *Ceropegia bulbosa* var. *lushi*.

CONCLUSION

The present study successfully established an efficient and reproducible protocol for the in vitro propagation of *Ceropegia bulbosa* var. *lushi*, a threatened medicinal plant of significant therapeutic value. Among the explants tested, nodal segments exhibited the highest regeneration potential, while MS medium supplemented with 1.6 mg L^{-1} BAP and 0.2 mg L^{-1} IBA proved optimal for multiple shoot induction and shoot proliferation. Callus induction was most effective on MS medium containing 0.5 mg L^{-1} BAP and 1.0 mg L^{-1} IAA, whereas efficient in vitro rooting was achieved using IBA-supplemented medium. Regenerated plantlets were successfully acclimatized with a high survival rate under field conditions, demonstrating the reliability of the developed protocol. The formation of healthy tubers during culture further confirmed the regenerative capacity of the propagated plants. This protocol offers a practical approach for rapid clonal multiplication, ex situ conservation, and sustainable utilization of *C. bulbosa* var. *lushi*. Furthermore, it provides a valuable platform for future studies on germplasm preservation, genetic improvement, and large-scale production of pharmaceutically important secondary metabolites.

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Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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Ethics Statement - NA

Informed Consent - NA

Data Availability
All data generated or analyzed during this study are included within this published article.

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