



# Rapid Multiplication Techniques of Black Pepper (*Piper nigrum* L.): A Comprehensive Review

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

As global demand for high-quality spices continues to rise, black pepper (*Piper nigrum* L.) stands out for its culinary and medicinal importance. However, traditional propagation methods such as stem cuttings and seed sowing often suffers from slow growth, genetic variability, and disease susceptibility. To address these challenges, a range of rapid multiplication techniques (RMTs) has emerged, revolutionizing black pepper cultivation. Thus, this review helps to provide a comprehensive overview of key RMTs including serpentine layering, soil mound, bamboo and trench methods, raised bed propagation, and the use of low-cost mist chambers that have improved propagation efficiency, plant uniformity, and disease resistance to ensure enhanced

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production. Among these, serpentine layering and soil mound methods have shown high survival rates and shortened the juvenile phase, while bamboo and trench techniques offer cost-effective and scalable solutions. Additionally, the integration of biotechnological innovations such as micropropagation (tissue culture), genome editing via CRISPR/Cas9, speed breeding, and marker-assisted selection (MAS) has further advanced black pepper production. These approaches have not only increased multiplication rates but also facilitated the development of climate resilient, disease resistant cultivars. Despite these advantages, widespread adoption remains limited due to high setup costs and technical barriers. Therefore, future success depends on collaborative efforts between researchers, policymakers, and extension services to ensure equitable access and training. By combining traditional practices with scientific advancements, the black pepper industry can transition toward a more sustainable, high-yielding future.

**Keywords:** *Black pepper; rapid multiplication; micropropagation; CRISPR/Cas9; sustainable cultivation.*

## 1. INTRODUCTION

Black pepper (*Piper nigrum* L.), often hailed as the "king of spices," holds a special place in kitchens and markets around the world, not just for its sharp, pungent flavor, but also for its medicinal value, largely thanks to compounds like piperine (Singletary, 2010). Originating from the tropical regions, this woody perennial vine has traditionally been propagated through stem cuttings or seeds. However, these methods are often slow and inconsistent, and plants grown from seed can vary genetically and are more prone to diseases like *Fusarium* wilt (Bhuyan et al., 2015; Nair & Dutta, 2007). To overcome these challenges, researchers and growers have turned to rapid multiplication techniques. These methods help ensure uniformity, quicker plant establishment, and stronger disease resistance. Vegetative propagation using runner shoot cuttings remains common because it preserves genetic traits and boosts survival rates (Ranjith & Nagaraj, 2016; Khatke et al., 2024). But advances in biotechnology particularly tissue culture are increasingly being used to enhance propagation without over relying on field grown mother plants (Aziz et al., 2019). At the same time, the studies of how the plant responds to environmental and pathogenic stress are helping fine tune these methods for even better results (Nirmal et al., 2016). As a result, rapid multiplication techniques have become central to modern black pepper cultivation, overshadowing traditional propagation practices with scientific progress (Ee & Shang., 2017). These approaches are not just making farming more sustainable; they're also helping meet growing global demand, fueled by black pepper's well documented antioxidant, anti-inflammatory, and antimicrobial properties (Meixner, 2019; Singletary, 2010).

## 2. CHALLENGES IN TRADITIONAL PROPAGATION TECHNIQUES OF BLACK PEPPER

Traditional propagation methods for black pepper (*Piper nigrum* L.), while essential for its propagation are accompanied with limitations that hinders the productivity and sustainability. These challenges arises from the biological, environmental and operational constraints, which are increasingly becoming problematic in the face of rising global demand and climate variability.

### 2.1 Limited Availability of Quality Planting Material

Vegetative propagation *via* stem cuttings from runner shoots is the most commonly practiced traditional method, it also ensures genetic fidelity to the parent plant (Nair & Gupta, 2003). However, reliance on field grown mother plants restricts the availability of healthy and disease-free cuttings. Over time, repeated harvesting depletes plant vigor which eventually leads to reduced rooting efficiency and poor survival rates of the propagated saplings. This scarcity of robust planting material causes delay in establishing new plantations. Recent works highlight that subpar seed quality and limited access to elite genotypes further compound this issue, particularly in regions like Indonesia and India (Tarigan et al., 2023).

### 2.2 Disease Susceptibility

Traditionally propagated black pepper is highly vulnerable to soil borne pathogens such as *Fusarium oxysporum* and *Phytophthora capsici*, which cause root rot and wilting. For example,

Brazil, once a leading producer, experienced yield declines exceeding 50% due to fungal infections linked to traditional practices (de Castro *et al.*, 2016). Traditional methods lack integrated disease-management protocols, leaving crops exposed to recurrent infections as compared to the new advanced methods (Abbasi *et al.*, 2010). The absence of resistant varieties in conventional propagation exponentially increases these losses. (de Castro *et al.*, 2016).

### 2.3 Genetic Variability in Seed Propagation

The method of seed based propagation often introduces significant genetic heterogeneity due to cross-pollination which results in inconsistent fruit quality, alkaloid content (e.g., piperine), and agronomic performance (Shivakumar *et al.*, 2020). This variability undermines the commercial value, as buyers prioritize uniformity in flavor and bioactive compound profiles (Singletary, 2010). These genetic instability in seed derived plants hinders the efforts to standardize some certain cultivation practices, particularly in regions like Kerala, India, where diverse agroclimatic conditions further amplify variability (Tarigan *et al.*, 2023).

### 2.4 Slow Growth and Maturation

Black pepper vines propagated through traditional cuttings usually require 3-4 years to reach the productive stage, delaying returns on investment for farmers (Kandiannan *et al.*, 1998). The prolonged juvenile phase increases exposure to environmental stress factors such as droughts or pest outbreaks, further jeopardizing yield stability. Studies from South India link slow growth to nutrient-depleted laterite soils, which are common in traditional cultivation zones (Varghese & Ray, 2024).

### 2.5 Labor-Intensive Practices

Traditional methods demand meticulous manual labor for activities like cutting selection, nursery management, and pest control to achieve good productivity. Small scale growers, particularly in developing nations, often lack resources to sustain these practices which often leads to undersired outcomes (Aziz *et al.*, 2019). In regions like Kerala, the labor costs alone accounts for over 60% of production expenses, discouraging adoption of intensive cultivation methods for small and marginal farmers. (Tarigan *et al.*, 2023).

**Table 1. Comparative analysis of Rapid Multiplication Techniques (RMTs) as compared to Traditional Techniques**

| Parameter            | Traditional Methods                                   | Rapid Multiplication Techniques                              | References   |
|----------------------|---|--|--|
| Multiplication Ratio | Low (1:10 per mother plant)                           | High (1:40 via rapid clonal propagation)                     | Khandekar <i>et al.</i> , (2004); Thapa <i>et al.</i> , (2021)   |
| Time to Maturity     | 3-4 years to full productivity                        | Reduced to 1-2 years (soil mound method)                     | Khandekar <i>et al.</i> , (2004); Varghese & Ray, (2024)         |
| Labor Intensity      | High (manual nursery management)                      | Reduced by 30-50% (e.g., trench method)                      | Thapa <i>et al.</i> , (2021)                                     |
| Cost Efficiency      | Lower benefit-cost ratio (1:1)                        | Higher benefit-cost ratio (2.1:1 for soil mound method)      | Khandekar <i>et al.</i> , (2004)                                 |
| Rooting Success Rate | 50-60% (soil-dependent)                               | 80–85% (serpentine layering)                                 | Karmawati <i>et al.</i> , (2020); Thapa <i>et al.</i> , (2021)   |
| Disease Resistance   | Vulnerable to <i>Fusarium</i> and <i>Phytophthora</i> | Improved resistance (sterile media, CRISPR-edited cultivars) | Khew <i>et al.</i> , (2022); Tarigan <i>et al.</i> , (2023)      |
| Scalability          | Limited to small-scale farms                          | Suitable for large-scale production                          | Khandekar <i>et al.</i> , (2004) ; Deepak <i>et al.</i> , (2022) |
| Genetic Uniformity   | Moderate (runner cuttings)                            | High (tissue culture, clonal propagation)                    | Thapa <i>et al.</i> , (2021); <i>et al.</i> , (2022)             |

## 2.6 Environmental Dependency and Soil Degradation

Black pepper is a crop that requires specific tropical conditions with high humidity and well drained soils. The adverse change in climate has induced shifts in rainfall patterns and temperature which has disrupted these requirements, reducing the efficacy of traditional propagation in historically suitable regions (Varghese & Ray, 2024). Soil degradation, particularly in Kerala's laterite soils, has been linked to unsustainable practices such as excessive chemical fertilizer use and monocropping, which reduce organic carbon and nutrient retention (Karmawati et al., 2020).

## 2.7 Limited Scalability and Technological Gaps

Traditional propagation techniques often struggles to meet global demand due to its low multiplication ratio (1:10 for cuttings) compared to modern techniques like tissue culture (1:40) (Thankamani et al., 2004). The reliance on outdated practices disrupts the adoption of biotechnological advances, such as in vitro shoot regeneration or liquid culture systems for metabolite production (Nirmal et al., 2016). For instance, tissue culture protocols for *Piper nigrum* remain underutilized despite their potential for disease-free mass propagation (Abbasi et al., 2010).

## 3. RAPID MULTIPLICATION TECHNIQUES IN BLACK PEPPER

Due to the ever increasing global demand of black pepper for use in various culinary and pharmaceutical purposes, the need of Rapid multiplication Techniques (RMT) has become the need of the hour to meet the production demands of the market. RMTs not only increases the rate of multiplication in terms of propagation but also helps to enhance the total production and quality of the produce which greatly enables the farmers to get more market access and achieve enhanced income.

### 3.1 Serpentine Layering: Simple, Efficient, and Reliable

Serpentine layering is a simple and farmer friendly method that allows multiple plants to be produced from a single vine (Anandaraj et al., 2014) and is very commonly practiced method. It is a superior method as compared to seed multiplication for rapid multiplication in *Piper*

*nigrum* (Sasikumar et al., 2008). This technique involves bending a healthy runner shoot and burying several nodes in the soil, which encourages each buried section to root and sprout independently. As per a comparative varietal trial conducted by Thapa et al. (2021), the Panniyur-1 variety achieved an impressive 93% survival rate after 30 days and 88.33% after 90 days, with an average yield of over 51 new plants annually. In comparison, Panniyur-2 and Pournami showed lower survival rates of 78-82% and fewer propagated layers (35-40 per year). This method is especially effective in tropical climates like India's and can work well across different soil types, from laterite to clay-loam. It also reduces the labor burden by up to 25% compared to standard stem cuttings (Thapa et al., 2021). Supporting this, Waman et al. (2023) also reported a 100% survival rate using serpentine layering in woody pepper vines within just 47-75 days of operations.

### 3.2 Soil Mound Method: Boosting Root Growth and Efficiency

The soil mound method takes propagation a step further by creating raised beds with a mix of compost, sand, and cocopeat. This mixture helps to improve the air circulation and water retention, encouraging stronger and healthier roots. Khandekar et al. (2004) found that this technique gave a benefit-cost ratio of 2.1:1 at 15 cm plant spacing and produced 40% more runner shoots per area compared to bamboo-based propagation. One major advantage of this method is that it reduces the juvenile phase of black pepper from the usual 3-4 years to under 2 years which eventually helps the farmers to start the harvest sooner. It is particularly useful in labor scarce regions like Kerala, where it has been shown to cut labor costs by 30%. The method also increases plant length, node count, and root development, especially when organic inputs like vermicompost are added (Bhuyan et al., 2015; Ranjith & Nagaraj, 2016).

### 3.3 Bamboo Method: Simple-Tech, High Impact

In the bamboo or split bamboo method, the split sections of bamboo or PVC pipes are filled with a mixture of coir dust and cow dung or any other available rooting mixture, then tilted over nursery beds at a 45° angle to encourage good drainage. Usually, stands are made to hold the split bamboo or PVC pipes at the desired angle. Then single-node cuttings are inserted into slits filled with the rooting mixture and under optimal

conditions, they root in about 30-45 days. Each split bamboo/PVC can support about 15-20 cuttings, making it a cost-effective solution for large-scale propagation (Ranjith & Nagaraj, 2016). In a comparative experiment, bamboo split method was observed to be more superior over other methods of propagation with respect to all the characters (Hanumanthappa et al., 2016). This method is particularly suitable for nurseries or farmers with limited access to expensive propagation equipment.

### 3.4 Trench Method: Deep Roots, Strong Starts

This method involves digging trenches that are 2-3 meters long and with width and depth of about 45-60 cm, ideally during the monsoon or just before the onset of monsoon. These trenches are then filled with a mixture of topsoil, compost, FYM and sand to provide a loose, fertile base that supports strong root growth. Besides helping the roots to thrive, trench planting also supports the integration of live supports plants like *Gliricidia* or *Erythrina*, upon which the black pepper vines can climb and grow to maturity. Anandaraj et al., (2014) reported that this technique significantly improved vine growth and even accelerated fruiting as compared to mound or pit planting. It also helps to control waterlogging and soil erosion, especially in sloped or hilly terrains.

### 3.5 Low Cost Mist Chambers: Controlled Environments for Consistent Results

Simple and budget friendly mist chambers offer a practical solution for propagating black pepper plants in environment with semi controlled conditions. It is typically assembled using readily available materials such as bamboo and UV-stabilized polyethylene. To maintain the desired humidity and to facilitate timely irrigation, either fogging nozzles or hand sprayers are used inside

the structures. This helps to create and maintain an ideal condition with desired humidity and moderate temperatures (25-30°C) that black pepper cuttings require to root. These low cost mist chambers increases the chances of rooting, reduces plant mortality, and makes it possible to propagate plants throughout the year, even in regions where advanced nursery infrastructure is lacking. Aryan (2012) and Mustakim et al. (2022) both highlighted their affordability and effectiveness, especially for small and marginal farmers. With a view to augment the production of planting materials in black pepper by exploiting the potential of the protected structures, Sujatha et al. (2016) in an experiment reinforced the advantages black pepper multiplication under protected structures.

### 3.6 Rapid Multiplication in Raised Beds: Fast, Healthy, and Scalable

The Rapid Multiplication Technique (RMT) using raised beds has gained significant traction as a practical nursery method for propagating black pepper. The beds are prepared with well drained, fertile soil mixed with sand and Farm yard manure (FYM), under partial shade. The lateral shoots with 2-3 nodes from the desired plants are cut and treated with fungicides and rooting hormones like indole-3-butyric acid (IBA), which are then planted close together. The moisture and soil temperature in the nursery bed is maintained using plastic sheets or banana leaves as mulches and under such favourable conditions, rooting typically occurs within 4-6 weeks. The young plants are then hardened off and transplanted in the main field. This approach is widely used in commercial nurseries for producing uniform, disease free planting material (Ravindran & Nair, 2000). Additionally, application of biocontrol agents (*Pseudomonas* and *Trichoderma*) has shown to increase the rooting and shooting of black pepper cuttings and also reduced the seedling mortality rate in the nursery (Ee & Shang, 2017).

**Table 2. Advantages of Rapid Multiplication Techniques (RMTs)**

| Technique           | Advantages                                   | Limitations                              | References   |
|---------------------|--|--|--|
| Serpentine Layering | High survivability (93%), genetic uniformity | Labor-intensive node management          | Aziz et al., 2019; Thapa et al., 2021              |
| Soil Mound Method   | 40% higher productivity, cost-effective      | Requires nutrient-rich media             | Hanumanthappa et al., 2016; Khandekar et al., 2004 |
| Genome Editing      | Disease resistance, abiotic stress tolerance | High technical expertise required        | Nirmal et al., 2016; Khew et al., 2022             |
| Speed Breeding      | Reduces breeding cycle by 60%                | Energy-intensive controlled environments | Kandiannan et al., 2020; Khew et al., 2022         |

### 3.7 Biotechnological Innovations: Pushing the Boundaries

#### 3.7.1 Micropropagation (Tissue Culture)

Micropropagation is an advanced tissue culture technique which has emerged as a reliable method for high rate of multiplication coupled with the additional advantage of obtaining disease free planting material which makes it a viable alternative to conventional propagation (Nirmal Babu & Minoo, 2003). This technique involves the use of sterile culture of explants typically sourced from shoot tips, nodal segments, or meristematic tissues placed in a nutrient media enriched with specific plant growth regulators. In the context of black pepper, micropropagation effectively addresses the constraints of conventional stem cutting methods, which offer limited multiplication efficiency. For instance, despite the potential for disease free mass propagation of black pepper through tissue culture, it remained largely underutilized (Abbasi et al., 2010) which gave rise to the use of standard protocols utilizing Murashige and Skoog (MS) medium, supplemented with cytokinins such as 6-Benzylaminopurine (BAP) for shoot proliferation, and auxins like Indole-3-butyric acid (IBA) for rooting, have shown consistent success in the rapid multiplication of black pepper (de castro et al., 2016). Umadevi et al., (2015) developed a meristem culture protocol for the rapid production of the plantlets in the liquid medium containing Murashige and Skoog (MS) medium with 0.1 mg L<sup>-1</sup> kinetin and 0.5 mg L<sup>-1</sup> GA<sub>3</sub>, subsequently, direct shoot induction in ½ MS with 3 mg L<sup>-1</sup> BA and 1 mg L<sup>-1</sup> IAA followed by shoot growth and development in ½ MS +0.5 mg L<sup>-1</sup> Indole butyric acid. Apart from boosting propagation speed, micropropagation also ensures uniformity, enhanced vigor, and year-round availability of planting material, while significantly reducing vulnerability to foot rot disease caused by *Phytophthora capsici* (Nirmal et al., 2016; Afroz 2020; Deepak et al., 2022).

#### 3.7.2 Genome Editing Using CRISPR/Cas9

The development of genome editing tools such as CRISPR/Cas9 has revolutionized the ways in which black pepper can be improved, particularly in terms of its rapid multiplication. The effectiveness of this technique in editing disease-susceptibility genes, including PcAr3, which increases resistance to *Phytophthora capsici* in

cultivars like Semongok Emas, was demonstrated by Khew et al. (2022). Its efficacy was demonstrated by further field trials, which revealed a striking 70% decrease in disease incidence. Genome editing has the ability to improve features like drought tolerance and increase piperine production, which is in line with the expanding demands of the spice industry and helps fulfill the rising demand for premium planting materials.

#### 3.7.3 Speed Breeding

Speed breeding is an innovative method that accelerates the time taken to develop new plant generations by fine tuning and manipulating environmental elements such as temperature and exposure to light. Generally, in case of black pepper it can take over 2 decades for a single breeding cycle to complete which makes it challenging to obtain high quality and genetically stable planting materials. However, Khew et al. (2022) discovered that the duration of the lengthy breeding cycle may be reduced to just 5-7 years by controlling the environmental conditions, such as a 22-hour exposure to light at 25°C. The breakthrough of this technique has led to the rapid development of exceptional cultivars in Malaysia, such as Semongok Aman, which stands out for its resilience in areas with erratic rainfall and salinized soils.

#### 3.7.4 Marker-Assisted Selection (MAS): Targeted Genetic Advancement

Marker-Assisted Selection (MAS) is an innovative breeding method that greatly enhances the viability of the planting materials, which employs molecular markers to identify and select disease-resistant genotypes early in the propagation process. Specific single nucleotide polymorphisms (SNPs) linked to resistance to *Fusarium solani*, a soil-borne fungus that affects black pepper, were found by Khew et al. (2022). With the implementation of MAS in nurseries, there has been an astounding 45% decrease in seedling mortality in high-risk locations, particularly in Sarawak, Malaysia. This has also increased the availability of disease-free planting materials which is essential for preserving and carefully distributing traditional and genetically varied cultivars with desired characteristics (Deepak et al., 2022).

### 4. CONCLUSION

Rapid multiplication techniques and other biotechnology breakthroughs have helped to

overcome many of the difficulties associated with conventional black pepper multiplication methods over the years. We can now create consistent, disease-free planting material more quickly thanks to these new, creative methods, which eventually helps the plants grow earlier and provide more high-quality material. More significantly, these methods give both commercial growers and small-scale farmers a huge edge by improving resistance to major illnesses and reducing expenses. A turning point has been reached with the development of cutting-edge technologies such as genome editing, speed breeding, and marker-assisted selection (MAS), which have made it possible to produce robust, high-yielding cultivars that are better suited for climate-resilient production. However, despite the obvious benefits, these technologies haven't been extensively embraced yet because of their high upfront prices, requirement for technical know-how, and lack of training opportunities, all of which act as obstacles to their wider use and use. In the future, closer collaboration between researchers, politicians, and agricultural outreach programs is essential. We have a great chance to create a more resilient and sustainable black pepper sector that can flourish in our constantly shifting climate by combining traditional farming knowledge with state of the art technology.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

#### COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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