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Advances in propagation and conservation of medicinal species: A review

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ABSTRACT

As many medicinal species are on the verge of extinction from their natural habitats, due to global climatic change, over-exploitation, anthropogenic activities, destructive harvesting, natural calamities, low seed viability, pests and diseases etc. Hence, there is an urgent need for conservation of medicinal plants. This review examined the propagation of medicinal plants through sexual (seed), vegetative (stem and root cuttings), and *in vitro* micro-propagation methods. Considering this the current review reported that root and stem cutting treated with different plant growth regulators is the most effective practices for propagation of medicinal plants. Application of plant growth regulators has been shown to significantly improve propagation efficiency: IBA (1000 ppm) achieved 86–96% rooting and establishment, NAA and IAA (500–2000 ppm) yielded 70–90% rooting success, and GA_3 (200–1000 ppm) enhanced seed germination to 85–97%. Promoting propagation and *ex situ* conservation can help ensure sustainable supply, preserve genetic diversity, and support commercialisation of endangered medicinal plant species.

Keywords: Conservation, Dormancy, *In vitro* micro-propagation, Tissue culture

India has 28% of endemic plants, which is an exclusive feature of available plant diversity (pib.gov.in). Medicinal plants are traditionally used of herbal medicines which is documented in various Indian texts such as Ayurveda, Unani, Siddha and Homeopathy (Pal and Shukla 2003). As per National Medicinal Plants Board (NMPB), out of 1,178 pharmaceutical plants species are involved in global and domestic trade, with of 242 species have an annual demand of up to 100 Mt/year (Yadav 2019).

India harbours around 6,198 medicinal plant species, which are extensively utilized by traditional healers and local communities for treating a wide range of physical and mental ailments, preventing diseases, and supporting cultural and spiritual practices, highlighting their immense ecological, medicinal, and socio-economic importance (Kharat and Mokat 2020, Kharat and Mokat 2022). The herbal industry uses large number of phyto-chemical compounds (secondary metabolites) synthesized by medicinal plants. However, several medicinal plants are on the verge of extinction from their natural habitats due to overexploitation, anthropogenic activities and natural calamities (Chandrakar 2014, Ramakrishnan *et al.* 2017, FRLHT 2018, Kharat and Mokat 2020, Mokat and Kharat 2022**)**. Presently, 9,493 registered companies/units are dependent on the wild sources of medicinal plants for fulfilling their demands but 1,255 medicinal plants are listed as non-available from the wild source (Biswas *et al.* 2017).

The total of 335 medicinal plant species in India are currently classified as threatened, reflecting the significant pressure on their natural populations due to overexploitation and habitat loss (Shankar 2019). Presently 9,493 registered companies/units are dependent on the wild sources of medicinal plants for fulfilling their demands but 1,255 medicinal plants are listed as non-available from the wild source (Biswas *et al.* 2017). Thus, Indian medicinal plants continue to experience high demand both nationally and internationally, with certain species such as *Andrographis paniculata* (2,197 tonnes), *Withania somnifera* (9,12 tonnes), and *Saraca asoca* (10,724 tonnes), *Bacopa monnieri* (6,622 tonnes), *Ocimum tenuiflorum* (5,403 tonnes), *Tinospora cordifolia* (2,933 tonnes), *Phyllanthus amarus* (2,985 tonnes), *Santalum album* (1,073 tonnes), *Asparagus racemosus* (16,659 tonnes), *Rauvolfia serpentina* (589 tonnes), *Piper longum* (6,280 tonnes), *Decelepis hamiltonii* (200 tonnes) and *Hemidesmus indicus* (1,000 tonnes) with annual increment 20–25 % in average ranking among the most sought-after for their therapeutic and commercial value (Goraya and Ved 2017, Kharat and Mokat 2020).

The current herbal drug market in India is estimated at around ₹40 billion, and it is projected that demand will increase by approximately 16% over the next three to four years (Semwal *et al.* 2019). However, the present production

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of Ayurvedic herbs falls short of meeting this growing market requirement, creating significant supply gaps that have, in turn, led to instances of adulteration and compromise in the quality of products within the Ayurvedic drug supply chain, highlighting the urgent need for sustainable cultivation, quality control, and standardised propagation practices to ensure both the availability and authenticity of these highly valued medicinal resources (Kharat and Mokat 2020). The ever-increasing global demand for medicinal plant raw materials for traditional medicine, nutraceuticals, and pharmaceutical industries has exerted immense pressure on wild plant populations, often resulting in unsustainable harvesting practices that degrade natural habitats and accelerate the decline of many species (Somashekhar and Sharma 2002, Ved *et al.* 2020). This dependency on wild collection, coupled with limited commercial cultivation of high-value medicinal taxa, has further exacerbated the risk of local extinction and genetic erosion in several priority species (Sharangi 2018, Kala 2019).

Conservation and sustainable utilisation of medicinal plant genetic resources therefore require proactive strategies, including the development of effective propagation protocols, *ex situ* conservation through nursery production, and integration of propagation methods into formal cultivation systems to reduce pressure on natural populations (Ved and Goraya 2008, Jain and Mudgal 2010). Propagating prioritised medicinal plant species in well-managed nurseries not only preserves valuable genomic stocks but also generates readily available planting material for large-scale cultivation, thereby helping to shift production away from destructive wild harvesting and toward sustainable agro-based systems. However, the widespread adoption of medicinal plant cultivation has been hindered by a lack of comprehensive information on nursery management, propagation methods, and agro-techniques, limiting the establishment of profitable cultivation systems (Kirthikar and Basu 1980, Singh *et al.* 2017). Addressing these gaps through targeted research, extension, and policy support is essential to ensure long-term conservation, enhance livelihoods of local communities, and safeguard critical plant resources for future generations.

Although most industries actively approach farmers and tribal communities to obtain high-quality raw materials for medicinal plant-based products but, the desired quality and quantity are often not met due to the lack of standardized planting materials. Therefore, the development and implementation of effective propagation techniques to produce Quality Planting Materials (QPMs) have become highly essential, to ensure the availability of healthy, genetically uniform, and disease-free plants, support large-scale cultivation, reduce pressure on wild populations, and ultimately strengthen the supply chain for both domestic and commercial herbal industries.

The present review integrates experimental findings related to seed dormancy breaking, germination enhancement, adventitious rooting, survival rates, and the effect of plant growth regulators (PGRs) on propagation efficiency.

Propagation of medicinal plants: The continuous and rapidly growing interest in the commercialization of medicinal plants, combined with unskilled, unsustainable, and often irrational overharvesting practices, has severely impacted the natural populations of these valuable species, leading to decline in their numbers and pushing many into categories of endangered, vulnerable, and threatened status (Li *et al.* 2019). The rapid depletion of important medicinal plants from natural resources has been further exacerbated by escalating global demand, with numerous species now facing localized extinction due to overexploitation and habitat loss (Veeraiah and Reddy 2012, Pandey 2012). One of the most practical and sustainable approach to meet the rising demand for medicinal plants, while maintaining ecological balance, is to enhance their availability through well-planned and scientifically guided cultivation practices. By adopting the most effective propagation techniques whether through seeds (sexual propagation), vegetative cuttings, or *in vitro* (micropropagation) methods (Table 1) threatened and high-demand species can be conserved, their populations restored, and their supply made consistently accessible for medicinal, commercial, and research purposes. However, the success of widespread cultivation across managed landscapes relies heavily on a thorough understanding of species-specific seed dormancy patterns, growth requirements, and optimized propagation protocols that incorporate both conventional and biotechnological methods (Kala *et al.* 2020, Gupta and Verma 2022).

Techniques in sexual propagation: Propagation of medicinal plants through seeds is widely regarded as one of the most effective and natural methods for ensuring species survival and maintaining genetic diversity. Numerous studies have shown that seeds exhibiting physical dormancy often require specific pre-treatments to break dormancy and enhance germination. These treatments may include physical methods such as dry heat, hot or cold-water soaking, stratification, or freezing, as well as chemical treatments using plant growth regulators like gibberellic acid (GA_3), potassium nitrate (KNO_3), thiourea, and acids, among others. These pre-sowing treatments help overcome inhibitory seed coats, stimulate metabolic activity, accelerate germination time, and significantly improve germination rates, thereby facilitating successful cultivation and sustainable utilization of medicinal plant species (Karim *et al.* 2020, Rahimi and Afshari 2021).

Water is an important component in the external environment for the stimulation of seed sprouting. At room temperature, seed soaking in water helps remove inhibitors, soften the seed coat, and enhance germination percentage and speed (Hartmann and Kester 1979, Li *et al.* 2019). Ghosh and Sen (1988) reported that *Zizyphus mauritiana* seeds treated with 1% KH_2PO_4 improved the germination percentage up to 88%. Guava seeds before sowing soaked in water for 12 h gave 98% germination (Singh and Soni 1974). Naidu and Mastan (2001) reported that 79–81% germination was observed when *Pterocarpus santalinus* seeds were soaked in water for 6 days. Gupta (2003)

Tabel 1 Comparison of major propagation methods used in medicinal plants

Propagation method	Types and treatments	Advantages	Limitations	Applications
Seed propagation (Sexual type)	Water soaking, hot water treatment, acid scarification, GA_3 treatment	Maintains genetic diversity; low cost; simple	Seed dormancy; low viability; genetic variability	Species with viable seeds and large-scale cultivation
Vegetative propagation (stem/root cuttings) (Asexual type)	Hardwood, semi-hardwood, softwood cuttings treated with IBA, IAA, NAA	True-to-type plants; rapid multiplication; uniformity	Species-specific hormone optimization required; seasonal dependency	Commercial multiplication and conservation of elite genotypes
Micropropagation (tissue culture) (*in vitro* type)	MS medium supplemented with BAP, IBA, NAA, KIN	Mass multiplication; disease-free plants; year-round production	High cost; technical expertise required; soma-clonal variation risk	Rare, endangered, and high-value medicinal plants

reported that *Rauvolfia serpentina* seeds soaked in cold water overnight gave 86% seeds germination while 40% seed germination was observed in control. According to Srivastava *et al.* (2011), seeds of *Aconitum heterophyllum* treated with IAA (500 ppm) recorded high seed germination (97.17%). Rasebeka *et al.* (2014) observed that concentrated H_2SO_4 and hot water treatment for three species, viz. *Acacia erioloba, A. tortilis* and *A. nigrescens* were significant for increasing seed germination. Amusa (2011) found significant result in cold water pre-treatment for *Afzelia Africana* while *Pongamia pinnata* seeds soaked in hot water for 30 min were documented germination percentage up to 100% (Kumar 2007).

Recent research has highlighted the effectiveness of various pre-sowing seed treatments whether in hot and cold water enhancing germination and overcoming seed dormancy across a range of medicinal plants, thereby aiding mass propagation efforts (Venudevan *et al.* 2025).

Renard and Clerk (1978) reported that *Impatiens balsamina, Brassica rapa, Lavendula unguistifolia* and *Viola odorata* seeds were treated with GA_3 gave excellent germination. *Eucalyptus* seeds gave well sprouting and vigour index after treated with GA_3 at 100 ppm (Bhattacharya *et al.* 1991). Vanangamudi and Vanangamudi (2003) found that IAA and succinic acids were very effective for enhancing seed germination and vigour of *Tamarindus indica*. The treatment of KCl on *Medicago sativa* seeds were recorded up to 96% germination (Azhdari *et al.* 2010). Patil *et al.* (2018) investigated that *Syzygium cuminii* seeds treated with water soaked for 24 h boiling water (100°C) for 5 sec, GA_3 at 200 mg/L for 10 min, KNO_3 at 0.5% for 10 min and thiourea at 0.5% for 10 min resulted in very high percentage of seed germination.

Recent research has reinforced the effectiveness of stratification and combined treatments for difficult-to-germinate medicinal seeds. For example, cold stratification followed by GA_3 treatment significantly improved germination in *Angelica sinensis* and *Dendrobium* spp. (Zeng *et al.* 2019, Xu *et al.* 2021). Similarly, pre-sowing soaking with KNO_3 and thiourea enhanced germination in *Nigella sativa* and *Withania somnifera* compared to water treatment alone (Kumar *et al.* 2020, Sharma and Singh 2022). These studies underscore the importance of tailoring seed pre-treatments to species-specific requirements to achieve optimal propagation outcomes.

Kundu *et al.* (2020) reported that *Saraca asoca* seeds do not exhibit dormancy and are capable of germinating across a wide temperature range of 15–50°C, with optimal germination occurring over a period of 25 days at 30–50°C; however, seeds exposed to temperatures below 30°C showed reduced germination rates, which were influenced by factors such as temperature fluctuations, seed moisture content, and the level of seed maturity. Proper storage of seeds at 15°C with controlled moisture content was found to significantly extend their viability for up to four years, highlighting the importance of understanding species-specific physiological requirements for successful propagation and long-term conservation. Venkatesh *et al.* (1997) reported that seeds of *Pongamia pinnata* soaked in 100 ppm IBA for 12 h gave 66.7% germination and maximum root length. Misiha and El-Ashry (1991) noted the effect of H_2SO_4 and GA_3 on seed germination of *Magnolia grandiflora*. The maximum seed sprouting percentage was observed in seeds treated with 1000 ppm of GA_3. Somashekhar and Sharma (2002) reported that *Boerhavia diffusa, Cassia absus, Catharanthus roseus, Eclipta prostrata, Phyllanthus amarus, Plumbago rosea, Plumbago zeylanica, Psoralea corylifolia, Achyranthes aspera, Solanum nigrum* etc. These medicinal plant's seeds gave 60–90% germination within 4–12 days. Lalithkumar (2008) reported that *Withania somnifera, Ocimum sanctum, Andrographis paniculata* and *Catharanthus roseus* plant's seeds treated with GA at 250 ppm have given 77.9%, 74.9%, 82.0% and 71.0% germination respectively.

Sedghi *et al.* (2010) reported that the soaking of *Andrographis paniculata* seeds in 200 ppm GA_3 improved seed germination by 89%. Salvi *et al.* (2015) also found significant results of GA_3 which showed minimum number of days required for seed germination in *Rauvolfia serpentina.* Madhushree *et al.* (2018) documented that *Saraca asoca,* seeds were treated with GA_3 200 ppm and highest germination rate was 2.95%. Gupta (2003) obtained

92% seeds germination in *Embelia ribes* which were treated with GA_3 100 ppm while 48% seed germination in control.

Aparna *et al.* (2018) noted in *Embelia ribes* that the seeds over control treated with GA_3 750 ppm showed maximum germination percentage and seeds treated with GA_3 750 ppm showed maximum seedling vigour. *Podophyllum hexandrum* seeds treated with GA_3 (Gibberellic acid) 200 ppm helped to break seed dormancy (Kharkwal *et al.* 2008). Masoodi *et al.* (2000) also indicated that *Ulmus wallichiana* seeds treated with GA_3 100 ppm showed 71% germination while 48% germination as matched to control. Phatak *et al.* (2017) studied the different concentrations of GA_3 treatments for *Rauvolfia* and recorded best results on seed germination in GA_3 (1000 mg/L) treatment.

Vegetative propagation: Vegetative propagation plays a crucial role in the domestication and large-scale cultivation of plants, offering an effective alternative to seed propagation for species that are difficult to grow from seeds or have low germination rates. Vegetative propagation is widely recognized as a common and highly effective practice for the large-scale multiplication of medicinal plants, as it allows for the rapid production of genetically uniform and high-quality planting material. It also preserves the desirable traits of parent plants, reduces the dependency on seeds which may have low viability or dormancy issues and plays a vital role in meeting the increasing domestic and international demand for medicinal species while simultaneously contributing to their conservation, sustainable cultivation, and long-term availability for pharmaceutical, traditional, and commercial applications (Arumugam *et al.* 2020).

Through stem and root cuttings (Asexual propagation): Among the various vegetative techniques, cutting is one of the most widely employed methods due to its simplicity, cost-effectiveness, speed, and convenience, allowing for the rapid multiplication of a wide range of plant species while ensuring uniformity, maintaining the desirable traits of the parent plant, and facilitating the conservation and commercial production of medicinal, horticultural, and economically important plants (Wilson 1993). Stimulation of adventitious rooting to stem cuttings through application of auxins is a powerful tool in many forest plant species. The exogenous application of auxins promotes rooting in stem cuttings in several species (Hartmann *et al.* 2007). Somashekhar and Sharma (2000) reported in *Bacopa monnieri, Coleus forskohlii, Plumbago rosea, Plumbago zeylanica* the propagation by stem cuttings and *Acorus calamus, Curculigo orchioides* through rhizomes, *Aloe barbadensis, Eclipta prostrata, Chlorophytum borivilianum* propagated through root sucker were highly successful.

Ghasolia *et al.* (2013) developed a novel approach for propagating the medicinal plant *Bacopa monnieri* (Brahmi) using cyanobacterial media. Cyanobacterial extracellular products (CEP) significantly improves shoot proliferation, rooting, chlorophyll content, and nutrient accumulation in brahmi compared to conventional media, while also evaluating the effects of various plant growth regulators for efficient and cost-effective mass propagation.

Stem cuttings are classified into different types namely hardwood, semi-hardwood, softwood and herbaceous etc. based on their physical stage of development of the wood, from which they are prepared. Hardwood cuttings showed maximum redevelopment ability as compared to semi-hardwood cutting and softwood cutting (Waman *et al.* 2019). Mokat *et al.* (2022) reported that the propagation *Rauvolfia serpentina* (L.) Benth. ex Kurz through stem and root cuttings is an effective method for conserving this valuable species, as it not only facilitates the rapid multiplication of healthy and genetically uniform plants but also helps reduce pressure on wild populations, ensures the availability of quality planting material for medicinal and commercial use, and provides a practical approach to *ex situ* conservation, supporting both sustainable cultivation practices and long-term preservation of its genetic resources. Silva *et al.* (2020) investigated the effects of different types of stem cuttings as well as varying light intensities on the vegetative propagation of *Aloysia citriodora*, revealed that hardwood cuttings exhibited the highest survival and rooting rates compared to softwood and semi-hardwood cuttings, regardless of the light conditions, highlighting the importance of selecting the appropriate cutting type for successful propagation, improving plant establishment, and ensuring the large-scale cultivation and conservation of this medicinally and commercially valuable species. Madhushree *et al.* (2018) reported that the *Saraca asoca,* plant hard wood cuttings treated with IBA 2000 ppm showed the 33.41% positive effect on rooting and 33.70% on sprouting.

Micro-propagation technique through tissue culture: The Murashige and Skoog (MS) medium (1962) has become one of the most widely used and foundational culture media in various plant tissue culture techniques due to its comprehensive nutrient composition that supports growth and development of a diverse range of plant species; however, the success and responsiveness of tissue culture often vary significantly among species, as recalcitrance in culture can be influenced by multiple factors, including the type of plant, the use of specific organic extracts, and the nature, combination, and concentration of growth regulators or hormones applied, all of which critically determine the efficiency of callus induction, shoot regeneration, root formation, and overall micropropagation outcome (Erica 2000).

Reddy *et al.* (2001) reported that *in vitro*-derived shoots cultured on MS medium supplemented with auxins such as indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) successfully induced root formation, with IBA proving to be particularly more effective in promoting robust root development, thereby demonstrating its critical role in enhancing the efficiency of micropropagation, supporting the establishment of healthy plantlets, and facilitating the large-scale clonal multiplication of medicinally and economically important plant species through tissue culture techniques. Molnar *et al.* (2011) reported that plant cell and tissue culture involve growing isolated plant parts on a nutrient medium that substitutes for their natural environment. The

Table 2 Vegetative propagation techniques in medicinal Plants

Plant species	Method	Growth Regulator	Rooting/ sprouting Response	Reference
Bacopa monnieri	Stem cuttings	Cyanobacterial media/ PGRs	Improved shoot proliferation, rooting, chlorophyll	Ghasolia *et al.* (2013)
Saraca asoca	Stem cuttings	IBA 2000 ppm	33.41% rooting, 33.70% sprouting	Madhushree *et al.* (2018)
Premna integrifolia	Stem cuttings	IBA 1000 ppm	86% rooting, 96.5% field establishment	Sharma *et al.* (2011)
Hemidesmus indicus	Stem and root cuttings	IAA 2000 ppm	Moderate rooting (~60%), high sensitivity to auxin levels	Murthy and Pullaiah (2005)
Tinospora govaniana	Rhizome cutting	NAA 100–250 ppm	71.4–90.4% rooting, 85–100% survival	Chauhan *et al.* (2020)
Tinospora cordifolia	Stem cuttings	IBA 100–500 ppm	Good rooting at 100 ppm	Rakshe and Mokat (2018)
Berberis aristata	Stem cuttings	IBA 2500–7500 ppm	Enhanced rooting, high uniformity	Ali *et al.* (2008)
Carissa edulis	Stem cuttings	IBA 100–500 ppm	Good rooting	Pascaline *et al.* (2010)
Asystasia schimperi	Stem cuttings	IBA 100–500 ppm	Good rooting	Pascaline *et al.* (2010)
Toddalia asiatica	Stem cuttings	IBA 100–500 ppm	Good rooting	Pascaline *et al.* (2010)
Salacia fluminensis	Stem cuttings	IBA 100 ppm	80–85% rooting	Soares *et al.* (2011)
Salacia chinensis	Stem cuttings	IAA 1000–5000 ppm	Maximum rooting at 5000 ppm	Mokat *et al.* (2012)
Hibiscus sabdariffa	Softwood and semi-hardwood cuttings	IBA / NAA	Rooting improved by auxin treatment	Govinden-Soulange *et al.* (2009)
Salacia oblonga	Stem and root cuttings	IBA 200–300 ppm	Maximum shoot/root response	Deepak *et al.* (2016)

success of these cultures depends on factors such as the choice of explant, the composition of the medium, and the physical conditions. Since fully synthetic media do not always support optimal growth, natural substances can enhance culture performance. Extracts and biomass from algal strains, including those from the Mosonmagyaróvár Algal Culture Collection (MACC), have been found to stimulate cell growth and plant regeneration. Using these natural compounds can therefore improve the efficiency of *in vitro* plant culture across different species.

According to Chauhan *et al.* (2020), among the various explants of *Trillium govanianum* cultured on MS medium only shoots tip and complete shoot buds showed effective *in vitro* regeneration by producing shoots and callus-like structures. Isah and Umar (2020) reported that while the seeds of *Chonemorpha fragrans* can be utilized for clonal propagation, the species exhibited very slow growth and poor sprouting capacity, and its low rooting efficiency significantly limits successful asexual propagation through conventional stem cuttings; as a result, *in vitro* propagation has emerged as a practical and effective alternative, providing a reliable means for rapid clonal multiplication, ensuring the production of healthy and uniform plantlets, and offering a valuable approach for large-scale cultivation, conservation of genetic resources, and sustainable utilization of this medicinally important but propagation-challenged species.

Komalavalli and Rao (2000) reported that *in vitro* propagation of *Gymnema sylvestre* is significantly influenced by explant type, seedling age, culture medium, growth regulators, organic additives, and antioxidants. Maximum shoot multiplication was achieved using 30-day-old axillary node explants on MS medium supplemented with BA, kinetin, NAA, malt extract, and citric acid, while rooting was most effective on half-strength MS medium with IBA. The regenerated plantlets were successfully acclimatized to soil, demonstrating the effectiveness of optimized tissue culture techniques for large-scale propagation and conservation of this medicinal plant.

Mehalaine and Chenchoun (2021) highlighted the urgent need for alternative propagation strategies due to the overexploitation of medicinal plants, focusing on four Lamiaceae spp. (*Thymus algeriensis*, *Rosmarinus officinalis*, *Marrubium vulgare*, and *Salvia officinalis*) in Algeria. Their study demonstrated that micropropagation using 10–15 cm cuttings under greenhouse conditions was highly effective for *S. officinalis* with 100% regeneration, while other species showed lower success rates. *In vitro* micropropagation on MS medium supplemented with IAA and Kinetin significantly enhanced callus formation and shoot regeneration in *T. algeriensis*, *R. officinalis*, and *M. vulgare*, although organogenesis remained limited, and *S.*

officinalis showed no *in vitro* organogenic response. The findings suggested that micropropagation is the most effective approach for *S. officinalis*, whereas *in vitro* techniques offer a viable option for conserving and propagating the other species. Overall, the study underscores the importance of combining conventional and biotechnological methods to ensure sustainable production, conservation, and potential pharmaceutical use of medicinal plants.

Asexual propagation has proven to be one of the most effective techniques for the multiplication of *Stevia* plants, particularly through the use of stem cuttings, as it allows for the rapid production of genetically uniform and high-quality plantlets; studies have shown that micropropagation can achieve up to 85% success in generating healthy plantlets, while conventional stem cuttings result in approximately 60% propagation efficiency, and seed-based propagation remains comparatively lower, ranging between 25.5% and 40%, highlighting the superiority of asexual and tissue culture methods in ensuring faster, reliable, and large-scale propagation of this economically and medicinally important species (Ma Claudia *et al.* 2020).

Plant cells are inherently totipotent, meaning they have the capacity to develop into complete plants through embryo formation. Plant tissue culture takes advantage of this totipotency by growing plant cells *in vitro* on synthetic media, supplemented with plant growth regulators, including natural hormones and their synthetic analogues, to control and promote growth as needed. Research has shown that *in vitro* culture, especially micropropagation, is highly effective for medicinal plants, facilitating rapid plant multiplication, increased production of valuable secondary metabolites, and the conservation of diverse species, including rare, threatened, and endangered plants. While *in vitro* propagation offers significant advantages and wide-ranging applications in both research and commercial production, many medicinal plants still lack fully optimized and standardized protocols, which restricts their large-scale commercialization (Singh and Singh 2021).

Bais *et al.* (2000) studied *Decalepis hamiltonii* plant axillary bud culture produced maximum number of 12.8 shoots on MS + BA 8.87 mM + NAA 2.69 mM. The micro-shoots promoted maximum roots for treatment of 40 pMAgNCb and may be inhibition of ethylene action for promotive effects of AgNC>3 on rooting. Sudha and Seeni (2001) studied *Decalepis arayalpathra* leaf and inter-nodal ex-plants of *in vitro* elevated shoot cultures in MS medium with 2.5 mg/L 6- BA, 0.5 mg/L 2-ip and 0.5 mg/L NAA. Shoot cultures were well-kept on MS agar medium combined 0.5 mg/L BA, 0.05 mg/L 2-ip and 0.05 mg/L NAA. Incubated in entire darkness in partial forte MS medium added with 0.5 mg/L IBA and 0.2 mg/L NAA preferred initiation of roots in 89% of the cultures on leaf explants.

Arumugam and Thirumal (2011) investigated the *in vitro* micropropagation of *Hemidesmus indicus* by using nodal and leaf explants. Nodal and leaf explants produced callus on media containing 2,-D, 2,4,5-T, 2ip, NAA, BAP, IAA and KIN. The maximum frequency of callus was present in the leaf ex-plants treated with IAA, NAA and IBA. Rhizo-genesis was detected in leaf ex-plants ample in auxin treatment, viz. 2,4-D and 2,4,5-T and shoot was induced from the callus of nodal ex-plants on MS medium with 2,4,5-T, KIN and BAP. Gopinath *et al.* (2014) reported that when axillary buds of *Artemisia annua* were cultured on MS medium supplemented with 1.5 mg/L BAP and 0.5 mg/L NAA, they exhibited efficient and well-organized shoot initiation, and subsequent addition of 1.0 mg/L IBA to the medium facilitated strong and structured root development, enabling the formation of healthy, robust plantlets which, when acclimatized and transferred to field conditions, achieved a high survival rate of 85%, demonstrating the effectiveness of this *in vitro* propagation protocol for large-scale multiplication and potential commercial cultivation of this medicinally important species.

Tripterygium wilfordii explants, cultured on a MS medium containing with BAP (2.0 mg/L) and NAA (0.1 mg/L) obtained new axillary shoots. Cultured on MS medium with IBA (0.2 mg/L) and activated charcoal (0.5 mg/L) were shoots rooted more than 92% reported by Chen (2009). Govinden-Soulange *et al.* (2009) reported that in the *in vitro* culture of *Hibiscus sabdariffa*, nodal explants grown on MS medium supplemented with BAP and KIN successfully initiated multiple shoots, and when individual shoots containing two nodes were separated, rooted, and gradually acclimatized on MS medium as well as a 1:1 mixture of soil and sterile peat, they exhibited vigorous growth within three weeks and developed into robust plantlets that became fully suitable for transplantation under field conditions within five to six months, demonstrating the efficiency of this tissue culture protocol for large-scale propagation and potential commercial cultivation of this medicinally and economically important species. Dakah *et al.* (2014) found that *in vitro* propagation of *Ziziphora tenuior* cultured on MS medium supplemented with growth hormones such as NAA or IBA in combination with 0.5 mg/L kinetin resulted in a high frequency of callus formation, reaching 70.2%, indicating the effectiveness of this hormonal treatment in stimulating undifferentiated tissue growth and providing a reliable method for large-scale propagation and conservation of this medicinally important species.

Lucas aspera explants cultured on MS medium, when supplemented with cytokinins such as BAP, kinetin, or 2-iP at concentrations ranging from 0.5–5.0 mg/L, either alone or in combination with auxins like IAA, IBA, or NAA at 0.1–1.0 mg/L, demonstrated significant potential for shoot regeneration, highlighting the critical influence of both the type and concentration of plant growth regulators on *in vitro* morphogenesis, as well as the synergistic effects of cytokinins and auxins in promoting multiple shoot induction, elongation, and overall regeneration efficiency in this medicinally important species. The explants induced multiple shoots in higher frequency on MS medium with 1.5 mg/L BAP and 0.1 mg/L NAA and highest frequency of rooting was observed on MS medium with 0.1 mg/L IBA (Vijendra *et al.* 2017). Srikun (2017) reported *Strobilanthe*

stonkinensis Lindau shoot explants were sterilized by concentration NaOCl of 1.2% for 10 min and 0.6% for 15 min, which produced 70% good developing vigorous shoots. Shoot reproduction was the best on MS medium combination with 16 mM BA for 4 week obtained highest shoot elongation. High frequency rooting was obtained on MS medium with 7.5 mMIBA. Plantlets were transferred to pots and this plantlet 100 % survival.

Role of plant growth regulators in propagation: Plant growth regulators (PGRs) are essential for optimizing propagation, whether for rooting, shoot multiplication, or breaking seed dormancy. Auxins like IBA, IAA, and NAA are primarily used for rooting, while cytokinins like BAP and kinetin stimulate shoot proliferation. Gibberellins such as GA_3 enhance seed germination and seedling vigour.

An experiment was conducted by Sharma *et al.* (2011) to evaluate the effect of different plant growth regulators on the rooting of *Premna integrifolia* cuttings, a rare medicinal species. Cuttings treated with growth regulators showed significantly improved root and shoot development compared to the untreated control. The highest rooting percentage (86%) was observed in cuttings treated with 1000 ppm IBA, compared to 67.66% in the control. Other growth parameters, including the number of roots per cutting, fresh and dry root weight, sprouting percentage, and field establishment, were also highest in cuttings treated with 1000 ppm IBA (96.5%), followed by those treated with 2000 ppm IBA (93.2%).

Rao *et al.* (2000) observed enhanced rooting in *H. indicus* when treated by using the 'quick dip' method in different concentrations of rooting hormones, viz. IBA, IAA and NAA. Root growth was very slow without hormone treatments. Vegetative propagation of *Hemidesmus indicus* through stem and root cuttings has generally proven to be unsatisfactory, even after the application of various treatments, as the species exhibits low rooting and poor shoot development; experimental studies in which stem cuttings were briefly dipped for 2 min in different concentrations of auxins such as IAA, IBA, and NAA (ranging from 500–5000 ppm) revealed that only IAA at 2000 ppm was effective in inducing a moderate rooting response of approximately 60%, while higher concentrations of auxins tended to inhibit root initiation, underscoring the species-specific sensitivity to hormone levels and highlighting the need for careful optimization of propagation protocols to achieve successful asexual multiplication and sustainable cultivation of this medicinally valuable plant (Murthy and Pullaiah 2005).

The study by Chauhan *et al.* (2020) investigated the asexual propragation in *T. govanianum* using the whole rhizomes with incissions at apical, middle and distal sections of rhizomes. The result showed that rhizomes with apical portion showed good survival efficiency (71.4–90.4%) when treated with NAA 250 ppm. However, the whole rhizomes with incission at apical portion showed comparatively higher survival efficiency (85.7–100%) when treated with NAA 100 ppm Rakshe and Mokat (2018) studied that *Tinospora cordiflia* and *Tinospora sinesis* stems cuttings treated with IBA 100, 200, 300, 400 and 500 ppm and control, good results were observed in control and 100 ppm.

Ali *et al.* (2008) reported that the application of different concentrations of indole-3-butyric acid (IBA), specifically 2500, 5000, and 7500 ppm, had a significant positive effect on the vegetative propagation of *Berberis aristata*, enhancing rooting success in cuttings taken from various regions of the plant, including apical, sub-apical, and basal sections, thereby demonstrating the critical role of auxin concentration and cutting position in improving rooting efficiency, ensuring the production of healthy and uniform plantlets, and supporting large-scale cultivation and conservation efforts for this medicinally important species. Endangered plants such as *Carissa edulis, Asystasia schimperi* and *Toddalia asiatica* when propagated by stem cuttings treated with different concentrations of IBA (100-500 ppm) showed good results (Pascaline *et al.*2010).

Soares *et al.* (2011) studied the *S. fluminensis* the stem cuttings treated with IBA at 100 ppm and observed best rooting percentages of 80% at 20–30ºC under as well as 85% at 30ºC in the dark. Govinden-Soulange *et al.* (2009) studied the *Hibiscus sabdariffa* softwood and semi hardwood cuttings by using medium containing soil, compost and rocks and after dipping in IBA, NAA reported rooting more effective in cuttings treated with auxins. *Salacia chinensis* stem cutting treated with Kerdix and IAA with different concentrations of 1000, 2000, 3000, 4000 and 5000 ppm noted significant results on stem cutting treated with IAA 5000 ppm (Mokat *et al.* 2012).

Ayanoglu *et al.* (2008) investigated that propagation of plants by using stem cuttings in *Salvia indica*, *Helichrysum plicatum* subsp. *plicatum* DC. and *Satureja thymbra* using different concentrations of IBA were very successful. Similar were the results in *H. plicatum* stem cuttings: 100, 250, 500, 1000 and 2000 ppm and 100, 250, 500, 1000, 2000 and 4000 ppm for *Salvia indica* and *Satureja thymbra.* The good rooting ratios were observed in 250 and 1000 ppm IBA for *H. plicatum,* 1000 and 2000 ppm IBA for *S. thymbra* and *S. indica*. Deepak *et al.* (2016) studied vegetative propagation of *Salacia oblonga* by using roots and stems with leaves and without leaves with different concentrations of IBA (0–500 ppm). The ex-plants of stem with leaves and without leaves showed maximum shooting response with 300 ppm IBA and explant roots presented highly response with 200 ppm IBA.

The rapid commercialization and escalating demand for medicinal plants have placed immense pressure on natural populations, leading to habitat loss, declining genetic diversity, and increasing numbers of threatened species. India's rich repository of medicinal flora, deeply embedded in traditional healthcare systems and cultural heritage, requires urgent and strategic conservation interventions. Sustainable utilization cannot rely solely on wild harvesting; instead, it must be supported by scientifically validated propagation techniques and organized cultivation practices.

Seed propagation, when supported by appropriate

Table 3 Comparative role of plant growth regulators in medicinal plant propagation

Growth regulator	Category	Primary function	Reported effects
IBA (Indole-3-butyric acid)	Auxin	Root induction	Enhanced rooting percentage and root length
IAA (Indole-3-acetic acid)	Auxin	Rooting and seed germination	Moderate rooting response; improves germination
NAA (Naphthalene acetic acid)	Synthetic auxin	Root and callus induction	Callus formation; rhizogenesis
GA_3 (Gibberellic acid)	Gibberellin	Breaking seed dormancy	Increased germination percentage and seedling vigour
BAP (6-Benzylaminopurine)	Cytokinin	Shoot multiplication	Multiple shoot induction
Kinetin (KIN)	Cytokinin	Shoot proliferation	Enhanced shoot regeneration in vitro

pre-sowing treatments and growth regulator applications, ensures species survival and genetic variability. Vegetative propagation techniques such as stem and root cuttings enable rapid multiplication of elite and high-demand species while maintaining genetic fidelity. *In vitro* micropropagation, supported by optimized culture media and plant growth regulators, offers significant advantages for mass multiplication, germplasm conservation, and restoration of threatened taxa. The combined application of these propagation strategies, along with nursery management, quality planting material production, and farmer-level adoption of standardized agro-techniques, is essential to reduce pressure on wild populations.

Future efforts should prioritize research-driven protocol optimisation, capacity building among farmers and tribal communities, policy support for medicinal plant cultivation, and the establishment of organized supply chains. By integrating conservation, cultivation, and commercialization in a balanced framework, it is possible to safeguard medicinal plant genetic resources, ensure sustainable industrial supply, enhance rural livelihoods, and preserve these invaluable biological assets for future generations.

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