A comprehensive review on propagation techniques of the medicinal herb *Ocimum tenuiflorum* L.

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Abstract:

***Ocimum tenuiflorum* L. (holy basil) is an economically important medicinal plant widely valued for its diverse therapeutic properties and applications in traditional medicine, food, cosmetics, and pharmaceuticals. The growing global demand for holy basil and its essential oil has driven interest in efficient propagation methods to ensure a consistent supply of high-quality planting materials. This review provides a comprehensive analysis of sexual propagation, clonal propagation, and tissue culture techniques, critically evaluating their advantages, limitations, and scalability for commercial production. Key production bottlenecks, including low seed germination uniformity, limited rooting efficiency in cuttings, and high tissue culture costs, are identified, and strategies are proposed to optimise propagation efficiency and reduce costs. These insights provide a foundation for future breeding programs, sustainable cultivation, and industrial applications of *O. tenuiflorum*.**

Keywords: **clonal propagation, holy basil, *Ocimum tenuiflorum*, sexual propagation, tissue culture.**

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1. Introduction

Ocimum tenuiflorum L. (holy basil), a perennial shrub belonging to the family Lamiaceae, is a highly valued medicinal and aromatic plant widely used in traditional medicine, pharmaceuticals, cosmetics, and functional foods. Rich in bioactive compounds, particularly essential oils, *O. tenuiflorum* has demonstrated diverse therapeutic properties, including anti-inflammatory, antimicrobial, antioxidant, anticancer, and anti-ulcer activities [1-3]. The essential oil content of *O. tenuiflorum* typically ranges from approximately 0.3% in fresh material to 0.5% in dried leaves, with eugenol (>70%), methyleugenol (≈12%), and β-caryophyllene identified as the predominant constituents [4]. Recent comprehensive phytochemical and pharmacological analyses of *Ocimum* species have revealed considerable diversity in essential oil composition, emphasising the major roles of eugenol, methyl eugenol, and *β*-caryophyllene in determining biological activity [5]. These findings highlight the economic and therapeutic significance of producing high quality, chemically consistent plant material through standardised and reliable propagation techniques.

The global holy basil essential oil market was valued at approximately USD 14.5 million in 2024 and is projected to grow at a compound annual growth rate (CAGR) of 10.1% through 2033, reflecting rising demand in the pharmaceutical, nutraceutical, and personal care industries [6]. The growing commercial utilisation of *O. tenuiflorum* and its essential oil underscores its expanding economic importance and the need for efficient, large-scale propagation systems. Several studies have reported that the commercial cultivation of *O. tenuiflorum* has generated significant economic benefits for farming communities in India, Egypt, and other producing countries [7, 8]. Originating in India, this species is now widely cultivated across Asia, North America,

and Europe, with key production regions in the United States, Canada, Iran, Thailand, and Vietnam [2, 3, 8].

In Vietnam, holy basil is increasingly cultivated in provinces (before the administrative merger) such as Quang Tri, Nghe An, Thanh Hoa, Ha Nam, Bac Giang, and Phu Tho to supply raw materials for medicine, healthcare products, cosmetics, and natural perfume production [9, 10]. However, large-scale production faces several critical challenges. Among these, the lack of genetically uniform, high-quality planting materials remains a major bottleneck, as the species is primarily propagated through seed sowing. While this method is simple and inexpensive, it often results in genetic variability, inconsistent seedling quality, and lower essential oil yields.

To meet the increasing market demand and ensure sustainable cultivation, it is essential to develop and optimise efficient propagation techniques, including sexual propagation, vegetative propagation, and tissue culture propagation. A systematic evaluation of each method's strengths, limitations, and practical applications is required to identify strategies that balance cost-effectiveness and product uniformity.

Despite the growing economic and medicinal significance of *O. tenuiflorum*, there has been no comprehensive review systematically comparing available propagation techniques and assessing their industrial applicability. This review addresses this gap by (i) synthesising current research on propagation methods, (ii) analysing the advantages and limitations of each approach, and (iii) proposing future perspectives to enhance propagation efficiency. These insights aim to serve as a valuable reference for researchers, farmers, and industry stakeholders involved in the sustainable production and commercialisation of holy basil.

2. Role of propagation

2.1. Sexual propagation by seeds

Seed propagation is one of the simplest and most widely practised methods for *O. tenuiflorum*. Successful seed-based propagation depends on several factors affecting germination such as temperature, viability, and dormancy status [11]. While inexpensive and technically simple, seed propagation often produces variable seedlings that differ in morphology and bioactive compound content, leading to inconsistent yield and oil quality.

Table 1. Effects of different pre-sowing treatments on germination of *O. tenuiflorum.*

Method/ Treatment	**Key parameters** (concentration, temperature, duration, spectrum)	**Environment**	**Reported effect on germination/seedling vigour**	**References**
GA_3 seed soaking	GA_3 0.05-0.1% for 24 h; alternating 20-30°C	Controlled chamber	Highest germination >90% and greatest seedling Vigor; fastest radicle emergence	[12]
KNO_3 priming+LED light spectrum	KNO_3 0.2-0.4%; LED spectra (white, green, red/blue)	Plant factory with artificial lighting (PFAL) system	0.4% KNO_3 under green LED enhanced germination (>85%) and seedling growth	[13, 14]
Hot-water treatment	Immersion 65°C for 5-10 min	Nursery setting	Moderate improvement (~70%); low cost option, but risk of seed damage if >10 min	[15]

Mechanical scarification	Gentle sandpaper abrasion before sowing	Nursery/lab tests	Germination 65-75% depending on seed lot; results inconsistent across cultivars	[15]
Acid scarification (H_2SO_4)	Immersion 30-60 sec then rinsing in distilled water	Laboratory	Marked increase (80-90%) but requires strict safety control and timing	[15]
Combined treatments (GA_3+KNO_3 or hot water)	Alternating temperature regimes with combined treatments	Growth chamber	Synergistic effects; combined treatments consistently outperformed single ones	[12, 15]
Thermal treatment	Exposure to 50ºC for 2 h (before sowing)	Laboratory	Highest germination rate at 50°C; higher temperatures reduced viability	[10]
Temperature regime optimisation	20-40°C gradient tested on multiple cultivars	Growth chamber	Optimum germination at 25-30°C; >35°C caused abnormal seedlings	[16, 17]
Baseline (control)	Untreated seeds under ambient lab conditions	Laboratory	Lower germination (50-70%); slower and uneven seedling emergence	[12, 15]

Results summarised in Table 1 demonstrate that different pre-sowing treatments significantly influenced the germination percentage, seedling vigour, and radicle emergence rate of *O. tenuiflorum* seeds. Among the tested methods, gibberellic acid (GA_3) seed soaking (0.1% for 24 h) under alternating temperatures of 20-30°C resulted in the highest germination rate (>90%), superior seedling vigour, and the fastest radicle emergence, as reported by S. Singh, et al. (2024) [12]. These findings are consistent with the established role of GA_3 in breaking seed dormancy by promoting α-amylase activity, which facilitates starch hydrolysis and enhances embryo growth.

Following GA_3 treatment, KNO_3 priming (0.4%) combined with green LED illumination in a plant factory system showed germination rates above 85% [13, 14]. KNO_3 is known to act as an osmopriming agent, improving water uptake and providing nitrate signals that stimulate cytokinin biosynthesis, which in turn promotes seed germination. Interestingly, seedling vigour was further enhanced under green LED light, likely due to its effects on phytochrome-mediated photomorphogenesis. These findings suggest that integrating nutrient priming with optimised light spectra could represent a scalable, controlled environment strategy for large-scale propagation of *O. tenuiflorum*.

Thermal treatments also improved germination efficiency. Hot water treatment at 65°C for 5-10 min moderately improved germination (70%), but longer exposure led to a risk of seed damage [15]. Similarly, thermal pretreatment at 50°C for 2 h yielded a marked improvement in germination rates, with T.H. Luong, et al. (2019) [10] reporting optimal performance at this temperature. These findings suggest that moderate heat stress likely softens the seed coat, enhances water permeability, and accelerates metabolic activation. However, excessively high temperatures (>75°C) were associated with a significant decline in germination, likely due to protein denaturation and embryo damage.

Recent studies have also highlighted the benefits of combined dormancy-breaking techniques. Treatments that integrate GA_3 soaking with KNO_3 priming or hot water exposure consistently produced better germination and seedling vigour compared with single treatments, suggesting a synergistic effect among hormonal-, chemical-, and temperature-based methods [12, 15]. Additionally, experiments on temperature regimes confirmed that the optimal temperature for germination is between 25 and 30°C, whereas higher temperatures above 35°C increased abnormal seedling development and reduced establishment success [16, 17].

In summary, GA_3 soaking and KNO_3 priming are the most effective and reproducible strategies for improving *O. tenuiflorum* germination and establishing uniform seedlings for large-scale production. However, integrating these treatments with controlled environmental conditions, including temperature optimisation and LED-assisted germination systems, can further enhance success. These findings are highly relevant for commercial propagation where uniform seedlings directly contribute to higher biomass accumulation and increased essential oil yield.

2.2. Clonal propagation by stem cutting

Clonal propagation through stem cuttings is a simple and cost-effective method that produces uniform progeny genetically identical to the mother plant. The success of rooting depends on multiple factors, including the cultivar, the physiological stage of the mother plant, environmental conditions, and the application of plant growth regulators (PGRs) [18].

Several studies have demonstrated that the application of auxins, particularly indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA), significantly enhances rooting performance. For instance, treating cuttings with IBA at 1000 ppm increased rooting success up to 90%, indicating its strong influence on root initiation [19]. Similarly, S. Achapjee, et al. (2015) [20] reported that both the type of cutting and auxin

concentration are critical: 20 cm-long apical cuttings treated with IAA achieved the highest rooting rate (82.48%), whereas IBA treatment resulted in a moderate rate (62.28%). Moreover, 20 cm middle cuttings treated with IAA at 2000 ppm showed superior growth compared to apical cuttings, demonstrating that cutting position affects overall plant performance.

Table 2. Effects of different approaches in clonal propagation by stem cutting of *O. tenuiflorum.*

Approach/ treatment	**Substrate used**	**Hormonal/ biostimulant treatment**	**Rooting success (%)**	**Shoot growth/ survival (%)**	**Reference**
Softwood stem cuttings (10-12 cm)	Sand+FYM (1:1)	IBA 2000 ppm (quick dip, 5 sec)	78	72	[19]
Semi-hardwood cuttings	Sand+Soil+Vermicompost (1:1:1)	IBA 1000 ppm basal treatment	65	60	[18]
Softwood cuttings with auxin mix	Cocopeat+Vermiculite (1:1)	IBA 1000 ppm+NAA 500 ppm	82	80	[21]
Softwood cuttings with seaweed extract	Perlite+Cocopeat (1:1)	IBA 2000 ppm+seaweed biostimulant	85	84	[21]

Nodal cuttings cultured *in vitro*	Half-strength MS medium	IBA 0.5 ppm	90	80	[19]
Apical cuttings	Sand	IBA 1000 ppm	75	82	[9]
Control (no hormonal treatment)	Sand	None	40	35	[21]

As summarised in Table 2, softwood cuttings treated with IBA (2000 ppm)+seaweed extract achieved 85% rooting and 84% survival [21], while IBA (1000 ppm)+NAA (500 ppm) in a cocopeat-vermiculite (1:1) substrate yielded 82% rooting and 80% survival. In contrast, untreated controls achieved only 40% rooting and 35% survival.

In vitro nodal cuttings cultured on half-strength MS (Murashige and Skoog) medium with IBA (1.0 mg/l) recorded 90% rooting and 80% shoot growth [19], outperforming soil based methods. These results emphasise that integrating auxins, nutrient rich substrates, and biostimulants can significantly enhance clonal propagation efficiency and uniformity. Conversely, semi-hardwood cuttings treated with IBA (1000 ppm) in a sand+soil+vermicompost (1:1:1) mixture achieved only 65% rooting and 60% survival, suggesting that tissue maturity influences rooting ability and that higher auxin concentrations or alternative biostimulants may be required [18].

Overall, the evidence suggests that the integration of IBA or IAA treatments, nutrient-rich substrates, and biostimulant supplementation significantly improves the efficiency of clonal propagation in *O. tenuiflorum*. These optimised approaches can be effectively applied in large-scale nursery programs to ensure uniform plant quality and

a sustainable supply of raw materials for medicinal and essential oil industries [18, 19, 21].

2.3. Propagation via cell tissue culture (in vitro)

Tissue culture-based micropropagation offers a highly efficient and reliable method for producing genetically uniform, disease-free, and high-quality planting material of *O. tenuiflorum*. This approach overcomes the limitations of conventional seed germination and stem cutting propagation, which are often constrained by low germination rates, seasonal dependence, and inconsistent rooting success. *In vitro* techniques have been widely applied for large-scale commercial production, germplasm conservation, and genetic improvement of medicinal plants [19].

The success of *in vitro* propagation largely depends on several factors, including the explant type, nutrient medium composition, PGRs, and environmental conditions such as light, temperature, and humidity [22]. In *O. tenuiflorum*, nodal segments, shoot tips, and leaf explants are commonly used as starting materials due to their high morphogenic potential and reduced contamination risk [23].

Table 3. Effects of different approaches in propagation via tissue culture of *O. tenuiflorum*

Explant type	PGR combination	Shoots/explant	Regeneration (%)	Reference
Nodal segments	BAP+NAA	9.5	89	[19]
Nodal culture	BAP+IBA	6.8	94	[23]
Leaf explants	BAP+NAA	5.4	85	[24]
Shoot tips	TDZ+NAA	5.8-7.2	88-96	[22, 25]
Callus-mediated regeneration	2,4-D+BAP	4.2	80	[18]

Nodal explants	Bioreactor (BAP+IBA)	10.5	95	[25]

The results in Table 3 highlight that *in vitro* micropropagation of *O. tenuiflorum* can be highly efficient when the type of explant, basal medium, and PGRs are optimised. Among all approaches, nodal explant culture on MS medium supplemented with 6-Benzylaminopurine (BAP)+IAA or IBA consistently achieved regeneration efficiencies above 90% and number of shoot range 4.2-10.5 shoots per explant [19, 22]. The use of thidiazuron (TDZ) at low concentrations (0.2 mg/l) resulted in the highest shoot proliferation rate (7.2 shoots/explant) reported so far, indicating TDZ's strong cytokinin-like activity [25]. Similarly, S. Patel, et al. (2020) [24] demonstrated that leaf explants could also regenerate shoots via indirect organogenesis, although this process was less efficient than nodal culture. Furthermore, Y. Zhang, et al. (2024) [25] reported significant advances in bioreactor assisted systems, achieving a 95% regeneration efficiency and generating more than 10 shoots per explant. This technique offers a scalable solution for industrial scale mass propagation of *O. tenuiflorum*, meeting the growing demand for uniform, high-quality planting material in the medicinal plant industry.

Overall, auxin-cytokinin balance plays a pivotal role in optimising shoot induction and rooting. While BAP remains the most commonly used cytokinin, combining it with IBA or NAA at low concentrations produces superior results. Callus-mediated regeneration, although useful for somatic embryogenesis, was comparatively less efficient due to slower shoot elongation rates [18].

3. Analysis, evaluation, and application orientation

Variation in essential oil composition among propagated plants may result from somaclonal variation or culture induced stress. These observations reinforce the need

for optimised propagation systems that preserve both genetic identity and metabolic uniformity.

The efficient propagation of *O. tenuiflorum* is crucial for ensuring the sustainable production of high-quality planting material for medicinal, nutraceutical, and industrial purposes. A comparative assessment of three primary propagation techniques, namely sexual propagation by seeds, clonal propagation by stem cuttings, and tissue culture, was carried out to evaluate their efficiency, limitations, and potential applications as summarised in Table 4 and Fig. 1.

Table 4. Comparison of different propagation techniques for *O. tenuiflorum*.

Criteria	**Seed propagation**	**Clonal propagation (Stem cuttings)**	**Tissue culture (*In vitro*)**
Genetic uniformity	Low; high variability in morphology, yield, and oil composition	High; maintains true to type traits	Very high; produces genetically stable clones
Germination/rooting success	50-70% (often reduced by dormancy)	65-85% with IBA treatment	>90% with optimised PGR protocols
Multiplication rate	Low	Moderate	Very high (>10 shoots per explant in bioreactor-assisted systems)
Disease management	Risk of seed borne pathogens	Risk of disease transfer from mother plants	Produces disease free plants
Technical requirement	Minimal infrastructure; farmer friendly	Requires basic nursery setup and rooting management	Requires sterile laboratories, trained personnel, and advanced facilities

Cost effectiveness	Low cost, highly accessible	Moderate cost; suitable for nurseries	High initial cost but cost effective for large scale production
Scalability	High for bulk cultivation, but uniformity compromised	Limited scalability due to labour intensity	Highly scalable with automation and bioreactor integration
Suitable applications	Germplasm conservation, basic cultivation, breeding programs	Elite cultivar multiplication, medium scale production, phytochemical uniformity	Industrial scale essential oil production, pharmaceutical grade propagation, germplasm preservation
Time required to obtain plantable seedlings	45-60 days	60-75 days	>150 days
References	[10, 12, 16]	[9, 18, 19, 21]	[22-25]

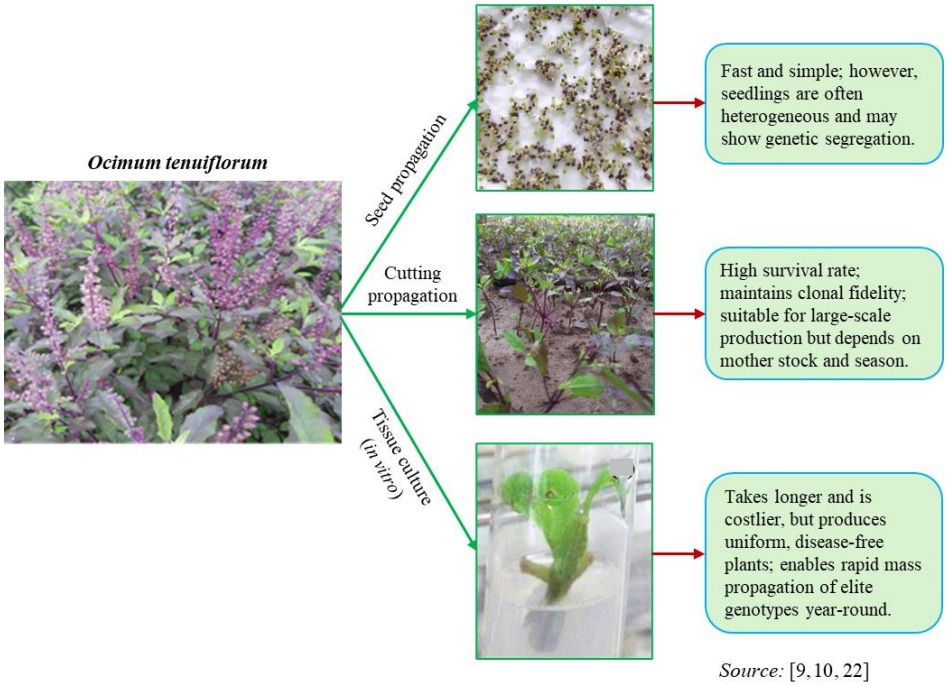

Fig. 1. Schematic diagram of propagation methods of *Ocimum tenuiflorum* L.

Seed propagation remains the simplest and most economical technique, suitable for germplasm conservation and breeding but limited by low germination rates and high trait variability [10, 12, 16].

Clonal propagation ensures uniformity and consistency in essential oil content, ideal for elite cultivar multiplication, though its scalability is constrained by labour intensity and disease risk [9, 19, 21].

Tissue culture provides the highest efficiency, producing disease-free, genetically stable, and chemotype consistent plants. TDZ assisted nodal cultures and bioreactor based systems achieve regeneration rates above 95% [22-25]. However, the method demands advanced facilities and technical expertise.

Consistency in essential oil composition is crucial for pharmaceutical and food grade applications. Studies have shown that variations in propagation techniques and environmental conditions can alter metabolite profiles among *Ocimum* species [5].

Thus, optimising propagation protocols that preserve chemotype stability is essential to maintain product quality across cultivation cycles.

To enhance production efficiency and scalability, an integrated propagation strategy is recommended. The first step involves using seed propagation for the selection of elite genotypes to maintain genetic diversity and identify plants with desirable traits. In the second step, *in vitro* micropropagation can be applied to rapidly multiply disease free and high value clones while maintaining uniformity in morphology and phytochemical composition. In the final step, large-scale field production can be achieved economically by using stem cutting propagation, which enables the distribution of uniform planting material to farmers and nurseries.

This integrated approach combines the strengths of all three techniques, thereby improving the quality, consistency, and scalability of *O. tenuiflorum* cultivation. It provides a sustainable framework that balances genetic diversity, phytochemical stability, cost effectiveness, and production efficiency, supporting both small scale growers and industrial producers.

4. Conclusions

The optimal propagation method for *O. tenuiflorum* depends on production objectives, available resources, and intended applications. Seed propagation is simple and economical but limited by genetic variability among seedlings. Stem cutting propagation with IBA treatment provides higher uniformity and survival rates, making it suitable for nursery level production. In contrast, tissue culture propagation is more resource-intensive yet ensures genetic stability and produces disease free plants that meet the requirements of high value industrial uses.

Future studies should focus on optimising substrate composition, hormonal combinations, and seasonal conditions, as well as developing standardised and cost effective tissue culture protocols. The integration of conventional propagation with

controlled environment cultivation and bioreactor assisted micropropagation will be essential to achieve sustainable, large-scale production of high-quality *O. tenuiflorum* planting materials.

CRediT author statement

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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