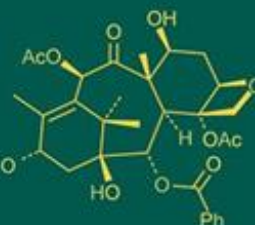
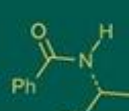
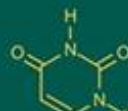
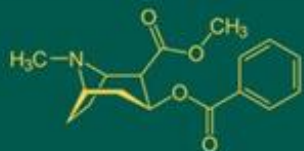


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(7): 233-243
www.biochemjournal.com
Received: 10-06-2026
Accepted: 18-07-2026
Published: 25-07-2026

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Tissue culture-based techniques for the conservation and growth of *Angelica glauca*

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7Sc.8973>

Abstract

Angelica glauca, a high-value Himalayan medicinal herb, faces rapid population decline due to overexploitation, habitat degradation and poor natural regeneration. This paper provides a comprehensive review of tissue culture-based techniques developed for the conservation and propagation of *A. glauca*. The study highlights the plant's botanical characteristics, ethnobotanical importance and phytochemical richness, emphasizing the urgent need for sustainable in vitro approaches. Various tissue culture methods-including callus induction, organogenesis, and somatic embryogenesis-are discussed as effective strategies for large-scale plant regeneration. Protocols for explant sterilization, shoot and root induction, and hardening are summarized, along with advancements in synthetic seed production and cell suspension cultures for enhanced secondary metabolite synthesis. The review also explores the use of molecular markers to ensure genetic fidelity among regenerated plantlets and evaluates cryopreservation techniques for long-term germplasm conservation. Furthermore, challenges such as somaclonal variation, optimization of growth regulators, and scalability are addressed, with insights into future prospects involving genetic engineering and bioreactor-based production systems. Overall, this review underscores the pivotal role of plant tissue culture in safeguarding *Angelica glauca*, offering sustainable solutions for its conservation, commercial propagation, and continued utilization in the pharmaceutical and herbal industries.

Keywords: *Angelica glauca*, Tissue Culture, Micropropagation, Conservation, secondary metabolites, Cryopreservation

1. Introduction

Medicinal and aromatic plants (MAPs) of the Himalayan region are recognized for their immense ecological, economic, and therapeutic significance. These plants contribute to traditional medicine systems, local livelihoods, and global pharmaceutical industries. Due to unsustainable overexploitation, habitat degradation and climate change, the population density and distribution of MAPs in high altitude regions is significantly declined (Singh *et al.*, 2024) [41]. The Western Himalaya is a major biodiversity hotspot, characterized by a high richness of endemic MAPs such as *Aconitum heterophyllum*, *Angelica glauca*, *Dactylorhiza hatageria*, *Nardostichys jatamansi*, *Picrorhiza kurroa*, *Sassurea ovballata* etc. However, a substantial knowledge gap remains of their abundance, ecological status and conservation requirements (Singh *et al.*, 2024; Thapliyal *et al.*, 2024) [41, 42]. Furthermore, traditional propagation methods fail to meet the increasing demand for these resources due to seasonal limitations, low seed germination rates and poor natural regeneration (Mehrotra & Khan, 2022) [24]. Consequently, biotechnological interventions, particularly tissue culture, offer viable solutions for sustainable propagation, conservation and metabolite production of these endangered and economically valuable species (Rai *et al.*, 2022; Dey 2004) [32, 9]. Among these critical species, *A. glauca* serves as a paramount candidate for review due to its high commercial demand and the urgent need to bridge the gap between its traditional utility and modern biotechnological conservation strategies.

1.1 Botanical and taxonomic overview of *angelica glauca*

Angelica glauca Edgew., belonging to the family Apiaceae, is an aromatic perennial herb endemic to the Himalayan region. The genus *Angelica* comprises over 60 species distributed across temperate regions worldwide, many of which are valued for their medicinal and aromatic properties (Wang *et al.*, 2024) [44]. Morphologically, *A. glauca* is characterized by tall, hollow stems, pinnately compound leaves, and umbellate inflorescences bearing small white flowers. The roots are thick, aromatic, and constitute the primary source of pharmacologically active

compounds. Taxonomic revisions have revealed substantial morphological diversity among *Angelica* species, emphasizing the need for accurate identification and systematic classification to aid conservation and biotechnological studies (Wang *et al.*, 2024) ^[44]. Phytochemical analyses indicate that *A. glauca* and other *Angelica* species possess diverse bioactive compounds, including coumarins, flavonoids, and essential oils, which contribute to their therapeutic potential (Mishra & Paliwal, 2025; Sarker & Nahar, 2004) ^[25, 39]. These features underscore both the botanical significance and the pharmacological value of *A. glauca*, justifying focused conservation and propagation efforts.

1.2 Ethnobotanical importance and traditional uses

Ethnobotanical studies highlight the importance of *A. glauca* in traditional Himalayan medicine. Local communities utilize the roots and rhizomes to treat digestive disorders, respiratory ailments, and inflammatory conditions (Savkin *et al.*, 2013). The plant is also incorporated into Ayurvedic and Unani formulations for its carminative, stimulant, and expectorant properties. Beyond medicinal applications, its aromatic roots are used in perfumery and as flavoring agents (Savkin *et al.*, 2013). Emphasizing that documenting ethnobotanical knowledge is crucial for both biodiversity conservation and the preservation of cultural heritage (Schultes, 1997) ^[40]. The

traditional use of *A. glauca* not only reflects its medicinal and economic importance but also underscores the urgent need for sustainable harvesting practices and conservation interventions to prevent further depletion of wild populations (Savkin *et al.*, 2013; Schultes, 1997).

1.3 Phytochemistry and bioactive compounds

The medicinal properties of *A. glauca* are largely attributable to its rich phytochemical profile (Table 1). It contains bioactive compounds such as coumarins, terpenoids, flavonoids, and essential oils, which exhibit antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective activities (Mishra & Paliwal, 2025; Schultes, 1997) ^[25, 40]. Detailed phytochemical investigations provide insights into the structural diversity and therapeutic potential of these molecules, which are also critical for pharmaceutical and nutraceutical applications (Ranjha *et al.*, 2023) ^[35]. Modern extraction, isolation, and identification techniques, including chromatography and spectrometry, have enabled precise characterization of these compounds, facilitating their utilization in standardized formulations (Altemimi *et al.*, 2017) ^[4]. Understanding the phytochemistry of *A. glauca* is therefore essential not only for medicinal applications but also for biotechnological interventions aimed at enhancing the production of secondary metabolites.

Table 1: Major Phytochemical Constituents of *Angelica glauca* and Their Biological Activities.

Phytochemical Class	Phytoconstituents	Extraction / Identification Methods	Biological Activities	References
Coumarins	Umbelliferone, Esculetin, Bergapten, Psoralen	Methanolic/ethanolic extraction; HPLC, LC-MS	Antioxidant, anti-inflammatory, hepatoprotective, antimicrobial	(Mishra & Paliwal, 2025; Ranjha <i>et al.</i> , 2023)
Flavonoids	Quercetin, Kaempferol derivatives	Solvent extraction; HPLC-UV, LC-MS/MS	Antioxidant, anti-inflammatory, cytoprotective	(Ranjha <i>et al.</i> , 2023)
Terpenoids	α -Pinene, β -Pinene, Limonene, Caryophyllene	Steam distillation (essential oil), GC-MS	Antimicrobial, anti-inflammatory, bronchodilatory	(Mishra & Paliwal, 2025)
Essential Oils	Umbelliferone-rich oil, monoterpenes, sesquiterpenes	Hydro-distillation; GC-MS	Antioxidant, antimicrobial, aromatic therapeutic uses	(Mishra & Paliwal, 2025; Ranjha <i>et al.</i> , 2023)
Phenolic Compounds	Ferulic acid, Caffeic acid	Solvent extraction (methanol); HPLC	Strong antioxidant, anti-aging, hepatoprotective	(Ranjha <i>et al.</i> , 2023; Altemimi <i>et al.</i> , 2017)
Phthalides	Ligustilide, Butylidenephthalide	Ethanol extraction; GC-MS	Spasmolytic, neuroprotective, anti-inflammatory	(Altemimi <i>et al.</i> , 2017)
Polysaccharides	Acidic polysaccharides (unspecified)	Hot-water extraction; FT-IR	Immunomodulatory, antioxidant	(Ranjha <i>et al.</i> , 2023)

1.4 Conservation status and need for tissue culture approaches

Angelica glauca is currently threatened due to overharvesting, habitat fragmentation, and limited natural regeneration, which have led to significant population declines in the Western Himalaya ((Singh *et al.*, 2024; Thapliyal *et al.*, 2024; Dey, 2004) ^[41, 42, 9]. Conventional propagation is hindered by low seed germination rates, dormancy, and climatic restrictions (Mehrotra & Khan, 2022) ^[24]. Plant tissue culture offers a reliable method for *ex situ* conservation and mass propagation of endangered medicinal species (Oseni *et al.*, 2018) ^[28]. *In vitro* techniques, such as callus culture, organogenesis, and somatic

embryogenesis, allow for rapid multiplication of disease-free, genetically uniform plants under controlled conditions (Oseni *et al.*, 2018; Tripathi *et al.*, 2021) ^[28, 43]. These approaches also enable the preservation of genetic resources through germplasm storage and cryopreservation (Kumar *et al.*, 2026) ^[19]. Kumar *et al.* emphasized that integrating tissue culture with conservation strategies is critical for safeguarding Himalayan medicinal plants like *A. glauca* while ensuring sustainable supply for pharmaceutical and commercial purposes. Thus, tissue culture-based propagation represents a key tool in bridging conservation goals with the increasing demand for medicinal plants.

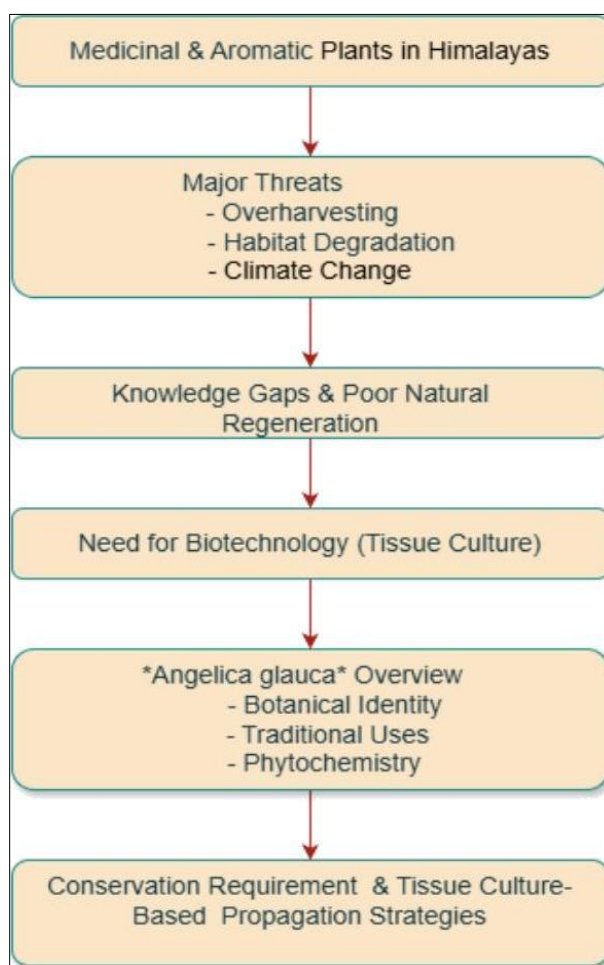


Fig 1: Flowchart of the Introduction and Background of *Angelica glauca*

2. Plant tissue culture: an overview

Plant tissue culture is a pivotal biotechnological tool that enables the growth and multiplication of plant cells, tissues, or organs under controlled *in vitro* conditions. This approach not only provides a reliable method for the rapid propagation of economically important plants but also plays a critical role in the conservation of endangered species (Oseni *et al.*, 2018; Tripathi *et al.*, 2021) ^[28, 43]. The technique involves the aseptic culture of plant material on nutrient media supplemented with appropriate growth regulators, under controlled environmental conditions such as light, temperature, and humidity.

2.1 Principles of plant tissue culture

The fundamental principle of plant tissue culture is totipotency, which posits that every living plant cell retains the genetic potential to regenerate into a complete plant under suitable conditions (Niazian, 2019) ^[27]. Exploiting this property, plant cells, tissues, or organs can be induced to develop callus, shoots, or roots through the precise manipulation of nutrients, phytohormones, and environmental factors. Key stages in tissue culture include explant selection, sterilization, initiation of culture, organogenesis or somatic embryogenesis, and hardening of regenerated plantlets (Oseni *et al.*, 2018; Niazian, 2019) ^[28, 27].

Auxins and cytokinins are the primary growth regulators used to direct the developmental pathway of cultured cells. A higher auxin-to-cytokinin ratio generally promotes root induction, while a higher cytokinin-to-auxin ratio favors shoot proliferation. Additionally, factors such as media composition,

pH, carbohydrate source, and light regime significantly influence tissue growth and differentiation. Understanding these principles is essential for successful micropropagation and for optimizing *in vitro* regeneration protocols of medicinal plants like *Angelica glauca* (Niazian, 2019) ^[27].

2.2 Importance of *in vitro* techniques for medicinal plants

In vitro propagation techniques are particularly crucial for medicinal plants that face challenges such as overharvesting, low seed viability, or habitat degradation. Controlled laboratory-based propagation ensures a reliable and continuous supply of plant material, independent of seasonal or environmental constraints (Ahlawat *et al.*, 2024) ^[1]. These techniques also facilitate the production of disease-free and genetically uniform plantlets, which is essential for maintaining the quality and efficacy of bioactive compounds (Oseni *et al.*, 2018; Tripathi *et al.*, 2021) ^[28, 43].

Furthermore, *in vitro* culture provides an opportunity to enhance the production of secondary metabolites through elicitation or metabolic engineering, thereby increasing the medicinal value of propagated plants (Ranjha *et al.*, 2023; Altemimi *et al.*, 2017) ^[35, 4]. Techniques like callus culture, organogenesis, and somatic embryogenesis not only enable rapid multiplication but also serve as platforms for biotechnological research aimed at improving the yield and quality of therapeutic compounds (Ahlawat *et al.*, 2024) ^[1]. The application of tissue culture in medicinal plants like *A. glauca* ensures both sustainable utilization and conservation of

valuable genetic resources (Kumar *et al.*, 2026; Ahlawat *et al.*, 2024)^[19, 1].

2.3 Role of tissue culture in conservation and commercial propagation

Tissue culture plays a dual role in *ex situ* conservation and commercial propagation of endangered and high-value medicinal plants. By enabling the multiplication of plants from small explants, it reduces the need for destructive wild harvesting, thereby supporting ecological conservation (Kumar *et al.*, 2026; Ahlawat *et al.*, 2024)^[19, 1]. Cryopreservation and *in vitro* germplasm storage further enhance long-term conservation by maintaining genetic stability of rare or threatened species (Kumar *et al.*, 2026)^[19].

Commercially, tissue culture allows large-scale production of uniform and disease-free plants for the pharmaceutical, nutraceutical, and herbal industries. Micropropagation ensures rapid availability of high-quality planting material, which is especially valuable for plants with slow natural regeneration, such as *Angelica glauca* (Ahlawat *et al.*, 2024)^[1]. Integration of tissue culture with conventional cultivation and bioreactor-based production systems provides a sustainable model for meeting industrial demand while preserving natural populations (Ahlawat *et al.*, 2024; Husen & Pant, 2024)^[1, 13].

2.4 Comparative effect of pgrs on high altitude species

The morphogenesis of plant tissues *in vitro* is fundamentally governed by the type, concentration, and ratio of Plant Growth Regulators (PGRs) employed in the culture medium. The

balance between auxins and cytokinins is particularly critical for high-altitude Himalayan species, which often exhibit physiological dormancy and slow growth rates due to their adaptation to extreme climatic conditions. As illustrated in the classical model by Skoog and Miller, a higher ratio of cytokinins generally promotes shoot organogenesis, while a higher ratio of auxins favors root development or callus induction (Hnatuszko-Konka *et al.*, 2021)^[12]. However, for endangered alpine herbs, specific hormonal regimes are required to overcome recalcitrance. As summarized in Table 2, somatic embryogenesis - a preferred route for mass propagation and synthetic seed production is frequently achieved using strong auxins such as 2,4-Dichlorophenoxyacetic acid (2,4-D). For instance, Pandey *et al* reported that the synergistic application of 2,4-D and NAA was essential for the induction of embryogenic callus in *A. glauca*. Similarly, in *Paris polyphylla*, high concentrations of 2,4-D combined with Kinetin (Kn) significantly enhanced somatic embryo yield (Raomai *et al.*, 2014)^[36]. In contrast, protocols targeting direct organogenesis typically rely on cytokinin-rich media. Studies on *Picrorhiza kurroa* demonstrated that Thidiazuron (TDZ) pretreatment effectively induced profuse shoot multiplication (Patil *et al.*, 2016)^[31], while Khan *et al.* utilized a combination of BAP, NAA and Gibberellic Acid (GA₃) to achieve multiple shoot induction in *Saussurea costus*. This comparative analysis highlights that while the fundamental principles of tissue culture are universal, the specific PGR requirements must be tailored to the species' ecological and physiological background to ensure successful conservation and commercial propagation.

Table 2: Summary of published *in vitro* regeneration protocols for selected high-altitude medicinal and aromatic plants, showing the culture process, principal plant growth regulator (PGR) combinations and concentrations, key morphogenic outcomes, and primary references. Abbreviations: 2,4-D = 2,4-dichlorophenoxyacetic acid; NAA = naphthaleneacetic acid; Kn = kinetin; BAP = 6-benzylaminopurine; BA = benzyladenine; TDZ = thidiazuron; ABA = abscisic acid; GA₃ = gibberellic acid

Species	Process	PGR combination & concentration	Key result(s)	Ref.
<i>Angelica glauca</i>	Somatic embryogenesis	3 µM 2,4-D; 2 µM NAA + 2 µM BA	86.7% callus induction; 100% embryogenic callus; bipolar embryos	(Pandey <i>et al.</i> , 2011)
<i>Viola canescens</i>	Somatic embryogenesis	0.15 mg/L 2,4-D + 0.05 mg/L Kn; 1.5 mg/L ABA; 0.10 mg/L BAP	Up to 19.15 embryos/clump; 86.7% plantlet conversion	(Khajuria <i>et al.</i> , 2021)
<i>Picrorhiza kurroa</i>	Organogenesis	0.5 µM TDZ (15 d pretreatment)	Profuse shoot multiplication; 100% survival after acclimatization	(Patil <i>et al.</i> , 2016)
<i>Aconitum heterophyllum</i>	Somatic embryogenesis	Auxin-dominant regimes (e.g., 2,4-D + Kn)	Somatic embryos from callus; plant regeneration achieved	(Giri <i>et al.</i> , 1993)
<i>Swertia chirata</i>	Somatic embryogenesis	Auxin + cytokinin combinations	High-frequency embryogenesis and regeneration	(Jha <i>et al.</i> , 2011)
<i>Nardostachys jatamansi</i>	Somatic embryogenesis	16.1 µM NAA + 1.16 µM Kn; 9.30 µM Kn + 1.34 µM NAA	Optimized embryo-to-plantlet conversion	(Mathur, 1993)
<i>Saussurea costus</i>	Organogenesis	2.0 mg/L BAP + 1.0 mg/L NAA + 0.25 mg/L GA ₃	82% shoot bud initiation; multiple shoots	(Khan <i>et al.</i> , 2021)
<i>Valeriana wallichii</i>	Organogenesis / Somatic embryogenesis	2,4-D or NAA; Kn 1.0 mg/L + NAA 0.25 mg/L	Callus induction and shoot regeneration	(Mathur & Ahuja, 1991)
<i>Paris polyphylla</i>	Somatic embryogenesis	2.0 mg/L 2,4-D + 1.0 mg/L Kn	Up to 63 embryos per g callus	(Raomai <i>et al.</i> , 2014)

3. Micropropagation of angelica glauca

Micropropagation offers an efficient and reliable approach for the large-scale propagation of endangered and medicinally important species such as *Angelica glauca*. Through controlled *in vitro* conditions, it ensures the production of genetically stable and disease-free plants, overcoming limitations of conventional propagation methods such as low seed germination and seasonal dependency (Oseni *et al.*, 2018; Samridha & Chandra, 2024)^[28, 38]. The micropropagation process involves multiple stages explant selection, sterilization,

callus induction, shoot and root regeneration, and hardening each of which requires specific optimization to achieve maximum efficiency (Samridha & Chandra, 2024; Bhatt *et al.*, 2024)^[38, 6].

3.1 Explant selection and sterilization protocols

The success of micropropagation largely depends on the type of explant used and the sterilization protocol adopted. For *A. glauca*, explants such as leaf segments, petioles, nodal explants, and root segments have been successfully used for culture

initiation (Samridha & Chandra, 2024) ^[38]. Younger, actively growing tissues are preferred due to their high meristematic activity and responsiveness to *in vitro* conditions (Oseni *et al.*, 2018) ^[28]. Sterilization typically involves a multistep process including surface washing with detergent, treatment with 70% ethanol, and exposure to sterilizing agents such as mercuric chloride (0.1%) or sodium hypochlorite (5%) for short durations, followed by thorough rinsing with sterile distilled water (Samridha & Chandra, 2024; Bhatt *et al.*, 2024) ^[38, 6]. Samridha and Chandra (2024) ^[38] emphasized the importance of optimizing sterilization exposure time to minimize tissue injury while ensuring complete microbial decontamination. Establishing aseptic culture conditions is essential to prevent contamination by fungi and bacteria, which are common in explants collected from wild or field-grown plants. Proper selection of explants and efficient sterilization not only ensure successful culture establishment but also maintain tissue viability for subsequent morphogenic responses.

3.2 Callus induction and organogenesis

Callus induction serves as the foundational step for indirect regeneration pathways, providing a dedifferentiated mass of cells that can be manipulated toward either organogenesis or somatic embryogenesis. In *A. glauca*, the induction efficiency and morphological nature of the callus are significantly regulated by the explant maturity and the exogenous hormonal regime, particularly by the effect of auxins.

Research indicates that auxins, specifically 2,4-Dichlorophenoxyacetic acid (2,4-D) and α -Naphthaleneacetic acid (NAA) are indispensable for initiating cell division in *A. glauca* explants. Pandey *et al.* (2011) ^[29] reported maximum callus induction frequencies (86.7%) when leaf explants were cultured on Murashige and Skoog (MS) medium supplemented with 3 μ M 2,4-D. The resulting callus is typically described as friable and varying in color from creamy white to pale yellow, a morphology indicative of high embryogenic potential. While 2,4-D is effective for induction, prolonged exposure can inhibit subsequent differentiation, therefore, protocols often necessitate subculturing onto media with reduced auxin concentrations or the addition of cytokinins like 6-Benzyladenine (BA) to promote further development (Bhatt *et al.*, 2024) ^[6]. Following successful induction, the differentiation of callus into organized structures requires a precise shift in the auxin-to-cytokinin ratio.

Somatic embryogenesis: For embryogenic pathways, the combination of auxins and cytokinins is critical. The synergistic effect of 2 μ M NAA and 2 μ M BA has been observed to facilitate the transition from proliferation to differentiation, resulting in the formation of bipolar somatic embryos (Pandey *et al.*, 2011) ^[29]. Bhatt *et al.*, (2024) ^[6] highlighted that manipulating phytohormone concentrations, light intensity, and carbohydrate sources significantly influences callus texture and growth rate. Organogenic Calli with friable morphology are preferred for subsequent regeneration stages. Periodic subculturing at 4–6 week intervals is essential to maintain cellular viability and prevent browning due to phenolic oxidation. The induction of organogenesis from callus marks a key step toward developing complete plantlets under controlled *in vitro* conditions.

3.3 Direct and indirect shoot regeneration

Shoot regeneration in *A. glauca* can occur through either direct organogenesis from explant tissues or indirectly via callus intermediates (Lone *et al.*, 2024) ^[21]. Direct shoot regeneration avoids genetic variations that may arise during callus-mediated regeneration, thus maintaining clonal fidelity. Nodal and shoot tip explants are most commonly used for direct regeneration, cultured on MS medium fortified with cytokinins such as BAP and kinetin. Lone *et al.* (2024) ^[21] also reported that an optimal concentration of BAP (1–2 mg/L) combined with a low level of auxin (0.5 mg/L NAA) significantly enhances shoot multiplication frequency in Himalayan medicinal plants, including *Angelica* species.

For indirect shoot regeneration, callus tissues derived from leaf or petiole explants are transferred to media with reduced auxin and elevated cytokinin levels to trigger shoot bud differentiation (Bhatt *et al.*, 2024; Lone *et al.*, 2024) ^[6, 21]. Light intensity and photoperiod play crucial roles in chlorophyll synthesis and shoot elongation. Multiple shoot formation can be achieved within 4–6 weeks, followed by elongation on hormone-free or reduced-strength MS media. The high regeneration frequency achieved through these protocols demonstrates the adaptability of *A. glauca* to *in vitro* morphogenesis (Lone *et al.*, 2024) ^[21].

3.4 Root induction and hardening

Once healthy shoots are obtained, they are transferred to rooting media for rhizogenesis. Root induction in *A. glauca* is typically achieved using half-strength MS medium supplemented with auxins such as indole-3-butyric acid (IBA) or naphthalene acetic acid (NAA) (Deepika, 2024) ^[8], also demonstrated that 1.0 mg/L IBA yielded the highest root induction rate, producing long and well-branched roots within three weeks. Sucrose concentration and medium strength significantly affect root initiation and elongation, with reduced nutrient levels often enhancing rooting efficiency. After sufficient root development, the plantlets are subjected to hardening the gradual acclimatization of *in vitro* grown plants to ex vitro conditions. Plantlets are first transferred to a soil–sand–peat mixture (1:1:1) under high humidity and low light, followed by gradual exposure to natural light and ambient humidity (Oseni *et al.*, 2018; Deepika, 2024) ^[28, 8]. The survival rate of hardened plants can exceed 80% when acclimatization is properly managed. Rooted and hardened plants are then transplanted into field conditions, contributing to large-scale conservation and reintroduction programs for *A. glauca*.

3.5 Case studies and reported protocols

Several successful *in vitro* propagation protocols have been reported for *A. glauca* (Table 3), demonstrating reproducible regeneration and genetic stability. Rawat *et al.* (2018) ^[37] developed a robust regeneration system involving nodal explants cultured on MS medium supplemented with BAP (2.0 mg/L) and NAA (0.5 mg/L), yielding high-frequency shoot multiplication. The regenerated plantlets exhibited no significant somaclonal variation as confirmed through molecular marker analyses (RAPD and ISSR), indicating genetic stability across regenerants. Phytochemical profiling of *in vitro* raised plants revealed consistency in the production of essential metabolites compared to mother plants, validating the reliability of tissue culture-derived propagation for maintaining both genetic and biochemical fidelity (Rawat *et al.*, 2018) ^[37]. These findings align with previous studies emphasizing the integration of micropropagation and molecular tools for

conservation of endangered Himalayan MAPs (Kumar *et al.*, 2026; Bhatt *et al.*, 2024) ^[19, 6]. The standardized protocols developed for *A. glauca* thus provide a foundation for its

commercial-scale propagation, genetic conservation, and sustainable utilization.

Table 3: Optimized protocol for the in vitro propagation of *A. glauca* via direct and indirect organogenesis, detailed by developmental stage.

Stage	Key Steps	Media / Hormones	Outcome
Explant Selection & Sterilization	Young leaf/petiole/nodal explants; detergent wash → 70% ethanol → HgCl ₂ (0.1%) or NaOCl (5%)		Clean, viable explants
Callus Induction	Explants cultured; subculture every 4–6 weeks	MS + 2,4-D (1–2 mg/L) + BAP/Kinetin (0.5–1 mg/L)	Friable, organogenic callus
Shoot Regeneration (Direct)	Nodal/shoot tip explants cultured	MS + BAP (1–2 mg/L) + small NAA	Multiple shoots; high fidelity
Shoot Regeneration (Indirect)	Callus shifted to cytokinin-rich medium	MS + BAP (1–2 mg/L) + low NAA	Shoot buds in 4–6 weeks
Root Induction	Healthy shoots transferred to 1/2 MS	1/2 MS + IBA (1 mg/L)	Strong roots in 3 weeks
Hardening	Transfer to soil: sand: peat (1:1:1); gradual light exposure		>80% survival, field-ready plants

4. Somatic embryogenesis and synthetic seed production

Somatic embryogenesis (SE) represents a critical *in vitro* regeneration pathway, enabling the formation of embryos from somatic (non-reproductive) plant cells. This process not only facilitates large-scale clonal propagation but also serves as a foundation for synthetic seed technology, genetic transformation, and cryopreservation of elite genotypes (Oseni *et al.*, 2018; Tripathi *et al.*, 2021; Kumar *et al.*, 2026) ^[28, 43, 19]. In the context of *Angelica glauca*, somatic embryogenesis offers an efficient route for mass propagation and germplasm conservation, particularly when conventional propagation methods fail due to poor seed viability or environmental constraints (Mehrotra & Khan, 2022; Dey, 2004) ^[24, 9].

4.1 Stages of somatic embryogenesis

Somatic embryogenesis progresses through distinct developmental stages: induction, proliferation, maturation, and germination. Initially, explant tissues such as leaf, hypocotyl, or callus are cultured on auxin-rich media (commonly containing 2,4-D or NAA), which induces the formation of embryogenic callus (Gray *et al.*, 1991) ^[11]. These embryogenic cells subsequently undergo differentiation into globular, heart, torpedo, and cotyledonary embryos, mimicking the stages of zygotic embryogenesis. The maturation phase is often supported by cytokinin-enriched media, leading to well-formed embryos capable of germinating into complete plantlets (Gray *et al.*, 1991) ^[11].

Gray, Purohit, and Trigiano (1991) ^[11] emphasized that the synchronization of these stages is crucial for obtaining uniform embryo development, as variations in plant growth regulators (PGRs), light, and temperature can significantly affect embryogenic response. In medicinal plants such as *Angelica* spp., successful somatic embryogenesis provides a reproducible system for generating genetically stable clones, facilitating both *ex situ* conservation and secondary metabolite production (Mishra & Paliwal, 2025; Oseni *et al.*, 2018; Kumar *et al.*, 2026) ^[25, 28, 19].

4.2 Factors affecting embryogenesis efficiency

The efficiency of somatic embryogenesis depends on several interrelated factors, including explant type, physiological state of donor tissue, nutrient composition, and PGR concentration (Krishna Kumar & Thomas, 2012) ^[18]. They also reported that auxin-to-cytokinin ratio plays a pivotal role in embryogenic

callus induction and embryo maturation. Similarly, the presence of activated charcoal, amino acids, and vitamins in culture medium enhances embryo quality and reduces morphological abnormalities.

Environmental parameters such as light intensity, temperature, and humidity also influence embryogenesis efficiency, as do endogenous hormonal levels and oxidative stress management (Oseni *et al.*, 2018; Krishna Kumar & Thomas, 2012) ^[28, 18]. For *A. glauca*, optimizing these conditions is essential for high-frequency embryogenesis, which is a prerequisite for downstream applications like synthetic seed development and germplasm conservation (Kumar *et al.*, 2026) ^[19]. Furthermore, embryogenic cultures can serve as ideal starting materials for cell suspension cultures, allowing scalable metabolite production under controlled bioreactor conditions (Mishra & Paliwal, 2025; Tripathi *et al.*, 2021) ^[25, 43].

4.3 Synthetic seed technology: applications and limitations

Synthetic seed technology, also known as artificial seed production, is a biotechnological innovation that encapsulates somatic embryos or other propagules in a protective gel matrix (commonly sodium alginate) to simulate true seeds (Ara, Jaiswal & Jaiswal, 2000) ^[5]. They too highlighted that this approach combines the advantages of clonal propagation with the storage and transport convenience of seeds, making it particularly useful for rare or endangered species.

In *A. glauca*, synthetic seeds derived from somatic embryos could enable year-round propagation, easier exchange of germplasm, and enhanced survival rates during field transplantation (Kumar *et al.*, 2026; Gray *et al.*, 1991) ^[19, 11]. However, limitations persist, including desiccation sensitivity, reduced conversion rates to plantlets, and variability in encapsulation materials (Ara, Jaiswal & Jaiswal, 2000) ^[5]. These challenges can be mitigated by optimizing encapsulation matrices, incorporating growth promoters, and controlling moisture and temperature conditions during storage.

Synthetic seed systems also hold promise for integrating with cryopreservation and tissue culture protocols to create long-term conservation frameworks for Himalayan medicinal plants (Singh *et al.*, 2024; Kumar *et al.*, 2026) ^[41, 19]. By merging somatic embryogenesis and synthetic seed technology, researchers can achieve scalable propagation while maintaining genetic fidelity, offering a sustainable strategy for conserving *A. glauca* and similar endangered taxa.

5. Cell suspension culture and secondary metabolite production

The production of bioactive compounds through *in vitro* culture systems has emerged as a sustainable and controlled alternative to field-based cultivation of medicinal plants. Cell suspension cultures, elicitation techniques, and bioreactor-based systems play a crucial role in enhancing the yield of secondary metabolites while preserving genetic resources of endangered species such as *Angelica glauca*. These advanced tissue culture methods provide high scalability, reproducibility, and opportunities for metabolic engineering (Mehrotra & Khan, 2022; Oseni *et al.*, 2018) ^[24, 28].

5.1 Establishment of cell suspension cultures

Cell suspension culture involves the growth of dispersed plant cells in a liquid medium under aseptic and controlled environmental conditions. It serves as a vital system for large-scale biomass and secondary metabolite production, especially in species where conventional propagation and extraction are limited due to conservation constraints (Oseni *et al.*, 2018; Ali & Abbasi, 2013) ^[28, 2]. Typically, friable callus tissues are first induced on solid medium containing appropriate concentrations of auxins and cytokinins, followed by their transfer into liquid medium where continuous agitation ensures aeration and homogenous distribution of nutrients (Ali & Abbasi, 2013) ^[2]. Ali and Abbasi (2013) ^[2] demonstrated the successful establishment of cell suspension cultures in *Artemisia absinthium* L., achieving significant accumulation of phenolics, flavonoids, and other antioxidant compounds under optimized growth conditions. Similar approaches can be adopted for *Angelica glauca*, considering its high content of coumarins and essential oils (Mishra & Paliwal, 2025; Sarker & Nahar, 2004) ^[25, 39]. Suspension cultures offer the advantage of continuous metabolite harvesting, scalability for industrial production, and adaptability to various stress treatments for enhancing yield. Therefore, establishing robust cell suspension systems is an essential step toward commercial exploitation and conservation of *A. glauca*'s bioactive resources (Oseni *et al.*, 2018; Ali & Abbasi, 2013) ^[28, 2].

5.2 Enhancement of secondary metabolites through elicitation

Elicitation is a strategic approach used to stimulate the biosynthetic pathways of secondary metabolites by mimicking biotic or abiotic stress conditions *in vitro*. Elicitors can be classified as biotic (e.g., fungal extracts, yeast extract, chitosan) or abiotic (e.g., heavy metals, UV radiation, jasmonic acid, salicylic acid), each triggering defense-related metabolic responses that upregulate key enzymes involved in secondary metabolite biosynthesis (Narayani & Srivastava, 2017) ^[26]. Narayani and Srivastava (2017) ^[26] highlighted that elicitation significantly enhances the accumulation of valuable compounds by activating plant defense mechanisms and secondary metabolism (Narayani & Srivastava, 2017) ^[26]. The technique has been effectively utilized in several medicinal plants to boost the production of coumarins, alkaloids, and terpenoids metabolites also present in *A. glauca* (Mishra & Paliwal, 2025; Ranjha *et al.*, 2023) ^[25, 35]. Integrating elicitation into suspension culture protocols allows for fine-tuning of metabolite profiles, thereby improving both yield and quality without altering the genetic stability of cultured cells ^[8, 13]. In the case of endangered Himalayan species, elicitation not only increases productivity but also reduces dependence on wild harvesting, aligning with

conservation and sustainability goals (Kumar *et al.*, 2026; Narayani & Srivastava, 2017) ^[19, 26].

5.3 Bioreactor-based production

Bioreactor-based culture systems represent the most advanced stage of *in vitro* metabolite production, enabling large-scale cultivation of plant cells under optimized and controlled conditions. These systems provide automated regulation of parameters such as pH, temperature, oxygen, and nutrient supply, ensuring consistent growth and metabolite synthesis (Pandey *et al.*, 2019) ^[30].

They demonstrated the successful use of bioreactors for *Ocimum basilicum* suspension cultures, where elicitation and aeration control significantly enhanced triterpenoid yields (Pandey *et al.*, 2019) ^[30]. Applying similar bioreactor principles to *Angelica glauca* can facilitate continuous production of valuable compounds like coumarins and essential oils, which are pharmacologically and commercially important (Mishra & Paliwal, 2025; Altemimi *et al.*, 2017) ^[25, 4]. Bioreactors also minimize contamination risks and allow scale-up from laboratory to industrial levels, making them essential tools for sustainable production of secondary metabolites (Mehrotra & Khan, 2022; Oseni *et al.*, 2018; Pandey *et al.*, 2019) ^[24, 28, 30]. Furthermore, integrating bioreactor systems with elicitation strategies can synergistically improve yields by providing both optimized growth conditions and biochemical stimulation. Such integrated biotechnological platforms ensure efficient utilization of *A. glauca*'s metabolic potential while contributing to *ex situ* conservation and reducing exploitation pressure on wild populations (Ali & Abbasi, 20123; Narayani & Srivastava, 2017; Pandey *et al.*, 2019) ^[26, 30].

6. Genetic fidelity and molecular characterization

Maintaining genetic uniformity is a critical aspect of any tissue culture-based propagation system, especially when the goal is to conserve endangered medicinal species like *Angelica glauca*. Micropropagation techniques, though efficient for large-scale plant production, can sometimes induce somaclonal variations due to prolonged culture duration, exposure to growth regulators, or stress conditions during *in vitro* manipulation (Oseni *et al.*, 2018; Tripathi *et al.*, 2021) ^[28, 43]. Therefore, ensuring genetic fidelity of regenerated plants through molecular characterization has become an indispensable component of plant tissue culture research (Biswas & Kumar, 2023) ^[7].

6.1 Importance of genetic stability in micropropagation

Genetic stability in micro-propagated plants ensures that the regenerated clones maintain their true-to-type characteristics, including morphological, physiological, and phytochemical traits (Biswas & Kumar, 2023) ^[7]. During the tissue culture process, especially through callus or somatic embryogenesis routes, genetic alterations such as point mutations, chromosomal rearrangements, and epigenetic modifications may occur. These changes can alter plant morphology or secondary metabolite profiles, reducing their commercial and medicinal value (Oseni *et al.*, 2018; Tripathi *et al.*, 2021) ^[28, 43]. Biswas and Kumar (2023) ^[7] highlighted that the assessment of genetic fidelity using molecular markers provides a reliable and reproducible means of verifying clonal uniformity. The application of DNA-based molecular tools such as Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), and Amplified Fragment Length Polymorphism

(AFLP) has revolutionized the detection of genetic variation at the molecular level. Such markers are essential for confirming that tissue-cultured plants of *A. glauca* are genetically identical to their mother plants, ensuring the success of conservation and propagation programs.

6.2 Molecular markers for clonal fidelity assessment

The use of molecular markers has emerged as one of the most powerful and precise approaches to assess genetic fidelity among *in vitro* regenerated plants (Alizadeh *et al.*, 2015)^[3]. They as well reported that molecular markers such as ISSR, RAPD, Simple Sequence Repeats (SSR), and Sequence-Related Amplified Polymorphism (SRAP) are widely used to detect genetic variations in micropropagated horticultural and medicinal plants. These techniques allow for high-throughput screening and can detect even minute genetic changes that may not be visible morphologically. Among these, ISSR markers are particularly valuable because they target highly polymorphic regions between microsatellite loci, providing both reproducibility and sensitivity. Studies on medicinal plants have consistently demonstrated that tissue culture-derived plants analyzed through these markers show high genetic uniformity when optimized protocols are used (Alizadeh *et al.*, 2015; Biswas & Kumar, 2023)^[3, 7]. For *A. glauca*, integrating molecular marker-based fidelity assessment within micropropagation protocols will enhance confidence in large-scale propagation and ensure the conservation of genetic integrity.

6.3 Reported studies on *A. glauca*

Although comprehensive genetic fidelity studies on *Angelica glauca* remain limited, methodologies developed in related medicinal species provide valuable insights. Kumar and Agrawal (Kumar & Agarwal, 2017)^[20] conducted genetic diversity and population structure analysis using ISSR and SRAP markers in *Simarouba glauca*, a bioenergy crop, demonstrating their efficiency in detecting intra- and inter-population variations. The results emphasized the importance of combining different marker systems for a more accurate understanding of genetic relationships (Kumar & Agrawal, 2017)^[20]. Similarly, the application of such markers can be adapted for *A. glauca* to monitor clonal fidelity, assess genetic diversity among natural populations, and aid in selecting elite genotypes for micropropagation. Integrating these molecular approaches with biochemical profiling will not only validate the genetic uniformity of regenerated plants but also strengthen future biotechnological conservation strategies for Himalayan medicinal flora (Alizadeh *et al.*, 2015; Kumar & Agarwal, 2017)^[3, 20].

7. Cryopreservation and germplasm conservation

The long-term preservation of endangered medicinal plants such as *Angelica glauca* is vital for maintaining genetic diversity, ensuring future availability of elite germplasm, and supporting sustainable utilization. Conventional seed storage and field gene banks are often insufficient for alpine species due to climatic limitations, recalcitrant seed behavior, and high maintenance costs (Singh *et al.*, 2024; Thapliyal *et al.*, 2024)^[41, 42]. Therefore, *in vitro* conservation and cryopreservation techniques have emerged as effective tools for safeguarding valuable plant genetic resources (Oseni *et al.*, 2018; Kumar *et al.*, 2026)^[28, 19]. These approaches enable the maintenance of viable tissues, cells, and embryos under controlled conditions,

significantly reducing the loss of genetic material over time (Rajasekharan & Sahijram, 2015)^[34].

7.1 *In vitro* conservation techniques

In vitro conservation refers to the storage of plant germplasm under aseptic, controlled laboratory conditions, using techniques that slow down growth and metabolism to prolong viability (Rajasekharan & Sahijram, 2015)^[34]. They emphasized that *in vitro* methods, such as slow-growth storage, minimal growth media, and osmotic regulation, are particularly suitable for medicinal and endangered species. By manipulating temperature, light intensity, and osmotic stress, plant tissues can be maintained in a semi-dormant state for extended periods without subculturing.

In the case of *Angelica glauca*, these techniques can preserve shoot cultures or callus tissues derived from elite genotypes. Such germplasm can later be regenerated for propagation or secondary metabolite extraction. *In vitro* conservation also offers the advantage of disease-free maintenance and genetic stability when combined with molecular marker analysis (Sarker & Nahar, 2013; Oseni *et al.*, 2018)^[28]. Moreover, it provides a complementary approach to field conservation efforts, particularly for Himalayan medicinal plants that face threats from overexploitation and environmental changes (Singh *et al.*, 2024; Kumar *et al.*, 2026)^[41, 19].

7.2 Cryopreservation protocols for long-term storage

Cryopreservation involves the storage of plant tissues at ultra-low temperatures, typically in liquid nitrogen at -196°C , effectively halting all metabolic activities and cellular processes (Kantha & Engelmann, 1944). This method is widely regarded as the most reliable approach for long-term preservation of germplasm without genetic alteration. Kantha and Engelmann (1994)^[15] highlighted several cryopreservation techniques such as vitrification, encapsulation–dehydration, and droplet-freezing, which are applicable to shoot tips, somatic embryos, pollen, and callus cultures.

For *A. Glauca*, cryopreservation can be used to store shoot apices or embryogenic cultures derived from tissue culture, ensuring long-term genetic security. The process generally includes preculture, cryoprotection using agents like DMSO or glycerol, rapid freezing in liquid nitrogen, and thawing followed by regeneration. The success of this technique depends on minimizing ice crystal formation and optimizing post-thaw recovery media. Integration of cryopreservation with micropropagation ensures year-round availability of healthy plantlets and genetic material for research and reintroduction programs (Rajasekharan & Sahijram, 2015; Kantha & Engelmann, 1944)^[34].

7.3 Relevance for endangered himalayan medicinal plants

High-altitude Himalayan medicinal plants, including *Angelica glauca*, are increasingly threatened by unsustainable harvesting and climate-induced habitat loss (Singh *et al.*, 2024; Thapliyal *et al.*, 2024; Raina & Gautam, 2020)^[41, 42, 33]. Traditional conservation approaches, such as seed banking and *in situ* protection, are limited due to short growing seasons, unpredictable weather, and low seed viability. As Raina and Gautam (2020)^[33] emphasize, biotechnological conservation techniques, including *in vitro* and cryogenic storage, are indispensable for protecting the genetic resources of these species (Raina & Gautam, 2020)^[33].

For Himalayan flora, cryopreservation not only ensures long-term storage of valuable germplasm but also supports reintroduction and restoration programs in degraded ecosystems. Moreover, integrating molecular tools for genetic fidelity assessment (as discussed in Section 8) ensures the preservation of true-to-type germplasm (Sarker & Nahar, 2024; Kumar *et al.*, 2026; Raina & Gautam, 2020) [19, 33]. In this way, cryopreservation and *in vitro* storage collectively contribute to sustainable conservation strategies, aligning with global efforts to protect biodiversity hotspots like the Indian Himalayas.

8. Challenges, opportunities, and future perspectives

Despite the considerable progress achieved in plant biotechnology and tissue culture techniques, several scientific and practical challenges still hinder the efficient propagation and conservation of *Angelica glauca*. The integration of advanced biotechnological tools with traditional conservation practices offers vast potential for sustainable utilization and genetic improvement of this endangered Himalayan species.

8.1 current gaps in tissue culture research on *A. glauca*

Although numerous studies have demonstrated the potential of tissue culture for the propagation of medicinal plants, there remains a significant lack of standardized and optimized protocols for *Angelica glauca* (Oseni *et al.*, 2018; Kumar *et al.*, 2026) [28, 19]. Existing reports on micropropagation are limited, and results often vary due to differences in explant source, media composition, and hormonal concentrations. Factors such as high contamination rates, callus browning, and low rooting efficiency are frequent constraints during *in vitro* culture of *A. glauca*.

Another major limitation is the insufficient molecular data related to genetic diversity, biochemical pathways, and genome structure of this species, which restricts the development of targeted tissue culture strategies. The absence of a comprehensive genetic database also limits the identification of elite genotypes for *in vitro* conservation. Moreover, most studies have been restricted to laboratory-scale research, with very few efforts directed toward scaling up propagation or field-level acclimatization. Addressing these research gaps requires interdisciplinary collaboration between plant biotechnologists, conservationists, and ecologists to develop species-specific and cost-effective micropropagation systems that can ensure both genetic stability and commercial viability.

8.2 Potential for genetic engineering and genome editing

The application of genetic engineering and genome editing technologies represents one of the most promising frontiers for improving *A. glauca* propagation, conservation, and bioactive compound yield. With the advent of molecular tools such as *Agrobacterium*-mediated transformation, RNA interference (RNAi), and CRISPR-Cas9-based genome editing, it is now possible to precisely manipulate key genes associated with secondary metabolite biosynthesis and stress tolerance.

For example, the regulation of genes involved in coumarin and flavonoid biosynthesis could be enhanced to increase the yield of medicinal compounds in cultured tissues. Moreover, transgenic or edited lines could be developed with improved rooting capacity, reduced senescence, or enhanced resistance to microbial contamination during *in vitro* culture. However, the successful application of these technologies to *A. glauca* will require fundamental research on its genomic structure, metabolic pathways, and gene expression networks. Integrating

omics-based approaches (genomics, proteomics, and metabolomics) with tissue culture studies could accelerate the identification of candidate genes for targeted manipulation. Furthermore, ethical, ecological, and biosafety considerations must be addressed before large-scale deployment of genetically modified lines for commercial or conservation purposes.

8.3 Commercialization and sustainable utilization

Commercialization of *Angelica glauca* through tissue culture-based production systems presents both an opportunity and a challenge. On one hand, micropropagation offers the potential for mass-scale production of disease-free planting material, ensuring consistent supply to pharmaceutical and cosmetic industries. On the other hand, high production costs, lack of infrastructure, and limited awareness among local farmers and herbal industries hinder widespread adoption (Kumar *et al.*, 2026) [19].

Establishing plant tissue culture industries in the Himalayan region, supported by government and private initiatives, can provide livelihood opportunities to rural communities while promoting biodiversity conservation. Furthermore, integrating bioreactor-based cultivation systems can enhance the yield of valuable secondary metabolites in a cost-effective manner. Sustainable utilization also requires a balanced approach between economic exploitation and ecological preservation. Implementing certification systems for tissue-cultured plants, promoting public-private partnerships, and incorporating indigenous knowledge can strengthen the conservation-commercialization linkage. In addition, future efforts should focus on developing eco-friendly cryo-banking, bioinformatics-driven germplasm databases, and community-based conservation programs to ensure long-term survival and sustainable use of *A. glauca* and other Himalayan medicinal plants (Rajasekharan & Sahijram, 2015; Raina & Gautam, 2020) [34, 33].

9. Conclusion

Angelica glauca, a rare and valuable plant from the Himalayas, has a lot to offer in terms of medicine and commerce, but it is suffering from overuse, poor living conditions, and no natural growth to speak of. Propagation and conservation of the plant through tissue culture techniques may be the only scientific way to save its genetic diversity and use it sustainably. By doing this, *in vitro* culture techniques such as micropropagation, callus induction, and somatic embryogenesis have already proved the large-scale production of elite disease-free plantlets with genetic fidelity and high metabolite yield. However, the efforts in cryopreservation, germplasm storage, and molecular marker-assisted selection have led to *A. glauca* becoming a long-term conservation candidate; thus, it will be available to the future generations. A lot of work still needs to be done on the standardization of protocols, the optimization of growth media, and the scalability of tissue culture systems to achieve this. The use of cutting-edge biotechnological approaches including CRISPR-Cas9 gene editing, metabolic profiling, and bioreactor production of *A. glauca* in large volumes open up new avenues to increase both the total amount of drug produced and the plant's resistance to adverse environmental conditions. On the other hand, conservation efforts for the future have to shift their focus from lab-scale studies to the full-scale, field-level implementations involving local communities, government policy makers, and the herbal industry. The creation of tissue culture and cryo-banks in the Himalayan region could change

the situation regarding conservation and the generation of livelihoods. The interaction between biotechnology, conservation biology, and sustainable commercialization will eventually determine the fate of *Angelica glauca*, transforming it from an endangered species into a living proof of how science can collaborate with nature to provide effective solutions in the current era of ecological unpredictability.

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