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Micropropagation and Secondary Metabolite Enhancement in Endangered Leguminous Climbers

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Abstract:

The growing need for plant-derived medicinal compounds has highlighted the urgency of preserving endangered medicinal plants, many of which are threatened by over-exploitation, habitat loss, and global warming. Of these, leguminous climbers such as Clitoria ternatea, Mucuna pruriens, and Abrus precatorius are of special significance owing to their high content of bioactive secondary metabolites like alkaloids, flavonoids, and terpenoids, which are in great demand in the pharmaceutical sector for their medicinal value. But the existence of these species is threatened, and thus there is a compelling need for efficient conservation measures. Micropropagation, a tissue culture technique, has also been identified as a viable option to overcome this problem by facilitating the quick, large-scale propagation of these climbers under in vitro conditions, thus providing genetic uniformity and minimizing the reliance on wild populations. In addition, improvement in optimizing micropropagation techniques not only favors sustainable propagation of these plants but also improves the production of secondary metabolites using elicitors, plant growth regulators, and environmental stresses, ultimately improving their medicinal value. This review explores different micropropagation methods and procedures to increase secondary metabolite yield in leguminous climbers, with special reference to the dual significance of these methodologies for maintaining biodiversity and fulfilling the increasing market demand for quality plant-based pharmaceutical products.

Keywords: Micropropagation, Endangered Leguminous Climbers, Secondary Metabolites, Conservation Strategies, Pharmaceutical Applications, Plant Tissue Culture

1. INTRODUCTION

The increasing need for plant-derived medicinal compounds has shed light on the conservation of medicinal plants, some of which are threatened by overexploitation, habitat destruction, and poor seed viability [1]. Leguminous climbers like Clitoria ternatea, Mucuna pruriens, and Abrus precatorius are prized for their varied pharmacological activities, among which is their rich diversity of secondary metabolites such as alkaloids, flavonoids, and terpenoids. Since these species are under threat of extinction, the necessity for proper conservation measures increases. Micropropagation, a method that entails the growth of plant tissues in a controlled environment,

provides an effective solution to grow these endangered plants quickly while ensuring genetic homogeneity. Apart from conserving these species, micropropagation methods can be engineered to increase the yield of secondary metabolites, which are responsible for their medicinal value [2]. This review discusses the techniques adopted for the micropropagation of the leguminous climbers and the strategies to enhance production of secondary metabolites, as well as their importance for conservation and commercialization. The amalgamation of the above techniques has the potential to preserve biodiversity while fulfilling the increasing needs of the pharmaceutical industry.

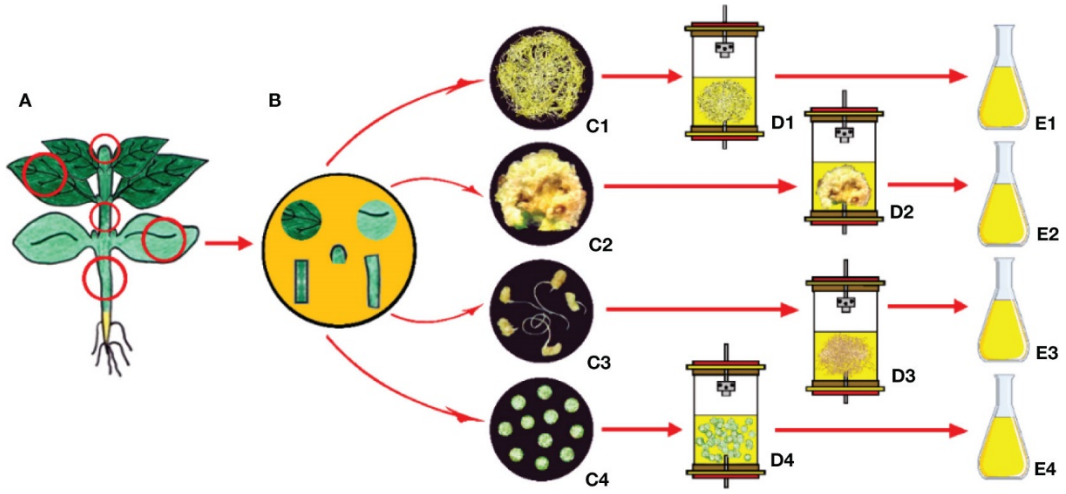

Figure 1: Production Of Secondary Metabolites [3]

1.1.Background Information and Context

Conservation and sustainable use of medicinal plants have increasingly become essential as a result of the growing need for plant-derived bioactive compounds and the high rate at which most species are facing extinction. Among these, leguminous climbers like Clitoria ternatea, Mucuna pruriens, and Abrus precatorius are of great medicinal importance because of their dense phytochemical content such as alkaloids, flavonoids, and other secondary metabolites [4]. Nonetheless, low seed viability, habitat loss, and overexploitation challenge their survival in the wild. Micropropagation, a novel plant tissue culture technology, holds the key here to the speedy and mass multiplication of these endangered plants for conservation as well as commercial production.

1.2.Objectives of the Review

This review aims to achieve several key objectives:

- To Explore and Optimize Micropropagation Techniques

- To Enhance the Production of Secondary Metabolites
- To Contribute to Conservation and Commercialization Efforts.

1.3.Importance of the Topic

The relevance of this issue is the fact that it serves a two-way purpose: supporting biodiversity preservation and fulfilling drug needs through sustainable processes. With the combination of micropropagation with secondary metabolite improvement, industries and scientists are able to devise effective protocols in conserving genetic resources and unleashing the maximal therapeutic values of these useful plants [5]. This method is responsible for the sustainability of medicinal plant conservation and production, providing a realistic solution to the current problems of environmental degradation and overharvesting of threatened species.

2. IN VITRO PROPAGATION AND METABOLITE ENHANCEMENT IN MEDICINAL PLANTS: PROGRESS AND CHALLENGES

Various studies have made progress in vitro culture methods for medicinal crops such as Clitoria ternatea, Mucuna pruriens, and Abrus precatorius by using nodal segment culture, somatic embryogenesis, and specially designed media formulations with growth regulators such as BAP and NAA to facilitate shoot and root development [6]. Such micropropagation methods allow large-scale cultivation and preservation, particularly of pharmacologically useful species. Moreover, secondary metabolite synthesis has been maximized through elicitation methods—methyl jasmonate and UV stress—eliciting the plant's defense system, which enhances yields of bioactive compounds such as alkaloids and phenolics. Even with the advantages of generating genetically uniform, high-quality plantlets and precious metabolites, there are challenges, such as somaclonal variation, variable metabolite yields, and scaling environmental controls [7]. Mitigation of these constraints is essential to the commercial potential and sustainable utilization of these biotechnological approaches.

Table 1: Summary of Literature on Medicinal Climber Conservation [8]

Author	Study	Focus Area	Methodology	Key Findings
Deepa and Thomas (2020) [9]	In vitro strategies for the conservation of Indian medicinal climbers	Conservation of endangered Indian medicinal climbers	Tissue culture-based techniques (organogenesis, somatic embryogenesis)	Highlighted the importance of controlled environments for genetic stability, emphasized organogenesis and somatic

				embryogenesis for conservation.
Gupta and Kumar (2022) [10]	Role of Biotechnology in Genetic Improvement of Clitoria ternatea	Genetic improvement and bioactive compound production in Clitoria ternatea	Somatic embryogenesis, callus culture, genetic manipulation	Demonstrated the role of biotechnology in enhancing growth, yield, and pharmacological value through genetic improvement.
Huy et al. (2018) [11]	Molecular biodiversity convergence with biogeography and ethnobotany of rare and endangered medicinal plants	Biodiversity, biogeography, and ethnobotany of medicinal plants from northern Vietnam	Molecular biodiversity analysis, ethnobotanical data integration	Identified high-value climbers at risk due to overharvesting and habitat destruction; suggested combining genetic and ethnobotanical data for conservation.
Isah (2020) [12]	Nodal segment explant type and preconditioning influence in vitro shoot morphogenesis in Ginkgo biloba	In vitro shoot morphogenesis and plant regeneration	Tissue culture, explant type and preconditioning treatments	Found that explant type and preconditioning conditions significantly impacted shoot regeneration in Ginkgo biloba, with implications for medicinal climbers.
Kanthaliya et al. (2021) [13]	Biology and biotechnological strategies for conservation management of	Conservation and management of Pueraria tuberosa, a	Micropropagation, cryopreservation, genetic improvement	Emphasized the importance of biotechnological strategies like micropropagation

	Pueraria tuberosa	medicinal liana		and cryopreservation for the sustainable propagation of Pueraria tuberosa.

2.1.Key Research Studies

Many researchers have concentrated on improving in vitro propagation methods for medicinal plants such as Clitoria ternatea, Mucuna pruriens, and Abrus precatorius. Clitoria ternatea has been efficiently propagated by nodal segment culture, which gives rise to homogeneous plantlets, and somatic embryogenesis has facilitated the regeneration of whole plants from somatic tissues [14]. The application of the different combinations of cytokinins (e.g., BAP) and auxins (e.g., NAA) has been found effective in increasing shoot proliferation and root development. In the same manner, Mucuna pruriens has also been aided through tissue culture techniques specific to initiate shoot and root growth in facilitating its mass cultivation because of its pharmacological activity. In addition, the impacts of media compositions, growth regulators, and culture conditions have been investigated for the propagation of this legume species. In Abrus precatorius, secondary metabolite production has been researched, showing that alkaloid production can be greatly increased through the application of stress conditions like UV light or methyl jasmonate treatment [15]. These approaches have been successful in boosting the yield of the bioactive compounds, pointing to the significance of secondary metabolite production optimization in mass production of the bioactive compounds in controlled environments.

2.2.Methodologies and Findings

Micropropagation methods mostly consist of axillary shoot proliferation, somatic embryogenesis, and callus formation. For axillary shoot proliferation, nodal explants are grown on growth media that have plant growth regulators like BAP and NAA to induce the formation of shoots, which can be further subculture for propagation [16]. Somatic embryogenesis allows the production of genetically uniform plants through the induction of somatic tissues to develop embryos, whereas callus formation comprises the growth of undifferentiated masses of cells from different plant tissues, which can subsequently be regenerated into plants under optimized conditions. In secondary metabolite synthesis, elicitation and environmental stress are crucial factors. Elicitation, either by biotic or abiotic elicitors such as fungal elicitors or methyl jasmonate, induces the defense process of the plant, increasing the yield of bioactive

metabolites such as alkaloids and phenolic compounds. Environmental conditions including light intensity, temperature, and media composition can also play a pivotal role in optimizing metabolite production, with decreased light intensity being found to increase phenolic compound production in Mucuna pruriens, adding to its medicinal value [17].

2.3.Critically Evaluating Strengths and Weaknesses

The main advantage of micropropagation is its potential to generate large quantities of genetically similar plantlets in a short time, an important aspect for the preservation of rare species and the large-scale production of high-quality plants, particularly in the case of rare species where seed propagation may be inefficient or not feasible. Despite that, micropropagation has its limitations, such as the possibility of somaclonal variation, which results in genetic inconsistencies that can influence the uniformity of the yields of plants, especially in commercial production [18]. Also, high shoot multiplication rates are challenging in some species, which makes scalability difficult, particularly among slow-growing species. In secondary metabolite increase, despite the potential of elicitation techniques to enhance metabolite levels, regulation of the environmental conditions to attain optimal levels is intricate and not easy to maintain at industrial levels. Secondary metabolite production is also subject to high variability, with varying plant cultures producing variable amounts and qualities of the desired product, which renders large-scale manufacturing uneconomical and unreliable. In general, although tissue culture and secondary metabolite improvement represent great potential, overcoming challenges for somaclonal variation, shoot multiplication, and environmental control will be necessary in order to best optimize these technologies for commercial production.

3. EXPANSION OF MICROPROPAGATION TECHNIQUES

Selection of explant and optimal culture conditions are critical during micropropagation, using nodal segments, shoot tips, and leaf fragments as frequently applied, backed by nutrient-rich culture media and critical combinations of plant growth regulators (PGRs) such as auxins and cytokinins to enhance development of shoots and roots [19]. Culture environment, including photoperiod, temperature (commonly 20–25°C), light intensity, and humidity, has to be tightly controlled to favor normal growth. Production of secondary metabolites is promoted by certain PGRs, elicitation methods (for instance, methyl jasmonate or fungal extracts), and stress factors such as changed levels of nutrients or light. Micropropagation also facilitates germplasm preservation, reintroduction of endangered species to native environments, and bioremediation, presenting useful instruments for ecological restoration and biodiversity conservation.

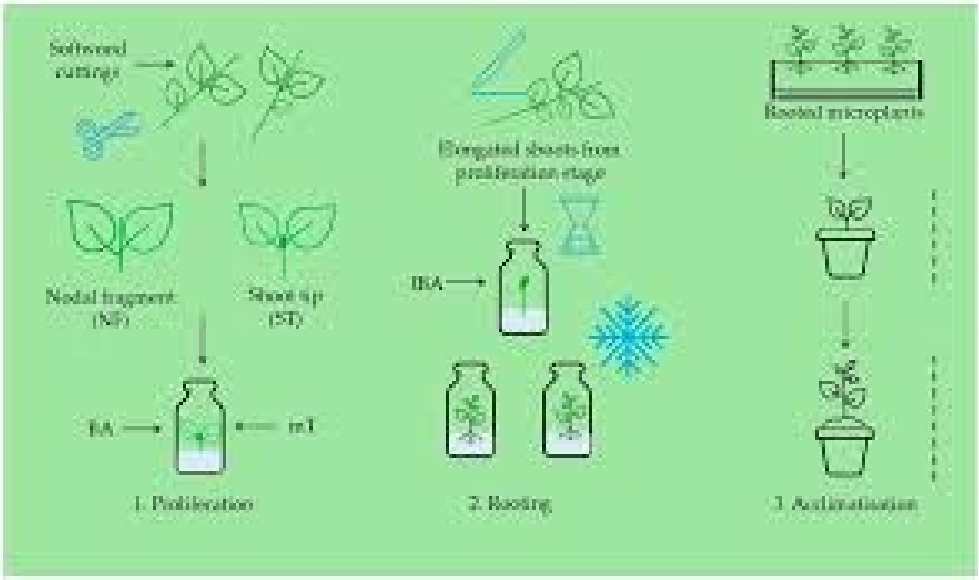

Figure 2: Micropropagation [20]

3.1.Explant Selection and Culture Conditions

Explant choice is the initial important step in micropropagation, as it can directly impact the success of the tissue culture process. In general, explants such as shoot tips, nodal segments, and leaf pieces are selected as they can initiate growth and differentiation under in vitro conditions [21]. Explant selection is based on the plant species, desired traits, and the objective of propagation, whether mass production or for gaining genetically uniform plants.

The culture medium should have all the necessary nutrients like carbon sources (e.g., sucrose), vitamins, amino acids, and macro and micro elements promoting plant growth. Plant growth regulators (PGRs), i.e., auxins and cytokinins, are also supplemented in certain concentrations to control cell division, differentiation, and morphogenesis. The PGR mixture is different in accordance with the plant species as well as tissue type being multiplied. For instance, auxins stimulate root growth, whereas cytokinins stimulate shoot growth [22].

3.2.Culture Environment

The environment is also important in micropropagation success. The photoperiod, temperature, and relative humidity within the culture chamber have to be regulated carefully to simulate ideal growing conditions for the targeted plant species. For example, most plants will need a 16-hour light/8-hour dark photoperiod for optimal growth, while others might have other light needs depending on where they grow naturally [23].

Light intensity is of special significance during shoot and root induction stages. Over-lighting can cause chlorosis to develop, whereas inadequate light may hamper photosynthesis and growth [24]. Temperature also has a marked influence on cell division and differentiation rates and should be kept in an optimal range—generally between 20°C to 25°C for most plants.

3.3.Enhancement of Secondary Metabolites

The yield of secondary metabolites in plants may be increased through optimizing the growth conditions and by the application of elicitation techniques [25].

1. **Plant Growth Regulators (PGRs)**: Apart from stimulating overall growth, certain PGRs may affect the production of secondary metabolites. For example, jasmonic acid or its derivatives are used to induce the production of secondary metabolites such as alkaloids, flavonoids, and terpenoids in some plant species. The ratio between auxins and cytokinins can also be adjusted to stimulate the production of certain metabolites of economic or medicinal importance.
2. **Elicitation Techniques**: To enhance secondary metabolite yield, abiotic or biotic stressors referred to as elicitors are usually employed [26]. Mechanical plant tissue damage or the use of chemical elicitors like methyl jasmonate can mimic stress, resulting in elevated secondary metabolite synthesis. Biotic elicitors like fungal extracts also stimulate plant defense mechanisms, stimulating their metabolic processes.
3. **Optimizing Culture Conditions for Secondary Metabolites**: Modified media or environmental stressors, including variations in light intensity, temperature, and nutrient supply, can also induce the production of secondary metabolites [27]. Reduction of nitrogen content in the culture medium, for instance, may enhance the accumulation of alkaloids in some plant species.

3.4.Applications in Conservation

1. **Germplasm Conservation**: Micropropagation also plays an important role in conserving threatened plant species by allowing the conservation of valuable genetic material. In vitro cultures permit the asexual multiplication of rare or threatened species without the problems related to seed-based propagation, such as seed dormancy or poor germinability [28]. In addition, cryopreservation methods can conserve plant tissue for long durations, allowing the maintenance of genetic diversity even in the presence of climatic changes or loss of habitats.
2. **Reintroduction into Natural Habitats**: Micropropagation success can also prove to be a crucial asset for habitat restoration work. In vitro-propagated plants can be reintroduced to their original habitats to restore the ecological balance [29]. It is particularly important for those species that are strongly threatened due to habitat loss or climate change. Tissue culture technology makes available high-quality, genetically variable plants for such restoration activities.
3. **Bioremediation and Ecosystem Services**: Apart from conservation, micropropagation can also be used in bioremediation ventures. There are some plants

that, once propagated and planted in polluted environments, tend to restore soil conditions by removing toxins or by stabilizing the ground [30]. In addition, the plants can participate in the revival of biodiversity through support for diverse ecosystem services, including pollination and soil health.

4. COMMERCIALIZATION AND SUSTAINABILITY IN BIOTECHNOLOGICAL PRODUCTION OF ENDANGERED CLIMBERS

The biotechnology-based mass propagation of vulnerable climbers using methods like micropropagation, somatic embryogenesis, and synthetic seed technology is a potential path towards conserving and marketing such plant species of high value [31]. Climbers are numerous which have medicinal, ornamental, or ecological values but are at risk owing to overharvesting, destruction of their habitat, and climatic fluctuations. Biotechnology allows the mass and fast propagation of climbers that are otherwise hard to propagate using conventional means, thereby providing a uniform supply of good-quality plant material for conservation and market needs.

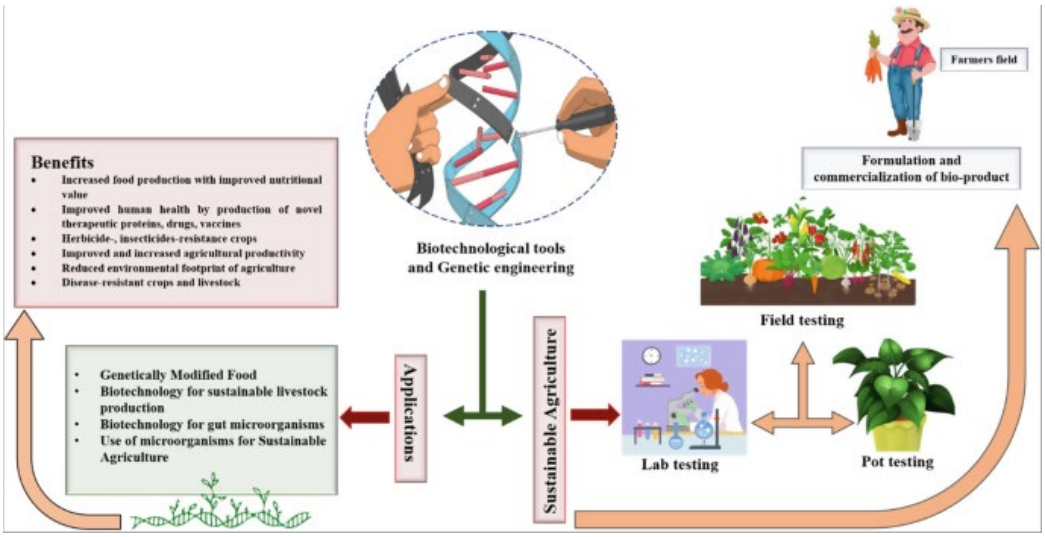

Figure 3: Biotechnological Tools and Applications in Sustainable Agriculture [32]

Commercialization of these biotechnologically grown climbers provides new avenues in the pharmaceutical, cosmetic, horticultural, and nutraceutical sectors. With increasing global demand for natural products and plant-derived medicines, threatened climbers like Tinospora cordifolia, Piper longum, and Gloriosa superba are of tremendous economic value. Tissue culture techniques enable the production of genetically homogeneous and disease-free plants, which are of quality standards for commercial purposes [33]. Furthermore, value-added products from these climbers—like herbal extracts, essential oils, and health supplements—can be processed, generating income streams for local communities and businesses.

But the process of commercialization should be compatible with sustainability values to avoid exploitation and ensure that these resources are available in the long term. Biotechnological

intervention should be complemented with conservation measures focusing on habitat conservation, in situ and ex situ conservation, and genetic diversity maintenance. Regulatory measures and ethical norms should control collection of germplasm, intellectual property rights, and benefit-sharing regimes to assure fair use and protection of biodiversity.

In addition, sustainable commercialization involves the creation of environmentally friendly cultivation procedures and community-based enterprise promotion. Through the use of local communities to cultivate and manage biotechnologically propagated climbers, conservation efforts can be complemented by socio-economic development. Education and capacity building programs can enable farmers and stakeholders to embrace biotechnology as well as maintain traditional knowledge and biodiversity. Therefore, if properly implemented, the biotechnological production of threatened climbers can be a model for sustainable development, reconciling ecological conservation with economic advancement [34].

5. DISCUSSION

The exploration of the findings concerning micropropagation and secondary metabolite enhancement in threatened leguminous climbers presents a number of key insights into the possibility of biotechnological interventions towards the conservation and commercialization of these precious species [35].

5.1. Interpretation and Analysis of Findings:

The in vitro culture methods of such species as Clitoria ternatea, Mucuna pruriens, and Abrus precatorius have proven remarkable advancement in generating genetically consistent plantlets via nodal segment culture and somatic embryogenesis [36]. Such methods make large-scale cultivation of endangered plants that are challenging to conserve via conventional seed practices possible. By maximizing growth conditions and plant growth regulators (PGRs) such as BAP and NAA, scientists have maximized shoot multiplication and root development. Moreover, elicitation methods involving agents such as methyl jasmonate and UV light have effectively increased the yield of bioactive secondary metabolites, including alkaloids, flavonoids, and terpenoids, which are important for pharmaceuticals [37]. Nonetheless, problems such as somaclonal variation, which triggers genetic inconsistencies, and scaling up production of secondary metabolites are not yet overcome. In addition to this, making these processes sustainable calls for reconciling commercial cultivation with the preservation of genetic diversity.

5.2. Implications for Conservation and Commercialization:

Micropropagation has great potential in the conservation of threatened leguminous climbers through fast multiplication, which ensures preservation of genetic material and facilitates

reintroduction of plants into natural habitats under threats from habitat loss and climate change. When combined with conservation measures like cryopreservation, tissue culture provides long-term conservation of genes for eventual restoration [38]. Commercially, the biotechnological multiplication of these climbers has potential for the pharmaceutical, nutraceutical, and cosmetic sectors, supplying high-quality, genetically similar, disease-free plants for production of medicinal and value-added products. Commercialization has to be carried out with caution so that there is no overexploitation, necessitating sustainable measures in sync with in situ and ex situ conservation. In addition, engaging local communities in cultivation and management can give economic rewards to conservation, enhance livelihoods, and maintain traditional agricultural knowledge while blending modern biotechnological approaches for better conservation strategies.

5.3. Future Directions and Challenges:

Although promising progress in micropropagation and secondary metabolite augmentation exists, numerous challenges exist in applying these methods on a larger scale. Non-predictable metabolite yields and the necessity for standardized elicitation methods are a major stumbling block to consistent and sustainable outcomes [39]. Besides that, handling somaclonal variation is essential in order to prevent genetic variability within propagated plants so that it has to be dealt with further using reliable methods of clonal propagation and genomic tools to ensure genetic integrity. Additionally, the environmental effect of bringing biotechnologically propagated plants into natural environments requires careful thought so as not to interfere with existing ecosystems [40]. In summary, though these biotechnological methods have great potential for conservation and commercialization, their long-term success will be a function of balanced strategy that unites sustainable methods, ethical considerations, and scientific research to secure the conservation of these precious plants and satisfy increasing demand for medicinal compounds from plants.

6. CONCLUSION

Micropropagation stands out as an effective and eco-friendly approach towards conservation and large-scale propagation of medicinally valuable leguminous climbers like Clitoria ternatea, Mucuna pruriens, and Abrus precatorius, which are highly prized for their medicinal significance secondary metabolites. The combination of more advanced tissue culture methods such as nodal culture, somatic embryogenesis, and callus induction with refined growth conditions and elicitor treatment has substantially boosted both plant recovery and bioactive compound yields. Although still-present challenges of somaclonal variation, lack of reproducibility in metabolite production, and scalability will eventually be overcome with further development of protocols and control of environments. Ultimately, this twofold strategy not only facilitates the conservation of endangered plant biodiversity but also contributes to

expanding pharma demand through sustainable cultivation of high-quality, plant-based therapeutic agents.

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