

Review Article

# Advancements in Cardamom (*Elettaria cardamomum* Maton) Breeding: Genetic Diversity, Biotech Strategies, and Future Directions

Mohammedsani Zakir Shehasen\* 

Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, Jimma, Ethiopia

## Abstract

Cardamom (*Elettaria cardamomum* Maton), often referred to as the 'Queen of Spices,' belongs to the Zingiberaceae family and is noted for its unique flavor and aroma. As an herbaceous perennial, cardamom thrives in the shaded environments of the Western Ghats in South India, where it exhibits significant genetic diversity necessary for breeding improvement. This paper explores the genetic resources of cardamom, including advances in breeding methodologies and biotechnological approaches aimed at enhancing productivity and quality. Key breeding strategies include clonal selection, hybridization, and mutation breeding, which have led to the development of several high-yielding and stress-resistant varieties. The integration of molecular techniques, such as marker-assisted selection and genetic transformation, offers prospects for tapping into cardamom's genetic diversity for future improvements. However, the genetic erosion of natural cardamom populations necessitates urgent conservation efforts to safeguard this valuable germplasm. This review emphasizes the importance of innovative breeding techniques and strategic direction for the sustainable enhancement of cardamom as a critical global spice crop.

## Keywords

Cardamom, Breeding Improvement, Genetic Diversity, Biotechnological Approaches, Sustainable Agriculture

## 1. Introduction

Cardamom (*Elettaria cardamomum* Maton), often referred to as the "Queen of Spices," is a true cardamom species that belongs to the Zingiberaceae family within the Scitaminae order. It is highly esteemed for its distinctive flavor and delightful aroma, making it one of the most important and valuable spices [1]. Typically, cardamom is cultivated in the shade of evergreen trees in the Western Ghats of South India, thriving at elevations between 600 and 1200 meters above sea level, with an average annual rainfall of 1500 to 4000 mm and temperatures ranging from 10 to 35 °C. The ideal conditions

for cardamom cultivation include a humid tropical climate and soil rich in organic matter [2]. While India is considered the native home of cardamom, the primary center of diversity for the genus is found in the Sarawak (Malaysia) and Borneo area, where eight species have been identified. Due to India being the origin of cardamom, its natural populations display a significant degree of variability in both quantitative and qualitative traits stemming from genetic and environmental factors [3]. Improvement programs focus on harnessing this genetic diversity to create elite genotypes tailored for various

\*Corresponding author: mohammedsani641@gmail.com (Mohammedsani Zakir Shehasen)

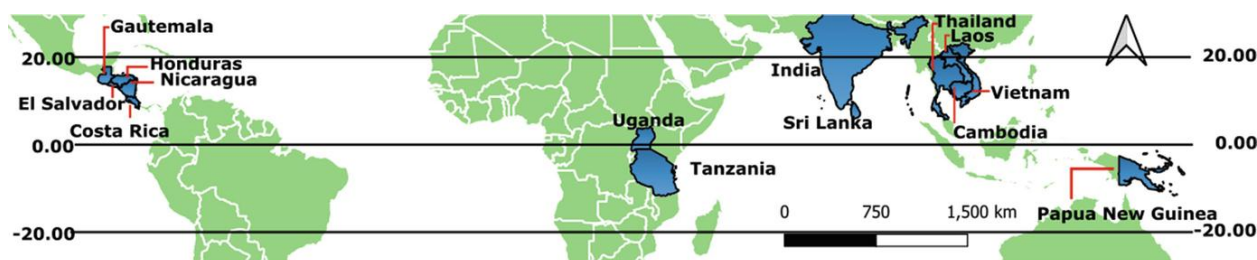
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agro-ecological zones, as well as hybrids with higher yields, better quality, and resistance to both biotic and abiotic stresses. To boost crop yield and enhance disease resistance, leveraging hybrid vigor is crucial. Cardamom, which can reproduce sexually and vegetatively, allows for heterosis breeding, and the genetic integrity of the resulting offspring can be preserved through vegetative methods [4, 5]. Major producers of

cardamom globally include Guatemala, Tanzania, Sri Lanka, El Salvador, Vietnam, Laos, and Cambodia [6]. *Elettaria cardamomum*, a highly valued spice, is native to South India's Western Ghats, with notable production also occurring in countries such as Indonesia, India, Guatemala, Nepal, Sri Lanka, Laos, Bhutan, Tanzania, Grenada, and Honduras [7]. Current cardamom production is represented in Figure 1.



Source: [7]

Figure 1. Cardamom producing countries.

Global production of cardamom is approximately 35,000 metric tons annually. Over the past twenty years, the consumption of cardamom has significantly risen worldwide. Key consumers include Middle Eastern nations, India, Pakistan, various European countries, the United States, and Japan. Notably, countries in the Middle East, such as Saudi Arabia and the United Arab Emirates, along with South-East Asian nations like India, contribute to over 60% of global consumption [6].

Cardamom is a perennial herb that can grow between 2 to 5 meters tall, featuring underground rhizomes and upright green stems (tillers) formed from leaf sheaths. Research into its vegetative growth indicates that suckers develop for approximately 18 months from the time they first appear [8]. As a shade-preferential plant, cardamom thrives best with 40-50% shade, which supports its growth and development [9].

Documented evidence shows that cardamom cultivation began in India in 1803, at which point the pods were collected from forests [10, 11]. This suggests that the selection of cardamom for improved agronomic traits has been ongoing for around 219 years. Initially, cultivation involved clearing the underbrush in forests where cardamom naturally flourished. This practice likely led to a gradual reduction of wild cardamom populations and their genetic diversity. The continuous cultivation of cardamom and selective breeding for specific morphological characteristics has resulted in the emergence of numerous landraces adapted to particular locations.

Small cardamom plants typically reach maturity in 20 to 22 months post-planting, with economic yields commencing in the third year and lasting for about 8 to 12 years for high-yield varieties, depending on management practices [12]. Like other cultivated plants, wild cardamom varieties (including disease-resistant strains [13] from abandoned plantations) may exhibit genetic variability and harbor alleles useful for

crop improvement initiatives. Genetic diversity assessments can be conducted through morphological, biochemical, and molecular markers [14]. Utilizing molecular techniques has enhanced the quality of biological diversity data, which is crucial for effective conservation strategies [15, 11].

## 2. Innovations and Approaches in Breeding

### 2.1. Goals for Enhancing Cardamom

Cardamom crop improvement methods include clonal selection, hybridization, poly cross-breeding, mutation breeding, and polyploidy breeding. The cardamom crop improvement program are as follows: 1) To develop more versatile plant varieties, 2) To develop high-yielding accessions with improved capsule characteristics, 3) To generate varieties that are resistant to biotic stresses, including fungi (rhizome rot), pests (thrips, root grubs, nematodes), and viral disease (katte), 4) Develop drought-resistant and high-yielding varieties [16].

### 2.2. Botany and Growth of Cardamom

Cardamom is a herbaceous perennial that grows from underground rhizomes, which can be propagated through rhizome division. It typically reaches heights of 2 to 5 meters. The leaves are lanceolate with an acuminate tip and exhibit a dark green color, while the aerial stem forms by encircling the leaf sheaths. Tillers sprout from the axils of the underground stem, with most vegetative buds developing during the monsoon season [16]. The inflorescence is characterized by a long panicle with racemose clusters that extend above the ground from the underground stem. Generally, two to four panicles

emerge from the swollen base of the tillers in most cultivars, with some cultivars featuring branched panicles. The flowers are white with a pink central lip, arranged in a loose spike that measures 30 to 60 cm in length and emerges from the rhizome [17]. Regenerative buds (panicles) typically develop over a span of 10 to 12 months [18].

The panicles grow above the soil and go through a direct development phase lasting about seven months. The growth patterns and characteristics of the panicles, along with the shape and size of the capsules, vary among different cardamom varieties. Flowers are grouped in clusters called cincinni and are supported by scale leaves. Each flower is bisexual, featuring three linear, persistent bracts, three unequal petals, and a longer lip with a violet tinge, three carpels, a single style, and a trilobular ovary with axile placentation containing multiple ovules in each carpel. Cardamom can bloom throughout the year from panicles formed in both the current and previous seasons, with peak flowering occurring over a

six-month period from May to October. The time from flower or bud initiation to full bloom typically ranges from 26 to 34 days, while capsule development takes about 110 to 120 days from the full bloom stage [19].

Flowering primarily occurs in the early morning, from 3:30 AM to 8:00 AM, followed by anthesis. Anther dehiscence occurs immediately after, with maximum pollen release occurring between 5:30 AM and 6:30 AM [20, 21]. The pollen grains are round, mostly single, and average about 87.6  $\mu\text{m}$  in diameter. Studies have shown that pollen viability drops to 6.5% after two hours of storage, and 0% after 6-8 hours [20], although cardamom pollen can be successfully stored in liquid nitrogen [22]. Cultivated cardamom varieties exhibit different growth habits for their panicles as well as varying capsule shapes and sizes. There are three classifications based on panicle shape: Mysore (erect panicle), Malabar (prostrate panicle), and Vazhukka (semi-erect panicle), which is a natural hybrid of the Mysore and Malabar varieties [23].



**Figure 2.** Over view of vegetative and productive progress of cardamom.

### 2.3. Breeding

The fundamental chromosome number for *Elettaria* is  $x = 12$ , with somatic chromosome counts of  $2n = 48$  or  $52$ . Cardamom is primarily cross-pollinated, leading to significant variability among seedling progenies. Germplasm is collected and preserved at several research institutions, including the Research Institute for Spices and Medicinal Plants in Bogor, Indonesia, the Indian Institute of Spices Research (IISR) in Calicut, and the All India Coordinated Research Project on Spices (AICRPS). Breeding efforts have focused on developing selections and hybrids to improve yield and enhance resistance to pests and diseases. Plants characterized by medium height and prostrate panicles are optimal for closer planting density (3000 plants per hectare), while robust plants with semi-erect panicles are better suited for low-density cultivation and intensive management practices (1000 plants per hectare). Various breeding techniques, such as clonal selection, hybridization, mutation breeding, polycrossing, polyploidy breeding, and tissue culture methods including micro propagation and soma clonal variation have contributed to the improvement of cardamom crops.

### 2.4. Pollination

Cardamom features bisexual flowers that are self-compatible, although cross-pollination is more prevalent. The primary pollinators are *Apis cerana* and *Apis dorsata*. Cardamom flowers typically bloom for 15 to 18 hours, with peak stigma receptivity and pollen viability occurring between 8 AM and 10 AM. Pollination during this window leads to approximately 72% fruit set, while receptivity gradually declines, resulting in a minimum fruit set of 24%. Bee foraging is most active during morning hours, contributing to the higher fruit set in cardamom. Observations of fruit set across different months indicate a significant percentage (50 to 59 percent) during June through September, attributed to the humid conditions prevalent at that time. Conversely, during the dry months from December to March, fruit set is minimal [24-27, 32].

### 2.5. Cytology

The somatic chromosome number of cardamom has been reported as  $2n = 48$  [33, 34], although [35] indicated a count of  $2n = 52$ . Variations in chromosome numbers have been

observed between the Mysore and Malabar varieties of cardamom, suggesting that both aneuploidy and structural changes in the chromosomes play a role in the differentiation of these varieties. Previous studies have suggested that cardamom has an amphidiploid origin derived from likely extinct wild species. Related genera, including *Globo*, *Balbifera*, *Phoemaria*, *Amomum* spp., and *Alpinia* spp., also exhibit a chromosome number of  $2n = 48$  and are believed to have evolved from a shared basic number of  $X = 12$  [36].

## 2.6. Collection and Conservation

The Indian Cardamom Research Institute (ICRI) houses the national germplasm conservatory for both small and large cardamom, featuring the largest collection globally, with 800 accessions of small cardamom and 300 accessions of large cardamom. This diverse genetic pool serves as the foundational resource for current and future cardamom crop development initiatives [37]. However, the genetic diversity of cardamom is rapidly diminishing due to habitat changes in the Western Ghats, necessitating systematic exploration and collection of germplasm. A significant collection of cardamom germplasm is preserved at the Indian Institute of Spices Research (IISR) in Myladumpara and at various centers involved in the All India Coordinated Research Project on Spices (Table 1).

As a vegetatively propagated crop, cardamom germplasm is primarily conserved in clonal repositories in the field, which is labor-intensive and vulnerable to threats such as pest outbreaks, diseases, and drought. Field repositories are also susceptible to diseases like 'katte' and rhizome rot, which can lead to considerable losses. To enhance the safety of germplasm collections, it is vital to integrate in vitro conservation alongside field gene banks. Nirmal Babu [38] has suggested a method for inducing slow growth in cardamom on half-strength Murashige and Skoog (MS) medium, supplemented with 15 g/L each of sucrose and mannitol, within screw-capped culture tubes incubated at  $22 \pm 2^\circ\text{C}$  with a 12-hour light/dark cycle and light intensity of 2500 lux. Under these conditions, cultures can be preserved for up to a year without the need for subculturing. The conserved plantlets can then be multiplied effectively, achieving high establishment rates and normal growth when planted.

Additionally, cryopreservation techniques utilizing liquid nitrogen at  $-196^\circ\text{C}$  are being explored for long-term storage of cardamom seeds and encapsulated, vitrified shoot tips [39]. In India, six research organizations, including ICAR-Indian Institute of Spices Research (IISR) in Kozhikode, the Cardamom Research Centre in Appangala, and ICRI in Myladumpara, amongst others, are actively working on cardamom improvement. These institutions are conducting routine surveys to identify and exploit desirable genetic traits from various germplasm accessions through both traditional and modern crop improvement methods. The Cardamom Research Station (CRS) in Pampadumpara has collected and

preserved 190 germplasm lines as of 2020. The International Plant Genetic Resources Institute (IPGRI), now known as Bioversity International, published cardamom descriptor descriptions in 1994 [40].

Cardamom exhibits significant natural variation due to its cross-pollinated nature and predominant seed propagation. This genetic diversity is crucial for developing new varieties that cater to the needs of farmers and consumers. Consequently, prioritizing the selection, conservation, assessment, and utilization of germplasm in breeding strategies is essential [8]. Although there are natural populations of cardamom in protected areas, in situ conservation is limited. Ex-situ conservation efforts in India are primarily conducted by the aforementioned six organizations, which are detailed in Table 2. Such ex-situ conservation relies on field gene banks for initial assessment and characterization of genetic resources, utilizing agronomical, morphological, and chemical traits for plant evaluation. Various morphological and chemical variations, as well as yield differences, have been observed in these collections [41]. However, ex-situ conservation is continually threatened by various biotic and abiotic stressors [23].

## 2.7. Genetic Resources and Cultivar Diversity

Cardamom plants are classified into three categories based on the morphology of their panicles. Key differences among these types are summarized in Table 1.

- 1) Mysore Type: This type thrives at elevations ranging from 900 to 1200 meters above sea level. The plants are robust, reaching heights of 3 to 4 meters, and feature completely erect panicles (Figure 3A). Significant populations can be found in Kerala and Karnataka. The cured capsules are ribbed, have three edges, and are slightly longer than those of the Malabar type, measuring approximately 21 mm in length [42].
- 2) Malabar Type: Best suited for lower elevations between 600 and 1000 meters above sea level, the Malabar type consists of medium-sized plants that grow to about 2 to 3 meters tall. These plants are less prone to thrips and shoot borer infestations. They are commonly found in low-rainfall regions of Kerala and Tamil Nadu. The cured capsules are typically round and about 18 mm long [42].
- 3) Vazhukka Type: The Vazhukka variety is a natural hybrid of the Mysore and Malabar types, benefiting from the strengths of both. It flourishes at elevations of 900 to 1200 meters above sea level. These plants are sturdy, with semi-erect panicles (Figure 3C), and produce large, globose or ovoid capsules [16, 23].

Based on factors such as adaptability, panicle characteristics, and fruit size and shape, cardamom is categorized into three botanical varieties: Malabar, Mysore, and Vazhukka. The distinctive attributes of these varieties are detailed in Table 1.



**Table 1.** Specific characteristics of the three varieties of cardamom.

Characters	var. Malabar	var. Mysore	var. Vazhukka
Adaptability	Lower altitudes 600-900m a.s.l.	Higher altitudes 900-1200 m a.s.l.	Wide range
Areas of cultivation	Karnataka	Kerala and parts of Tamil Nadu	Kerala
Plant growth	Medium	Robust	Robust
Panicles	Prostrate	Erect	Semi erect
Capsules	Round or oblong	Bold, elongated	Round to oblong
Leaf petiole	Short	Long	Long
Capsule colour at maturity	Pale/golden/yellow	Green	Green
Cultivation	Kerala and Tamil Nadu	Kerala and Karnataka	Kerala

Source: [43, 23]

## 2.8. Characterization and Evaluation

Cardamom germplasm exhibits a wealth of genetic diversity in terms of agronomic traits, yield characteristics, and quality attributes. A detailed descriptor for the characterization and documentation of cardamom germplasm was published by IPGRI [40]. Prasath [44] documented substantial variability in panicle length and yield per plant. Notable variations have been observed in features such as inflorescence branching, fruit (capsule) size and shape, leaf and plant pubescence, and the retention of green color [45]. Cardamom is valued for its volatile oil, which can range from 6.5% to 10.5%. The evaluation of germplasm has also identified two accessions (Acc.221 and Acc.218) that contain 7.8% essential oil, characterized by high levels of aroma-bearing constituents like alpha terpinyl and linalyl acetates, while showing a lower concentration of 1,8-cineole [41]. The Mysore genotype, PR-107, has been recognized for its superior quality due to its high ester content, including alpha terpinyl acetate, geranyl acetate, and linalyl acetate. Among 134 disease-resistant accessions collected from critical hot spots, 17 were identified as resistant to mosaic (katte virus) disease [13]. The effective utilization of germplasm hinges on thorough characterization and documentation of existing variability, coupled with their collection and conservation efforts. Genetically resistant cultivars have played a crucial role in integrated disease management (IDM).

A study was conducted to characterize 70 small cardamom genotypes at the ICAR-IISR Regional Station in Appangala (Kodagu District, Karnataka, India), assessing morphological traits and yield parameters, as well as identifying sources of resistance against diseases like rhizome rot and leaf blight. The experimental setup involved planting 70 genotypes in 2010 with a spacing of 2 x 2 m, replicated five times, with each clump representing a replication. Standard agricultural

practices were followed to cultivate the crop. Observations for vegetative and yield traits were documented for three consecutive years (from the third to fifth year of planting), and pooled data were analyzed. Correlation coefficients were calculated using the method outlined by [46]. The natural occurrences of rhizome rot and leaf blight diseases were monitored in August and September over three years (from the third to fifth year of planting). For each genotype, five clumps were assessed for leaf blight severity, employing a 1-6 disease rating scale: 1 = no symptoms, 2 = isolated spots on young leaves, 3 = sparse elongated spots on young and mature leaves, 4 = coalescing elongated spots affecting 25% of leaf area, 5 = extensive spots on all leaves affecting up to 50% of leaf area, and 6 = total infection of all leaves resulting in a blighted appearance. The percent disease index (PDI) was then calculated, classifying genotypes into categories: highly resistant (<10%), resistant (11-20%), moderately resistant (21-30%), moderately susceptible (31-40%), susceptible (41-50%), and highly susceptible (>51%).

A similar disease rating scale for rhizome rot was designed according to the number of infected tillers per clump. Disease incidence for each genotype was recorded in five clumps and categorized as follows: 1 = no infection, 2 = positive infection with advancing margins less than 1 cm and one tiller infected, 3 = prominent water-soaked patches infecting 2 to 5 tillers (25%), 4 = infection spreading to 50% of total tillers, and 5 = all tillers infected, resulting in plant decay or death. The corresponding PDI was computed, and genotypes were classified into groups: highly resistant (0.0 - 5.0%), resistant (5.1 - 10.0%), moderately susceptible (10.1 - 25.0%), susceptible (25.1 - 50.0%), and highly susceptible (>50%) [47].

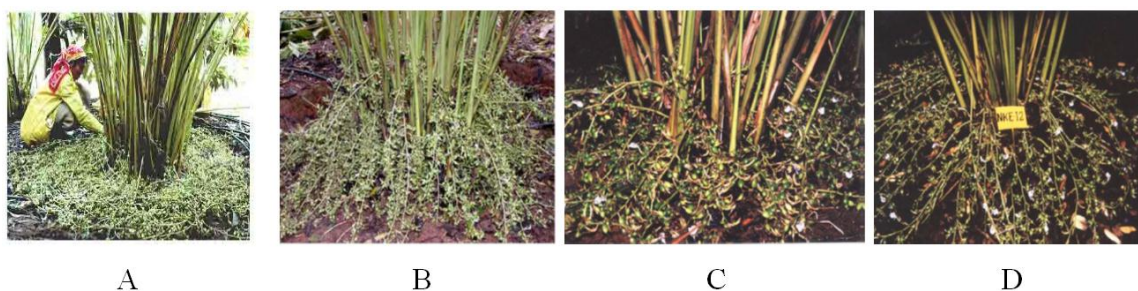
Significant variation was noted in morphological traits, including plant height, number of bearing tillers, capsules per plant, and fresh weight of capsules. Among the evaluated 70 small cardamom genotypes (data not shown), plant height ranged from 110 cm (IC 547208) to 310 cm (IC 547186). The

genotype IC 547214 exhibited the highest number of bearing tillers (18), closely followed by IC 547196 with 17.66 tillers. IC 547205 recorded the longest panicle length (119.4 cm) and the highest number of nodes per panicle (36.4). The maximum number of capsules (3516) and fresh weight of capsules per plant (3456.2 g) were recorded in genotype IC 547205, with IC 584093 following closely with 2276.6 capsules per plant and a fresh weight of 2182 g. Regarding capsule characteristics, the genotype IC 584096 produced the longest (2.18 cm) and broadest (1.48 cm) capsules, with the highest seed count (27.4), while the shortest (1.16 cm) and smallest (0.73 cm) capsules were found in genotypes IC 547281 and IC 584072, respectively [48].

## 2.9. Cardamom Improvement

The primary goals of cardamom breeding, in addition to maximizing yield, are to enhance resistance to biotic stresses such as viral diseases like 'katte' and 'kokke kandu,' as well as

fungal diseases including rhizome rot, clump rot, and capsule rot. Other key objectives include drought tolerance, developing plants with larger capsules containing a higher number of seeds per fruit, achieving a greater percentage of capsule dry recovery (over 22%), increasing the essential oil content, specifically  $\alpha$ -terpenyl acetate (which contributes to aroma and flavor), and creating varieties with broad adaptability. Cardamom breeding relies on selections from germplasm and open-pollinated offspring of well-known cultivars [36]. Currently, twelve high-yielding cardamom varieties have been released for cultivation (Table 2). Among them, IISR Vijetha is noted for its tolerance to the katte virus, while IISR Avinash and ICRI 4 show relative resistance to rhizome rot. The PV 1 variety features long and bold capsules, while CCS 1 is characterized by a compact growth habit, making it suitable for high-density planting (Figure 3A). Ongoing hybridization efforts between NKE, RR, extra bold, and Multi-branch types aim to develop desirable new varieties.



Source: [36]

**Figure 3.** Released varieties of Cardamom, A. CCS1: a high-yielding, compact plant variety; B. Green Gold: the most desirable selection among farmers; C. IISR Avinash: a variety resistant to rhizome rot; D. IISR Vijetha: a variety resistant to katte disease.

### 2.9.1. Clonal Selection

Clonal selection is the primary method used for developing new cardamom varieties. Improved cultivars emerge from the selection of exceptional plants found within landrace populations, which exhibit desirable traits such as higher yields and enhanced capsule features [49, 5, 23]. This success can be attributed to the heterozygous nature of the crop and the technique of clonal propagation, which enables the multiplication of selected plants. During the selection process, plants are evaluated in their natural environments, including fields and forests. Once identified, these plants undergo clonal multiplication, followed by preliminary evaluations, before entering more extensive comparative yield trials and multi-location assessments to verify their superiority and adaptability. Given that cardamom has specific agro-ecological

requirements, the improved selections consistently exceed local clones in both yield and capsule quality. All currently available improved varieties have been developed by selecting for these favorable traits, focusing on both qualitative and quantitative aspects as revealed through preliminary and comparative trials across multiple locations to validate the excellence of the chosen clones.

### 2.9.2. Hybridization

Inter-varietal hybridization was conducted among selected superior cultivars to develop lines exhibiting high yield, resistance to 'katte', and tolerance to drought conditions. Ongoing on-farm trials are being carried out with these varieties, and the most promising lines identified in these trials are detailed in Table 3.

**Table 2.** Released varieties of cardamom with yield and quality characteristics.

Sl. No.	Variety	Source	Yield (kg/ha)	Essential oil %	1,8 cine-ole %	$\alpha$ -Terpenyl acetate %	Capsule shape	Areas recommended for cultivation
1.	IISR Coorg Suvasini	IISR, CRC Appangala	409	8.7	42	37	Oblong	Kodagu&Hassan districts of Karnataka
2.	PV-1	KAU, Pampadumpara	260	6.8	33	46	Long	All cardamom tracts of Kerala & Karnataka
3.	Mudigere 1	UAS, Bangalore	275	8.0	36	42	Oval	Malnad region of Karnataka
4.	Mudigere 2	UAS, Bangalore	476	8.0	45	38	Round	Traditional cardamom growing Tracts of hill zones of Karnataka
5.	ICRI-1	ICRI, Myladumpara	325	8.3	29	38	Round	South Idukki zone of Kerala
6.	ICRI-2	ICRI, Myladumpara	375	9.0	29	36	Oblong	Vandanmettu & Nelliampathi zones of Kerala
7.	ICRI-3	ICRI, Myladumpara	439	6.6	54	24	Oblong	Hill zones of Karnataka
8.	ICRI-4	ICRI, Thadiyankudisai	455	6.4	--	--	Globose	Lower Pulneys in Tamil Nadu
9.	IISR Avinash	IISR, CRC, Appangala	847	6.7	30.4	34.6	Oblong	Rhizome rot infested areas
10.	IISR Vijetha 1	IISR, CRC, Appangala	643	7.9	44.9	23.4	Oblong	Moderate to high shaded mosaic infested areas
11.	PV-2	KAU, Pampadumpara	982	10.45	--	--	Long	Cardamom hill reserves of Kerala
12	Njallani Green Gold	Farmers Selection	1600	--	--	--	bold	All cardamom growing regions

Source: [36]

**Table 3.** Promising cardamom hybridization derived lines evolved at ICRI.

Hybrid combinations	Projected yield (kg/ha)
MCC 16 x MCC 40	610
MCC 61 x MCC 40	675
MCC 21 x MCC 16	650
MCC 21 x MCC 40	870
MCC 16 x MCC 61	800

Source: [36]

A significant number of crosses have been performed to combine traits for high yield and resistance against rhizome rot and cardamom mosaic diseases. These hybrids are cur-

rently being evaluated at the Indian Institute of Spices Research, Appangala. Notable positive heterosis has been observed at both the seedling and pre-bearing stages of the cardamom crosses [50]. Based on their performance, heterosis, and combining ability, 15 hybrid combinations have been selected for further assessment. Factors such as plant height, total tillers, bearing tillers, and yield per plant were found to be influenced by non-additive gene action [51].

### 2.9.3. Inter-Generic Hybridization

To enhance Katte resistance in cultivated cardamom using wild relatives, inter-generic crosses were conducted with *Ammomum subulatum*, *Alpinia neutans*, *Hedychium flavascens*, and *Hedychium coronarium* as male parents [24]. The cross with *A. neutans* resulted in the development of a few fruits, while in other instances, no fruit formation occurred. Compatibility barriers hindered the formation of fruits in these crossing combinations [45].

### 2.9.4. Mutation Breeding

Efforts have been undertaken to create genotypes of cardamom that are tolerant to the cardamom mosaic (katte) virus and drought, as well as to enhance quality. This involves treating cardamom seeds and rhizomes with various mutagens, including  $\gamma$ -rays, Nitrosomethyl Urea (NMU), Diethyl Sulphate (DES), and Ethyl Methyl Sulphate (EMS). However, no promising mutants have been identified to date.

### 2.9.5. Polyploidy Breeding

Polyploids in cardamom were created by treating sprouting seeds with a 0.5% aqueous solution of Colchicine [52]. The resulting polyploid lines displayed a greater number of epidermal cell layers, a thicker cuticle, and an increased wax coating on the leaves, which are traits commonly associated with drought tolerance in plants. Early cytological studies reported varying chromosome counts ( $2n = 48, 52$ ) in cardamom [33, 34, 35]. Observations of different chromosome numbers in the Mysore and Malabar varieties suggested that both aneuploidy and structural chromosomal changes have played roles in varietal differentiation [53]. Previous research indicates that cardamom has an amphidiploid origin stemming from wild species [36]. Given the strong correlation between ploidy levels and reproductive modes, understanding the genome size of specific species is crucial for developing hypotheses regarding their evolutionary potential and the processes shaping the genus [54]. Additionally, Ravindran [55] noted that anther and microspore cultures significantly expedite the development of haploids and diploids, thus greatly reducing the time required for these processes.

### 2.9.6. Biotechnological Approaches

#### (i). Micro Propagation

As a cross-pollinated crop, micropropagation is an effective method for producing true-to-type and virus-free planting materials from high-yielding clones. This study presents an efficient and reliable micropropagation protocol utilizing shoot tips as explants, which can be employed to develop elite planting materials. We evaluated the growth patterns of cardamom shoots across 45 different media treatments. The highest shoot proliferation was achieved on Murashige and Skoog (MS) medium enriched with  $4.4 \mu\text{M}$  6-benzylaminopurine (BAP) and  $2.32 \mu\text{M}$  kinetin (Kn), yielding an average of 5.83 shoots per explant. The longest shoots, measuring 6 cm, were produced on a medium containing  $0.44 \mu\text{M}$  BAP and  $2.32 \mu\text{M}$  Kn. The regenerated shoots demonstrated good rooting on full-strength MS basal medium, averaging 3.50 roots per explant with an average root length of 4.33 cm within four weeks. Following the acclimatization of robust plantlets, successful hardening was achieved, resulting in an impressive field survival rate of 80% [56].



Figure 4. Micro propagation of cardamom.

Cardamom was among the earliest crops to be commercially propagated through micro propagation (Figure 4) [57, 58]. Kumar [59] observed a successful transformation of immature floral buds into vegetative plantlets, along with the development of inflorescences, providing a reliable method to minimize contamination risks, especially since other sources often experience high contamination rates. The Spices Board conducted a field evaluation of tissue-cultured cardamom plants over an area of approximately 100 hectares, and the findings indicated that the performance of the micro-propagated plants was comparable to that of traditional suckers [60].

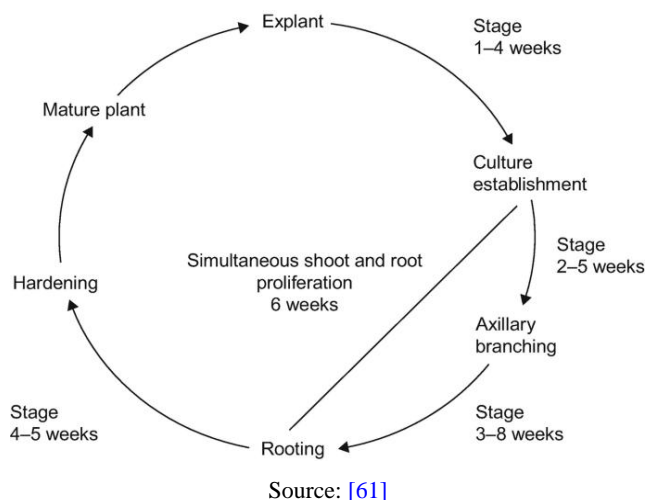


Figure 5. Plant regeneration and soma clonal variation on cardamom.

Successful regeneration of plantlets from the callus of seedling explants of cardamom has been documented (Figure 5) [62, 63]. High-frequency plant regeneration from callus cultures derived from rhizomes and vegetative buds facilitated the development of soma clonal variation, enabling the selection of advantageous genotypes. Notable morphological variation was observed among the soma clones, with several clones exhibiting tolerance to Katte [64, 65].

#### (ii). Anther Culture

Attempts at anther and microspore culture were conducted



by [55]. They reported successful callus induction and proliferation from cardamom anthers in MS medium supplemented with 0.1 mg/L TDZ. Subsequently, the swollen anthers were cultured in MS medium containing 0.5 mg/L 2,4-D and 0.1 mg/L TDZ. Plant regeneration was achieved from the anther-derived callus on MS medium formulated with 0.5 mg/L 2,4-D, 0.1 mg/L TDZ, 0.2% tryptone, and either 25% sucrose with 5% glucose or 15% sucrose with 15% glucose.

### (iii). Protoplast Culture

Protoplasts were successfully isolated from the leaf mesophyll tissues of cardamom, sourced from both in vitro-grown plantlets and cell suspension cultures, yielding approximately  $3.5 \times 10^5$  protoplasts per gram of leaf tissue. These protoplasts were effectively plated on culture media, allowing for their development into the microcalli stage [22].

### (iv). Synthetic Seeds

Embryogenic calli and in vitro-derived shoot buds of cardamom were encapsulated in a 5% calcium alginate solution to create synthetic seeds. These seeds showed the potential for storage for up to 9 months in MS medium, achieving a survival and germination rate of 75% [66].

### (v). Genetic Transformation

A preliminary investigation into the transformation of cardamom was conducted using the biolistic method to determine the optimal conditions for gene delivery, as well as to evaluate the efficiency of the plasmid vector pAHC 25 and the Ubi-1 promoter (from maize ubiquitin) for transformation and gene expression in embryogenic callus of cardamom. The bombarded callus tissue displayed transient expression of the GUS gene [28].

### (vi). Molecular Characterization

Molecular markers such as RAPD, PCR-RFLP, and ISSR polymorphism were utilized to profile 96 collections of significant cultivars, varieties, and related genera of cardamom, allowing for the development of genetic fingerprints and investigation of interrelationships. The resulting phylogram revealed that *Elettaria cardamomum* clusters closely with *Amomum subulatum* and *A. microstephanum*, highlighting *Amomum* as the genus most closely related to cultivated cardamom within the studied group. The analysis demonstrated that all examined varieties, promising lines, and landraces are distinct, with no duplicates present in the germplasm collections. Furthermore, the study revealed notable divergence between collections from Kerala and Karnataka, the two primary regions known for cardamom diversity, while the relatively lower divergence observed within populations is attributed to the open-pollinated nature of the seed origins (siblings). The findings suggest that adopting controlled breeding practices, rather than relying on selections from open-pollinated progeny, would enhance variability in

the cardamom germplasm [29]. Additionally, one putative RAPD marker was identified as being associated with resistance to katte in cardamom. A standardized protocol for isolating DNA from market samples of cardamom was developed, which can facilitate the identification of different commercial grades and detect potential adulterants [30].

### (vii). Poly Cross Breeding

Cardamom, being a cross-pollinated crop, benefits significantly from the poly cross-breeding approach for the development of superior varieties. In a designated plot, elite clones with predominantly desirable traits are cultivated together, while beehives are placed on the plot to facilitate pollination. This ensures optimal fruit set and maximizes the number of seeds produced per capsule. Researchers observed that while the anticipated yield per plant for the progeny of Mudigere-1 was 1663g, one selection from the poly cross seeds of Mudigere-1 actually achieved a yield of 2360g per plant, an impressive 44% increase. Additionally, another progeny from the line D 237 recorded a yield of 2670g per plant, representing a 60% increase in yield [31].

## 3. Future Line of Cardamom Breeding

Future breeding strategies for cardamom should focus on creating broadly adaptable varieties by integrating the diverse yield and quality traits found in different cultivars that thrive under changing climatic conditions. Establishing structured populations to identify key genes, along with their application in Marker-Assisted Selection (MAS), will enhance the efficiency of developing new varieties. Furthermore, generating virus-resistant lines through transgenic methods that utilize coat protein genes will aid in addressing viral challenges, while also fostering improvements in quality.

## 4. Conclusion

Despite being heralded as the "queen of spices," cardamom has seen limited advancements through both traditional and modern breeding methods. This scarcity of research presents a valuable opportunity for breeders worldwide. Previous breeding efforts primarily focused on creating more adaptable varieties and developing high-yielding accessions with enhanced capsule traits. There has also been an emphasis on producing varieties resistant to biotic stresses, such as fungi, insect pests, and viral diseases, as well as varieties that are drought-resistant. Moving forward, it is essential to implement more sophisticated breeding techniques, particularly in the area of quality improvement, which has yet to be thoroughly explored in cardamom.

## Abbreviations

IPGRI      International Plant Genetic Resources Institute

IDP	Integrated Disease Management
MS	Murashige and Skoog
BAP	Benzylaminopurine
μM	Micromol
NMU	Nitrosomethyl Urea
DES	Diethyl Sulphate
EMS	Ethyl Methyl Sulphate
IISR	Indian Institute of Spices Research
AICRPS	All India Coordinated Research Project on Spices

## Author Contributions

Mohammedsani Zakir Shehasen is the sole author. The author read and approved the final manuscript.

## Conflicts of Interest

The author declares no conflicts of interest.

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