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Review on Breeding Method and Achievements of Cardamom (*Elettaria cardamomum* Maton) and Future Prospects

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ABSTRACT

Cardamom (Elettaria cardamomum Maton) acclaimed as the 'Queen of Spices' is the true cardamom belonging to the family Zingiberaceae under the natural order Scitaminae. It is herbaceous perennial with underground rhizomes and aerial leafy stems made of leaf sheaths. Cardamom has bisexual flowers, self compatible but cross-pollination is more common. The somatic chromosome number of cardamom is reported to be 2n = 48. The genetic resource of cardamom is being eroded rapidly with the changes in habitat of the Western Ghats and needs systematic exploration and collection of germplasm. Cardamom germplasm exhibits rich genetic diversity for various agronomic, yield and quality attributing characters. Based on the adaptability, nature of the panicle, shape and size of fruits, the cultivated cardamom is grouped into three botanical varieties viz. Malabar, Mysore and Vazhukka. Cardamom is valued for its volatile oil which varies from 6.5 to 10.5 per cent. The major chemical constituents which impart sweet flavour to the oil are terpinyl acetate, linalyl acetate and linalool. Developing structured populations to tag important genes and using them in MAS will increase the efficiency of new varietal development. Developing virus resistant lines using coat protein genes through transgenic path way help in mitigating the viral problems. This paper highlighted cardamom breeding method, achievement and future line shortly.

Keywords: *achievement, biotechnologies, breeding, Cardamom, improvement*

INTRODUCTION

Cardamom *(Elettaria cardamomum* Maton) acclaimed as the 'Queen of Spices' is the true cardamom belonging to the family Zingiberaceae under the natural order Scitaminae. It is one of the most significant and profoundly valued spices. Cardamom is commonly developed underneath evergreen forest trees of Western Ghats of South India and it is a shade loving plant thriving well in elevations up to 600-1200m a.s.l under an average annual rainfall of 1500-4000mm and temperature range of 10 ^{o}35 C. Muggy tropical climate and soil wealthy in organic matter is ideal for cardamom cultivation. India is considered the local home of *Elettaria cardamomum* though the major centre of diversity for the genus is the Sarawak (Malaysia) and Borneo region, from where eight species have been listed. Natural population's cardamom now exists only in the evergreen forests of Western Ghats (Peter *et al*., 2007). Cardamom is herbaceous enduring (2-5 m in height) with underground rhizomes and elevated verdant stems (tillers) made of leaf sheaths. Studies on vegetative growth implied that suckers precede their growth for a period of about 18 months from the time of emergence (Madhusoodanan *et al*. 2002).

BODY OF THE TEXT

Botany and Growth of Cardamom

The advancement of regenerative buds (panicles) takes place in about 10 to 12 months (Krishnamurthy *et. al*., 1989). Inflorescence is a long panicle emerging from the underground stem, but comes up above the soil. The direct development of panicles stretches over a period of seven months. The growth habit of the panicles and the shape as well as the size of the capsules shifts in various developed varieties of cardamom. Flowers are arranged in bunches (known as *cincinni*) subtended by scale leaves. Flowers are bisexual, bracts linear, oblong and persistent, sepals 3, petals 3, unequal, lip longer with violet tinge carpels 3, style 1, ovary - trilocular, axile placentation, ovules-many in each carpel. Normally flowering in cardamom could be seen throughout the year on panicles formed during the current as well as in previous year. The peak flowering is spread over a period of six months from May to October.

The time required to a full bloom stage from flower/bud initiation ranges from 26 to 34 days and capsule development takes about 110 to 120 days from the full bloom stage (Parameswar and Venugopal, 1974). As a rule, most extreme number of flowers open during early hours of the day 3.30 - 8.00 AM immediately followed by the anthesis. The dehiscence of anthers took place immediately followed by anthesis with maximum pollen bursting between 5.30-6.30 AM (Pattanshetti and Prasad, 1972, KAU, 2001). The pollen grains were round and mostly found in single, measured on an average 87.6 µ in diameter. Studies on the viability of the pollen grains indicated only 6.5% viability after 2 hours of storage and 0% after 6-8 hours of storage (Pattanshetti and Prasad, 1972). However cardamom pollen can be stored successfully in liquid nitrogen (Geetha, 2002).

Pollination

Cardamom has bisexual flowers, self-compatible but cross-pollination is more common. *Apis cerana* and *Apis dorsata* are the predominant pollinators. Cardamom flowers remain in bloom for 15-18 hours and stigma receptivity and pollen viability were reported to be maximum during morning hours between 8 AM and 10 AM. Pollination during this time result about 72% fruit set. Thereafter, the stigma receptivity declined gradually giving in the minimum fruit set of 24%. The active foraging of bees is observed in the morning hours of the day providing higher fruit set in cardamom. The extent of fruit set noted in various months implied that there was high percentage of fruit set (50 to 59 percent) during June, July, August and September because of humid atmosphere that prevailed during this period. However, during the dry season from December to March, there was actually very little fruit set (Parameswar, 1973, Parvathi *and* Chandrappa, 1993, Belavadi *et al*., 1977, Belavadi and Parvathi, 1998, Belavadi *et al*., 2000).

Cytology

The somatic chromosome number of cardamom is reported to be 2n = 48 (Gregory, 1936; Sharma and Bhattacharya, 1959) while Chakravarti (1948) reported 2n=52. Variations in chromosome numbers were seen in Mysore and Malabar varieties of cardamom implied that aneuploidy as well as structural alterations in the chromosome contributed to the varietal differentiation. Earlier researchers have reported that cardamom is of amphidiploid origin from wild species, which are probably extinct. Allied genera such as *Globa, Balbifera, Phoemaria, Amomum* sp. and *Alpinia* spp also possess 2n=48 and are considered to be evolved from a common basic number, X=12 (Peter *et al*., 2007).

Collection and Conservation

The genetic resource of cardamom is being eroded quickly with the changes in habitat of the Western Ghats and wants systematic exploration and collection of germplasm. A good collection of cardamom is maintained at IISR, Indian cardamom Research Institute, Myladumpara and many centres of All India Coordinated Research Project on Spices (Table 1). Cardamom being a vegetatively propagated crop, germplasm is currently protected in clonal repositories in the field which is labour intensive and exposed to hazards such as outbreak of pests, diseases and drought. Field repositories of cardamom are also exposed to diseases such as *'katte'* and rhizome rot resulting in considerable loss. Therefore, *in vitro* conservation of germplasm, in addition to field gene banks, would give safety of germplasm collections. Nirmal Babu *et al*. (1999) and Geetha *et al*. (2002) indicated a method of inducing slow growth on half-strength Ms medium without growth regulators, supplemented with 15 g/L each of sucrose and mannitol in screw-capped culture tubes and incubation at 22±2 ^{o}C with photoperiod of 12 h light/12 h dark and a light intensity of 2500 lux. Cultures maintained under these conditions can be conserved for one year without sub-culture. Conserved plantlets multiplied normally and were planted with high percentage of establishment and normal growth. Long term storage by means of cryopreservation using liquid nitrogen at - 196 ^{o}C: Cryopreservation of cardamom seeds and encapsulated, vitrified shoot tips in liquid nitrogen (Ravindran *et al*., 2004).

Genetic Resources and Cultivar Diversity

Based on the adaptability, nature of the panicle, shape and size of fruits, the developed cardamom is categorized into three botanical varieties viz. Malabar, Mysore and Vazhukka (Sastri, 1952). The characteristic features of these varieties are given in Table 1.

Table1. *Specific characteristics of the three varieties of cardamom*

Characters	var. Malabar	var. Mysore	var. Vazhukka
Adaptability	Lower altitudes 600-900 m 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

Source: *Sastri, 1952*

Characterization and Evaluation

Cardamom germplasm indicate rich genetic diversity for various agronomic, yield and quality attributing characters. A detailed descriptor was published by IPGRI for characterization and documentation of cardamom germplasm (IPGRI, 1994). Prasath *et al.* (2001) reported high variability for panicle length and yield per plant. Variations have also been reported in significant characters like, branching of inflorescence, fruit (capsule) size, shape, leaf and plant pubescence and retention of green colour (Madhusoodanan *et al*., 1994). Cardamom is used for its volatile oil which varies from 6.5 to 10.5 percent. Evaluation of germplasm has also led to the identification of two accessions (Acc.221 and Acc.218), which contain 7.8 % essential oil. Its oil has high component of aroma bearing constituents such as alpha terpinyl and linalyl acetates and low concentration of 1, 8-cineole (Zachariah *et al.*, 1998). The Mysore genotype, PR-107 was found superior in quality because of high content of esters, such as alpha terpinyl acetate, geranyl acetate and linalyl acetate (Raj *et al.*, 2000). Seventeen accessions resistant to mosaic (katte virus) disease were identified among 134 disease escapes collected from hot spots (Venugopal, 1999).

Cardamom Improvement

The main focus of Cardamom breeding in addition to high yield are resistance to biotic stress viz., viral diseases like 'katte' and 'kokke kandu' and fungal diseases such as rhizome rot, clump rot and capsule rot; drought tolerance; plants with bold capsules with more number of seeds/fruit; higher percentage of capsule dry recovery (>22%), higher percentage of essential oils, α -terpenyl acetate which is responsible for the aroma and flavor and varieties with wide adaptability. Cardamom breeding depends on selections from germplasm and from open pollinated progenies of popular cultivars (Peter *et al*., 2007). Twelve high yielding varieties of cardamom were released for cultivation (Table 2). IISR Vijetha is katte virus tolerant line while IISR Avinash and ICRI 4 are relatively tolerant to rhizome rot. PV 1 has long and bold capsules. The variety CCS 1 has compact growth habit and is good for high density planting. (Fig.1). Hybridization between NKE, RR, extra bold and Multi-branch types are in progress with an aim to evolve desirable types.

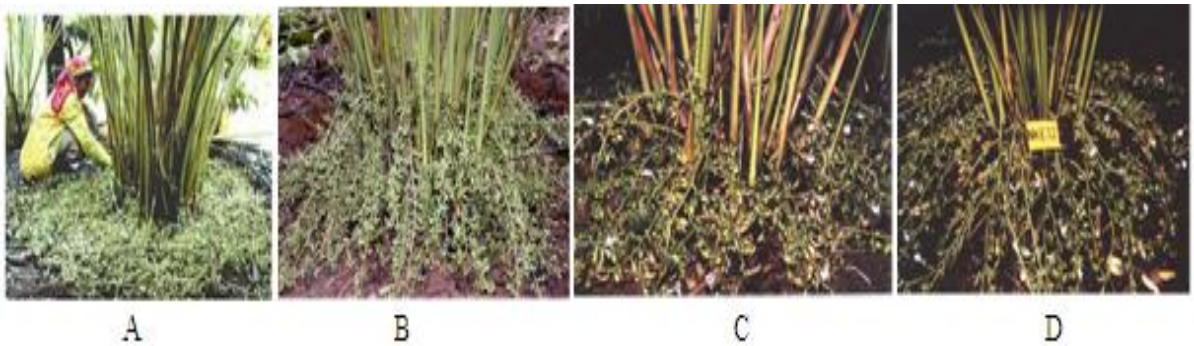

Fig1. *Important released varieties of Cardamom*

A. CCS I a high yielding short plant type; B. Green Gold the most sought after farmer's selection of cardamom, C. IISR Avinash a rhizome rot resistant variety; D. IISR Vijetha a katte resistant variety

Source: *Peter et al., 2007*

Clonal selection

All the existing improved varieties have been evolved by selection for desirable characters such as higher yield and superior capsule characters. Selection in cardamom is based on both qualitative and quantitative characters from preliminary, comparative yield trial and multi-location trials to confirm the superiority of the selected clone.

Hybridization

Inter-varietal hybridization was made between identified superior cultivars for deriving lines with high yield, *'katte'* resistance and drought tolerance. On farm trials of these varieties are in progress. The promising lines from these trials are given in Table 3.

Table2. *Released varieties of cardamom with yield and quality characteristics*

Sl. No.	Variety	Source	Yield (kg/ha)	Essential oil %	1,8 cineole %	α -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 Kerela
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: *Peter et al., 2007*

Table3. *Promising cardamom hybridization derived lines evolved at ICRI, Myladumpara*

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: *Peter et al., 2007*

A large number of crosses have been made to combine high yield and resistance to rhizome rot and cardamom mosaic diseases, which are currently under evaluation at Indian Institute of Spices Research, Appangala. Varying degrees of significant positive heterosis was recorded in both the seedling and pre bearing stage of cardamom crosses (Padmini *et al*., 2001). Based on *per se* performance, heterosis and combining ability, 15 hybrid combinations are short listed

for further evaluation. Plant height, total tillers, bearing tillers and yield per plant were under the influence of non additive gene action (Prasath and Venugopal, 2001).

Inter-generic Hybridization

In an effort to bring Katte resistance from wild relatives to cultivated cardamom , inter-generic crosses were made using *Ammomum subulatum*, *Alpinia neutans*, *Hedychium flavascence* and *Hedychium coronarium* as male parents (Parameshwar, 1977). Cross with *A.neutans* set a few fruits and in other cases no fruit formation was noticed. Compatibility barriers prevented the formation of fruits in these cross combination (Madhusoodanan *et al*., 1994).

Mutation Breeding

Effort has been made to develop genotypes tolerant to cardamom mosaic (*katte*) virus, drought, and better quality through treatment of cardamom seeds and rhizomes with different mutagens such as γ-rays and Nitrosomethyl Urea (NMU), Diethyl Sulphate (DES) and Ethyl Methyl Sulphate (EMS) but no desirable mutant could be identified so far.

Polyploidy Breeding

Polyploids were induced in cardamom by treating the sprouting seeds with 0.5 per cent aqueous solution of Colchicine (Sudharshan, 1989). The polyploidy lines exhibited increased layer of epidermal cells, thick cuticle and thicker wax coating on the leaves which are the general characters associated with drought tolerance in nature.

Biotechnological Approaches

Micropropagation

Being cross-pollinated crop, micropropagation is ideal for generating true to type and virus free planting material from high yielding clones.

Fig2. *Micropropagation of Cardamom*

Cardamom is one of the first crops where the micropropagation (Fig.2) was commercialized (Nadgauda *et al.*, 1983, Vatsya *et al.*, 1987). Kumar *et al.* (1985) reported successful conversion of immature floral buds to vegetative plantlets and inflorescences form an excellent source for reducing culture contamination especially since other sources are prone to high rate of contamination. Field evaluation of tissue cultured plants of cardamom in about 100 ha area was carried out by Spices Board and the results showed that the micropropagated plants performed at par with suckers (Kuruvilla *et al.*, 2005).

Plant Regeneration and Somaclonal Variation

Successful regeneration of plantlets from callus of seedling explants of cardamom was reported (Rao *et al.*, 1982; Nirmal Babu *et al.*,1993). High frequency plant regeneration from rhizome and vegetative bud-derived callus cultures was used for development of somaclonal variation and selection of useful genotypes from them. Good morphological variation was observed among the somaclones. A few clones tolerant to Katte were identified (Nirmal Babu *et al.*, 1997; Peter *et al.*, 2001).

Anther Culture

Attempts on anther and microspore culture were made by Ravindran *et al.* (2002). They reported Callus induction and proliferation from cardamom anthers in MS medium containing 0.1mgl^{-1} TDZ and thereafter the swollen anthers on MS medium containing 0.5 mgl^{-1} 2, 4-D and 0.1mgl^{-1} TDZ. Plant regeneration was obtained from anther derived callus on MS medium with 0.5 mgl^{-1} 2,4-D, 0.1 mgl^{-1} TDZ, 0.2% Trypton along with 25% sucrose and 5% glucose or 15% sucrose and 15% glucose.

Protoplast Culture

Protoplasts could be isolated successfully from leaf mesophyll tissues, collected from *in vitro* grown plantlets and cell suspension cultures of cardamom with a protoplast yield of 3.5 x 10^{5} / g of leaf tissue. These protoplasts could be successfully plated on culture media and made to develop to microcalli stage (Geetha *et al.*, 2000).

Synthetic Seeds

In cardamom embryogenic calli and *in vitro* developed shoot buds were encapsulated in 5% calcium alginate to develop synthetic seeds which could be stored upto 9 months in MS medium with 75% survival and germination (Sajina *et al.*, 1997).

Genetic Transformation

A preliminary study on transformation of cardamom was attempted using biolistic process to study the optimum conditions for gene delivery and the efficiency of the plasmid vector pAHC 25 and promoter Ubi-1 (maize ubiquitin) for transformation and gene expression in cardamom embryogenic callus. Transient expression of GUS gene was noticed in the bombarded callus tissue (Nirmal Babu *et al*., 1998).

Molecular Characterization

Molecular markers like RAPD, PCR - RFLP and ISSR polymorphism were used to profile 96 collections comprising important cultivars, varieties and related genera of cardamom to develop fingerprints and to study the inter-relationships. The phylogram showed that *Elettaria cardamomum* is clustered with *Amomum subulatum* and *A. microstephanum* indicating that *Amomum* is closest to cultivated cardamom among the genera studied. Among the released varieties, promising lines and land races the phylogram has indicated that all the genotypes are distinct from each other and there are no duplicates in germplasm collections. The study showed a clear divergence in Kerala and Karnataka collections, the two main regions of cardamom diversity and the comparatively less divergence within a population is because of the open pollinated seed origin (siblings) of the individual collections. The study also indicated that controlled breeding rather than selection from open pollinated progeny is to be preferred in cardamom to bring more variability in germplasm (Jayakumar *et al.*, 2005). One putative RAPD marker was also found to be associated with katte resistance in cardamom. A protocol for isolation of DNA from market samples of cardamom was standardized. This can be used to identify different grades of commercial cardamom and to identify adulterants (IISR, 2004).

Future Line of Cardamom Breeding

The future breeding strategies in cardamom need to develop widely adaptable varieties by bringing together the various yields and quality attributes distributed in different cultivars which perform well in changed climatic conditions. Developing structured populations to tag important genes and using them in MAS will increase the efficiency of new varietal development. Developing virus resistant lines using coat protein genes through transgenic path way help in mitigating the viral problems.

Conclusion

Generally little have been done on the improvement of cardamom through traditional and modern breeding mechanisms as it is the queen of spices. This limitation of research work on the crop can be used as a gap for the breeders in the world.

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