STUDIES ON VARIABILITY, GENETIC DIVERGENCE AND CROP IMPROVEMENT IN CARDAMOM (*Elettaria cardamomum* Maton)

Thesis submitted in part fulfilment of requirements for the Degree of Doctor of Philosophy in Botany of the University of Calicut

by

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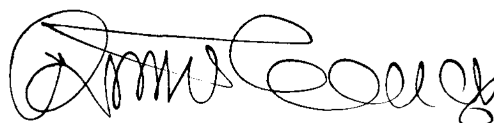
CERTIFICATE

Certified that this thesis entitled "**STUDIES ON VARIABILITY, GENETIC DIVERGENCE AND CROP IMPROVEMENT IN CARDAMOM (*Elettaria cardamomum* Maton)**", embodies the results of a piece of bona fide research work carried out as part fulfilment of requirements for the degree of **Doctor of Philosophy** in Botany of the University of Calicut by **Mr.V.V.Radhakrishnan** under my guidance and supervision and that no part of the thesis has been submitted for any other degree.

I further certify that all helps and sources of information availed of in this connection have been duly acknowledged.



Calicut University,
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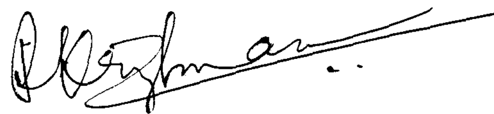
DECLARATION

This is to declare that the thesis entitled "**STUDIES ON VARIABILITY, GENETIC DIVERGENCE AND CROP IMPROVEMENT IN CARDAMOM (*Elettaria cardamomum* Maton)**" submitted by me for the degree of **Doctor of Philosophy** in Botany of the University of Calicut has not formed the basis for the award of any other degree/ diploma.



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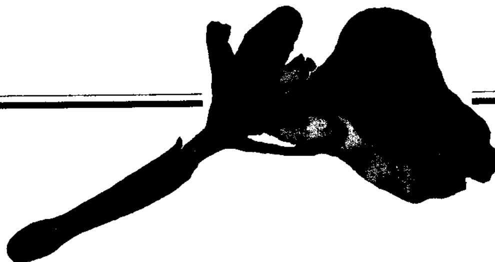
Dedicated to my

Mother (Late) V.V. Madhavi Amma

And

Grand father (Late) Naduvalath Valiya Raman Nair



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PREFACE

Cardamom, popularly known as the 'queen of spices' is extensively grown in the hilly regions of South India. It is valued for its flavour that spices the life of millions and for earning valuable foreign exchange. Cardamom breeding in India has advanced considerably during the past twenty five years. Even then, there are a number of bottlenecks and constraints to be addressed. The plant is presently propagated clonally by majority of farmers and it may lead to loss of variability in future. Evaluation and conservation of diversity and selection of new genotypes both for commercial cultivation and use as parents in breeding programmes may enhance the prospects of cardamom cultivation. The present study is a humble effort to review the works that have been carried out so far and also to make an attempt for the generation of some new information on the genetics and breeding of cardamom.

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INTRODUCTION

V.V. Radhakrishnan "Studies on variability, genetic divergence and crop improvement in cardamom (Elettaria Cardamomum Maton)" Thesis. Department of Botany, University of Calicut, 2003

Chapter 1

INTRODUCTION

Cardamom (*Elettaria cardamomum* Maton) is a perennial, herbaceous rhizomatous monocot, belonging to the family Zingiberaceae. It is a native of the moist evergreen forests of the Western Ghats of South India. The cardamom of commerce is the dried ripe fruit (capsule) of the cardamom plant. This is often referred to as the 'Queen of Spices' because of its very pleasant aroma and taste and is highly valued from ancient times. It is grown extensively in the hilly regions of South India at elevations of 600-1300 m as an under crop in forest lands. Cardamom is also grown in Sri Lanka, Papua New Guinea, Tanzania and Guatemala. Guatemala is incidentally the largest producer of cardamom now.

The 'Western Ghat forests' of the Malabar Coast of India is the centre of origin and the primary centre of diversity of cardamom. In ancient days, cardamom was collected mainly by tribal people as a forest produce. Later, by the end of 19th century, cardamom plantations came up in Western Ghat forest areas and also in Ceylon (Sri Lanka). The credit for starting organized cultivation of cardamom goes to the British planters. Later cardamom was introduced to Guatemala from Ceylon by 1920s and after the second world war cardamom production in Guatemala expanded considerably and by 1970s the country became the largest

commercial producer of cardamom eclipsing the monopoly of India.

In India, the area under cardamom has come down over the last one decade from 1,05,000 ha during 1987-88 to 72,451 ha during 1999-2000, while production has gone up from 3,200 tons during 1987-88 to 9,290 tons in 1999-2000 and further to 11,920 tons in 2002-2003. During the period, productivity has increased from 47 kg/ha to 173 kg/ha. Cardamom is mainly cultivated in three states in India namely, Kerala, Karnataka and Tamil Nadu. Kerala has got 59 per cent of area and contributes 70 per cent of production; Karnataka has 34 per cent of area and 23 per cent production, while Tamil Nadu has 7 per cent area as well as production. Most of the cardamom growing areas in Kerala are located in the districts of Idukki, Palakkad and Wayanad. In Karnataka, cardamom is grown in Coorg, Chikmagalur and Hassan Districts and to a lesser extent in North Kanara District. In Tamil Nadu, cardamom is cultivated at certain localities in Pulney and Kodai hills. In India, it is a small holders' crop with about 40,000 holdings covering an area of about 80,000 ha of cardamom (George and John, 1998).

The cardamom growing regions of South India lie within 8^0 and 30^0 N latitudes and 75^0 and $78^030'$ E longitudes. Cardamom growing areas are located at elevations ranging from 600-1300 m above mean sea level and these areas lie on both sides – the windward and leeward – of the Western Ghats which act as a barrier for the monsoon winds, thereby

determining the spatial distribution of rainfall. The rainfall pattern differs among the cardamom growing regions located in Kerala, Tamil Nadu and Karnataka (Nair *et al.*, 1991).

The productivity increase in recent years is due to the use of high yielding varieties and better agro-production technology. However, the export of cardamom has touched rock bottom during the same period. In 1985-86, cardamom export was 3,272 tons while in 1989-90 the quantity exported was only 173 tons. In 1994-95, the export was 251 tons and the current export is to the tune of 300 tons per year. The export earnings have come down from Rs. 53.46 crores in 1985-86 to Rs. 22.54 crores during 2002-2003 (Spices Board, personal communication).

Currently, Guatemala produces around 13,000-14,000 tons of cardamom annually. Cardamom cultivation has picked up in Papua New Guinea also. Here, cardamom is mainly grown in virgin forests with an evenly distributed rainfall throughout the year. The productivity is very high and present production is about 68-70 tons per year. Cardamom was introduced to Tanzania in the beginning of the twentieth century by German immigrants (Lawrence, 1978). Production is about 127 tons per year. Sri Lanka is another small producer, contributing about 75 tons/year. India has been the world's largest producer of cardamom until 1979-80, when Guatemala came to the scene as the major rival and world leader in cardamom production. Now about 90 per

cent of global trade in cardamom is the contribution of Guatemala (Ravindran and Madhusoodanan, 2002).

Cardamom is a major spice crop of India and improvement of the crop has attracted considerable attention after independence. The major thrust areas of cardamom breeding are (i) developing high yielding, location specific cultivars for the major cardamom growing areas, (ii) evolving high production technology for the major cardamom growing tracts, (iii) evolving lines tolerant or resistant to clump rot and virus diseases, (iv) selection/breeding for high quality and (v) evolving drought and heat resistant lines. Another area that has attracted much attention is post harvest technology, industrial utilization and product development.

Cardamom is used as an important flavour in a variety of food products, beverages, and oral formulations of medicines. In addition, cardamom is popular as an after food mouth flavourant in India. If India wants to recapture the lost glory in the production and trade of the 'Queen of Spices', there should be concerted attempts to achieve a quantum jump in productivity not only through high yielding varieties, but also through the process of constraint alleviation, mainly in respect of drought, pests and diseases. As the global requirements are growing, the future seems to be bright both for Guatemala and India if the research and development programmes are properly planned and organized.

The present study is an effort to survey the literature on the subject matter, to evaluate the genetic control,

variability, divergence, heritability, correlation and genetic advance of characters in cardamom, to analyze the performance of some of the improved genotypes, to attempt molecular characterization of some of those genotypes and also to identify promising lines suitable for future breeding programmes aimed at augmenting cardamom production and productivity.

REVIEW OF LITERATURE

V.V. Radhakrishnan “Studies on variability, genetic divergence and crop improvement in cardamom (Elettaria Cardamomum Maton)” Thesis. Department of Botany, University of Calicut, 2003

Chapter 2

REVIEW OF LITERATURE

2.1. Taxonomy

2.1.1. The genus and species

Cardamom belongs to the genus *Elettaria* of the monocotyledonous family Zingiberaceae. The genus name is derived from the Tamil root *Elettari*, meaning cardamom seeds. The genus consists of six species according to Mabberley (1987). Only *Elettaria cardamomum* Maton occurs in India, and this is the only economically important species of the genus. *Elettaria ensal* (Gaertn.) Abheywick. (*Elettaria major* Thaiw.), a native of Sri Lanka, is known as the Sri Lankan (Ceylon) wild cardamom, and it is a much larger and sturdier plant with a taste and flavour far inferior to the true cardamom. The Malaysian species, *Elettaria longituba* (Ridl.) Holtt. is a large perennial herb, its flowering panicles sometimes reaching a length of over 3 m with flowers appearing singly, and the cincinnus stopping flowering as soon as a fruit is formed (Holttum, 1950). The fruit is large and is not used. Sakai and Nagamasu (2000) listed seven species from Borneo (Indonesia). The related genera are *Elettariopsis* and *Cyphostigma*, both occurring in the Malaysia–Indonesia region. According to Holttum (1950) some of the species included under *Elettariopsis* and *Cyphostigma* resemble so closely to *Elettaria* and hence should come under the genus *Elettaria*.

Burtt and Smith (1983) have provided the taxonomic description for *Elettaria cardamomum*. The essential feature

of the inflorescence of *Elettaria* is a prostrate axis bearing two ranked sheaths with a cincinnus in the axil of each. The inflorescence is a racemose panicle of cincinni. Holttum (1950) is of opinion that Malaysian and Indian species of *Elettaria* represent parallel developments from different points of origin in the *Alpinia* stock.

The Malaysian species *Elettaria longituba* (Syn. *Elettariopsis longituba*) is one of the largest species of the genus. The fruits are large, but without any economic value (Holttum, 1950). Sakai and Nagamasu (2000) in their studies on Bornean Zingiberaceae have described six species of *Elettaria* (*Elettaria stolonifera, Elettaria kapitensis, Elettaria surculosa, Elettaria linearicrista, Elettaria longipilosa* and *Elettaria brachycalyx*).

The Sri Lankan wild cardamom *Elettaria ensal* (Gaertn.) Abheywickrama (*E.major* Thawaites) is morphologically similar to the true cardamom, but more robust, bearing erect panicles and much elongated fruits (Abheywickrama, 1959). However, Burtt (1980), as well as Burtt and Smith (1983) did not treat this as a separate species, but included it under *Elettaria cardamomum.* Abheywickrama (1959) treated this as a separate species because of its more robust nature and distinctly different fruit size. The chemical composition is also different. The two most important constituents of cardamom oil, 1,8- cineole and α - terpinyl acetate, are present only in traces in Sri Lankan wild cardamom (Bernhard, 1971). Rajapakse (1979) has also provided chemical evidence substantiating the distinct nature

of Sri Lankan wild cardamom. However, Burtt (1980) is of the opinion that these character differences are not sufficient enough to separate the Sri Lankan wild cardamom as a different species.

2.1.2. Varieties

Based on the nature of panicles, three varieties of cardamom are recognized (Sastri, 1952). The variety *Malabar* is characterized by prostrate panicles and variety *Mysore* possesses erect panicles. The third type, variety *Vazhukka* is considered a natural hybrid between the two and its panicle is semi erect or flexuous. In the case of *Malabar* variety, the plants are medium in size and attain 2-3 m height on maturity. The dorsal side of leaves may be pubescent or glabrous. Panicles are prostrate and the fruits are globose-oblong shaped. This variety is better suited to areas of 600-1200 m elevation. *Malabar* type is relatively less susceptible to thrips. It can thrive under low rainfall conditions. The plants of *Mysore* variety are robust and attain 3-4 m in height. Leaves are lanceolate or oblong-lanceolate, glabrous on both sides. Panicles are erect and capsules are ovoid, bold and dark green in colour. They are better adapted to altitudes ranging from 900-1200 m from mean sea level and thrive under assured, well distributed rainfall conditions. *Vazhukka* is considered to be a natural hybrid of the varieties *Malabar* and *Mysore* and exhibits various characteristics intermediate to these. Plants are robust like *Mysore*. Leaves are deep green, oblong-lanceolate or ovate; panicles semi erect (flexuous) and capsules bold and globose or ovoid. A number

of cultivars of cardamom have been recognized from India. They can be considered as ecotypes of the varieties *Mysore*, *Malabar* or *Vazhukka*. Most common among them are *Munjarabad*, *Bijapur*, *Kannielam*, *Makaraelam*, *Thara* and *Nadan* (Madhusoodanan *et al*., 2002).

Owen (1901) in his notes on cardamom cultivation in Ceylon mentions three varieties, which he calls the indigenous *Ceylon*, the *Malabar* and *Mysore*. He says that the first two can be easily distinguished by the colour of the stem. The *Malabar* plant is green or whitish at the base of the leaf or aerial stem, while in the *Ceylon* plant the base has a pink tinge. He also mentions that the *Mysore* form is robust, its panicles borne perpendicularly from the bulbs and the fruits grow in clusters of five or seven.

2.2. Anatomy

2.2.1. Anatomy of vegetative parts

Tomlinson (1969) and Mercy *et al.* (1977) carried out preliminary anatomical studies on aerial stem, rhizome, leaf sheath and root of cardamom plants. Aerial stem of leafy shoots has the typical monocot structure with numerous collateral endarch vascular bundles scattered in the ground parenchyma. Rhizome is sharply differentiated into an outer cortex and a central core by a plexus of irregular, congested vascular bundles. The sheathing base of the leaf has single layered upper and lower epidermis. Patches of hypodermal sclerenchyma occur associated with both upper and lower epidermal regions, but they are more prominent below the upper epidermis. Vascular bundles are arranged in a single

row alternating with large schizogenous air spaces. Cardamom leaf has the structure of a typical monocot leaf. Stomata are distributed mostly on the lower epidermis only. Stomatal frequency showed variations (Krishnamurthy *et al.*, 1989). Pillai *et al.* (1961) studied the root apical organization in many Zingiberaceae members including cardamom. They found that the root apical organization of cardamom is similar to that in *Alpinia*, *Hedychium* etc. and consists of three sets of structural initials, one each for the root cap and plerome and a common zone for dermatogen and periblem.

2.2.2. Floral anatomy

Gregory (1936) and Thompson (1939) were the first to study the floral anatomy of cardamom. Pai (1965) carried out a reinvestigation of cardamom floral anatomy, on which the following discussion is based. Two rings of bundles, each with six strands run in the floral axis beneath the ovary. The inner ring contains three large strands and three small radially flattened bundles alternating with each other. Based on the anatomical evidence Pai (1965) argued that the labellum in cardamom flower is a double structure.

2.3. Embryology

Mature anther wall consists of 6-11 layered epidermis, endothecium, four or five middle layers and a secretary tapetum. In many cases through division, the primary layer of the tapetum becomes biseriate. The cells of the sporogenous tissue develop into microspore mother cells and they later undergo meiosis producing isolateral tetrads. In microspores a generative cell is cut off near the wall, and later

it separates from the wall and comes to lie in the cytoplasm of the vegetative cell. At maturity the microspore develops warty projections on the wall. The mature pollen grains are 2-celled at the time of shedding (Panchaksharappa, 1966).

Ovules are anatropous, bitegmic and crassinucellate, borne on an axile placentum. The sporogenous cell enlarges into a megaspore mother cell, which undergoes meiosis forming megaspores. The chalazal spore enlarges and becomes the embryosac. Its nucleus undergoes three successive divisions resulting in the 8-nucleate embryosac. Rarely twin embryosacs were also observed (Panchaksharappa, 1966). The embryosac development is polygonum type. In a mature embryosac the antipodals are ephemeral, and the secondary nucleus is situated in the narrow chalazal caecum (Panchaksharappa, 1966). Philip (1968) investigated the cotyledon and epicotyl development in cardamom. The fruit is a capsule developed from an inferior ovary. Cardamom seed has externally an aril composed of a few layers of thin walled, elongated cells. The testa consists of an epidermis composed of elongated fusiform cells, about 100-250 μ long, square in sectional view, about 18 μ wide and 25 μ high. The inner seed coat consists of two layers; the inner one consisting of heavily thickened polygonal cells, about 15-25 μ in length and breadth and 30 μ high (Wallis, 1967). The kernel consists mostly of perisperm and a small endosperm. The endosperm surrounds a small, almost cylindrical embryo, which is made up of thin walled cells. Parry (1969) as well as Trease and

Evans (1983) also have provided brief descriptions of the histology of cardamom seeds.

Cardamom seeds when powdered give greyish brown powder with darker brown specks – it is gritty in texture having pleasant smell and flavour. The diagnostic characters of cardamom powder have been studied by Jackson and Snowdon (1990). The type of cardamom can be determined by counting the number of heavily thickened sclerenchymatous cells per square mm of a layer and using the standard figure for each type: Mysore – 3,310, Alleppey green – 3,790, Malabar – 4,600 (Willis, 1967). The Ceylon wild cardamom contains 3,020 sclerenchymatous cells per mm of layer.

2.4. Cytology

Gregory (1936) was the first to study the cytology of cardamom. According to him the basic chromosome number (x) is 12 and 2n = 48, indicating a balanced tetraploid nature. Reports of Ramachandran (1969) and Sudharshan (1989) also confirmed the findings. However, Chandrasekhar and Sampathkumar (1986) observed variation in the number as well as morphology of the chromosomes of var. *Mysore* and var. *Malabar* and concluded that aneuploidy as well as structural alterations in chromosomes has contributed to varietal differentiation. From a karyological standpoint, var. *Mysore* stands at a higher rank in the evolutionary ladder. Even the induced tetraploids (2n = 4x = 96) showed very limited chromosome abnormalities and had good pollen fertility and seed set (Sudharshan, 1989).

2.5. Physiology

2.5.1. Growth and flowering

Tillers emerge from axils of underground stem and from their bases vegetative buds emerge almost through out the year. However, majority of the vegetative buds are produced during January–March. The linear growth of tillers increases with the onset of South West monsoon and growth rate slows down with the cessation of rains. Linear growth pattern of tillers is similar in all cultivars. It takes almost 10 months for a vegetative bud to develop and a year for the panicle to emerge from the newly formed tillers (Sudharshan *et al.,* 1988). Kuruvilla *et al.* (1992) have carried out a round the year study on the phenology of tillers and panicles in the three varieties of cardamom.

Panicles emerge from the swollen bases of tillers. Rarely terminal panicles borne on the aerial shoots are also observed. Generally 2-4 panicles emerge from the base of a tiller. Pattanshetty and Prasad (1976) and Parameswar (1973) have made detailed observations on panicle production, its growth, age of flowering etc. Vegetative shoots require a period of 10-12 months to attain maturity for producing the reproductive buds and the newly emerging panicles take a period of 7-8 months for complete growth. Flowering in cardamom commences with the onset of monsoon. Pattern of flowering varies with regions (agro-climatic zones) and cultivars. Flowers appear on the panicles after four months and flowering continues for a period of six months. These panicles grow either erect (var. *Mysore*), prostrate (var.

Malabar) or in a semi erect (flexuous) manner (var. *Vazhukka*). Each inflorescence (panicle) possesses a long cane-like peduncle with nodes and internodes. Each node has a scale leaf in the axil of which flowers are borne on a modified helicoid cyme (cincinnus), thus forming a branched panicle. Multiple branching of panicles occurs in certain cultivars. In such cases the central peduncle undergoes further branching (secondary and tertiary branches) producing multi-branched panicles. Such branching is either present at the lower portion of the main peduncle or top portion alone or throughout the peduncle. Panicles bear leafy bracts on nodes and flowers are produced in clusters in the axils of bracts. Each cluster is a cincinnus (Holttum, 1950). A cincinnus is a modified cyme. It takes about 90-110 days for the first flower in a fresh panicle to open irrespective of the variety. The cincinni and capsules are formed during the 4th and 5th months respectively after panicle initiation (Kuruvilla *et al.*, 1992).

Flower opening commences from 3.30 am and continues till 7.30 am. Anther dehiscence is between 7.30 am and 8.30 am in the morning. Flowers wither within a day. Normally, flowering is seen all the year round on panicles produced in the previous year. Flowering is spread over a period of six months from May-October in India when majority of the cardamom areas receive the South West monsoon. Almost 75 per cent of flowers are produced during June-August. The time required to reach full bloom stage from flower bud initiation ranges from 25-35 days and

capsules mature in about 120 days from the full bloom stage (Krishnamurthy *et al.*, 1989).

2.5.2. Fruit set

A high percentage of fruits are shed before they reach maturity. Parameshwar and Venugopal (1974) observed about 80 per cent fruit drop. Various reasons are attributed to low fruit set and high fruit drop, such as climatic factors (temperature, wind, humidity), nutritional deficiencies, injuries, competition for resources, soil nutrient status, pests and diseases (Kuttappa, 1969) and physiological factors (Parameswar and Venugopal, 1974).

Growth regulators are useful in increasing fruit set. Krishnamurthy *et al.* (1989) investigated the effect of NAA, gibberellic acid and 2, 4-D on fruit set and fruit weight. Significant differences could be noticed in the case of fruit set. Tissue concentration of auxins was highest 36 h after pollination (315 mg/g) and then declined to 80 mg/g 30 days after pollination. The fall in auxin activity resulted in the formation of an abscission zone resulting in the shedding of immature capsules. Application of 40 ppm NAA or 4 ppm 2, 4-D decreased capsule drop and also led to an increase in yield (Vasanthakumar and Mohanakumaran, 1988). Gibberellic acid @ 25, 50, 100, 150, 200, 250 and 300 ppm and 2, 4-D @ 2-5 and 10 ppm increased panicle length, particularly gibberellic acid at 50 ppm. The highest fruit set was observed with 200 ppm gibberellic acid. (Pillai and Santhakumari, 1965). IBA and IAA failed to increase fruit set (Nair and Vijayan, 1973). Siddagangaiah *et al.* (1993)

studied the effect of chloromquat, daminozide, ethephon and maleic hydrazide on fruit set. Daminozide (1500 ppm), chloromquat (250 ppm) and ethephon (100 ppm) significantly enhanced tiller production and other vegetative characters when applied on 7 month old seedlings, but had little effect on other morphological characters.

2.5.3. Photosynthesis

Total leaf area is closely associated with total photosynthesis and dry matter accumulation. Hence accurate estimation of leaf area and canopy density is important. Korikanthimath and Rao (1983) have proposed a reliable method for leaf area estimation based on linear measurements of intact leaves followed by appropriate regression analysis. The leaf area factor differed among the varieties.

The light fraction that the leaves absorb has a direct bearing on crop growth and canopy development. Laboratory studies on photosynthetic efficiency in cardamom indicated that efficiency was greater at low light intensities than at higher ones. Photochemical process was favoured by low light compensation point. The translocation pattern showed that rhizome was the major sink followed by panicles and roots. Unlabelled leaves did not receive much of the labelled photosynthates from labelled leaves (Vasanthakumar *et al.*, 1989).

Kulandaivelu and Ravindran (1992) studied the photosynthetic activity of three cardamom genotypes, measured as the rate of O_2 liberated by isolated chloroplasts.

Results showed drastic reduction in photosynthetic rates in plants exposed to warm climate. As much as 60-80 per cent decrease in the level of total chlorophyll was noticed in all the three varieties tested.

Light requirement for cardamom nursery is about 55 per cent of the normal (Ranjithakumari *et al.*, 1993) and at this light intensity, growth and tiller production are the best.

2.5.4. Response to moisture stress

Cardamom tracts of Kerala and Karnataka experience a drought period of 4-5 months, and large scale losses occur as a result of drought, especially in Idukki, Palakkad and Wayanad Districts of Kerala. Identification of lines with resistance to moisture stress is an essential pre-requisite in improving cardamom cultivation in India. At the Regional Research Station in Mudigere, cardamom genotypes were screened with the aim of identifying drought tolerant clones. Clones differ in their drought susceptibility. Krishnamurthy *et al.* (1989) have reported the chlorophyll stability index (CSI) of the three varieties of cardamom (vars. *Malabar*, *Mysore* and *Vazhukka*) as well as the related taxa (*Hedychium flavescence* and *Amomum subulatum*). Among the three varieties, *Malabar* had the highest CSI percentage (43.14), *Mysore* had the lowest (24.5) and *Vazhukka* was intermediate in CSI percentage (31.14). Electrolyte leakage was 66.65 per cent in *Mysore*, 69.42 per cent in *Vazhukka* and 43.10 per cent in *Malabar*. Krishnamurthy *et al.* (1989) reported variations in leaf area index (LAI) recorded during the stress period. Measurements recorded for 3 years indicated

significant variations in LAI with respect to clones and climatic variations.

2.5.5. Dry matter accumulation and harvest index

Information on dry matter partitioning and harvest index is useful to breeders to set their methodology for developing desired ideotype. Krishnamurthy *et al.* (1989) studied the dry matter accumulation under drought spells over a period of 3 years. Significant variations were recorded among the clones studied. Dry matter accumulation (DMA) during the drought season of March-June varied from 161 g/plant to 279.5 g/plant. At the end of the drought spell (June) DMA ranged from 195 g/plant to 391 g/plant. The variations were highly significant. Korikanthimath and Mulge (1998) also studied dry matter partitioning and harvest index in cardamom. Dry matter content of the roots, rhizome, panicles, capsules, tillers and leaves of different clones varied significantly. Partitioning of photosynthates to various plant parts is controlled by the genetic make up of the genotype. Partitioning efficiency reflected in the per cent dry matter distribution towards capsules in clones such as Sel.9, Sel.7 etc. and their higher harvest index has clearly indicated their superior yielding ability, and their usefulness in crop improvement programmes.

2.6. Flower morphology

The flowers show typical zingiberaceous morphology. Flowers are bisexual, zygomorphic and open in succession from base towards apex. They are white in colour, trimerous, 30-35 mm long and with the central lip streaked blue or

violet. An oval and distinctly 3-lobed labellum is present. One fertile stamen is present and it is without connective appendages but prolonged into a short crest. Anthers are adnate to the filament, 0.6 to 0.7 cm long, 2-lobed and dehiscing vertically. The filament is distinct and slender and deeply grooved. The ovary is inferior and trilocular with axile placentation. The style is undivided and stigma is small and held above the anther. Structure of cardamom flower is pre-disposed for insect pollination as indicated by the prominent labellum, the stigma positioning above the anthers, and the presence of nectar glands. Fruits are ellipsoidal or almost spherical, non dehiscent, fleshy and green or pale green in colour and leathery when dry. The flower morphology does not vary with biotypes or varieties.

Pai (1965) has concluded from anatomical evidence that the labellum of cardamom flower is a double structure. He showed that the mid anterior strand in the labellum is a fusion product of the marginal traces of the two component members belonging to the inner androecial whorl. Parameswar (1973) and Parameswar and Venugopal (1974) are of the view that labellum is made up of three modified anthers. Biometrical observations did not reveal any difference among the three varieties in the mean length of flower and the length of different floral parts such as flower stalk, corolla tube, anther and style (Madhusoodanan *et al.*, 2002).

2.7. Palynology and pollination biology

Pollen grains are rich in starch and 2-celled at the time

of shedding. Moniliform refractive bodies are observed in some pollen grains. Exine develops warty projections that are spinescent (Panchaksharappa, 1966). Pollen fertility is maximum at full bloom stage and low at the beginning and the end of flowering periods (Venugopal and Parameswar, 1974). Size of pollen grains varies from 75 to 120 microns in different varieties of cardamom. Pollen grains loose their viability quickly and only 6.5 per cent remain viable up to 2 h and none after 6 h of storage (Krishnamurthy *et al.*, 1989).

Pollen fertility is comparatively lower during the early and late phases of flowering. Pollen grains germinate in 10 per cent sucrose solution and addition of 200 ppm boric acid improves germination and tube growth (Parameswar and Venugopal, 1974). Kuruvilla and Madhusoodanan (1988) found that 15 per cent sucrose and 150 ppm boric acid favoured pollen germination and tube growth and that the ideal temperature is 15-20^{0}C. Low concentration (5-10 ppm) of coconut water, gibberellic acid, cycocel and ethrel enhanced pollen germination and tube growth significantly (Kuruvilla and Madhusoodanan, 1988).

The structure of flowers is suited for cross pollination. Stigma is funnel shaped with cilia around a small cavity present on its top and is held significantly above the position of the anther so as to eliminate the chances of pollen mass from the same flower reaching the stigmatic surface in the natural course. Honey bees (*Apis cerana, Apis indica* and *Apis dorsata*) visit cardamom flowers during the flowering season for collecting nectar and pollen and they do help in

attaining over 90 per cent of pollination. The stigma remains receptive right from 4 am on the day of flower opening depending up on the environmental conditions. It is reported that receptivity of stigma is the highest between 8 am and 12 noon (Krishnamurthy *et al.*, 1989; Kuruvilla and Madhusoodanan, 1988). Parvathi *et al.* (1993) and Belavadi *et al.* (1998) noticed peak receptivity around 12 noon coinciding with peak pollinator activity. Stone and Willmer (1989) carried out a detailed study on pollination of cardamom in Papua New Guinea. There the most common foragers are *Apis mellifera*, and to some extent *Apis sapiens. Apis mellifera* showed a change in foraging behaviour over time, initially collecting pollen and switching over to nectar later in the day. Foraging starts around 7 am and reaches the peak around 10 am. The pollen collecting activity declined to a very low level by 12.30 pm. By this time the vast majority of stigmas get pollinated.

Belavadi *et al.* (1997) has carried out some interesting observations on cardamom flower structure and honey bee pollination. In cardamom flowers nectar is present in the corolla tube which is 23 ± 2.08 mm long (range 21.48 – 30.4 mm), through which the style passes. The honey bees (*Apis cerana* and *Apis dorsata*) despite their short tongue lengths (14.5 mm and 5.5 mm respectively) draw nectar up to 11.45 ± 2.65 mm and 11.65 ± 1.85 mm respectively. Controlled experiments using capillary tubes of similar dimensions showed that the depth of feeding by the two bee species corresponded to their tongue length when there was no style.

When a style is introduced, the depth of feeding increased with increase in style thickness. The presence of a style inside the corolla tube helped the bees to draw more nectar from the cardamom flowers.

The mean number of flowers per bush that open per day is 34.5. Pollen production per flower is reported to be 1.3 $\pm$ 0.2 mg, and this quantity gets reduced to 0.6 $\pm$ 0.2 after the visit of a bee, indicating that during the first foraging, about 50 per cent of the pollen is removed. Cardamom nectar has a pH of 7, and contains 55-100 m mol/l of glucose. The concentration of amino acids (at 8 am) is 3 mM. The nectar volume varied greatly over time. The initial volume, per flower at dawn is found to be around 1.6 μl, and by 11.00 the volume increases to 209 μl. This increase is due to active secretion by the nectaries at the base of the corolla tube. After foraging by a bee, the nectar volume falls rapidly (Stone and Willmer, 1989). In one location in Papua New Guinea the number of *Apis mellifera* visiting each flower was about 31 times in a day, while in another location the average visit was only around 10.3 in a day. The fruit set in the first area was much higher than that in the second area. Bee-pollinated fruits were found to contain an average of 11 seeds (Chandran *et al.*, 1983) in South India while in Papua New Guinea it was 13.8 (Stone and Willmer, 1989).

Belavadi *et al.* (1993, 1997, 1998), Parvathi *et al.* (1993) and Belavadi and Parvathi (1998) have carried out detailed studies on pollination ecology and pollination

biology of cardamom in a cardamom cropping system situation of Karnataka, India. Here the pollinator activity starts by 7.30 in the morning and continues till 6.30 in the evening, the peak being between 11.00 h–13.00 h. Bees start appearing on cardamom clumps when the temperature is around 21^{0}C. Individual foragers of *Apis cerana* made 4-7 trips to a single patch of flowers in a day and the number of flowers visited on each successive trip progressively increased. Individual forager visited 157-514 flowers in a day. On a clear sunny day a flower is visited as many as 120 times, on a cloudy day 57 times and a rainy day 20 times on the average. Mean number of flowers visited by a bee at a given clump is 12.32, when the mean number of flowers/clump is 30.

The above workers (Belavadi *et al.*, 1998; Belavadi and Parvathi, 1998) have also calculated the number of honey bee colonies required for effective pollination of cardamom. Based on a cardamom population of 3,000 plants/ha planted at 1.8 m apart, there will be approximately 60,000 flowers available for pollination daily. Based on the pollinator activity a minimum of three colonies/ha is needed, assuming that a colony will have about 5,000 foragers. Based on pollinator activity, an isolation distance of 15 m for seed production gardens has also been suggested (Belavadi *et al.*, 1993).

2.8. Fruits and seeds

The fruit is a capsule, globose or ellipsoid in nature, thin walled, smooth or with longitudinal ridges when ripe.

Varietal variations have been noticed in fruit shape. The fruit colour is green and turn golden yellow on ripening. Seeds are white when unripe turning brown on ageing and becoming black on maturity and their number per capsule varies between 10 and 20 in different genotypes. A thin mucilaginous membrane (aril) covers the seeds. The extent of fruit set is the highest when the atmospheric humidity is very high in the cardamom tract and setting is scanty in summer months even under irrigated condition. In general, fruit set percentage is high in young plants and when the plants surpass the economic life span, fruit setting declines to 50 per cent or even less (Madhusoodanan *et al.*, 2002).

2.9. Crop improvement

Use of genetically superior planting materials and cultivation adopting improved agrotechniques are the two important means to enhance crop productivity. The efforts carried out so far towards the collection and conservation of germplasm, study of genetic variability and efforts for genetic upgradation are reviewed below.

2.9.1. Germplasm

Cardamom, being a cross-pollinated crop and propagated through seeds and tillers, natural variability is fairly high. An assembly of diverse genetic stocks of any crop is the raw material from which a new variety can be moulded to suit the requirements of farmers and end users. Hence, collection, conservation, evaluation and exploitation of germplasm deserve utmost importance in breeding

strategies. In 1950s, two surveys were conducted in the cardamom growing areas in India, one to record the genetic resources and wild populations (Mayne, 1951) and the other to understand the geographical distribution of cardamom and the impact of environment on cardamom (Abraham and Thulasidas, 1958). Thereafter, explorations for germplasm collection have been made by as many as six organizations in India and the total number of accessions presently available with different centres is 1,350 (Madhusoodanan *et al.*, 1998; 1999). Earlier documentation was based on an old descriptor (Dandin *et al.*, 1981). A key for identification of various types has been formulated by Sudharshan *et al.* (1991). In 1994, a detailed descriptor for cardamom was brought out by the International Plant Genetic Resources Institute (IPGRI), Rome, Italy. Among the collections, genotypes with marker characters like terminal panicle, narrow leaves, pink coloured tillers, compound panicles, elongated pedicel etc. are available. Asexuality, cleistogamy and female sterility are a few of the variations observed among the collections.

Efforts to conserve cardamom genetic resources *in situ* are scanty even though natural populations occur in protected forest areas. In the Silent Valley National Park of Kerala State a sizeable population of cardamom plants exists under natural conditions (Madhusoodanan *et al.*, 2002).

Ex situ conservation of cardamom germplasm is being undertaken by four organizations in India namely the Indian Cardamom Research Institute, Myladumpara, Kerala; the

Indian Institute of Spices Research, Calicut, Kerala; the Kerala Agricultural University, Thrissur, Kerala and the University of Agricultural Sciences, Bangalore. *Ex situ* germplasm of cardamom is being maintained as field gene banks and they are used for preliminary evaluation, maintenance as well as for characterization. Characterization involves morphological as well as chemical characters. Many variations in morphological and chemical characters and yield have been recorded in these collections (Madhusoodanan *et al.,* 1994; Zachariah and Lukose, 1992; Zachariah *et al.,* 1998).

Ex situ germplasm is always at risk due to a variety of reasons, mainly biotic and abiotic stress factors. The prevalence of viral diseases is a serious threat to *ex situ* conservation of cardamom germplasm. An alternative is *in vitro* conservation and establishment of *in vitro* gene banks (Madhusoodanan *et al.*, 2002).

2.9.2. Genetic variability

Krishnamurthy *et al.* (1989) have classified the germplasm accessions of cardamom available at the Regional Research Station, Mudigere. They recognized 26 distinct types based on characters such as leaf pubescence, height and colour of aerial stem, panicle type etc.

At the Cardamom Research Centre of the Indian Institute of Spices Research in Kodagu District of Karnataka State, a study was carried out to assess the variability among 210 germplasm collections assembled from all major

cardamom growing areas by Padmini *et al.* (1999). The observations indicated that in general the variety *Vazhukka* and the variety *Mysore* are more robust than var. *Malabar*. The total number of tillers as well as bearing tillers per plant, leafy stem diameter and number of leaves are more in *Vazhukka* and *Mysore* than in *Malabar*. Characters such as panicle number, nodes per panicle, internodal length and capsule length exhibited high coefficient of variation (Padmini *et al.,* 1999).

Among the *Malabar* accessions, coefficient of variation was the highest for number of panicles per plant (78.8). In var. *Mysore* the characters with the highest variability are panicles per plant (74.8 per cent) and internodal length of panicles (69 per cent). Moderate variability existed for bearing tillers per plant, capsule breadth, capsule length and total tillers per plant (Padmini *et al.*, 1999). In var. *Vazhukka* the highest coefficient of variation was recorded for number of panicles per plant (74.5 per cent) followed by number of bearing tillers per plant (62 per cent). Moderate to low variability existed for capsule length, number of nodes per panicles, capsule breadth etc. (Padmini *et al.*, 1999). Observations on natural variations in morphological and yield parameters under the cardamom growing situation of Idukki District of Kerala were recorded by George *et al.* (1981). The highest variability was observed in panicle characters (Anonymous, 1958; 1986; 1987).

George *et al.* (1981) collected 180 accessions from the wild as well as from cardamom growing areas of Western Ghats. They could isolate distinct morphotypes and 12 ecotypes showing heritable adaptations. The following were some of the observations made by the above workers. Varieties *Mysore* and *Vazhukka* were more vigorous than *Malabar*, reaching even 6 m of height. One clone had very narrow leaves with only 3 cm width. In two accessions, tillers had characteristic pink and pale green colours. Though in general each tiller had two panicles, accessions with three and four panicles per tiller were present especially among the *Munjarabad* clones. Another known as 'Alfred clone' produced both basal and terminal panicles. Panicle length was highly variable among the accessions, ranging from 30-200 cm, the mean being 140 cm in varieties *Mysore* and *Vazhukka* and 80 cm in variety *Malabar*. Some accessions produced multiple branched panicles. Flower/fruit number varied from 4-36 per cincinnus. Variations were noticed in fruit shape as well. Acc. 1.175 had extra bold triangular fruits, Acc. 1.89 (*Munjarabad*) had round fruits, and Cv. PV-1, Acc. 1.4 and 1.36 (*Walayar* type) had elongated fruits where the length was more than double the diameter. Green capsule weight varied from 132 g to 40 g and seed number per fruit varied from 5-31.

Plants with multibranched panicles or compound panicles occur in small proportions in the segregating populations of certain lines such as Cl-37. Padmini *et al.* (2000) studied the variability among the compound panicle

types, which mostly have *Vazhukka* type of inflorescence. Among the compound panicles, proximal branching is more prevalent than the distal or entire branching types. The contribution of such branching towards yield (weight of fresh and dry capsules) varied from 12-41 per cent. Branching did not influence other yield or quality characters.

2.9.3. Genetic upgradation

Cardamom is amenable to both sexual and vegetative types of propagation and hence techniques such as selection, hybridization and mutation and polyploidy breeding are used for the genetic upgradation of the crop in India. Studies on certain aspects of crop improvement in cardamom have also been carried out in Sri Lanka (Melgode, 1938), Tanzania (Rijekbusch and Allen, 1971), Guatemala (Rubido, 1967) and Papua New Guinea (Stone and Willmer, 1989).

2.9.3.1. Selection for agronomic traits

Gopal *et al.* (1990; 1992) carried out correlation and path analysis study in cardamom. They reported that dry weight of capsules per plant was positively correlated with the other polygenic characters like height of tillers (0.88), productive tillers per plant (0.78), panicles per plant (0.998), capsules per panicle (0.998), fresh weight of capsules per plant (0.99), length of panicles (0.87), nodes per panicle (0.96) and internodal length (0.63) of panicle. Number of panicles per plant, fresh weight of capsules per plant, nodes per panicle and internodal length in the panicle showed significantly positive direct effects on yield. Panicles per

plant showed maximum direct effect on yield followed by fresh weight of capsules per plant. The above workers concluded that panicles per plant, fresh weight of capsules per plant, nodes per panicle and internodal length of panicle were useful characters for improvement of the yield of cardamom. Patil *et al.* (1997; 1998) also suggested use of traits like panicles per bearing tiller, panicles per clump, recovery ratio and capsules per panicle as criteria for selection for yield in cardamom. In a study using 12 genotypes these workers found that yield per clump had significant and positive correlation with capsules per panicle (0.967), cincinni per panicle (0.645), tillers per clump (0.639), panicle length (0.559), panicles per clump (0.537), bearing tillers per clump (0.340), vegetative buds per clump (0.309) and recovery ratio (0.224). Negative correlation was observed between fresh yield per clump and dry capsules per kg (-0.486). The above workers concluded that capsules and cincinni per panicle, bearing tillers and panicles per clump, panicle length and vegetative buds per clump are significant attributes primarily responsible for high yield in cardamom, and selection for improvement should be based on these attributes (Patil *et al.*, 1997; 1998).

Systematic evaluation of germplasm accessions in India during the 1980s resulted in the identification and release of a few elite clonal selections. Initial collections for desirable traits were made from planters' fields as well as from wild habitats based on certain parameters. In order to isolate elite clones, germplasm collections were subjected to

initial evaluation trials followed by comparative yield trials and multilocational testing under various agro-ecological conditions. Little studies have been taken up for selection of seedlings with precocity in bearing. The results available indicate that this phenomenon is not positively correlated with yield in cardamom (Madhusoodanan and Radhakrishnan, 1996). Selection for drought tolerance has also been attempted. The initial results indicated that the promising drought tolerant lines are low yielders.

So far, eleven selections are available in India for commercial cultivation. The performance of these selections is much superior to the conventionally used clones with regard to their yield and capsule characters. A sharp increase in national productivity of cardamom in India from 53 kg/ha (1989-90) to 173 kg/ha (1999-2000) has been recorded. It is significant that during 1988-89 the area under the crop was 81,113 ha but declined to 72,450 ha by 1999-2000 and the total production has increased to 11,920 tons (2002-2003) from 3,100 tons (1989-90). The major factors that contributed to this substantial increase in productivity and production are the use of improved clones during replanting the senile gardens and adoption of high production technology. Apart from the cardamom cultivars released from research institutes, some of the farmers have also carried out selection of superior clumps from populations. Cv. *Njallani Green Gold* is one such superior selection by a farmer, which has become very popular in Idukki District. This is the best yielding line now available, with high yield

potential. Growers have harvested up to 2,475 kg/ha dry cardamom from this under good management (John, 2000).

Korikanthimath *et al.* (1999) carried out a selection programme in the seedling progenies of Cl-37, using a population size of 5,000 plants. The yield ranged from 325 g/clump to 7,555 g/clump (wet weight). Out of this, 17.8 per cent were high yielders with a mean yield of 6,537 g of capsules/clump (wet weight). The above workers computed Bartlet index (BI) to find out earliness among the progenies and found that six lines were very early flowering types, but they were poor yielders, except one line that gave an yield of 5,156 g/clump (wet weight) and having a Bartlet index of 0.7085. In the study population, early lines were 29.7 per cent (BI: 0.6 – 0.7), medium types were 51.5 per cent (BI: 0.5 – 0.6), late types were 16.7 per cent (BI: 0.4 – 0.5) and very late types were 0.3 per cent (BI: less than 0.4). The strategy further involves the multiplication of the top 10 per cent and distribution to farmers after multilocational testing.

2.9.3.2. Selection for biotic stress tolerance

At the Cardamom Research Centre of the Indian Institute of Spices Research at Appangala (Karnataka), efforts have been made to survey and collect disease escapes from hot spot areas of *katte* disease. Collections of natural katte escape (NKE) lines from such surveys were then subjected to artificial inoculation through the use of insect vectors. The plants that have not taken up infection even after repeated screening were field evaluated again in a hot spot area. Some

of these resistant lines are also high yielding, comparable to released selections both in yield and quality (IISR, 1997).

One particular collection was shown to be resistant to rhizome rot caused by *Phytophthora* sp. This rhizome rot resistant line was tested in comparative yield trials along with the NKE lines. This line has given consistently good yield in all the years, the best among the lines tested giving 18 per cent more yield than the released high yielding variety. For further studies and improvement, the NKE lines were used in a diallele-crossing programme (IISR, 1998).

2.9.3.3. Selection for drought tolerance

Selection of cardamom genotypes was carried out using parameters such as relative water content, membrane leakage, stomatal resistance and specific leaf weight, and significant variation has been noted among cultivars (IISR, 1997).

2.9.3.4. Hybridization

Since cardamom is amenable to both sexual and vegetative propagation, hybridization is a very useful tool for crop improvement. The popular cardamom variety namely *Vazhukka* possibly originated as a natural cross between var. *Malabar* and var. *Mysore*. As only one species occurs in India, crossing in cardamom is confined to intra-specific level. Because of its perennial, cross pollinating and heterozygous nature, the conventional methods for evolving homozygous lines in cardamom are time consuming.

Various workers carried out both intergeneric and intervarietal hybridizations. The former one was with the intention of transferring disease resistance. Such attempts have not been encouraging except in a report of fruit set in a cross with *Alpinia neutans* (Parameswar, 1977). All other intergeneric crosses involving *Amomum*, *Alpinia, Hedychium* and *Aframomum* were sterile (Krishnamurthy *et al.*, 1989; Madhusoodanan *et al.*, 1990).

Intervarietal and inter-cultivar level hybridizations have been carried out for producing high yielding heterotic recombinants. A diallele cross involving six selected types with characters of early bearing, bold capsule, high yield, long panicle, leaf rot resistance and multiple branching was carried out and 30 cross combinations were made. All the hybrids were more vigorous compared to the parental lines (Krishnamurthy *et al.,* 1989). In another study, intervarietal hybridization has been carried out using different varieties of cardamom. This resulted in cross combinations of 56 F_1 hybrids. Evaluation of these hybrids led to the isolation of a few high yielding heterotic recombinants with an average yield of 470 to 610 kg/ha under moderate management (Madhusoodanan *et al.*, 1998; 1999).

2.9.3.5. Selection in polycross progenies

Chandrappa *et al.* (1998) carried out studies on the impact of selection in a polycross progeny population. Promising clonal selections of *Malabar* type cardamom

(including the ruling variety Mudigere-1) were grown in isolation, and open pollinated varieties of these selections were evaluated. In the case of 34 per cent of the progenies the average yield was found to be significantly higher than the average of the control variety (Mudigere-1). This yield increase varied from 1-149 per cent, and certain clones (691, 692, D -11, D - 19) were more promising than others. The above workers found that improvement of yield in cardamom could be more effectively achieved through a polycross breeding programme.

Chandrappa *et al.* (1998) found that based on the polycross progeny test, 38 per cent clones were poor in combining ability and hence can be rejected. They further suggested that lines with better combining ability (such as Mudigere-1, Cl-691, Cl-692, D-11, D-19, D-535, D-186, D-730 and D-18) and with 46-149 per cent higher yield compared to the means of the polycross progeny and the checks, may be used for restricted polycross nursery for isolating higher yielding selections. They also found that a population of more than 3,000 plants is essential for carrying out effective selection.

2.9.3.6. Polyploidy breeding

Polyploids ($2n = 4x = 96$) were successfully induced in cardamom by treating the sprouting seeds with 0.5 per cent aqueous solution of Colchicine (Sudharshan, 1987; 1989). The polyploid lines exhibited gigantism. Increased layer of epidermal cells, thicker cuticle and higher wax coating on the

leaves found in the induced polyploid lines are characters generally associated with drought tolerance in nature. The meiotic behaviour of induced polyploids was almost normal and the plants had reasonably good fertility. In all yield characters the tetraploids were reported to be inferior to the diploids (Anonymous, 1986).

2.9.3.7. Mutation breeding

Attempts for induction of desirable mutants using physical mutagens *viz.*, X-rays and gamma rays (^{60}Co source) and chemical mutagens (ethyl methane sulphonate and maleic hydrazide) have been made. Out of a large number of selfed and open pollinated progenies of M_1 plants, which did not take infection after repeated cycles of inoculation with *katte* virus vector, 12 plants were selected as tolerant to the disease (Bavappa, 1986). There are reports on the induction of sterility (Pillai and Santhakumari, 1965) and absence of macro-mutation in M_1 generation and its progeny (Anonymous, 1987). No desirable mutant could so far be developed in cardamom.

2.9.3.8. Biotechnological approaches

Biotechnological approaches have been used in the genetic upgradation and rapid propagation of cardamom recently. The major efforts and outcomes are presented below.

2.9.3.8.1. Micropropagation

Micropropagation offers tremendous scope for rapid vegetative propagation of elite clones or varieties eliminating systemic pathogens such as viruses. Replanting of senile, seedling raised plantations with selected high yielding clones multiplied through micropropagation can give 5-6 fold increase in the current average productivity of cardamom (Anonymous, 1996). Micropropagation technique can be used for the following applications in cardamom (Bajaj *et al.*, 1988): (i) increase in the propagation rate of plants; (ii) availability of plants throughout the year (in all seasons); (iii) protection of plants against pests and pathogens under controlled conditions; (iv) producing uniform clones and developing uniform productivity of secondary metabolites. In cardamom, various tissue culture approaches have been made use of, such as (i) through callusing, (ii) through adventitious bud formation and (iii) through enhanced axillary branching.

The first report of cardamom tissue culture was by Rao *et al.* (1982), who achieved regeneration of plants from callus cultures. Nadgauda *et al.* (1983) achieved a multiplication ratio of 1:3 when sprouted buds were cultured on MS medium supplemented with BAP (0.5 mg/l), Kn (0.5 mg/l), IAA (2 mg/l), calcium pantothenate (0.1 mg/l), biotin (0.1 mg/l) and coconut water (5 per cent). The plants were successfully rooted and grown in field. Kumar *et al.* (1985) reported direct shoot formation from inflorescence primordium when cultured using MS medium containing NAA, Kinetin and BAP; and they could also get plantlets rooted. Reghunath and

Bajaj (1992) have given a detailed treatment of micropropagation methods in cardamom. Lukose *et al.* (1993) used MS medium containing 20 per cent coconut water, 0.5 mg/l NAA, 0.2 mg/l IBA, 1.0 mg/l of 6 – benzyladenine and 0.2 mg/l kinetin. The plantlets rooted in White's basal medium containing NAA 0.5 mg/l and hardened in soil-vermiculite mixture. Other reports include those of Priyadarshan and Zachariah (1986), Vatsya *et al.* (1987), Priyadarshan *et al.* (1988), Reghunath (1989), Reghunath and Gopalakrishnan (1991), Nirmal Babu *et al.* (1997) and Pradipkumar *et al.* (1997). Presently commercial *in vitro* clonal multiplication of cardamom is being carried out by some biotech companies in India.

Reghunath and Bajaj (1992) have given the methodology for cardamom micropropagation in detail. They have tried various explants such as shoot primordium, inflorescence primordium, immature inflorescence segments and immature capsules and tested serial treatments with 95 per cent alcohol, 2-4 per cent sodium hypochlorite and 0.05 – 0.2 per cent mercuric chloride for decontamination of explants. Both MS and SH (Schenk – Hilderbrandt) media at half and full strengths were tested, along with auxins such as NAA, IAA and 2, 4-D alone and in combination. The cultures on 0.6 per cent agar were incubated at a light intensity of 1,000 lux and 16 h photoperiod. Maximum callus production was reported in MS medium supplemented with 4 mg/l NAA and 1mg/l BAP. This callus on subculturing on an auxin free medium having 3 mg/l BAP and 0.5 mg/l kinetin

started caulogenesis; each culture producing six-nine meristemoids, and they on culturing on the same medium produced shoots within 28 days. Coconut water (15 per cent) enhanced caulogenesis.

Priyadarshan *et al.* (1992) tested 17 media formulations using shoot primordia as explants. They got best results with MS medium fortified with IAA, BAP, kinetin, calcium pantothenate, biotin and coconut water. Reghunath and Bajaj (1992) outlined the culture method, using shoot and inflorescence primordial explants, and the media tested were MS and SH. SH medium was found to be better than full or half MS medium as it has given 31 per cent greater shoot dry weight. Liquid medium culture under agitation using a gyratory shaker produced 111.5 per cent more axillary branches than those cultured on semi-solid medium. The culture conditions include temperature of 23± 2°C, light intensity of 3,000 lux and 16 h photoperiod. The number of axillary branches was the highest in medium containing 4 mg/l BAP and 0.5 mg/l NAA. Axillary branch production was enhanced by coconut water. Var. *Mysore* and var. *Vazhukka* produced more axillary branches than var. *Malabar*. Three separate media have also been tested (IISR, 1997) for multiplication, sub culturing and rooting. Nirmal Babu *et al.* (1997) have given a detailed description about the culture conditions for micropropagation of cardamom.

The excised axillary shoots can be rooted on a semi-solid medium of half strength MS and 0.5 per cent activated

charcoal for one week, followed by subculturing in half MS medium containing 1.5 mg/l IBA under a light intensity of 3,500 lux (Reghunath and Bajaj, 1992). Rooted shoots were transferred to MS ½ liquid medium containing only mineral salts and were then shifted to green house for hardening. For planting, vermiculite – fine sand (1:1) was found to be the best giving 92 per cent establishment (Reghunath and Bajaj, 1992).

2.9.3.8.2. Exploitation of somaclonal variations

Callus regeneration protocols are important for generating somaclonal variations for future crop improvement use. An efficient system for callus regeneration is essential to produce large number of somaclones and such a system has been reported earlier by Rao *et al.* (1982) and was also standardized at IISR (Ravindran *et al.,* 1997). High amount of variability was noticed among the somaclones for morphological characters in the culture vessel itself (Ravindran *et al.,* 1997). The most striking morphological variant is a needle leaf variant with small needle shaped leaves that multiply and root profusely in the same medium (modified MS), but its rate of establishment in the nursery and field is reported to be low (Nirmal Babu, personal communication). Nirmal Babu and his colleagues have standardized cell culture system for large scale production of callus through somatic embryogenesis for enhancing genetic variability. The somaclones are being subjected to evaluation for virus resistance and other characters (Nirmal Babu, unpublished).

2.9.3.8.3. Field testing of TC plants

The first report on the field performance of TC plants was that of Lukose *et al.* (1993), though earlier Nadgauda *et al.* (1983) had mentioned about the field establishment of TC plants of cardamom. Lukose *et al.* (1993) carried out two statistically laid out trials to evaluate TC plants together with suckers and seedlings. Trial one was conducted with clone Cl-37 and trial two was with Cv. *Mudigere-1.* Variations observed among TC plants, suckers and seedlings were non-significant for most of the vegetative characters as shown by analyzing pooled data of four years. Yielding tillers, panicles/plant, green capsule yield and cumulative yield were significantly higher in TC plants, in both the trials. The earlier differences observed in growth characters disappeared in later years.

Sudharshan *et al.* (1997) reported the results of a large scale evaluation carried out by the Indian Cardamom Research Institute. In one instance the performance of eight high yielding micropropagated clones and open pollinated progenies were evaluated at 56 locations in an area of 37.5 ha. Variability was observed in the clonal population for vegetative characters. The overall variability in TC plants was 4.5 per cent as against 3 per cent in open pollinated seedling progenies for a given set of characters. Complete sterility was also reported in certain clones. Microcapsules were significantly more in TC plants, accounting for a major share of variation in these plants (8.4 per cent). However, in

spite of the occurrence of variations, TC plants yielded 34 per cent more than seedling raised plants. The causes of variations were attributed to adventitious bud formation during micropropagation *via* axiallary buds, genetic instability of adventitious meristem and tissue culture induced disorganization of meristems (Sudharshan *et al.,* 1997). Chandrappa *et al.* (1997) tested eight tissue cultured cardamom selections against their suckers and two local checks. The clones TC-5, TC-6 and TC-7 were found to be promising, and they differed among themselves for yield and yield attributes. TC-5 was the best, recording superior values for most observations.

2.9.3.8.4. ***In vitro* conservation**

In vitro conservation is an alternative method for medium term conservation. *In vitro* gene bank will be a safe alternative in protecting the genetic resources from epidemic diseases. Geetha *et al.* (1995) and Nirmal Babu *et al.* (1994; 1999a; b) have reported conservation of cardamom germplasm in *in vitro* gene bank by slow growth. The above workers carried out various trials to achieve an ideal culture condition under which the growth is slowed down to the minimum without affecting the physiology or genetic make up of the plant. Slow growth is achieved by the incorporation of agents for increasing the osmotic potential of the medium, such as mannitol. They found that half strength MS without growth regulators and with 10 mg/l each of sucrose and mannitol was the best for *in vitro* storage of cardamom under slow growth. By using the above medium in screw capped

vials the subculture interval could be extended to one year or more, when incubated in 22 ± 2°C at 2,500 lux of light and at 10 h photoperiod. Low temperature storage at 5°C and 10°C was found to be lethal for cardamom, as the cultures did not last more than three weeks (Geetha *et al.*, 1995).

2.9.3.8.5. Isolation and curing of protoplast

Protoplasts could be isolated from mesophyll tissues collected from *in vitro* grown plantlets, achieving an yield of 35 x 10^5/g of leaf tissue on incubation in an enzyme solution containing 0.5 per cent macerozyme R10, 2 per cent onozuka cellulase R10 and 9 per cent mannitol for 18-20 h at 25°C in dark (IISR, 1996; Geetha *et al.,* 2000). The yield of protoplasts from cell suspension culture was 1.5 x 10^5/g tissue, when incubated in 1 per cent macerozyme R10 and 2 per cent cellulase onozuka R10 for 24h at 25°C with gentle shaking at 53 rpm in dark. The viability of the protoplast was 75 per cent (mesophyll) and 40 per cent (cell suspension) respectively. The protoplast on culturing developed into microcalli (Geetha *et al.,* 2000).

2.9.3.8.6. Cryoconservation

Cryoconservation of cardamom seed was attempted by Choudhary and Chandel (1995). They tried to conserve seeds at ultra low temperature either (i) suspending seeds in cryovials in vapour phase of liquid nitrogen (-150°C) by slow freezing or (ii) by direct immersion in liquid nitrogen (-196°C) by fast freezing. The result showed that seeds possessing 7.7 – 14.3 per cent moisture content could be

successfully cryopreserved and they showed more than 80 per cent germination when tested after one year storage in vapour phase of liquid nitrogen (at –150°C).

2.9.3.8.7. Synthetic seeds

The first report of the production of synseeds by encapsulation of shoot tips in cardamom was by Ganapathy *et al.* (1994), who encapsulated shoot tips of the cardamom cultivar *Malabar* isolated from multiple shoots and encapsulated in 3 per cent (w/w) sodium alginate, with different gel matrices, and were subsequently cultured on MS medium. Sajina *et al.* (1997) reported the standardization of synseed production in many spices including cardamom. Synthetic seeds have many advantages over the normal micro propagation methods. This is an ideal system for conservation and exchange.

In spite of the fact that cardamom is a native of South India, and is being used for the last many centuries, there are many gaps existing in our understanding of this crop plant. No in depth study has gone into the botany of the crop. No information is available on the origin and interrelationships of cardamom, as it is represented by a single species in India. Alleviation of production constraints through conventional breeding or through molecular approaches will go a long way in increasing productivity and sustaining the production of this important spice crop.

MATERIALS AND METHODS

V.V. Radhakrishnan "Studies on variability, genetic divergence and crop improvement in cardamom (Elettaria Cardamomum Maton)" Thesis. Department of Botany, University of Calicut, 2003

Chapter 3
MATERIALS AND METHODS

The experimental investigations for the present study were carried out in the experimental farm of the Indian Cardamom Research Institute, Myladumpara, Idukki, Kerala, India during the period 1999-2002. The experimental field was located at an altitude of 1,068 m above MSL between 9° 53' N latitude and 77° 09' E longitude. The area enjoyed humid tropical climate. The experimental site had chocolate coloured forest loam soil with a pH of 5.5. The weather data of the area during the experimental period are presented in Tables 3.1 to 3.4.

The materials used in the case of the different experiments and the methodology followed are described in the following pages.

3.1. Phenology of tiller and panicle in cardamom

The materials used for the present study consist of the three cultivars of cardamom namely *Malabar*, *Vazhukka* and *Mysore*. Thirty plants of same age group of each cultivar were marked for studying panicle and tiller development. The emergence of panicles and tillers was recorded at monthly intervals and observations were made on the development and growth of thirty tillers and fifty panicles of each cultivar. Linear growth of tiller and panicle was also recorded at monthly intervals. Besides, fifty fresh panicles were marked in all the three cultivars and blooming was

recorded daily for assessing the percentage of flowering during various periods. The study was repeated for two seasons for concurrent results.

Table 3.1. Weather data of the study area for 1999

Month	Rainfall (mm)	Temperature (°C)		Relative Humidity (%)	Sunshine Hours
		Max.	Min.		
January	1.8	24.91	15.72	79.85	8.20
February	34.2	26.02	17.27	77.86	7.78
March	15.4	27.90	18.40	63.00	9.23
April	70.8	27.30	19.90	87.30	3.98
May	291.4	23.80	18.10	87.60	2.60
June	262.6	23.25	18.20	91.21	3.36
July	524.6	21.60	NA	93.50	1.59
August	104.2	24.50	NA	90.40	3.72
September	46.7	26.56	NA	80.90	5.80
October	424.2	25.00	NA	89.70	2.74
November	167.8	25.80	NA	75.60	7.41
December	23.4	24.10	NA	84.00	7.16

NA: Not available.

Table 3.2. Weather data of the study area for 2000

Month	Rainfall (mm)	Temperature (°C)		Relative Humidity (%)	Sunshine Hours
		Max.	Min.		
January	35.6	24.30	NA	83.50	7.98
February	235.5	24.80	NA	84.00	5.93
March	NIL	27.50	17.60	68.90	8.35
April	111.5	28.30	18.60	78.20	7.76
May	47.2	28.56	18.61	78.86	7.86
June	444.8	23.07	17.33	93.16	1.51
July	248.5	23.34	17.43	90.27	5.22
August	629.5	22.70	17.02	95.13	1.89
September	173.5	25.75	18.02	85.22	6.00
October	64.4	27.23	17.28	82.04	7.26
November	121.4	24.53	16.63	84.13	5.69
December	7.8	23.60	16.08	72.60	4.73

NA: Not available

Table 3.3. Weather data of the study area for 2001

Month	Rainfall (mm)	Temperature (°C)		Relative Humidity (%)	Sunshine Hours
		Max.	Min.		
January	9.20	24.10	16.23	79.47	7.16
February	34.00	26.19	16.30	78.73	7.48
March	2.70	28.20	17.73	70.40	8.85
April	319.20	27.50	18.35	76.80	6.84
May	147.00	27.90	21.86	85.50	6.78
June	430.30	23.08	19.98	83.29	1.47
July	495.60	23.20	16.68	89.42	2.56
August	325.80	23.58	17.43	95.27	2.58
September	113.70	26.59	17.61	82.42	6.77
October	336.60	25.50	17.30	85.30	4.50
November	259.28	24.80	17.30	85.20	6.20
December	16.00	23.60	15.70	86.80	6.80

Table 3.4. Weather data of the study area for 2002

Month	Rainfall (mm)	Temperature (°C)		Relative Humidity (%)	Sunshine Hours
		Max.	Min.		
January	NIL	24.50	15.90	89.90	9.90
February	61.40	24.40	16.00	87.00	8.10
March	12.40	28.48	17.13	80.00	9.29
April	55.70	33.61	18.29	76.04	8.32
May	164.00	27.52	19.75	86.28	5.88
June	264.30	25.39	19.63	97.63	2.96
July	170.20	24.40	18.03	99.68	3.51
August	349.20	22.60	16.50	93.35	5.55
September	94.40	26.55	16.42	83.42	7.20
October	308.60	25.70	17.29	89.60	4.10
November	113.60	24.60	16.10	88.37	4.80
December	6.80	24.40	16.60	84.20	6.96

3.2. Genetic control, variability, heritability, genetic advance and correlation of characters in cardamom

Ninety genotypes/accessions of cardamom collected from different parts of India and maintained in the germplasm conservatory of the Indian Cardamom Research Institute, Myladumpara, Idukki, Kerala, India have been used for the present experiment. Details of the genotypes are depicted in Table 3.5. The experiment was laid out in randomized block design with three replications and twelve plants per plot adopting 2.4 m x 2.4 m spacing. Suckers were used as planting materials and package of practices recommendations of the Spices Board, India was followed for cultivation. Observations on eighteen characters were recorded on the third, fourth and fifth years and averaged for analyzing the genetic variability of cardamom (Table 3.6).

3.2.1. Genetic control of the characters studied

Genetic control of the growth, yield and quality characters studied in cardamom has been analyzed based on the frequency distribution of the variables concerned. For the purpose, data on 270 plants belonging to 90 accessions were pooled and analyzed for the nature of variation of the characters.

3.2.2. Genetic variability

Genetic variability was analyzed with the help of analysis of variance and study of coefficient of variation (Tahir and Gupta, 2000).

Table.3.5. Genotypes/accessions used for the present study

Sl. No.	Accession No.	Source
1	MCC-129	Puthuthottam, Valparai, Tamil Nadu
2	MCC-130	Puthuthottam, Valparai, Tamil Nadu
3	MCC-131	Urulickal, Valparai, Tamil Nadu
4	MCC-139	Nalumukku, Simgampatty, Tamil Nadu
5	MCC-140	Kalakkad, Tamil Nadu
6	MCC-145	Simgampatty, Tamil Nadu
7	MCC-146	Kakachi, Simgampatty, Tamil Nadu
8	MCC-151	Kattappana, Kerala
9	MCC-152	Kattappana, Kerala
10	MCC-154	Kattappana, Kerala
11	MCC-155	Pampa, Kerala
12	MCC-156	Coonoor, Tamil Nadu
13	MCC-157	Coonoor, Tamil Nadu
14	MCC-159	Nilgiris, Tamil Nadu
15	MCC-160	Pachakanam, Kerala
16	MCC-161	Pachakanam, Kerala
17	MCC-162	Pottamkulam, Kadamakuzhy, Kerala
18	MCC-163	Kadamakuzhy, Kerala

19	MCC-167	Karithode, Kerala
20	MCC-168	Karithode, Kerala
21	MCC-169	Karithode, Kerala
22	MCC-170	Karithode, Kerala
23	MCC-171	Mananthavadi, Kerala
24	MCC-172	Mananthavadi, Kerala
25	MCC-173	Kalpetta, Kerala
26	MCC-174	Kalpetta, Kerala
27	MCC-175	Vythiri, Kerala
28	MCC-176	Kalpetta, Kerala
29	MCC-177	Meppadi, Kerala
30	MCC-178	Kalpetta, Kerala
31	MCC-179	Kalpetta, Kerala
32	MCC-180	Kalpetta, Kerala
33	MCC-181	Kalpetta, Kerala
34	MCC-182	Kalpetta, Kerala
35	MCC-183	Kalpetta, Kerala
36	MCC-184	Kalpetta, Kerala
37	MCC-185	Kalpetta, Kerala
38	MCC-186	Sugandhagiri, Pookode, Kerala
39	MCC-187	Sugandhagiri, Pookode, Kerala
40	MCC-188	Pookode, Kerala

41	MCC-189	Pookode, Kerala
42	MCC-191	Sugandhagiri, Pookode, Kerala
43	MCC-192	Sugandhagiri, Pookode, Kerala
44	MCC-193	Sugandhagiri, Pookode, Kerala
45	MCC-194	Sugandhagiri, Pookode, Kerala
46	MCC-201	Kailasappara, Kerala
47	MCC-241	Pottamkulam, Kadamakuzhy, Kerala
48	MCC-242	Pottamkulam, Kadamakuzhy, Kerala
49	MCC-243	Vellimala, Mali, Kerala
50	MCC-244	Vellimala, Mali, Kerala
51	MCC-245	Udumbanchola, Kerala
52	MCC-246	Udumbanchola, Kerala
53	MCC-247	Plamalakudy, Munnar, Kerala
54	MCC-248	Plamalakudy, Munnar, Kerala
55	MCC-250	Plamalakudy, Munnar, Kerala
56	MCC-253	Munnar, Kerala
57	MCC-254	Waterfalls, Anamalais, Tamil Nadu

58	MCC-255	Waterfalls, Anamalais, Tamil Nadu
59	MCC-262	Vellimala, Mali, Kerala
60	MCC-264	Vellimala, Mali, Kerala
61	MCC-266	Kadamakuzhy, Kerala
62	MCC-267	Pottamkulam, Kadamakuzhy, Kerala
63	MCC-268	Yercaud, Tamil Nadu
64	MCC-271	Yercaud, Tamil Nadu
65	MCC-272	Yercaud, Tamil Nadu
66	MCC-273	Yercaud, Tamil Nadu
67	MCC-274	Kolli Hills, Tamil Nadu
68	MCC-276	Kolli Hills, Tamil Nadu
69	MCC-278	Kolli Hills, Tamil Nadu
70	MCC-279	Kolli Hills, Tamil Nadu
71	MCC-281	Puliyanmala, Kerala
72	MCC-282	Puliyanmala, Kerala
73	MCC-283	Kalthotti, Kerala
74	MCC-284	Kalthotti, Kerala
75	MCC-286	Murukkadi, Kerala
76	MCC-288	Myladumpara, Kerala
77	MCC-292	Koottakuzhy, Kerala
78	MCC-299	Adurmalai, Kerala

79	MCC-300	Adurmalai, Kerala
80	MCC-309	Meghamalai Hills, Tamil Nadu
81	MCC-310	Meghamalai Hills, Tamil Nadu
82	MCC-311	Meghamalai Hills, Tamil Nadu
83	MCC-312	Meghamalai Hills, Tamil Nadu
84	MCC-314	Meghamalai Hills, Tamil Nadu
85	MCC-316	Gavi, Pachakanam, Kerala
86	MCC-319	Gavi, Pachakanam, Kerala
87	MCC-331	Gavi, Pachakanam, Kerala
88	MCC-334	Puliyanmala, Kerala
89	MCC-344	Puttady, Kerala
90	MCC-345	Puttady, Kerala

3.2.3. Heritability

Heritability of characters has been calculated as proposed by Singh and Choudhary (1985) and Singh and Narayanan (1993).

3.2.4. Genetic advance

Genetic advance of characters was calculated as given by Singh and Narayanan (1993).

3.2.5. Correlation of characters

Correlation of characters has been found out as given by Rangaswamy (1995).

Table 3.6. Characters of cardamom studied for variability analysis

Sl. No.	Character
1	No. of tillers/clump
2	Height of tiller (cm)
3	Number of leaves per tiller
4	No. of vegetative buds/clump
5	Leaf length (cm)
6	Leaf breadth (cm)
7	No. of bearing tillers/clump
8	No. of panicles/clump
9	Panicle length (cm)
10	Internodal length (cm)
11	No. of racemes/panicle
12	No. of capsules/raceme
13	No. of seeds/capsule
14	No. of capsules (dry) / kg
15	Percentage of 7mm and above sized capsules
16	Recovery percentage
17	Volatile oil content (%)
18	Yield /clump (dry weight in kg.)

3.3. Factor analysis in cardamom

Evaluation of germplasm and its utilization in breeding programmes are the two most important steps involved in the improvement of any crop. Evaluation includes biometric study of the morphological traits and related characters of the genotypes. One of the constraints in the successful and quick utilization of germplasm is the delay in characterization, evaluation and cataloguing of the germplasm. In the genetic

evaluation of the germplasm of cardamom, large number of morphological traits is recorded for estimation of genetic variance and correlation among these characters. The recording of data on such a large number of characters is time consuming, especially when large number of genotypes is evaluated. Application of multivariate data analysis has become a popular method mainly because it can provide information not otherwise accessible. In the present study, factor analysis by means of principal component analysis method was carried out to reveal such underlying factors in groups of related variables.

The experimental part of the study was undertaken in the Indian Cardamom Research Institute, Myladumpara, incorporating ninety genotypes of cardamom planted in a field evaluation trial in 1996 (Table 3.5). The genotypes were planted in randomized block design at a spacing of 2.4 m x 2.4 m with three replications and twelve plants per plot. The package of practices was adopted as per the recommendations of the Spices Board. Data on seventeen morphological, yield contributing and qualitative characters namely total tillers per clump, tiller height (cm), leaves per tiller, number of vegetative buds per clump, leaf length (cm), leaf breadth (cm), number of bearing tillers per clump, number of panicles per clump, panicle length (cm), internodal length (cm), racemes per panicle, capsules per raceme, seeds per capsule, recovery percentage, percentage of 7 mm and above sized capsules, number of dry capsules per kilogram and volatile oil content were recorded for three consecutive

years and averaged. Factor analysis was done using principal component analysis method as described by Harman, 1976.

3.4. Genetic divergence in cardamom

Eventhough statistically significant variability can be observed between different genotypes/accessions of a plant species, they can be grouped into different clusters of genetically closer accessions based on genetic divergence studies. Ninety accessions of cardamom maintained in the field gene bank of the Indian Cardamom Research Institute at Myladumpara, planted in 1996 constituted the material for the investigation (Table 3.5). An experiment was conducted for the purpose, in randomized block design with three replications and twelve plants per plot. Observations on eleven characters identified based on principal component analysis, namely leaf breadth, number of bearing tillers per clump, total tillers per clump, capsules per raceme, racemes per panicle, yield per clump, panicles per clump, internodal length, seeds per capsule, recovery percentage and number of dry capsules per kg were made consecutively in the third, fourth and fifth years and averaged. The data were subjected to multivariate analysis by using Mahalanobis generalized distance D^2 and the genotypes were clustered on the basis of minimum generalized distance using Tocher's method as given by Rao (1952).

3.5. Performance evaluation of selected clones of cardamom

The experiment was designed to identify the most promising clones from a group of elite genotypes maintained in the Indian Cardamom Research Institute, Myladumpara in a randomized block layout with three replications and twelve plants per plot adopting 3 m x 3 m spacing and planted in 1996. The materials used for the study include 17 genotypes namely ICRI-1, ICRI-2, MHC-10, MHC-13, MHC-18, MCC-40, MCC-21, MHC-22, MCC-200, MCC-73, MCC-85, MCC-260, MCC-346, MHC-27, MHC-26, MHC-24 and MHC-23. These genotypes were evolved from the germplasm maintained at the Indian Cardamom Research Institute, Myladumpara, Idukki, Kerala, through selection and hybridization. Package of practices recommendation of the Spices Board was followed for cultivation. Among the 17 genotypes, ICRI-1 and ICRI-2 are released clones. A brief description of the clones is given in Table 3.7.

Table 3.7. Clones used for performance evaluation

Sl. No.	Clone	Description
1	ICRI-1 (MCC-49)	A germplasm selection from an accession collected from Chakkupallam in Idukki District of Kerala. *Malabar* type with round, bold and dark green capsules. Under rainfed conditions, the yield reported is 325 kg/ha and under irrigated conditions, 656 kg/ha. Released for cultivation in 1992 by the Indian Cardamom

		Research Institute (ICRI), Myladumpara. Adapted to most of the parts of Idukki District, extending from Vandanmedu to Vandi-periyar. Previously known as "Veeraputhran".
2	ICRI-2 (MCC-61)	A germplasm selection from an accession collected from Pampadumpara in Idukki District. *Mysore* type with long, bold, parrot green capsules. Yield under rainfed conditions is 375 kg/ha and under irrigated conditions, 766 kg/ha. Anamalais in Tamil Nadu and Vandanmedu and Nelliampathy in Kerala are found good for ICRI-2. Released for cultivation in 1992 by ICRI, Myladumpara. Relatively tolerant to azhukal disease.
3	MHC-10	A hybrid of the germplasm collections, MCC-34 and MCC-4. Angular, bold and deep green capsules. Highly suited to Idukki and Nelliampathy hills of Kerala and Nilgiris of Tamil Nadu.
4	MHC-13	A hybrid of the germplasm collections, MCC-62 and MCC-34. Characterized by medium long capsules. The hybrid is well suited to Idukki and Nelliampathy hills of Kerala and Nilgiris of Tamil Nadu.
5	MHC-18	A hybrid of the germplasm collections, MCC-12 and

		MCC-35. It has characteristic deep green, angular bold capsules. Performance is fairly good even under rainfed conditions. The hybrid is well adapted to Idukki and Nelliampathy hills of Kerala.
6	MCC-40	A selection from the germplasm accession popularly known as "Walayar", collected from Mavadi in Idukki District. *Malabar* type with short panicles. Early bearing type with elongated long capsule. Relatively tolerant to drought.
7	MCC-21	Popularly known as Vazhukka LBC (Long Bold Capsule). Belongs to *Vazhukka* type. Collected from Kalthotti in Idukki District. Performs well even under moderate management. Highly adapted to Idukki tract. The capsules are long and angular bold.
8	MHC-22	A hybrid of MCC-49 (ICRI-1) and MCC-260 (Njallani land race). It has pendent or semierect panicles. Capsules are round, bold with deep green colour.
9	MCC-200	A collection from Koombanpara in Idukki District. A promising selection from the Preliminary Evaluation

		Trial (PET-I). It has angular bold capsules.
10	MCC-73	A collection from Santhanpara in Idukki District. A profusely bearing *Malabar* type with angular bold capsules.
11	MCC-85	Popularly known as Cinchona selection. A collection from Pampa in Pathanamthitta District. *Vazhukka* type selected from germplasm with angular bold capsules.
12	MCC-260	A land race popularly known as 'Njallani Green Gold'. It is a collection from Parakkadavu near Kattappana in Idukki District. *Vazhukka* type. The special feature of this clone is the robust nature with extra-bold capsules.
13	MCC-346	A germplasm selection from the accession collected from Vandi-periyar in Idukki district. *Vazhukka* type well adapted to all cardamom growing tracts of Kerala, Karnataka and Tamil Nadu.
14	MHC-27	A hybrid of MCC-260 (Njallani Green Gold land race) and MCC-61 (ICRI-2). The panicles are pendent type with long, angular bold, parrot green capsules.

15	MHC-26	A hybrid of MCC-260 (Njallani Green Gold land race) and MCC-49 (ICRI-1). The hybrid possesses pendent panicles having characteristic round, extra bold and parrot green capsules.
16	MHC-24	A hybrid of MCC-61 (ICRI-2) and MCC-49 (ICRI-1). It has pendent panicles with angular bold and parrot green capsules.
17	MHC-23	A hybrid of MCC-49 (ICRI-1) and MCC-345. It possesses pendent panicles with round bold deep green capsules.

Seventeen characters including seven growth characters, seven yield characters and three quality characters were studied in the 17 genotypes as shown in Table 3.8. The data were subjected to statistical analysis for bringing out the most promising selections/hybrids.

Table 3.8. Characters observed in the case of the evaluation of elite clones

Character	Method of observation
Growth characters	
1. Tillers per clump	The total number of tillers excluding the vegetative buds was observed.
2. Tiller height (cm)	The height of the tallest tiller was measured in cm scale.
3. Leaves per tiller	The number of leaves on the tallest tiller was counted.

4. Number of veg. buds	The total number of vegetative buds either without leaves or with one leaf was counted.
5. Number of bearing tillers	The total number of bearing tillers (tillers having panicles) in the clump was recorded.
6. Leaf length (cm)	The length of seventh leaf from the top of the pseudostem was recorded.
7. Leaf breadth (cm)	The width of the seventh leaf from the top of the pseudostem was measured. The width of the leaf at the middle portion was measured.
Yield characters	
1. Panicles per clump	The total number of panicles in each clump was counted.
2. Panicle length (cm)	The panicle length of three panicles in a clump was recorded and their mean value was worked out.
3. Racemes per panicle	The number of racemes on each panicle was noted down. Three observations of different panicles in a clump were recorded.
4. Seeds per capsule	The total number of seeds in a mature capsule was counted. Three observations on different capsules were recorded and their mean value was worked out.
5. Internodal length (cm)	The length between two nodes on a panicle was measured using centimetre scale.
6. Number of dry capsules per kg	The number of dry capsules in one kilogram was counted in each round of harvest and the mean value was worked out.

7. Yield per clump (kg)	Yield per clump in dry weight was recorded in each round of harvest and the values were added up at the end of the crop season to get the total yield per clump.
Quality characters	
1. 7 mm and above sized capsules (%)	In each round of harvest, the percentage of 7 mm and above sized capsule was determined by sieving the capsules through a 7 mm mesh. Mean value was worked out at the end of the crop season.
2. Recovery percentage	The driage was worked out in each round of harvest and the mean value calculated.
3. Volatile oil content	The volatile oil content (%) in the dry capsule was determined on whole pod basis (by using Clevenger apparatus).

3.6. Molecular characterization of selected clones of cardamom

3.6.1. Materials used

Molecular characterization of 14 genotypes of cardamom has been attempted with the help of RAPD technique for the present study. RAPD analysis was carried out in the year 2000 with the help of the Crop Improvement and Biotechnology Division of the Indian Institute of Spices Research, Calicut, India.

Two released high yielding varieties, ICRI-1 and ICRI-2; eight promising unreleased selections, MCC-12, MCC-21, MCC-40, MCC-16, MCC-73, MCC-85, MCC-260 and MCC-346 and four unreleased promising hybrids, MHC-

18, MHC-24, MHC-26 and MHC-27 were used for the present study.

3.6.2. Methodology followed

3.6.2.1. DNA isolation and quantification

Genomic DNA was successfully isolated from young and fresh leaves of cardamom plants using modified CTAB method of Ausubel *et al.* (1995) as detailed below.

1. Weighed samples (2 g) were ground in liquid nitrogen using prechilled mortar and pestle.

2. Transferred the fine powder of tissues to pre heated CTAB-extraction buffer (2ml) and incubated at 65°C, with intermittent mixing.

3. Added equal volumes of chloroform: isoamyl alcohol (24:1) (Merck). Mixed thoroughly, avoiding vigorous shaking.

4. Kept the Oakridge tubes on ice, centrifuged at 10,000 rpm for10 min. at 4°C.

5. Transferred the supernatant to fresh tubes with cut tips.

6. Added 2/3 volume of ice cold isopropanol (Merck) into the supernatant. Gently mixed by inversions, kept the solution for half an hour in refrigerator.

7. Carefully spooled the DNA using a sterile glass rod into 1,000 µl of 70% ethanol.

8. Spun at 5,000 rpm for 5 minutes at 4°C. Removed the supernatant inverted the tubes for 15 minutes to drain off excess alcohol and left the pellet to vacuum dry.

9. Added 500 µl of TE buffer into the pellet and dissolved the DNA completely.

3.6.2.2. Purification of genomic DNA

1. Added 40 ng of RNase (Genei, Bangalore) solution and incubated at 37°C for 1 hour.

2. Added equal volumes of phenol: chloroform: isoamyl alcohol (Sigma) 25:24:1 into the solution and mixed well.

3. Centrifuged at 12,000 rpm for 10 minutes at 4°C and pipetted out the upper aqueous phase into a fresh tube.

4. Extracted with equal volumes of chloroform: isoamyl alcohol (24:1) and centrifuged at 10,000 rpm and transferred the aqueous phase to fresh tubes.

5. Added 1/10 volume of sodium acetate (Qualigens) pH-5.3 and mixed by gentle tapping.

6. Precipitated by 0.8 volume of isopropanol and kept in 20°C for 1 hour for better precipitation.

7. Centrifuged at 10,000 rpm for 10 minutes at 4°C.

8. Discarded the supernatant and washed the pellet using 70% ethanol.

9. Vacuum dried the pellet for 5 minutes.

10. Completely dissolved in TE buffer.

3.6.2.3. Quantification of genomic DNA

Quantification in this experiment was done by standard ethidium bromide fluorescent quantification. λDNA (250 ng/μl) obtained from Genei, Bangalore was used as the standard as a final concentration of 25 ng/μl. The samples were run on 0.8 % agarose gel along with the λDNA standard and DNA quantity was estimated by comparing the band intensity with that of the standard.

3.6.2.4. Optimization of PCR components

Different combinations and concentrations of dNTPs, Taq polymerase and $MgCl_2$ were tested for good and consistent amplification of genomic DNA. Among the different combinations tested, 1X assay buffer (1.5 mM of $MgCl_2$, 150 μM of dNTPs, 1 U of Taq) gave good amplification with clear bands without any nonspecific banding. Hence, only this combination was used in further PCRs *i.e.*, for primer screening and RAPD analysis.

3.6.2.5. Optimization of PCR programming

The optimized reaction conditions for PCR that gave good amplification was as follows:

Cycle-1	94°C	for	2^1	Repeat-1
	40°C	for	1^1	
	72°C	for	2^1	
Cycle- 2	94°C	for	1^1	Repeat-30
	40°C	for	1^1	
	72°C	for	1^1	
Cycle-3	94°C	for	1^1	Repeat-1
	40°C	for	1^1	
	72°C	for	15^1	

3.6.2.6. Primer screening

A total of 100 operon primers (OPERON Technologies Inc., Alameda, California) were screened for amplification and polymorphism and eight primers found to be polymorphic were used in the present study (Table 3.9).

Table 3.9. Operon primers used for developing RAPD profiles in cardamom

Sl. No.	Primers	Sequence	% of GC
1	OPA 04	5'AATCGGGCTG 3'	60
2	OPA 16	5'AGCCAGCGAA3'	60
3	OPB 01	5'GTTTCGCTCC3'	60
4	OPB 17	5'AGGGAACGAG3'	60
5	OPC 10	5' TGTCTGGGTG3'	60
6	OPC 16	5'CACACTCCAG3'	60
7	OPF 07	5'CCGATATCCC3'	60
8	OPE 15	5'ACGCACAACC3'	60

3.6.2.7. Reaction mixture used for cardamom

The reaction mixture used for the present experiment is given below:

Component		Volume
1 X assay buffer	:	2.5μl
dNTPs 1 mM	:	3.75μl
$MgCl_2$ 10mM	:	1.25μl
Primer5 pmol	:	2μl
Taq DNA pol: 2u	:	0.5μl
DNA 10 ng	:	3μl
Double distilled water:		12μl
		25μl

The master mix for 15 reactions was prepared according to the above recipe. Taq DNA polymerase was added into the master mix as a final component. The mix was tapped gently for uniform mixing of all the components and 22μl of the solution was pipetted out into sterilized PCR tubes. DNA was added to each tube, centrifuged the tubes at 2,500 rpm for 2 minutes at 4°C. PCR reaction was carried out in MJ Research Thermocycler. Fractionation of DNA fragments after the polymerase chain reaction experiments was done by agarose gel electrophoresis. Standard agarose gels separate DNA fragments from 0.5 kb to 25 kb.

The process involved the following steps:

1) A gel was prepared with an agarose concentration appropriate for the size of DNA fragments to be separated.

2) The DNA samples were loaded in the sample wells and the gel was run at a voltage and for a time period that achieved optimal separation.

3) BioRad 1,000 gel documentation system was used for visualizing and documenting the data obtained from the stained gel. Ethidium bromide concentration of 0.5µg/ml was used.

3.6.2.8. Development of RAPD profiles

Eight OPERON primers were used for the studies. The isolated and purified DNA was subjected to PCR. The RAPD products were resolved in 2% agarose gel and the result was analyzed for the polymorphic bands in each of the genotypes.

The data obtained from the gels were scored for the presence and the absence of the bands. Later, cluster analysis of the genomic fingerprints was carried out using NTSYSpc2.0 software to analyze the genetic relationship among the genotypes being examined. Paired Affinity Indices (PAI) were calculated as per the following formula:

$$PAI = \frac{\text{Number of similar bands}}{\text{Total number of bands}} \times 100$$

RESULTS AND DISCUSSION

V.V. Radhakrishnan "Studies on variability, genetic divergence and crop improvement in cardamom (Elettaria Cardamomum Maton)" Thesis. Department of Botany, University of Calicut, 2003

Chapter 4
RESULTS AND DISCUSSION

The present experiments were carried out so as to study the phenology of tiller and panicle in cardamom, genetic control, variability, heritability, genetic advance and divergence of characters in cardamom, performance evaluation of selected clones and molecular characterization of some genotypes as described elsewhere. The major findings of the study are presented below under appropriate heads and discussed in the light of literature available.

4.1. Phenology of tiller and panicle in cardamom

Monthly observation on the emergence and growth of tillers and panicles in the case of thirty selected clumps each of same age group was recorded in the three cultivars of cardamom for the present study. Panicles usually emerge from the base of ten to twelve month old tillers, when they reach thirteen leaf stage. The present observation indicated that both tillers and panicles emerged round the year. Similar observations have been made by Sudharshan *et al.* (1988) and Kuruvilla *et al.* (1992). However, the frequency of production varied in different seasons. From pre-monsoon period to the beginning of active monsoon (*i.e.*, March – June), tiller production showed an increasing trend and thereafter a sudden decline. However, August to September months showed maximum tiller production (Tables 4.1, 4.2 & 4.3. and Fig. 4.1). Majority of the panicles emerged during

October to March. The pattern of emergence of tillers and panicles did not vary among the cultivars under the agro climatic conditions of Myladumpara. Earlier studies have also indicated that in cardamom, majority of the panicles emerge in the post monsoon and winter season (Pattanshetty and Prasad, 1976). The progressive growth of tillers continued over a period of 18 months in all the cultivars (Tables 4.1, 4.2 & 4.3). Linear elongation of tillers progresses with the onset of monsoon and the growth rate slows down with the cessation of rain. Reproductive buds are produced from the base of the tiller ten to twelve months after its emergence and these develop into panicles within one year. Generally two to four panicles are produced from each tiller. Soon after the development of panicles, linear growth rate of the sucker declines and the sucker withers away after the maturity of the panicle. These observations are in conformity with earlier studies (Pattanshetty and Prasad, 1976).

The growth pattern of the newly formed panicles is given in Tables 4.4, 4.5 & 4.6. Linear growth extended over a period of six months under Myladumpara conditions. According to Pattanshetty and Prasad (1976), the linear growth of the panicles extended over a period of seven months. The rate of growth was low in early stages (December to March) and it was very fast during April. This may be due to the interruption of dry season by pre-monsoon showers during March-April. It took 90 to 110 days for the first flower on a fresh panicle to bloom irrespective of

cultivars. The racemes and capsules are formed during 4th and 5th months respectively after panicle initiation. Capsule formation increased till August and thereafter declined slowly in all the cultivars of cardamom. Earlier workers have noticed flowering in cardamom throughout the year (Krishnamurthy *et al.*, 1989). In the present experiment also, flowering was observed in cardamom throughout the year and irrespective of cultivars, flowering was initiated on a fresh panicle during May and more than seventy per cent was over by August. The blooming span in general was six months.

The peak flowering and fruit set periods coincided in different cultivars of cardamom. Majority of the capsules were formed by the end of September irrespective of cultivars. It took about 120 to 135 days to form a mature capsule from a flower. Harvesting in cardamom generally starts in July and continues up to January/February in Kerala conditions. However, under irrigated situations, harvesting extends for another one month. Peak harvest was recorded from August to November in the present experiment.

Table 4.1. Tiller and panicle development and their growth pattern in Cv. *Malabar*

Month	Height of tiller (cm)	Number of leaves	Number of vegetative buds	Number of panicles
January	P	-	-	-
February	P	-	-	-
March	3.00	-	-	-
April	6.00	-	-	-
May	21.56	0.91	-	-
June	42.64	2.04	-	-
July	61.60	4.00	-	-
August	77.88	5.40	-	-
September	85.16	6.76	-	-
October	94.40	7.44	0.64	-
November	104.44	9.08	0.84	-
December	108.40	8.76	0.96	1.00
January	119.16	9.84	1.08	1.06
February	122.92	7.92	1.08	1.08
March	124.24	8.84	1.20	1.60
April	128.86	8.34	1.22	1.74
May	141.82	9.12	2.00	1.93
June	145.18	9.18	2.00	1.93
July	148.26	9.64	2.00	1.96
August	150.14	9.82	2.06	1.98
September	Linear growth of the tillers almost ceased			

P: Protuberance

Table 4.2. Tiller and panicle development and their growth pattern in Cv. *Mysore*

Month	Height of tiller (cm)	Number of leaves	Number of vegetative buds	Number of panicles
January	P	-	-	-
February	P	-	-	-
March	3.20	-	-	-
April	6.52	-	-	-
May	27.80	0.40	-	-
June	63.56	1.32	-	-
July	102.64	3.72	-	-
August	129.12	5.84	-	-
September	144.40	7.80	-	-
October	172.76	9.24	0.60	-
November	176.76	10.32	0.64	-
December	191.28	11.20	0.68	0.44
January	204.92	12.12	0.68	0.80
February	209.92	11.92	0.68	0.96
March	210.54	12.50	0.68	1.45
April	228.60	13.82	0.78	1.26
May	253.20	15.60	1.70	1.37
June	264.63	15.84	1.98	1.58
July	268.32	16.02	2.00	1.62
August	269.64	16.02	2.06	1.86
September	Linear growth of the tillers almost ceased			

P: Protuberance

Table 4.3. Tiller and panicle development and their growth pattern in Cv. *Vazhukka*

Month	Height of tiller (cm)	Number of leaves	Number of vegetative buds	Number of panicles
January	P	-	-	-
February	P	-	-	-
March	4.50	-	-	-
April	9.56	-	-	-
May	25.64	0.88	-	-
June	54.56	1.52	-	-
July	82.60	4.80	-	-
August	103.60	5.80	-	-
September	113.80	7.12	-	-
October	135.52	9.08	0.70	-
November	139.58	10.04	0.70	-
December	148.88	10.29	0.72	0.62
January	159.52	11.80	0.80	0.80
February	163.96	10.00	1.00	1.24
March	174.04	10.08	1.24	1.64
April	184.95	10.27	1.40	1.70
May	196.05	13.19	2.33	1.71
June	198.41	13.70	2.41	1.76
July	202.16	13.88	2.46	1.84
August	204.76	13.99	2.46	1.94
September	Linear growth of the tillers almost ceased			

P: Protuberance

Fig. 4.1. Frequency of tiller formation in cardamom cultivars

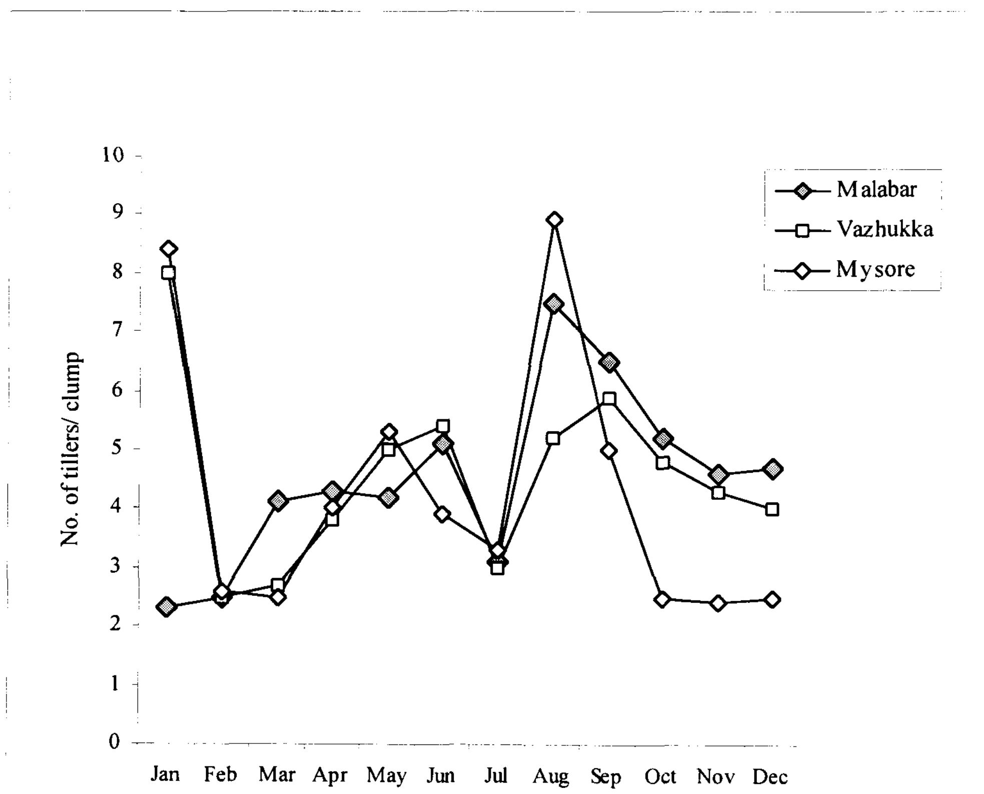


Table 4.4. Details of panicle development in Cv. *Malabar*

Month	Length of panicle (cm)	Number of racemes	Number of capsules
January	3.76	-	-
February	6.34	-	-
March	7.91	-	-
April	11.85	-	-
May	17.52	13.63	-
June	18.80	14.93	18.56
July	18.40	15.40	34.30
August	17.63	15.93	39.23
September	18.40	13.76	31.23
October	18.52	14.71	3.81
November	18.41	14.43	1.52
December	18.90	14.44	0.33

Table 4.5. Details of panicle development in Cv. *Vazhukka*

Month	Length of panicle (cm)	Number of racemes	Number of capsules
January	6.77	-	-
February	9.20	-	-
March	12.50	-	-
April	13.02	-	-
May	22.76	11.06	-
June	23.73	14.70	4.20
July	22.13	15.73	8.16
August	18.86	11.36	8.13
September	16.46	9.83	3.96
October	25.07	14.40	2.60
November	25.20	14.40	0.67
December	25.87	14.20	0

Table 4.6. Details of panicle development in Cv. *Mysore*

Month	Length of panicle (cm)	Number of racemes	Number of capsules
January	4.83	-	-
February	7.23	-	-
March	11.00	-	-
April	16.60	-	-
May	23.50	14.93	-
June	23.66	17.13	14.06
July	21.30	16.13	21.86
August	23.66	16.76	29.70
September	24.36	16.63	19.93
October	24.96	16.15	11.29
November	25.11	16.07	3.74
December	25.44	16.00	0.48

4.2. Genetic control, variability, heritability and correlation of characters in cardamom

Studies on genetic control of characters and their variability in cardamom were carried out utilizing ninety genotypes/accessions of cardamom collected from different parts of India and maintained in the germplasm repository of the Indian Cardamom Research Institute, Myladumpara (Table 3.5) as described elsewhere. Observations on eighteen variables with respect to growth, yield and quality were recorded on the third, fourth and fifth years, averaged and analyzed for the genetic control, variability, heritability and correlation of characters in cardamom (Table 3.6). The data on the above aspects are presented and discussed below under appropriate heads.

4.2.1. Genetic control of the characters under study

Genetic control of eighteen growth, yield and quality characters was analyzed from the pooled data of 270 plants belonging to ninety accessions. Observations were made for three consecutive years starting from the third year of planting and averaged in the case of each plant. The data were analyzed for frequency distribution in the case of each character so as to find out the mechanism of genetic control involved. Observations on the characters are presented and analyzed below.

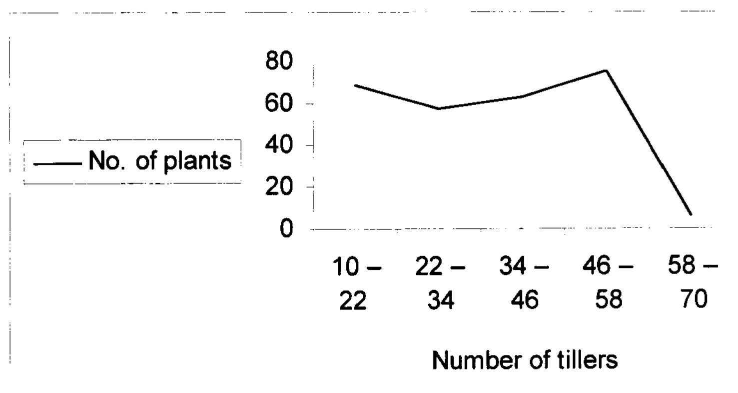
4.2.1.1. Number of tillers per clump

Number of tillers per clump in cardamom showed continuous variation as shown in Table 4.7 and Fig. 4.2, thus indicating polygenic control of the character. However, the frequency of the plants with lower number of tillers is higher and it may be due to the accumulation of recessive factors in higher numbers in the gene pool of the character.

Table 4.7. Frequency distribution of number of tillers per clump

Tillers per clump	Number of plants
10 – 22	69
22 – 34	57
34 – 46	63
46 – 58	75
58 – 70	6
Total	270

Fig. 4.2. Frequency curve of number of tillers per clump



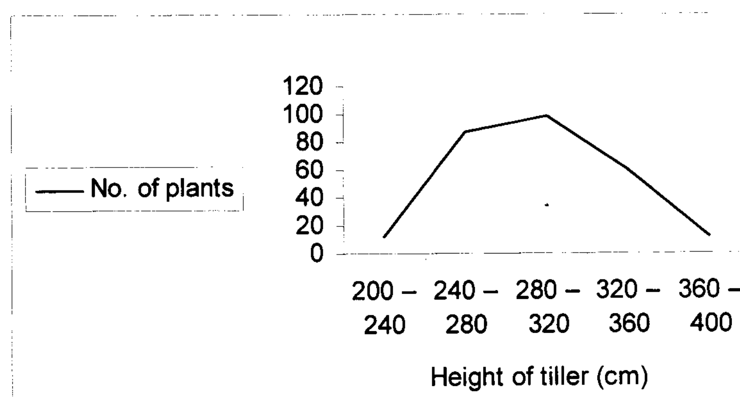
4.2.1.2. Tiller height

Tiller height in cardamom showed continuous frequency distribution and the curve was almost similar to the normal distribution curve, thus indicating polygenic control and balanced distribution of different allele combinations in the gene pool (Table 4.8 and Fig. 4.3).

Table 4.8. Frequency distribution of tiller height

Tiller height (cm)	Number of plants
200 – 240	12
240 – 280	87
280 – 320	99
320 – 360	60
360 – 400	12
Total	270

Fig. 4.3. Frequency curve of tiller height



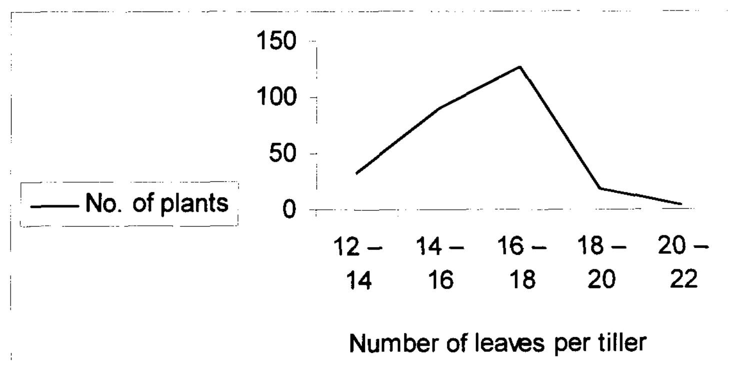
4.2.1.3. Number of leaves per tiller

The character shows continuous frequency distribution indicating its polygenic control. However, the frequency curve shows a fall towards the upper leaf number and it may be due to the influence of environment in the expression of the character or due to the presence of dominant alleles in a lower frequency than the expected (Table 4.9 and Fig. 4.4).

Table 4.9. Frequency distribution of number of leaves per tiller

No. of leaves per tiller	Number of plants
12 – 14	33
14 – 16	90
16 – 18	126
18 – 20	18
20 – 22	3
Total	270

Fig. 4.4. Frequency curve of number of leaves per tiller



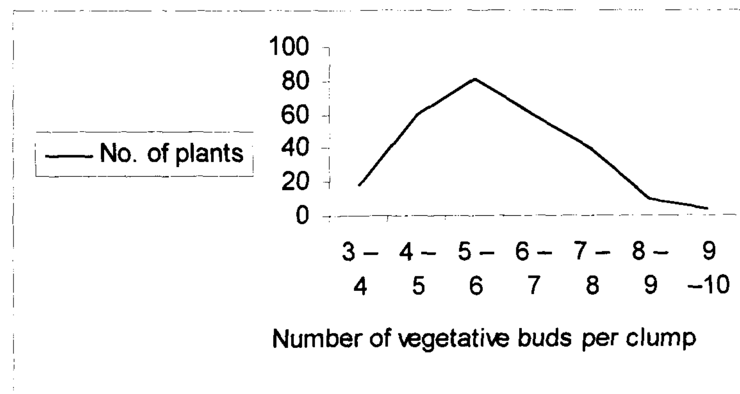
4.2.1.4. Number of vegetative buds per clump

Number of vegetative buds per clump also showed continuous frequency distribution with a fall in frequency towards the positive extreme of the character, indicating polygenic control and the influence of environment on the expression of the character (Table 4.10 and Fig.4.5).

Table 4.10. Frequency distribution of number of vegetative buds per clump

Number of vegetative buds per clump	Number of plants
3 – 4	18
4 – 5	60
5 – 6	81
6 – 7	60
7 – 8	39
8 – 9	9
9 –10	3
Total	270

Fig. 4.5. Frequency curve of number of vegetative buds per clump



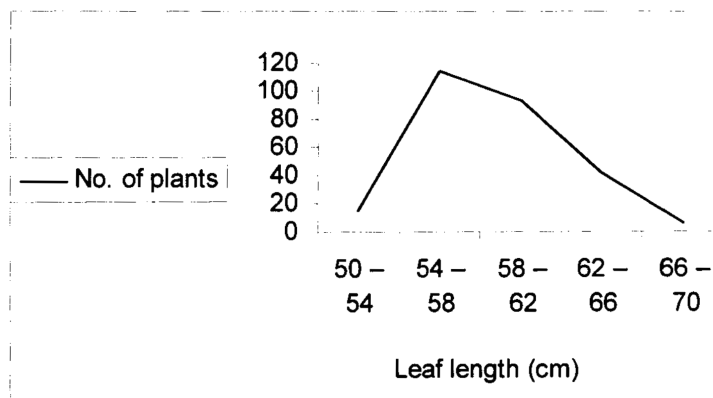
4.2.1.5. Leaf length

Leaf length showed continuous frequency distribution with the mode value of the character shifting to the second class of the distribution from the middle (third) class. This type of behaviour of the character might be due to the higher frequency of allele combinations with lower number of dominant alleles in the population (Table 4.11 and Fig. 4.6).

Table 4.11. Frequency distribution of leaf length

Leaf length (cm)	Number of plants
50 – 54	15
54 – 58	114
58 – 62	93
62 – 66	42
66 – 70	6
Total	270

Fig. 4.6. Frequency curve of leaf length



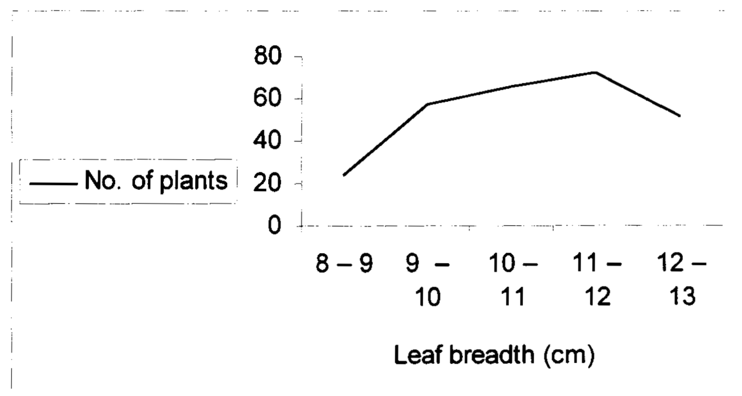
4.2.1.6. Leaf breadth

Frequency distribution of the character shows continuous variation, indicating its polygenic control. The curve shows a peak beyond the central class and it may be due to the accumulation of more number of dominant factors in the population (Table 4.12 and Fig. 4.7).

Table 4.12. Frequency distribution of leaf breadth

Leaf breadth (cm)	Number of plants
8 – 9	24
9 – 10	57
10 – 11	66
11 – 12	72
12 – 13	51
Total	270

Fig. 4. 7. Frequency curve of leaf breadth



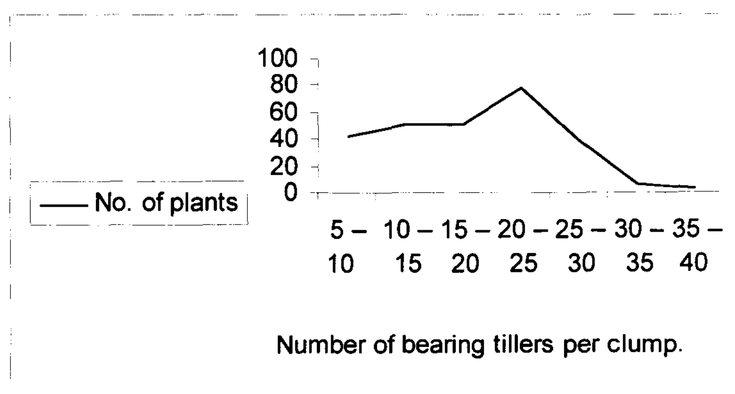
4.2.1.7. Number of bearing tillers per clump

The character shows continuous distribution, thus indicating the polygenic control of the character. The frequencies of the classes with lower number of bearing tillers per clump are higher and it may be due to the accumulation of recessive factors in the gene pool (Table 4.13 and Fig.4.8).

Table 4.13. Frequency distribution of number of bearing tillers per clump

Number of bearing tillers per clump	Number of plants
5 – 10	42
10 – 15	51
15 – 20	51
20 – 25	78
25 – 30	39
30 – 35	6
35 – 40	3
Total	270

Fig. 4.8. Frequency curve of number of bearing tillers per clump



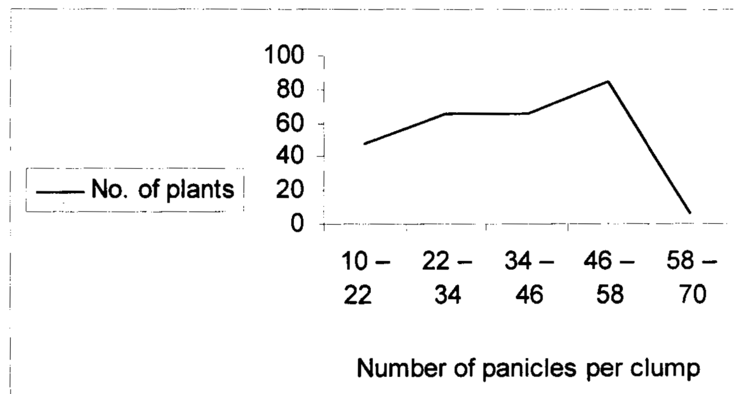
4.2.1.8. Number of panicles per clump

Number of panicles per clump shows continuous distribution indicating the polygenic control of the character. However, the distribution of allele combinations shows deviation from the expected frequencies (Table 4.14 and Fig. 4.9).

Table 4.14. Frequency distribution of number of panicles per clump

Number of panicles per clump	Number of plants
10 – 22	48
22 – 34	66
34 – 46	66
46 – 58	84
58 – 70	6
Total	270

Fig. 4.9. Frequency curve of number of panicles per clump



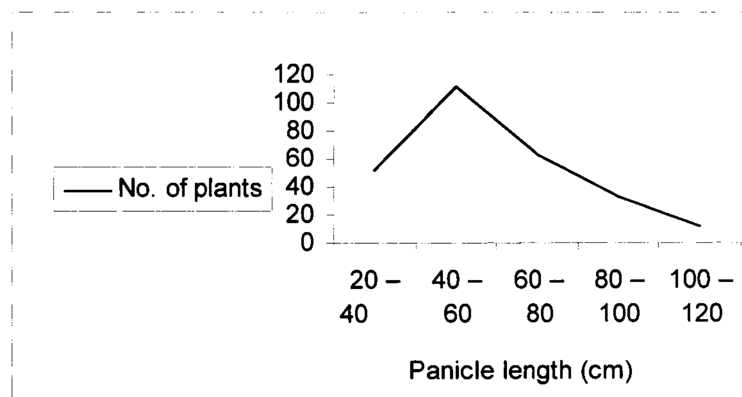
4.2.1.9. Panicle length

The character shows continuous frequency distribution indicating the polygenic control of the character. The mode class is not the central but the preceding one, thus showing that more number of plants expresses the character in the medium and low range. It may be due to the higher frequency of recessive alleles in the gene pool or to some extent environmental influence on the expression of the character (Table 4.15 and Fig. 4.10).

Table 4.15. Frequency distribution of panicle length

Panicle length (cm)	Number of plants
20 – 40	51
40 – 60	111
60 – 80	63
80 – 100	33
100 – 120	12
Total	270

Fig. 4.10. Frequency curve of panicle length



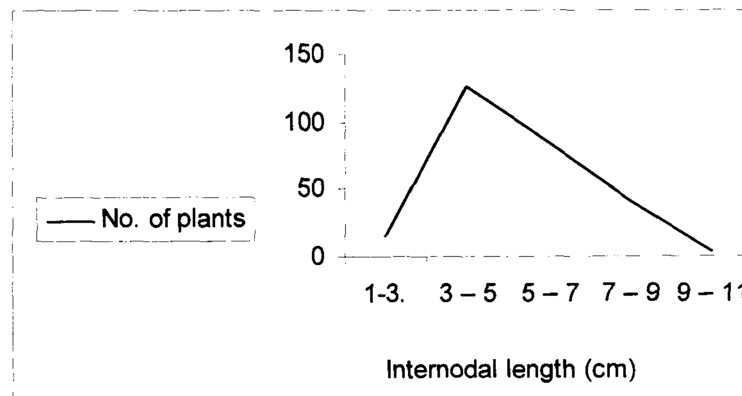
4.2.1.10. Internodal length

Internodal length of cardamom showed continuous frequency distribution indicating polygenic control of the character. Highest frequency of plants was shown by the second frequency class and it may be due to the accumulation of more number of recessive factors than expected (Table 4.16 and Fig. 4.11).

Table 4.16. Frequency distribution of internodal length

Internodal length (cm)	Number of plants
1 - 3	15
3 - 5	126
5 - 7	84
7 - 9	42
9 - 11	3
Total	270

Fig. 4.11. Frequency curve of internodal length



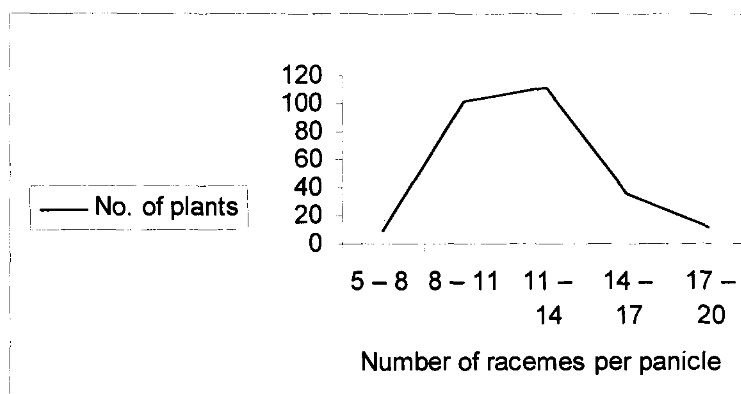
4.2.1.11. Number of racemes per panicle

Number of racemes per panicle showed continuous frequency distribution that was almost normal thus indicating polygenic inheritance and almost uniform distribution of allele combinations (Table 4. 17 and Fig. 4.12).

Table 4.17. Frequency distribution of number of racemes per panicle

Number of racemes per panicle	Number of plants
5 – 8	9
8 – 11	102
11 – 14	111
14 – 17	36
17 – 20	12
Total	270

Fig. 4.12. Frequency curve of number of racemes per panicle



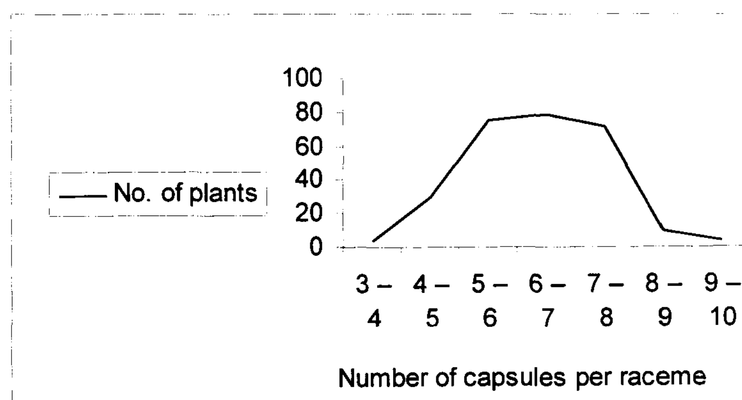
4.2.1.12. Number of capsules per raceme

The character showed continuous and normal frequency distribution indicating polygenic control of the character and balanced distribution of allele combinations (Table 4.18 and Fig. 4.13).

Table 4.18. Frequency distribution of number of capsules per raceme

Number of capsules per raceme	Number of plants
3 – 4	3
4 – 5	30
5 – 6	75
6 – 7	78
7 – 8	72
8 – 9	9
9 – 10	3
Total	270

Fig. 4.13. Frequency curve of number of capsules per raceme



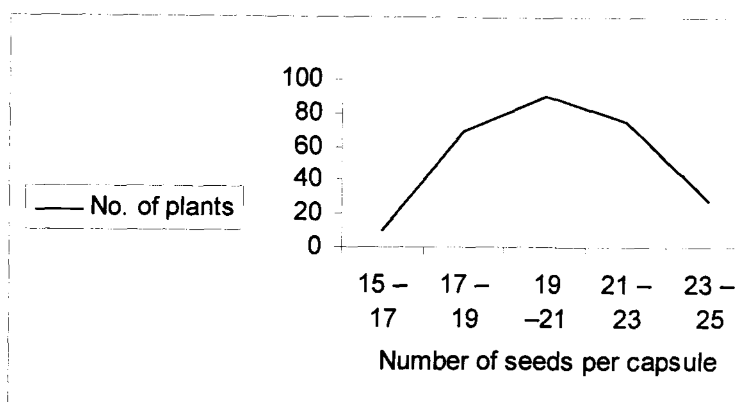
4.2.1.13. Number of seeds per capsule

Number of seeds per capsule showed normal frequency distribution indicating its polygenic control and balanced distribution of alleles and allele combinations in the gene pool (Table 4.19 and Fig. 4.14).

Table 4.19. Frequency distribution of number of seeds per capsule

Number of seeds per capsule	Number of plants
15 – 17	9
17 – 19	69
19 –21	90
21 – 23	75
23 – 25	27
Total	270

Fig. 4.14. Frequency curve of number of seeds per capsule



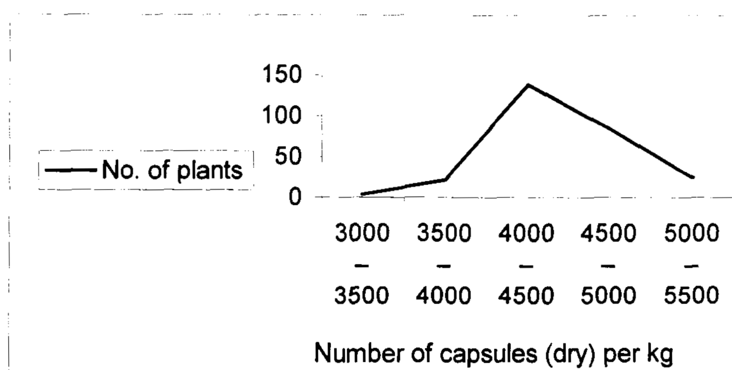
4.2.1.14. Number of capsules (dry) per kilogram

The character showed continuous frequency distribution indicating the polygenic control of the character. However, the character showed an accumulation of more number of recessive factors in the gene pool as evidenced by the increased frequency of low weight capsules (Table 4.20 and Fig. 4.15).

Table 4.20. Frequency distribution of number of capsules (dry) per kilogram

Number of capsules (dry) per kg	Number of plants
3000 – 3500	3
3500 – 4000	21
4000 – 4500	138
4500 – 5000	84
5000 – 5500	24
Total	270

Fig. 4.15. Frequency curve of number of capsules (dry) per kilogram



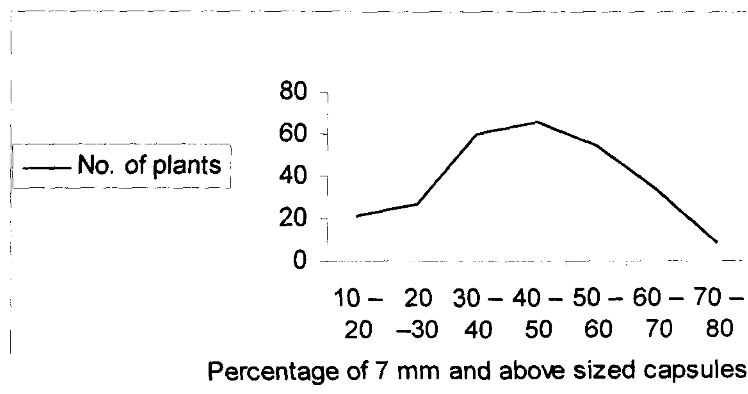
4.2.1.15. Percentage of 7 mm and above sized capsules

The character showed continuous distribution indicating polygenic control. The frequency distribution was normal except for the fact that there was slight decline in frequency in the case of plants with low percentage of bold capsules (Table 4.21 and Fig. 4.16).

Table 4.21. Frequency distribution of percentage of 7 mm and above sized capsules

Percentage of 7 mm and above sized capsules	Number of plants
10 – 20	21
20 –30	27
30 – 40	60
40 – 50	66
50 – 60	54
60 – 70	33
70 – 80	9
Total	270

Fig. 4.16. Frequency curve of percentage of 7 mm and above sized capsules



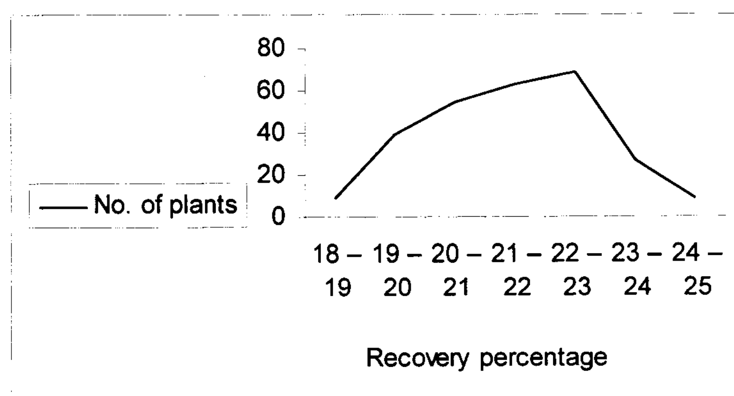
4.2.1.16. Recovery percentage

Recovery percentage or driage has shown continuous frequency distribution indicating polygenic control of the character. However, the peak of the frequency curve showed a shift beyond the central class and then it showed an abrupt decline thus indicating unequal distribution of dominant and recessive alleles controlling the character in the gene pool (Table 4.22 and Fig. 4.17).

Table 4.22. Frequency distribution of recovery percentage

Recovery percentage	Number of plants
18 – 19	9
19 – 20	39
20 – 21	54
21 – 22	63
22 – 23	69
23 – 24	27
24 – 25	9
Total	270

Fig. 4.17. Frequency curve of recovery percentage



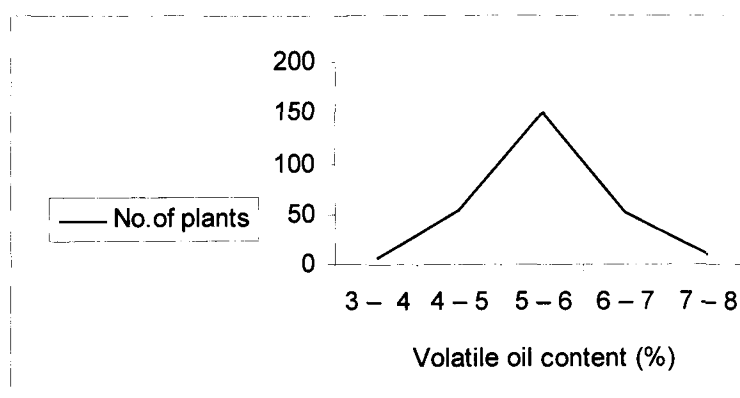
4.2.1.17. Volatile oil content (%)

Volatile oil content of capsules showed normal frequency distribution. The frequency distribution behaved as per statistical expectation thus indicating the fact that no considerable degree of selection has taken place in the population as far as the character is considered (Table 4.23 and Fig. 4.18).

Table 4.23. Frequency distribution of volatile oil content

Volatile oil content (%)	Number of plants
3 – 4	6
4 – 5	54
5 – 6	150
6 – 7	51
7 – 8	9
Total	270

Fig. 4.18. Frequency curve of volatile oil content



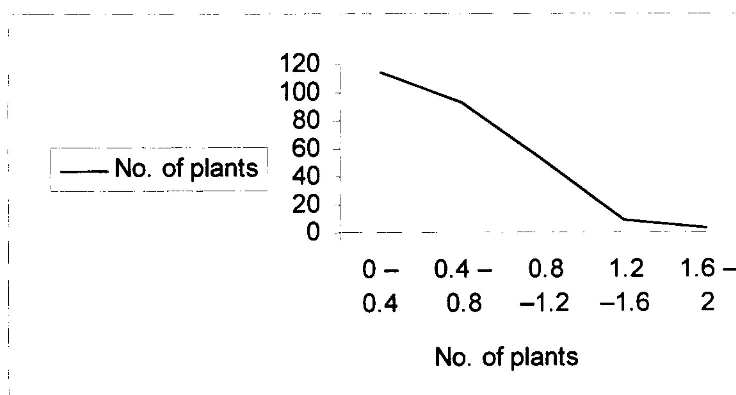
4.2.1.18. Yield per clump (kg)

The character showed continuous frequency distribution indicating polygenic control. However, the frequencies of the classes with higher yield per clump are low in the population under study. This shows that there is predominance in the frequency of recessive factors controlling the character in the gene pool of the population (Table 4.24 and Fig. 4.19).

Table 4.24. Frequency distribution of yield per clump

Yield per clump (kg)	Number of plants
0 – 0.4	114
0.4 – 0.8	93
0.8 –1.2	51
1.2 –1.6	9
1.6 – 2	3
Total	270

Fig. 4.19. Frequency curve of yield per clump



Analysis of eighteen growth, yield and quality characters as shown above has indicated polygenic control of such characters in cardamom. However, the distribution of dominant and recessive alleles contributing towards the character and character combinations deviate from the expected frequencies except in the case of a few characters. Such deviations are expressed mainly by number of tillers per clump, number of leaves per tiller, leaf length, leaf breadth, number of bearing tillers per clump, number of panicles per clump, panicle length, internodal length, number of dry capsules per kilogram, percentage of 7 mm and above sized capsules and yield per clump. Among these, number of tillers per clump, number of leaves per tiller, number of panicles per clump, panicle length, internodal length, number of dry capsules per kilogram and yield per clump showed higher frequency of expression of recessive phenotype thus evidencing the higher frequency of recessive alleles in the gene pool. Leaf breadth, seeds per capsule and recovery percentage showed higher frequency towards the dominant characters thus indicating the higher frequency of dominant alleles in the gene pool. However, characters like racemes per panicle, number of capsules per raceme, number of seeds per capsule and volatile oil content showed frequency distribution more similar to the expected model of normal distribution showing that in those characters there was almost balanced distribution of alleles and allele combinations.

The above analysis of quantitative characters in the cardamom germplasm studied shows that in the case of very

few characters only there is balanced distribution of dominant and recessive factors and in the case of others there is accumulation of either dominant or recessive alleles thus twisting the balance. However, in the case of some characters, the twist is towards the desirable phenotype. Even then, such an imbalanced distribution of alleles is not desirable in the case of a field gene bank and it should be upgraded incorporating all the desirable forms available.

The above analysis can not be considered fool proof mathematically because the plants analyzed were developed from clones and not from seeds. Moreover, clonal propagation by the farmers for years might have resulted in the elimination or reduction of frequencies in the case of many allelic forms that may apparently appear economically less important. Such allelic forms, especially contributing towards quantitative character complexes may have pleiotropic effects and they may prove highly useful under critical situations of gene erosion or susceptibility to pests, diseases, drought etc.

Polygenic control of agronomic characters has already been reported in the case of plantation crops (Dharmaraj and Sreenivasan, 1992; Sreenivasan and Santharam, 1993; Nikhila *et al.*, 2002; Raghu *et al.*, 2003), cereals (Paramasivan and Sreerangasamy, 1988; Shobha, 1993; Mohanan, 1996) and medicinal plants (Khandalkar *et al.*, 1993; Misra *et al.*, 1998; Chandramohanan, 2002; Jayasree, 2002).

4.2.2. Genetic variability

Analysis of variance has been carried out to find out the significance of differences in morphological characters in the case of the ninety accessions studied. The mean square values showed that the genotypes under study differed significantly for all the eighteen characters thus indicating significant differences in the genetic constitution of the accessions (Table 4.25).

Table 4.25. Anova for the eighteen characters studied for genetic variability analysis

Variables	Sources of variation			
	Replicates (df: 2)	Treatments (df: 89)	Error (df: 178)	Total (df:269)
1.Leaves per tiller	0.159	6.793*	1.994	3.569
2.Tiller height	2643.026	4046.226**	336.090	1580.759
3. Vegetative buds	1.293	5.220**	2.828	3.608
4.Leaf breadth	0.180	4.413**	0.615	1.869
5. Leaf length	18.239	36.535**	13.020	20.839
6. Bearing tillers	8.826	144.919**	1.998	49.335
7. Tillers per clump	15.211	557.738**	3.762	187.133
8. Capsules per raceme	0.217	3.692**	0.327	1.439
9. Racemes per panicle	0.451	17.122**	3.271	7.833
10. Yield per clump	0.017	0.331**	0.002	0.111
11. Panicles per clump	60.548	535.585*	11.252	185.097
12. Panicle length	1.659	1220.263**	53.071	438.860
13. Internodal length	0.070	9.279**	0.237	3.227
14. Seeds per capsule	0.541	11.833**	1.286	4.770
15. 7 mm capsules (%)	2.711	662.972**	1.692	22.488
16. Volatile oil content (%)	0.000	1.735**	0.000	0.574
17. Dry recovery (%)	1.595	5.975**	0.757	2.490
18. Number of capsules per kg	21077.615	401665.000**	20953.623	146914.406

* and ** indicate significance at 5% and 1% respectively

The mean, range, standard deviation and phenotypic and genotypic coefficients of variation in respect of the growth, yield and quality attributes of cardamom are presented in Tables 4.26, 4.27 and 4.28. The number of capsules per kilogram had maximum range of performance (3307.69 to 5246.67) with a mean value of 4419.77 followed by tiller height (217.33 cm to 380 cm) with a mean value of 297.64 cm and panicle length (27.33 cm to 118.67 cm) with an average of 59.52 cm. The minimum range of performance (3.33 % to 7.33 %) with a mean of 5.29 % was observed for volatile oil content, thus indicating the involvement of lower number of alleles in the control of this character. Differential variability of quantitative characters has been reported by earlier workers in rice (Shobha, 1993; Mohanan, 1996), coffee (Nikhila *et al.*, 2002; Raghu *et al.*, 2003) and medicinal plants (Misra *et al.*, 1998).

4.2.3. Heritability of characters

Polygenic characters show different levels of heritability in organisms based on their response to environmental impacts. Computation of PCV, GCV and heritability percentage of characters can provide an idea of the extent of environmental impact on them, thus providing an estimate of inheritance of characters that can be expected from parent to progeny which is very essential in identifying superior genotypes and plant types for agronomic purposes. PCV, GCV and broad sense heritability percentage were calculated in the present study for the purpose (Tables 4.26, 4.27 and 4.28).

Table 4.26. Variability analysis of growth characters of cardamom

Character	Range	Mean	SD	GCV	PCV	HB (%)	GA (%)
Total tillers	10-69.33	34.89	13.34	38.95	39.34	98.00	79.43
Tiller height (cm)	217.33-380	297.64	36.73	11.82	13.32	79.00	21.58
Leaves/ tiller	12.33-20.33	15.86	1.51	7.97	11.95	45.00	10.96
Vegetative buds per clump	3- 9	5.60	1.32	15.94	33.98	21.99	15.39
Leaf length (cm)	52.4-69.57	58.73	3.49	4.77	7.78	37.58	6.02
Leaf breadth (cm)	8.50-12.93	10.76	1.21	10.46	12.75	67.29	17.67
Bearing tillers per clump	5.67-35.67	18.30	6.95	37.71	38.49	95.97	76.10

SD: Standard deviation; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; HB: Heritability broad sense; GA: Genetic advance

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Table 4.27. Variability analysis of yield characters of cardamom

Character	Range	Mean	SD	GCV	PCV	HB (%)	GA (%)
Panicles per clump	12-62.67	36.09	13.36	36.64	37.79	93.95	73.15
Panicle length (cm)	27.33-118.67	59.52	20.17	33.14	35.33	88.00	64.05
Internodal length (cm)	1.96-10.07	5.17	1.76	33.59	34.88	92.71	66.62
Racemes per panicle	6.59-18.68	11.77	2.39	18.25	23.85	58.53	28.76
Capsules per raceme	3.89-9.46	6.33	1.10	16.76	19.05	77.45	30.39
Seeds per capsule	15.67-24.39	20.30	1.99	9.23	10.79	73.21	16.28
Number of capsules per kg	3307.69-5246.67	4419.77	360.97	8.07	8.71	85.83	15.39
Yield per clump (kg)	0.12-1.66	0.56	0.33	59.20	59.73	98.24	120.88

SD: Standard deviation; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; HB: Heritability broad sense; GA: Genetic advance

Table 4.28. Variability analysis of quality characters of cardamom

Character	Range	Mean	SD	GCV	PCV	HB (%)	GA (%)
7 mm capsules (%)	16-73.67	44.02	14.95	33.64	33.77	99..24	69.04
Dry recovery %	18.47-24.53	21.42	1.41	6.46	7.38	69.68	10.59
Volatile oil content (%)	3.33-7.33	5.29	0.76	14.38	14.38	100	29.62

SD: Standard deviation; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; HB: Heritability broad sense; GA: Genetic advance

Phenotypic coefficient of variation (PCV) was slightly higher than the genotypic coefficient of variation (GCV) in all the cases except in volatile oil content indicating the involvement of environment at least to some extent in the expression of the characters. However, in the case of volatile oil content of capsules, it is seen that the environment has no effect on the expression of the character as indicated by the same PCV and GCV values.

Broad sense heritability of the characters ranged from 100% to 21.99%. The highest heritability value (100%) was observed for volatile oil content followed by percentage of 7mm and above sized capsules (99.24%) and total tillers per clump (98%), showing that these characters are influenced by environment to a very low extent. The minimum estimate of heritability was observed for number of vegetative buds per clump (21.99%) indicating the influence of environmental factors on bud and tiller production. Similar studies have been undertaken in coriander by Tripathi *et al.*, 2000.

4.2.4. Genetic advance

The genetic advance of characters in per cent of mean was calculated in the case of the characters studied, so as to find out the utility of the character in crop improvement programmes. Genetic advance was found to be the maximum for yield per clump (120.88%) followed by total tillers per clump (79.43%), number of bearing tillers per clump (76.10%) and panicles per clump (73.15%). Genetic advance

in per cent of mean was low in the case of leaves per tiller (10.96%), recovery percentage (10.59%) and leaf length (6.02%) (Tables 4.26; 4.27 & 4.28). This shows that selection of superior genotypes in cardamom can be based on characters like yield per clump, tillers per clump, number of bearing tillers per clump and panicles per clump in that order. Tripathi *et al.* (2000) have undertaken similar works in coriander.

4.2.5. Correlation of characters in cardamom

Success of any breeding programme depends upon the efficiency of selection. Selection cannot be carried out on the basis of a single character since most of the agronomic characters are polygenic in nature, influenced by the environment and related to one another. Correlation studies have been done in the case of the growth, yield and quality characters of cardamom so as to understand the relationship between characters and also to identify the most suited characters that can be targeted for in selection and other improvement programmes. The present study revealed that yield per clump was significantly and positively correlated with twelve other agronomically important characters (Table 4.29). Positive correlation of yield per clump has been found with leaf breadth and internodal length of the panicle also, even though the coefficients are not statistically significant.

Correlation studies have indicated that total tillers per clump, bearing tillers per clump, panicles per clump, panicle length, racemes per panicle and capsules per raceme can be

used as the most important variables for selection. Number of leaves showed positive and significant correlation with tiller height, leaf breadth, number of bearing tillers, total tillers per clump, number of capsules per raceme, number of panicles per clump, panicle length, internodal length and percentage of 7 mm and above sized capsules.

Tiller height showed positive and significant correlation with number of bearing tillers per clump, total tillers per clump, capsules per raceme, panicles per clump, panicle length, internodal length and percentage of 7 mm and above sized capsules.

Bearing tillers per clump had significant and positive correlation with number of tillers per clump, capsules per raceme, racemes per panicle, panicles per clump, panicle length, volatile oil content and percentage of 7 mm and above sized capsules.

Total tillers per clump showed positive and significant correlation with capsules per raceme, racemes per panicle, panicles per clump, panicle length, volatile oil content and percentage of 7 mm and above sized capsules.

Significant and positive correlation was observed between panicles per clump, panicle length, internodal length and percentage of 7 mm and above sized capsules.

Number of racemes per panicle had significant and positive correlation with panicles per clump, panicle length, volatile oil content and percentage of 7 mm and above sized capsules.

Number of panicles per clump showed positive and significant correlation with panicle length, volatile oil content and percentage of 7 mm and above sized capsules.

Panicle length showed positive and significant correlation with internodal length, volatile oil content and percentage of 7 mm and above sized capsules.

Significant and positive correlation was found between volatile oil content and percentage of 7 mm and above sized capsules, recovery percentage, number of seeds per capsules and number of capsules per kilogram.

Percentage of 7mm and above sized capsules and recovery percentage had significant and positive correlation between number of capsules per kilogram and number of seeds per capsules.

However, significant and negative correlation has been observed between number of racemes per panicle and internodal length of the panicle and volatile oil content and number of capsules per kilogram.

Table 4.29. Correlation of characters in cardamom.

	Leaves/ tiller 1	Tiller height 2	V.buds/ clump 3	Leaf breadth 4	Leaf length 5	B.tillers / clump 6	Tillers/ clump 7	Capsuls / raceme 8	Race-mes/ panicle 9	Panicles/ clump 11	Panicle length 12	Internodal length 13	V. oil (%) 14	7mm capsules (%) 15	Recovery (%) 16	Seeds/ capsule 17	Capsules/ kg 18	Yield /clu-mp 10
1	1.000																	
2	0.609**	1.000																
3	-0.010	-0.066	1.000															
4	0.226*	-0.103	0.097	1.000														
5	-0.002	0.051	-0.158	-0.142	1.000													
6	0.309**	0.503**	-0.155	-0.054	-0.001	1.000												
7	0.292**	0.488**	-0.151	-0.061	-0.033	0.995**	1.000											
8	0.453**	0.449**	0.099	0.113	0.061	0.582**	0.574**	1.000										
9	-0.047	0.071	-0.114	0.012	-0.205	0.490**	0.509**	-0.193	1.000									
11	0.296**	0.506**	-0.147	-0.052	0.005	0.988**	0.985**	0.566**	0.463**	1.000								
12	0.300**	0.483**	-0.042	-0.056	0.001	0.438**	0.434**	0.228*	0.278**	0.422**	1.000							
13	0.311**	0.380**	0.048	-0.035	0.086	0.090	0.079	0.293*	-.326**	0.087	0.788**	1.000						
14	-0.025	0.088	-0.142	0.030	-0.168	0.343**	0.346**	0.164	0.251*	0.369**	0.225*	0.102	1.000					
15	0.228*	0.300**	-0.134	0.032	-0.010	0.714**	0.712**	0.557**	0.269*	0.714**	0.272**	0.097	0.322**	1.000				
16	0.084	-0.040	-0.133	0.122	0.023	0.180	0.168	0.161	0.114	0.175	0.075	0.034	0.363**	0.167	1.000			
17	0.087	-0.087	-0.104	0.194	0.063	0.202	0.192	0.153	0.120	0.177	0.069	0.034	0.336**	0.169	0.858**	1.000		
18	0.059	0.169	-0.025	-0.204	0.148	-0.064	-0.071	-0.047	-0.195	-0.047	-0.005	0.069	-0.242*	-0.324**	-0.014	-0.011	1.000	
10	0.341**	0.485**	-0.113 (NS)	0.024 (NS)	-0.029 (NS)	0.955**	0.955**	0.572**	0.620**	0.937**	0.460**	0.030 (NS)	0.362**	0.687**	0.216*	0.219*	-0.133 (NS)	1.000

*and ** indicate significance at 5% and 1% respectively.

4.3. Factor analysis in cardamom

Factor analysis refers to the statistical technique whose common objective is to represent a set of variables in terms of a smaller number of hypothetical variables. Hence, the complexity of recording, analyzing and interpreting a host of multivariate data in field experiments, especially in a perennial crop like cardamom can be lessened by adopting factor analysis whereby the breeder benefits from this technique, to concentrate on selected independent lead characters which also represent other characters related by their similarity in inheritance and expression. This will be of great importance in the development of core collections of cardamom germplasm from large gene pools available with different conservation agencies.

Factor analysis is the most common method used for identifying the factors of divergence in multivariate data. Factor analysis has been carried out in the present study in the case of seventeen morphological, yield contributing and qualitative characters recorded from ninety accessions of cardamom maintained in the cardamom germplasm of the Indian Cardamom Research Institute, Myladumpara (Table 3.5.). The study enabled to get a set of reduced number of new orthogonal variables. Further, the above set of variables identified six factors that accounted for 78.09% of the variability produced by the seventeen variables (Table 4.30). It is to be noted that this method estimated only mathematical associations among the multivariates and hence the results should be supplemented with biological interpretations of

these associations based on logics. All these variables were grouped with respect to various factors identified and lead variables were identified based on the factor loadings for each character (Tables 4.31& 4.32). In the present study, the first factor was found to be associated with number of bearing tillers per clump, total tillers per clump, percentage of 7 mm and above sized capsules and number of capsules per raceme with factor loadings of 0.944, 0.938, 0.801 and 0.708 respectively. Number of bearing tillers per clump, number of tillers per clump, number of panicles per clump and number of capsules per raceme may be considered as four independent traits as the factor seems to have more or less equal control in their inheritance. All these four variables can be expected to behave as a cluster in their inheritance.

The second factor was found to be associated with a set of quality characters *viz.*, seeds per capsule, recovery percentage and volatile oil content with factor loadings 0.942, 0.936 and 0.428 respectively. Among the variables, seeds per capsule and recovery percentage are the most important indicators of quality in cardamom and these may be considered as two independent traits. The third factor was also associated with yield characters *i.e.*, internodal length and panicle length. Here, internodal length of panicle was found to have the maximum factor loadings of 0.939 and it could be identified as the marker character.

The fourth factor was associated with only one yield character *viz.*, racemes per panicle (0.818) and fifth factor

was associated with growth characters *viz.*, leaf breadth (0.717), vegetative buds per clump (0.526) and leaves per tiller (0.474). Here, the maximum factor loading was for leaf breadth, identifying it as the prominent character among the three variables. Factor six was associated with a single character *i.e.*, dry capsules per kilogram, which can be considered as an independent trait. Thus, the dimension of data could be reduced to ten variables, controlled by six factors, which can thus very well present the structure of the whole data from the complete set of variables. This assumes importance in the context of evaluating large number of germplasm accessions.

Interrelationship of polygenic characters may be due to the pleiotropic nature of the alleles involved in the control of such characters and each allele pair contributing towards a particular aspect of expression of the character. A concept of character complexes can be developed based on the analysis of such relationships of variables.

Factor analysis has been used for the analysis of interrelationship of variables and grouping of characters by earlier workers (Rao *et al.*, 1981; Saji *et al.*, 2002).

Table 4.30. Percentage variability observed in the Factor analysis

Factor	Eigen value	Percentage variance	Cumulative percentage
1	4.260	27.178	27.178
2	2.142	12.600	39.778.
3	2.105	12.381	52.159
4	1.563	9.192	61.351
5	1.443	8.489	69.840
6	1.402	8.247	78.088

Table 4.31. Factor loadings of the pooled characters

Sl. No.	Characters	F1	F2	F3	F4	F5	F6
1	Tillers/ clump	**0.939**	0.101	0.103	0.215	-0.108	-0.007
2	Tiller height	0.563	-0.133	0.462	-0.031	0.079	0.420
3	Leaves/ tiller	0.417	0.065	0.308	-0.152	**0.474**	0.421
4	Vegetative buds/ clump	-0.129	-0.213	0.032	-0.027	**0.526**	-0.045
5	Leaf length	0.054	0.067	-0.039	-0.594	-0.475	0.143
6	Leaf breadth	-0.010	0.252	-0.108	0.019	**0.717**	-0.096
7	Bearing tillers/ clump	**0.944**	0.113	0.109	0.188	-0.110	0.006
8	Panicles/ clump	**0.938**	0.104	0.106	0.179	-0.117	0.002
9	Panicle length	0.294	0.042	**0.880**	0.207	-0.079	0.007
10	Internodal length	0.017	0.027	**0.939**	-0.271	0.039	-0.007
11	Racemes/ panicle	0.363	0.090	-0.102	**0.818**	-0.139	-0.067
12	Capsules/ raceme	**0.708**	0.087	0.160	-0.467	0.273	-0.014
13	Seeds/ capsule	0.093	**0.942**	-0.009	-0.014	0.028	-0.003

14	Recovery percentage	0.085	**0.936**	0.018	-0.001	-0.004	-0.002
15	Percentage of 7 mm and above sized capsules	**0.801**	0.087	0.044	-0.069	-0.004	-0.358
16	Number of dry capsules/ kg	-0.117	0.033	0.034	-0.057	-0.217	**0.827**
17	Volatile oil content	0.256	**0.428**	0.232	0.242	-0.100	-0.448

Table 4.32. Distribution of various characters among the six factors identified

Factor	Characters associated
1	**Bearing tillers/clump, Tillers/clump, Panicles/ clump,** Percentage of 7 mm and above sized capsules, **Capsules per raceme**
2	**Seeds per capsule, Recovery percentage,** Volatile oil content
3	**Internodal length,** Panicle length
4	**Racemes per panicle**
5	**Leaf breadth,** Vegetative buds per clump, Leaves per tiller
6	**No. of dry capsules per kilogram**

4.4. Genetic divergence in cardamom

The present analysis was carried out so as to group the genotypes under study into different clusters based on the genetic distance between them, since the genotypes showed significant differences between them in relation to the characters studied (Table 4.25). Principal component analysis was carried out for the purpose and six principal components were identified which could contribute 76.44% variability (Table. 4.33). The main contributors towards the majority of variations observed in the data related to eleven growth and yield attributes such as leaf breadth, bearing tillers per clump, total tillers per clump, capsules per raceme, racemes per panicle, yield per clump, panicles per clump, internodal length, seeds per capsule, recovery percentage and number of capsules per kilogram. Data on these eleven variables were subjected to multivariate analysis using Mahalonobis D^2 statistics.

Table 4.33. Latent roots, percentage of variance and cumulative variance observed in principal component analysis

Principal component	Latent roots	Percentage variance	Cumulative variance
PRIN – I	5.79	32.18	32.18
PRIN – II	2.27	12.61	44.79
PRIN – III	1.87	10.36	55.15
PRIN – IV	1.55	8.63	63.78
PRIN – V	1.24	6.87	70.65
PRIN – VI	1.04	5.79	76.44

The ninety genotypes were grouped into eight clusters based on minimum generalized distance using Tocher's

method as given by Rao (1952). Clusters I, II, III, IV, V, VI, VII and VIII comprised of 28, 28, 8, 22, 1, 1, 1 and 1 genotypes respectively (Table 4.34).

Table 4.34. Cardamom genotypes clustered based on D^2 statistics

Cluster Number	Accessions	Total
I	MCC-131, MCC-139, MCC-145, MCC- 151, MCC-155, MCC-159, MCC-163, MCC-169, MCC-177, MCC-178, MCC-180, MCC-182, MCC-191, MCC-193, MCC-201, MCC-248, MCC-253, MCC-264, MCC-266, MCC-278, MCC-279, MCC-282, MCC-288, MCC-300, MCC-312, MCC-331, MCC-344, MCC-345	28
II	MCC- 129, MCC-146, MCC-154, MCC-156, MCC-157, MCC-161, MCC-167, MCC-171, MCC-172, MCC-174, MCC-176, MCC-181, MCC-184, MCC-185, MCC-194, MCC-245, MCC-262, MCC-267, MCC-272, MCC-283, MCC-284, MCC-286, MCC-292, MCC-310, MCC-311, MCC-314, MCC-316, MCC-319	28
III	MCC-170, MCC-173, MCC-242, MCC-243, MCC-244, MCC-271, MCC-281, MCC-309	8
IV	MCC- 130, MCC-140, MCC-160, MCC-162, MCC-168, MCC-175, MCC-179, MCC-183, MCC-186, MCC-187, MCC-188, MCC-189, MCC-192, MCC-247, MCC-250, MCC-255, MCC-268, MCC-273, MCC-274, MCC-276, MCC-299, MCC-334	22
V	MCC-241	1
VI	MCC-254	1
VII	MCC-152	1
VIII	MCC-246	1

Intra and inter cluster D^2 values of the eight clusters are presented in Table 4.35. Results pertaining to the maximum, minimum and mean values of different characters of the genotypes in each cluster and the cluster means for the eleven characters studied in different clusters are presented in Tables 4.36 to 4.41. The intra cluster distance was 8.15 in

cluster I, 8.63 in cluster II, 10.11 in cluster III and 10.30 in cluster IV, thus showing maximum intra cluster distance between genotypes in cluster IV and minimum intra cluster distance in cluster I, when clusters with single genotypes are excluded. The distance between clusters was found to be maximum between clusters VI and VIII (48.58) followed by I and VIII (42.55) and III and VI (37.63) respectively. The minimum distance was recorded between clusters V and VII (9.83) thus indicating lesser genetic divergence between the clusters.

Genotypes belonging to the most distant clusters can be utilized for hybridization programmes so as to bring out the combination of superior alleles belonging to distant gene pools since such combinations may result in the production of better and promising hybrids.

Misra *et al.* (1990), Sodani *et al.* (1990), Indira (1994) and Srivastava *et al.* (2000) have undertaken similar studies in dahlia, taramira, capsicum and coriander respectively.

Table 4.35. Average intra and inter cluster distances in cardamom

Cluster	Cluster							
	I	II	III	IV	V	VI	VII	VIII
I	8.15							
II	21.15	8.63						
III	31.78	15.02	10.11					
IV	13.69	15.62	24.86	10.30				
V	26.64	11.66	12.88	21.97	0			
VI	10.97	27.65	37.63	17.40	33.46	0		
VII	22.44	12.48	17.91	19.97	9.83	29.55	0	
VIII	42.55	24.80	14.69	35.79	19.41	48.58	24.84	0

Table 4.36. Extremes and means of genotypes in cluster I

Sl. No.	Characters	Maximum	Acc. No.	Minimum	Acc. No.	Mean
1	Leaf breadth	12.93	MCC-344	8.73	MCC-180	10.83
2	Bearing tillers/ clump	17.67	MCC-312	6.00	MCC-182	11.84
3	Tillers/ clump	31.67	MCC-312	11.67	MCC-182	21.67
4	Capsules/ raceme	7.33	MCC-159	3.89	MCC-182	5.61
5	Racemes/ panicle	15.16	MCC-182	6.89	MCC-159	11.03
6	Yield/ clump	0.37	MCC-312	0.14	MCC-169	0.26
7	Panicles/ clump	35.33	MCC-312	12.33	MCC-182	23.83
8	Internodal length	5.96	MCC-248	2.47	MCC-151	4.22
9	Seeds/ capsule	23.11	MCC-178	16.33	MCC-131	19.72
10	Recovery %	23.50	MCC-193	18.67	MCC-131	21.09
11	Capsules/ kg	5116.33	MCC-266	3776.33	MCC-159	4446.33

Table 4.37. Extremes and means of genotypes in cluster II

Sl. No.	Characters	Maximum	Acc. No.	Minimum	Acc. No.	Mean
1	Leaf breadth	12.23	MCC-262	8.63	MCC-156	10.48
2	Bearing tillers/ clump	27.00	MCC-185	18.67	MCC-292	22.84
3	Tillers/ clump	50.67	MCC-185	37.33	MCC-292	44.00
4	Capsules/ raceme	8.88	MCC-156	5.44	MCC-154	7.16
5	Racemes/ panicle	16.28	MCC-161	9.87	MCC-286	13.08
6	Yield/ clump	0.98	MCC-161	0.53	MCC-286	0.76
7	Panicles/ clump	54.67	MCC-185	38.67	MCC-129	46.67
8	Internodal length	7.19	MCC-172	2.32	MCC-262	4.76
9	Seeds/ capsule	23.50	MCC-284	17.44	MCC-167	20.47
10	Recovery %	24.13	MCC-161	18.47	MCC-129	21.30
11	Capsules/ kg	5218.67	MCC-283	3998.33	MCC-174	4608.50

Table 4.38. Extremes and means of genotypes in cluster III

Sl. No.	Characters	Maximum	Acc. No.	Minimum	Acc. No.	Mean
1	Leaf breadth	11.90	MCC-244	9.53	MCC-170	10.72
2	Bearing tillers/ clump	32.00	MCC-309	26.33	MCC-173	29.17
3	Tillers/ clump	60.00	MCC-309	51.67	MCC-173	55.84
4	Capsules/ raceme	8.22	MCC-243	5.22	MCC-173	6.72
5	Racemes/ panicle	18.68	MCC-173	12.15	MCC-243	15.42
6	Yield/ clump	1.33	MCC-309	1.03	MCC-173	1.18
7	Panicles/ clump	60.00	MCC-242	51.33	MCC-244	55.67
8	Internodal length	8.50	MCC-343	2.39	MCC-281	5.45
9	Seeds/ capsule	24.39	MCC-244	15.67	MCC-170	20.53
10	Recovery %	24.53	MCC-244	19.47	MCC-170	22.00
11	Capsules/ kg	4654.00	MCC-243	3691.33	MCC-170	4172.67

Table 4.39. Extremes and means of genotypes in cluster IV

Sl. No.	Characters	Maximum	Acc. No.	Minimum	Acc. No.	Mean
1	Leaf breadth	12.73	MCC-187	8.50	MCC-274	10.62
2	Bearing tillers/clump	24.00	MCC-186	11.00	MCC-187	15.67
3	Tillers/ clump	20.33	MCC-140	18.33	MCC-299	29.17
4	Capsules/ raceme	8.66	MCC-299	4.89	MCC-276	6.78
5	Racemes/ panicle	14.70	MCC-273	6.59	MCC-299	10.65
6	Yield/ clump	0.51	MCC-274	0.24	MCC-299	0.38
7	Panicles/ clump	40.33	MCC-186	21.67	MCC-187	31.00
8	Internodal length	10.07	MCC-179	3.14	MCC-276	6.92
9	Seeds/ capsule	24.22	MCC-274	17.50	MCC-160	20.86
10	Recovery %	23.47	MCC-274	19.03	MCC-140	21.25
11	Capsules/ kg	5246.67	MCC-189	3994.00	MCC-273	4620.34

Table 4.40. Means of genotypes in clusters V, VI, VII and VIII

Sl. No.	Characters	Maximum	Acc. No.	Minimum	Acc. No.	Mean
1	Leaf breadth	12.73	MCC-187	8.50	MCC-274	10.62
2	Bearing tillers/ clump	24.00	MCC-186	11.00	MCC-187	15.67
3	Tillers/ clump	20.33	MCC-140	18.33	MCC-299	29.17
4	Capsules/ raceme	8.66	MCC-299	4.89	MCC-276	6.78
5	Racemes/ panicle	14.70	MCC-273	6.59	MCC-299	10.65
6	Yield/ clump	0.51	MCC-274	0.24	MCC-299	0.38
7	Panicles/ clump	40.33	MCC-186	21.67	MCC-187	31.00
8	Internodal length	10.07	MCC-179	3.14	MCC-276	6.92
9	Seeds/ capsule	24.22	MCC-274	17.50	MCC-160	20.86
10	Recovery %	23.47	MCC-274	19.03	MCC-140	21.25
11	Capsules/ kg	5246.67	MCC-189	3994.00	MCC-273	4620.34

4.41. Cluster means of eleven characters in cardamom.

Characters	I	II	III	IV	V	VI	VII	VIII
Leaf breadth	10.88	10.57	11.03	10.49*	12.43	12.93**	11.77	11.27
Bearing tillers/ clump	10.73	23.24	29.00	16.97	24.67	5.67*	25.00	35.67**
Tillers/ clump	19.88	44.77	55.08	32.35	49.33	10.00*	48.67	69.33**
Capsules/ raceme	5.42	6.88	7.23	6.32	9.46**	4.66*	5.77	7.44
Racemes/panicle	11.21	12.40	15.29	10.06*	10.49	10.84	15.64	18.08**
Yield/clump	0.25	0.76	1.19	0.41	0.96	0.12*	0.83	1.66**
Panicles/clump	21.46	46.37	55.54	33.35	49.00	12.00*	46.67	62.27**
Internodal length	4.29	4.68	6.17	6.71**	3.18	6.56	1.96*	5.31
Seeds/capsule	19.88*	20.09	21.34	20.42	22.28	20.17	22.50	23.28**
Recovery percentage	21.07	21.47	21.85	21.36	23.30	22.47	22.30	23.70**
Capsules per kg	4360.96	4448.32	4276.00	4596.41**	3771.67	4356.33	3307.67*	4021.67

* indicates minimum mean value

** indicates maximum mean value

4.5. Performance evaluation of selected clones of cardamom

Performance evaluation of a group of seventeen selected clones maintained in the Indian Cardamom Research Institute, Myladumpara was carried out as a part of the present study so as to identify the most promising clones among them. ICRI-1, ICRI-2, MHC-10, MHC-13, MHC-18, MCC-40, MCC-21, MHC-22, MCC-200, MCC-73, MCC-85, MCC-260, MCC-346, MHC-27, MHC-26, MHC-24 and MHC-23 were the clones studied for the purpose (Table 3.7 and Figs. 4.20 – 4.36). The growth, yield and quality characters recorded in the clones studied are presented and discussed below (Tables 4.42, 4.43, 4.44, 4.45 & 4.46).

4.5.1. Growth characters

4.5.1.1. Total tillers per clump

The variation in total number of tillers per clump in the case of the 17 clones studied was found to be highly significant. The number of tillers ranged between 63 and 123.67 with the highest number of tillers per clump in MCC-200. It showed significant variation from ICRI-1, ICRI-2, MHC-13, MHC-10, MHC-18, MHC-24, MCC-260, MCC-346, MHC-23, MHC-22, MHC-27 and MHC-26. ICRI-2, MHC-10, MHC-13, MHC-18, MCC-40, MCC-21, MCC-200, MCC-73, MCC-85 and MHC-24 showed significantly higher tiller number when compared with MHC-26 which showed the lowest tiller number among the seventeen clones studied. MCC-85, MCC-40, MCC-73 and MCC-21 showed only non significant reduction in tiller number when compared to MCC-200.

Fig. 4.20. ICRI-1

Fig. 4.21. ICRI-2

Fig. 4.22. MHC-10

Fig. 4.23. MHC-13

Fig. 4.24. MHC-18

Fig. 4.25. MCC-40

Fig. 4.26. MCC-21

Fig. 4.27. MHC-22

Fig. 4.28. MCC-200

Fig. 4.29. MCC-73

Fig. 4.30. MCC-85

Fig. 4.31. MCC-260

Fig. 4.32. MCC-346

Fig. 4.33. MHC-27

Fig. 4.34. MHC-26

Fig. 4.35. MHC-24

Fig. 4.36. MHC-23

Table 4.42. Growth characters of the seventeen elite clones studied

Sl. No.	Clone	Total tillers	Tiller height (cm)	Leaves/ tiller	Veg. buds/ clump	Leaf length (cm)	Leaf breadth (cm)	Bearing tillers/ clump
1	ICRI-1	83.67	243.50	15.33	4.67	53.91	9.35	45.67
2	ICRI-2	90.00	273.33	16.67	6.67	55.66	10.52	60.00
3	MHC-10	97.17	271.33	15.00	5.67	56.89	8.71	63.83
4	MHC-13	101.00	298.33	17.83	9.17	56.16	10.66	55.17
5	MHC-18	96.67	279.33	16.17	7.83	63.55	11.61	62.67
6	MCC-40	109.67	304.33	16.83	6.83	54.72	9.13	61.50
7	MCC-21	104.00	282.00	17.67	11.00	59.66	11.77	57.83
8	MHC-22	67.67	278.17	16.67	8.50	58.66	12.44	36.67
9	MCC-200	123.67	258.50	14.83	9.67	56.33	9.39	69.83
10	MCC-73	108.33	290.33	17.50	6.67	57.22	10.72	61.83
11	MCC-85	112.00	282.33	16.83	11.33	55.66	9.72	54.50
12	MCC-260	84.50	268.67	16.83	4.33	55.66	10.33	48.67
13	MCC-346	82.00	322.50	17.00	11.50	60.99	11.16	52.33
14	MHC-27	64.83	281.00	16.33	4.33	56.99	10.83	31.00
15	MHC-26	63.00	298.50	16.17	2.67	62.33	12.44	39.67
16	MHC-24	92.33	293.67	16.50	4.50	59.55	12.05	57.50
17	MHC-23	81.00	290.00	15.33	9.00	62.99	12.28	43.83
	CD(5%)	22.62	26.80	NS	3.57	3.91	1.08	15.11

4.5.1.2. Tiller height

There is high significance in variation among the 17 clones with respect to tiller height. The tiller height ranged between 243.5 cm and 322.5 cm. MCC-346 showed the highest value and it showed significant positive difference from all clones except MCC-40, MHC-26 and MHC-13. ICRI-1, MCC-200 and MCC-260 had low tiller height and ICRI-2, MHC-10, MHC-13, MHC-18, MCC-40, MCC-21, MHC-22, MCC-73, MCC-85, MCC-346, MHC-27, MHC-26, MHC-24 and MHC-23 differed significantly from ICRI-1 which showed the lowest value (Table 4.42).

4.5.1.3. Leaves per tiller

There is no significant difference among the 17 clones studied, with respect to leaves per tiller. It ranged from 14.83 to 17.83. The highest number of leaves per tiller was noticed in MHC-13 whereas MCC-200 had the lowest (Table 4.42).

4.5.1.4. Vegetative buds per clump

The total number of vegetative buds per clump differed significantly among the genotypes studied. The maximum number of vegetative buds was produced by MCC-346 and the minimum by MHC-26. The number of vegetative buds ranged between 2.67 and 11.50 and the variable in the case of MCC-85, MCC-21, MCC-200, MHC-13, MHC-23 and MHC-22 were on par with MCC-346. The clones ICRI-1, ICRI-2, MHC-18, MCC-40, MCC-73, MHC-10, MHC-24, MCC-260 and MHC-27 showed significant increase in the number of vegetative buds over MHC-26 (Table 4.42).

4.5.1.5. Bearing tillers per clump

There is highly significant variation among the seventeen clones in the case of the character. MCC-200 possessed the highest number of bearing tillers per clump and it differed significantly from ICRI-1, MCC-85, MCC-346, MCC-260, MHC-23, MHC-26, MHC-22 and MHC-27. ICRI-2, MCC-21, MHC-10, MHC-18, MCC-73, MCC-40, MHC-24 and MHC-13 are on par with MCC-200. The number of bearing tillers per clump ranged between 31 and

69.83 with the lowest number of bearing tillers observed in MHC-27 (Table 4.42).

4.5.1.6. Leaf length

Mean leaf length of the clones under study showed highly significant variation with a range from 53.91 cm to 63.55 cm. The leaves of MHC-18 showed the maximum leaf length and minimum value was observed in ICRI-1 (Table 4.42).

4.5.1.7. Leaf breadth

The leaf breadth of the seventeen clones also showed highly significant variation and it ranged between 12.44 cm and 8.71 cm. The clones MHC-22 and MHC-26 had the maximum leaf breadth and MHC-10 had the minimum (Table 4.42).

4.5.2. Yield characters

Variability in yield parameters is important for developing high yielding clones of crop plants (George *et al.*, 1981). In the present investigation, considerable variability was observed for yield parameters resulting in the selection of elite clones (Table 4.43).

4.5.2.1. Panicles per clump

Number of panicles is a major yield contributing parameter in cardamom (Gopal *et al.*, 1992; Sudharshan *et al.*, 1989). The seventeen clones studied presently showed highly significant variation in the case of the character.

MCC-200 showed the highest mean value of 129.67. The character in the case of the clones ICRI-2, MCC-73, MHC-10, MHC-24, MHC-18, MCC-40, MCC-21, MCC-85, MHC-13 and MCC-260 was on par with that of MCC-200 and in the case of ICRI-1, MCC-346, MHC-26, MHC-23, MHC-22 and MHC-27, there was significant reduction. MHC-27 had the lowest number of panicles per clump and ICRI-1 and MHC-22 also showed similar values. The mean value of panicles per clump of the clones ranged from 56 to 129.67 (Table 4.43).

Table 4.43. Yield characters of the seventeen elite clones studied.

Sl. No.	Clone	Panicles/ clump	Panicle length (cm)	Racemes/ panicle	Capsules/ raceme	Seeds/ capsule	Number of capsules/ kg	Yield (kg/ha)
1	ICRI-1	64.67	38.49	16.77	6.44	19.00	4487.67	875.00
2	ICRI-2	113.83	49.77	16.22	7.66	20.33	4231.67	960.00
3	MHC-10	124.67	54.55	15.78	6.11	19.33	4243.00	750.00
4	MHC-13	108.67	53.00	14.55	9.05	18.00	4679.67	1441.67
5	MHC-18	115.83	66.44	17.78	8.22	16.33	4674.67	1320.00
6	MCC-40	114.17	45.66	21.22	7.99	22.00	4762.00	1141.67
7	MCC-21	110.83	49.89	17.11	8.77	19.33	4279.67	1205.00
8	MHC-22	70.50	66.77	23.22	8.61	21.33	4255.33	1161.00
9	MCC-200	129.67	63.89	16.33	5.33	22.00	4324.67	901.00
10	MCC-73	127.83	69.44	21.66	8.27	23.67	4578.67	1886.67
11	MCC-85	110.67	53.66	15.55	6.55	20.67	4745.33	986.67
12	MCC-260	100.83	55.11	19.77	8.72	23.33	4301.33	1391.67
13	MCC-346	93.00	83.66	24.55	7.44	20.00	4259.67	1103.33
14	MHC-27	56.00	54.77	17.89	7.55	20.33	4219.67	906.67
15	MHC-26	90.33	63.11	20.55	8.77	23.00	4221.33	1986.67
16	MHC-24	117.83	54.11	18.55	7.78	20.33	4200.00	1651.67
17	MHC-23	88.33	66.11	20.66	6.66	20.00	4408.33	1230.00
	CD(5%)	29.12	13.69	3.71	1.24	3.61	181.70	350.20

4.5.2.2. Panicle length

The mean values of panicle length of the seventeen clones showed highly significant variation. MCC-346 showed the highest value (83.66 cm) and it differed significantly from all other clones. ICRI-1 possessed the shortest panicle (38.49 cm) (Table 4.43).

4.5.2.3. Racemes per panicle

There is highly significant variation in the case of the character among the clones studied. The mean values ranged from 14.55 to 24.55. The highest number of racemes per panicle was noticed in MCC-346 and the lowest number in MHC-13. MCC-346 differed significantly from all clones except MCC-40, MHC-22 and MCC-73 (Table 4.43).

Table 4.44. Yield performance of the seventeen elite clones studied

Sl. No.	Clone	Yield (kg/ha)	Percentage increase over ICRI-1	Percentage increase over ICRI-2
1	ICRI-1	875.00	--	-8.85
2	ICRI-2	960.00	9.71	--
3	MHC-10	750.00	-14.28	-21.87
4	MHC-13	1441.67	64.76	50.17
5	MHC-18	1320.00	50.85	37.50
6	MCC-40	1141.67	30.47	18.92
7	MCC-21	1205.00	37.71	25.52
8	MHC-22	1161.00	32.68	20.93
9	MCC-200	901.00	2.97	-6.14
10	MCC-73	1886.67	115.61	96.52
11	MCC-85	986.67	12.76	2.77
12	MCC-260	1391.67	59.04	44.96
13	MCC-346	1103.33	26.09	14.93
14	MHC-27	906.67	3.54	-5.55
15	MHC-26	1986.67	127.04	106.94
16	MHC-24	1651.67	88.76	72.04
17	MHC-23	1230.00	14.57	28.12

4.5.2.4. Capsules per raceme

The number of capsules per raceme showed highly significant variation among the seventeen clones studied. MHC-13 showed the maximum number of capsules per raceme and it differed significantly from ICRI-1, ICRI-2, MHC-24, MHC-27, MCC-346, MHC-23, MCC-85, MHC-10

and MCC-200. MHC-26, MCC-21, MCC-260, MHC-22, MCC-73, MHC-18 and MCC-40 are on par with MHC-13. The lowest number of capsules per raceme was observed in MCC-200. The mean value of the character ranged from 5.33 to 9.05 in the different clones studied (Table 4.43).

4.5.2.5. Seeds per capsule

Seeds per capsule differed significantly among the clones studied. The number of seeds was maximum in MCC-73 and minimum in MHC-18. The mean value of number of seeds of the seventeen clones ranged between 16.33 and 23.67. ICRI-2, MCC-73, MCC-260, MHC-26, MCC-40, MCC-200, MHC-22, MCC-85, MHC-27 and MHC-24 also showed significantly higher number of seeds per capsule when compared with the other clones (Table 4.3).

4.5.2.6. Number of capsules per kilogram

There is highly significant variation among the seventeen clones studied in the case of the character. The number of dry capsules per kilogram was the highest in MCC-40 and lowest in MHC-24. The mean values of the character in different clones ranged from 4200 to 4762 (Table 4.43).

4.5.2.7. Yield per hectare

Being a commercial crop, very high attention is paid for yield evaluation in cardamom (George *et al.*, 1981). Yield per hectare was extrapolated in the case of the seventeen clones under study and the character showed highly

significant variation among the clones. MHC-26 recorded the highest yield and the lowest yield was observed in MHC-10. The mean values of the character ranged between 750 kg/ha and 1986.67 kg/ha. Percentage increase in yield over ICRI-1 and ICRI-2 was worked out since ICRI-1 and ICRI-2 are released varieties (Table 4.44). The seventeen clones were classified in to three groups based on the per hectare yield of dry capsules (Table 4. 45). From this, it can be seen that the most promising selection among the seventeen clones is MCC-73 and the most promising hybrids are MHC-26 and MHC-24. The highest yield was obtained in MHC-26 (1986.67 kg/ha) and the yield was the lowest in MHC-10 (750 kg/ha). However, MCC-73 and MHC-24 are also on par with MHC-26 with respect to yield whereas all other clones except MCC-73 and MHC-24 showed significantly lower yield when compared to MHC-26.

The clones MHC-26, MCC-73 and MHC-24 have shown their potential as high yielders in the present study. The hybrid, MHC-26 which has been found to be the best yielder among the clones evaluated, has recorded 127.04% increase over ICRI-1 and 106.94% increase over ICRI-2. Since cardamom is highly heterogenous and heterozygous, vegetative propagation is suggested to produce 'true to type' planting materials (Nadgauda *et al.,* 1983). Large scale cultivation of these high yielding clones by adopting high production technology supported by modern technologies of propagation would substantially enhance production and productivity of cardamom.

Table 4.45. Yield based grouping of the elite clones studied

Group I (>1500 kg/ha)	Group II (1000-1500 kg/ha)	Group III (<1000 kg/ha)
MHC-26	MHC-13	MCC-85
MCC-73	MCC-260	ICRI-2
MHC-24	MHC-18	MHC-27
	MHC-23	MCC-200
	MHC-22	ICRI-1
	MCC-40	MHC-10
	MCC-346	
	MCC-21	

Identification of MHC-26, MCC-73 and MHC-24 as three high yielding clones with significantly higher crop production when compared to released varieties like ICRI-1 and ICRI-2 could be used for further evaluations and field trials and release as new varieties.

4.5.3. Quality characters

4.5.3.1. Percentage of 7 mm and above sized capsules

Cardamom capsules having 7 mm and above size get premium price in the market. It is one of the important qualitative characters for selection of elite clones. Percentage of 7 mm and above sized capsules of the clones under study showed highly significant variation (Table 4.46). The mean values ranged from 38.77 % to 72.53 %. MHC-26 showed the highest value (72.53 %) and MCC-260 and MHC-24 also were on par with MHC-26. The lowest value was observed in

MCC-40 (38.77). MHC-26 and MCC-260 differed significantly from all other clones except MHC-24.

Table 4.46. Quality characters of the seventeen clones studied

Sl. No.	Clone	% of 7 mm & above sized capsules	Recovery (%)	Volatile oil content (%)
1	ICRI-1	61.20	20.40	6.40
2	ICRI-2	53.53	20.12	6.70
3	MHC-10	39.47	19.68	6.00
4	MHC-13	44.30	20.90	7.20
5	MHC-18	50.87	20.22	6.80
6	MCC-40	38.77	20.75	6.33
7	MCC-21	58.53	18.64	5.67
8	MHC-22	41.57	19.05	6.50
9	MCC-200	46.27	20.00	6.00
10	MCC-73	57.57	18.95	7.33
11	MCC-85	52.53	21.10	6.50
12	MCC-260	69.33	20.35	5.33
13	MCC-346	55.60	21.42	6.67
14	MHC-27	47.47	23.83	6.60
15	MHC-26	72.53	20.90	7.00
16	MHC-24	66.77	20.15	6.20
17	MHC-23	54.13	20.79	6.90
	CD(5%)	9.32	-	-

4.5.3.2. Recovery percentage

The recovery percentage or driage showed highly significant variation among the seventeen clones studied. MHC-26 recorded the highest recovery and the lowest

recovery was observed in MCC-21. The mean value of driage ranged from 18.64% to 23.83% (Table 4.46).

4.5.3.3. Volatile oil content

The mean values of volatile oil content showed highly significant variation. MCC-73 showed the highest value (7.33%) and MCC-260 possessed the least volatile oil content (5.33%) (Table 4.46).

The high yielding clones identified above have been found to be superior in terms of quality also as seen from the present experiment. Hence, further screening and release protocols in the case of the above clones will result in the development of better quality clones.

4.6. Molecular characterization of selected genotypes of cardamom

Molecular characterization of fourteen selected genotypes of cardamom was carried out presently by RAPD analysis so as to find out their molecular relationship. The genotypes analyzed included two released varieties, ICRI-1 and ICRI-2; eight elite unreleased selections, MCC-12, MCC-21, MCC-40, MCC-16, MCC-73, MCC-85, MCC-260 and MCC-346 and four promising unreleased hybrids, MHC-24, MHC-18, MHC-26 and MHC-27.

High molecular weight genomic DNA was isolated from fresh leaves of the cardamom genotypes using modified

CTAB method (Ausubel *et al.*, 1995), as described elsewhere. The dissolved DNA samples were run on 0.8% agarose gel to test the quantity and quality. High amount of RNA contamination was observed in most of the lines. An aliquot of 50 μl was made from the DNA stock, 40 ng of RNase was added and incubated for one hour to remove the RNA contamination completely.

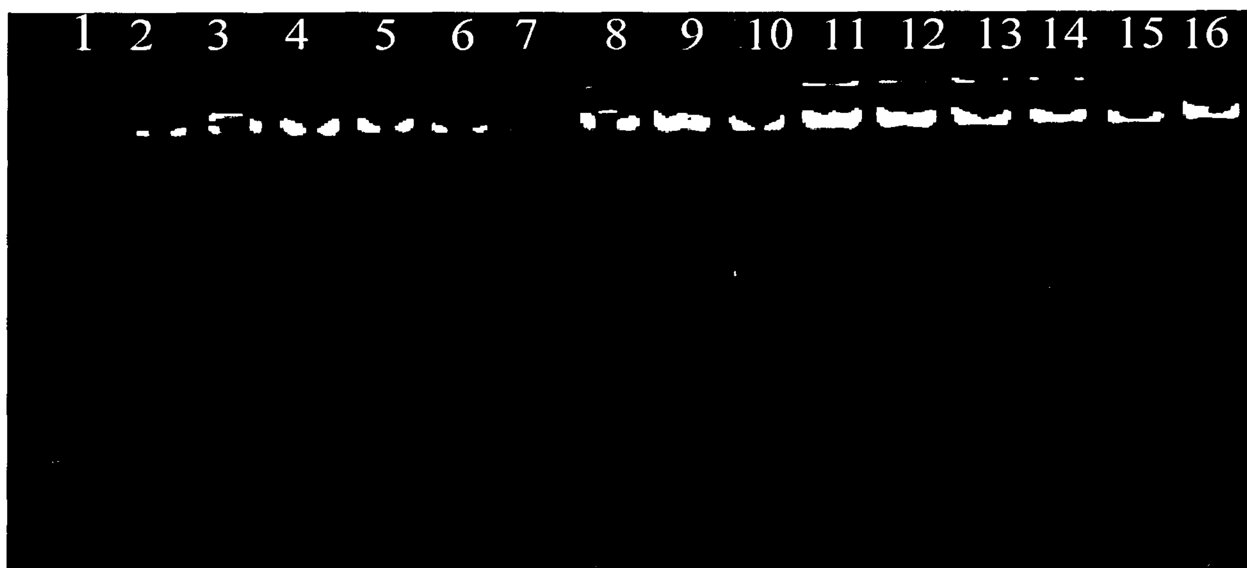
The DNA samples after RNase treatment were diluted to 250 μl using sterile TE buffer. This was done because during purification the minute volumes were found to be difficult to handle. This also prevented considerable DNA loss during the protocol. The extractions with phenol: chloroform (24:1) and phenol: chloroform: isoamyl alcohol (25:24:1) followed by sodium acetate isopropanol precipitation yielded moderately good amount of unsheared DNA.

The purified DNA dissolved in 40 μl of TE buffer was quantified in a standard agarose gel (0.8%). λDNA was used at the concentrations of 25, 50, 75, 100 and 150 ng. 2 μl of the DNA solution was added to each well. 1hr run at the voltage of 65V gave good separation for the comparison of the sample DNA with the standard molecular weight marker (Fig. 4.37). The estimated quantity of the genomic DNA after comparison is given in Table 4.47.

Table 4.47. Concentrations of DNA isolated from different genotypes of cardamom

Sl. No	Cardamom genotype	Quantity of DNA (ng/μl)
1	ICRI-1	200
2	ICRI-2	150
3	MCC-12	150
4	MCC-21	75
5	MCC-40	25
6	MCC-16	200
7	MCC-73	200
8	MCC-85	150
9	MCC-260	200
10	MCC-346	200
11	MHC-24	150
12	MHC-18	150
13	MHC-26	125
14	MHC-27	100

PCR conditions were followed as described elsewhere. All the DNAs quantified were diluted in order to get a uniform concentration of 10 ng per microlitre. All the DNAs after dilution showed the uniform band intensity 30 ng, when separated in 0.8% agarose gel indicating the uniform concentration among the diluted samples.



1. Lambda DNA 50 ng; 2. Lambda DNA 100 ng; 3 to 16. DNAs isolated from ICRI-1, ICRI-2, MCC-12, MCC-21, MCC-40, MCC-16, MCC-73, MCC-85, MCC-260, MCC-346, MHC-24, MHC-18, MHC-26, MHC-27.

Fig. 4.37. Genomic DNAs isolated from the fourteen genotypes of cardamom studied.

4.6.1. RAPD polymorphism with various operon primers

1. OPA 16: Four bands were scored for this primer at 0.5 kb, 0.8 kb, 1.1 kb and 1.5 kb regions. All the bands were found to be monomorphic with all the genotypes studied (Fig. 4.38).

2. OPA 04: This primer gave good amplified product with polymorphic bands at 0.52 kb, 0.75 kb, 0.85 kb and 1kb regions. 0.52 band was present in all the genotypes except in MHC 26 and MHC 27. 0.75 kb region was amplified in ICRI-1, ICRI-2, MCC-21, MCC-40, MCC-16, MHC-24, MHC-18, MHC-26 and MHC-27. 0.85 locus was amplified only in MCC-21, MCC-16, MHC-24, MHC-26 and MHC-27. MCC-73, MCC-85 and MCC-260 were characterized by the absence of the band at 1kb region (Fig. 4.39).

3. OPB 17: There were only two loci scored for this primer *viz*., at 0.8 kb and 1kb regions (Fig. 4.40). Band at 1 kb region showed polymorphism with ICRI-2 and MCC-40. Genotype MHC-27 could not be amplified with this primer indicating the failure of reaction in that well.

4. OPB 01: A total number of five loci were scored with this primer. A band at 0.5 kb region was present in all the genotypes studied. MHC-26 was distinguished from other genotypes with the absence of a band at 1.2 kb region. Large number of polymorphic bands were observed in some of the genotypes. Bands at 0.6 kb, 0.75 kb and 1kb were absent in MCC-16, MCC-73, MCC-85, MCC-260 and MCC-346. In

MCC-21, MHC-26 and MHC-27 only two *viz.*, 0.75 kb and 1 kb regions were absent (Fig. 4.40).

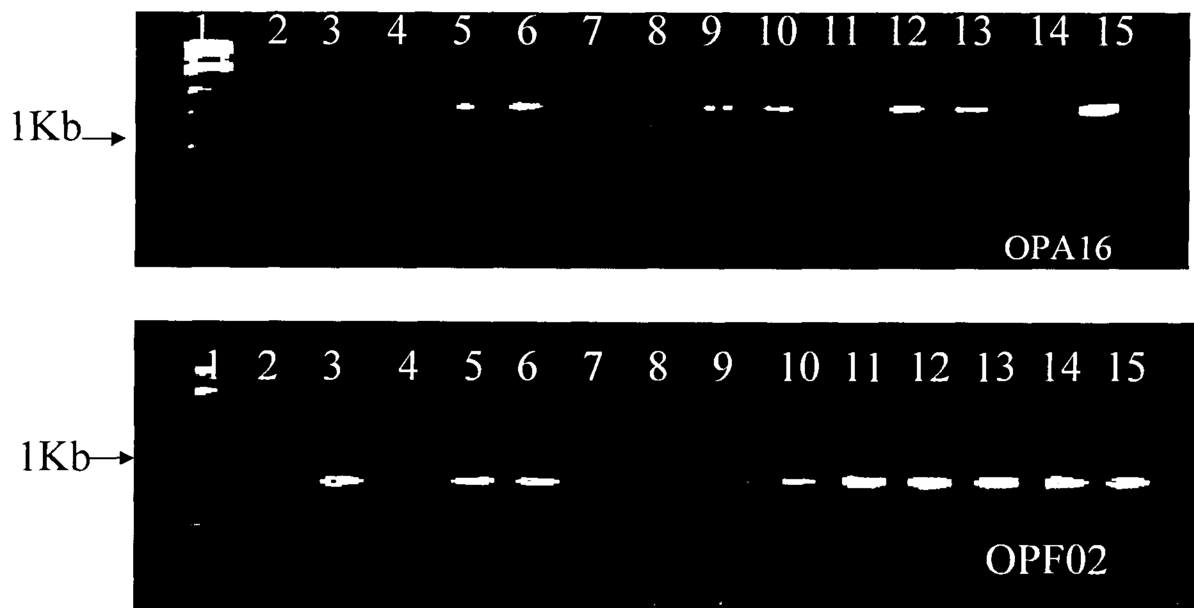
5. OPC 10: This primer gave good polymorphic bands with most of the genotypes. The reaction failed with MHC-26. Less intense bands obtained with ICRI-1 and ICRI-2, made the proper scoring of the bands a little difficult. MCC-12 was characterized with a unique band at 1 kb region with this primer. A total number of 9 loci were scored with this primer (Fig. 4.41).

6. OPC 16: The genotypes MHC-18 and MHC-26 failed with this primer. A total number of 10 bands were obtained with this primer. The patterns obtained for MHC-27 and MCC-21 were similar. Bands in the case of MCC-16, MCC-73, MCC-85, MCC-260, MCC-346 and MHC-24 were found to be uniform in pattern (Fig. 4.41).

7. OPF 02: This primer did not give good pattern with the genotypes studied. Only a single band could scored with this primer (Fig. 4.38).

8. OPE 15: This primer showed a lot of non specific bands with increased background. Only four loci could be scored with this primer (Fig. 4.39).

Among the eight primers studied, the most informative primers were OPC 10, OPC 16 and OPA 04 as evidenced by the above observation.

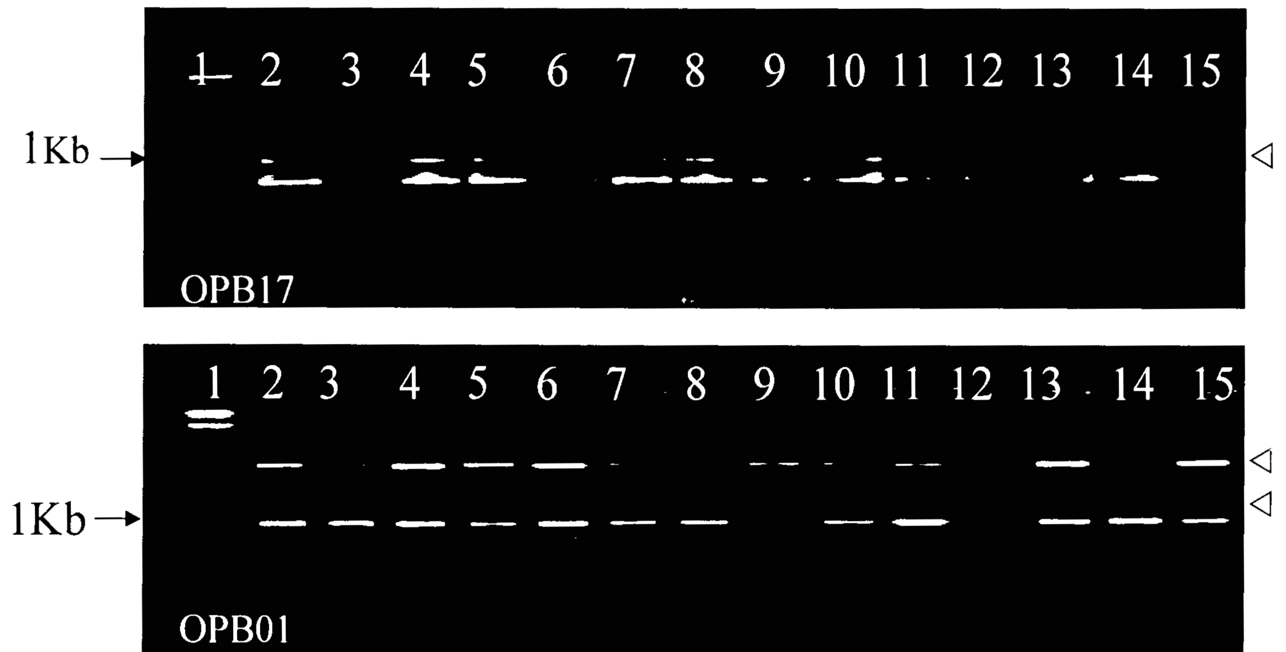


1 Kb ladder; 2. ICRI-1; 3. ICRI-2; 4. MCC-12; 5. MCC-21; 6. MCC-40; 7. MCC-16; 8. MCC-73; 9. MCC-85; 10. MCC-260; 11. MCC-346; 12. MHC-24; 13. MHC-18; 14. MHC-26; 15. MHC-27.

Fig. 4.38. RAPD polymorphism in the cardamom genotypes studied with operon OPA 16 and operon OPF 02

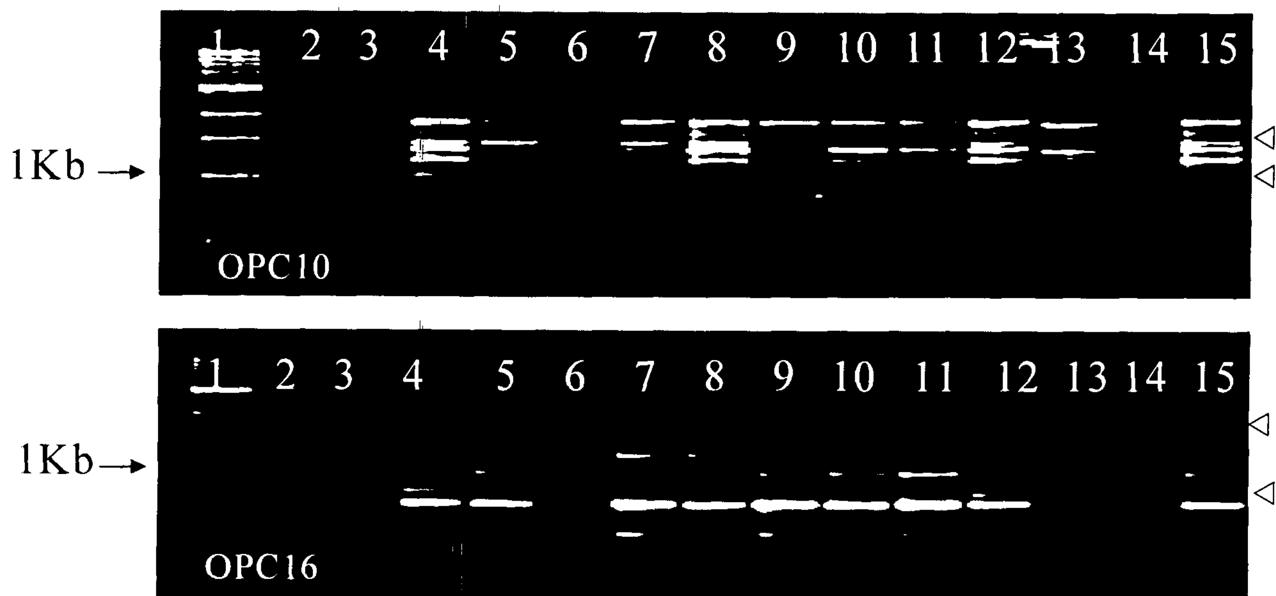
1 Kb ladder; 2. ICRI-1; 3. ICRI-2; 4. MCC-12; 5. MCC-21; 6. MCC-40; 7. MCC-16; 8. MCC-73; 9. MCC-85; 10. MCC-260; 11. MCC-346; 12. MHC-24; 13. MHC-18; 14. MHC-26; 15. MHC-27.

Fig. 4.39. RAPD polymorphism in the cardamom genotypes studied with operon OPE 15 and operon OPA 04



1 Kb ladder; 2. ICRI-1; 3. ICRI-2; 4. MCC-12; 5. MCC-21; 6. MCC-40; 7. MCC-16; 8. MCC-73; 9. MCC-85; 10. MCC-260; 11. MCC-346; 12. MHC-24; 13. MHC-18; 14. MHC-26; 15. MHC-27.

Fig. 4.40. RAPD polymorphism in the cardamom genotypes studied with operon OPB 17 and operon OPB 01



Kb ladder; 2. ICRI-1; 3. ICRI-2; 4. MCC-12; 5. MCC-21; 6. MCC-40; 7. MCC-16; 8. MCC-73; 9. MCC-85; 10. MCC-260; 11. MCC-346; 12. MHC-24; 13. MHC-18; 14. MHC-26; 15. MHC-27.

Fig. 4.41. RAPD polymorphism in the cardamom genotypes studied with operon OPC 10 and operon OPC 16

4.6.2. Similarity among the genotypes

Among the fourteen genotypes studied, the highest similarity was observed between ICRI-1 and ICRI-2 (95%) and hence these two genotypes were grouped together as a separate cluster. MCC-16, MCC-73, MHC-24, MHC-13, MCC-85 and MCC- 260 were clustered together with a similarity of 80- 90%. The lowest similarity was observed between ICRI-2 and MCC-21 (63%). Among the hybrids, MHC-24 and MHC-18 were found to be very close to MCC-16 and MCC-73. MHC-10 showed the highest similarity with MCC-73 (95%) and the lowest similarity could be observed with ICRI-2 (72%). The other hybrid, MHC-18 showed the highest similarity to MCC-73 (91%) and the lowest similarity to ICRI-2 (71%). MHC-27 was found to be close to MCC-21 with 85% of similarity. This genotype showed the least similarity with ICRI-2 (67%). The genotype MHC-26 did not show considerable similarity with any other genotypes. The detailed similarity indices calculated as per the formula given elsewhere is given in Table 4.48. The dendrogram of diversity among various cardamom genotypes is given in Fig. 4.42.

The above study showed that comparatively less amount of RAPD polymorphism could be observed among the cardamom genotypes studied. Four major clusters of genotypes were formed in the present study. ICRI-1 and ICRI-2 formed a cluster with 95% of similarity whereas MCC-12 and MCC-40 formed a group with 88% of similarity. MCC-16, MCC-73, MHC-24, MHC-18, MCC-

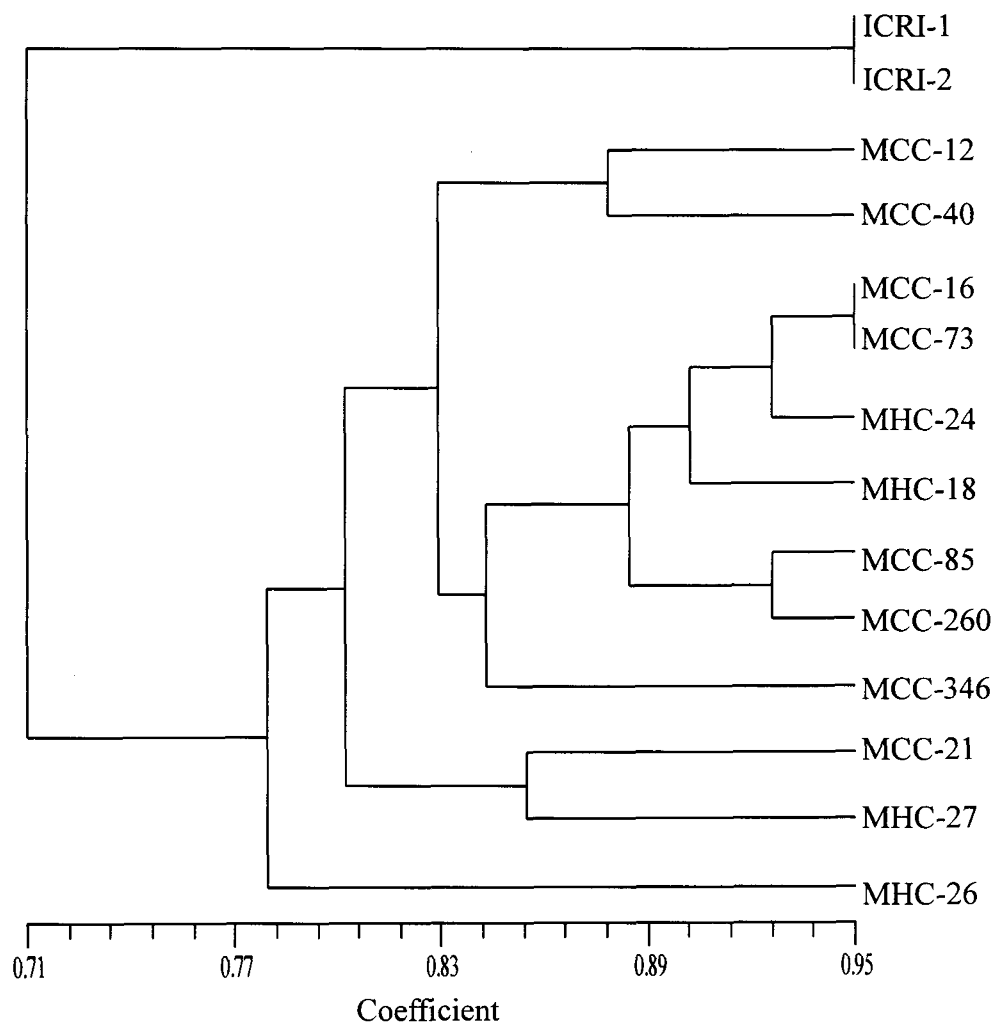
260 and MCC-346 were grouped together in the third cluster and MHC-26 formed a distinct cluster.

Table 4.48. Percentage of similarity among the fourteen genotypes of cardamom studied

	ICRI 1	ICRI 2	MCC 12	MCC 21	MCC 40	MCC 16	MCC 73	MCC 85	MCC 260	MCC 346	MHC 24	MHC 18	MHC 26	MHC 27
ICRI 1	X													
ICRI 2	95	X												
MCC 12	76	71	X											
MCC 21	67	63	77	X										
MCC 40	78	84	88	73	X									
MCC 16	72	73	85	86	88	X								
MCC 73	71	72	81	85	83	85	X							
MCC 85	71	72	76	77	83	85	90	X						
MCC 260	69	70	83	78	80	88	92	93	X					
MCC 346	72	71	80	81	83	80	85	86	88	X				
MHC 24	76	72	85	86	83	90	95	85	88	86	X			
MHC 18	76	71	85	81	83	90	91	90	88	81	90	X		
MHC 26	65	64	73	74	76	83	83	73	76	78	83	78	X	
MHC 27	71	67	76	85	74	81	84	76	78	81	90	80	78	X

Even though ICRI-1 and ICRI-2 are two morphologically distinct genotypes as well as released varieties, they could not be differentiated based on RAPD polymorphism. More studies to analyze the genotypes of cardamom using different types of molecular characterization techniques may yield more valuable information on the variability of genotypes at the molecular level. Such molecular approaches may provide new tools in identifying the genetic status of the genotypes so that they can be grouped based on such differences and distant genotypes can be identified for future breeding programmes.

Fig. 4.42. Dendrogram of diversity in fourteen cardamom genotypes studied for molecular characterization.



Recent years have witnessed the utilization of such techniques to assess the variability in different crops and to utilize such variability for breeding purposes (Fukuoka and Hosaka, 1992; Mulcathy *et al.*, 1993; Nagaraju *et al.,* 2003).

SUMMARY AND CONCLUSION

V.V. Radhakrishnan "Studies on variability, genetic divergence and crop improvement in cardamom (Elettaria Cardamomum Maton)" Thesis. Department of Botany, University of Calicut, 2003

Chapter 5

SUMMARY AND CONCLUSION

The present study on variability, genetic divergence and crop improvement in cardamom (*Elettaria cardamomum* Maton) has been carried out so as to review the available literature on cardamom breeding, to find out the genetic potential and variability of cardamom and also to suggest certain additional measures that can be adopted for the conservation of cardamom germplasm and breeding of elite materials. The field work was carried out in the experimental farm of the Indian Cardamom Research Institute, Myladumpara, Kerala, India during 1999-2002. The observations were analyzed for the phenology of tiller and panicle in cardamom, genetic control of the agronomic characters of cardamom, genetic variability, heritability, genetic advance and genetic divergence of characters in cardamom, and performance evaluation of certain selected clones. An attempt has also been made to analyze the nature of variability based on molecular characterization.

The present experiment revealed periodical variation in tiller and panicle development and it did not show much difference in relation to varietal variations. The peak flowering and fruit set period also coincided in different cultivars. Majority of the capsules were formed by the end of

September irrespective of cultivars and it took 120-135 days to form mature capsules.

The analysis of genetic control of growth, yield and quality characters showed that all of them varied quantitatively indicating their polygenic control. The study revealed that in the case of a very few characters only there is balanced distribution of dominant and recessive factors in the germplasm analyzed and in the case of others there is accumulation of either dominant or recessive alleles thus twisting the balance. Eventhough in some cases the twist is towards desirable phenotypes, such an imbalanced distribution of alleles is not desirable in the case of a field gene bank.

Study of genetic variability indicated differential variability among the characters studied. The number of capsules per kilogram had maximum range of variation followed by tiller height and panicle length. Minimum range was shown by volatile oil content. Higher degree of variability indicates involvement of more number of alleles.

Study of heritability of characters revealed higher phenotypic coefficient of variability when compared to genotypic coefficient of variability in all the cases except volatile oil content indicating the involvement of environment to some extent in the expression of characters. However, volatile oil content did not show such an influence of environment in its expression. Broad sense heritability values

ranged from 21.99 per cent to 100 per cent. The highest value was shown by volatile oil content followed by the percentage of 7 mm and above sized capsules and total tillers per clump indicating that these characters are influenced by environment to a low extent. Minimum estimated heritability was observed for number of vegetative buds per clump indicating the influence of environmental factors on bud production.

The genetic advance of the characters was calculated so as to find out the utility of the characters in crop improvement programmes. Genetic advance was found to be the maximum for yield per clump followed by total tillers per clump, number of bearing tillers per clump and panicles per clump. Genetic advance was low in the case of leaves per tiller, recovery percentage and leaf length. This shows that selection of superior genotypes in cardamom can be based on characters like yield per clump, tiller per clump, number of bearing tillers per clump and panicles per clump in that order.

Correlation studies have been carried out in the case of the growth, yield and quality characters in cardamom so as to understand the relationships between characters and also to identify the most suited characters that can be targeted for in selection and improvement programmes. The present study revealed that the yield per clump was significantly and positively correlated with twelve other agronomically important characters. Correlation studies have indicated that total tillers per clump, bearing tillers per clump, panicles per clump, panicle length, racemes per panicle and capsules per

raceme can be used as the most important variables for selection.

Factor analysis is a technique used to represent a set of variables in terms of a smaller number of variables. The study enabled to get a set of reduced number of new orthogonal variables. Further, the above set of variables identified six factors that accounted for 78.09 per cent of the variability produced by the seventeen variables studied. Among the six factors, the first factor was found to be associated with number of bearing tillers per clump, total tillers per clump, percentage of 7 mm and above sized capsules and number of capsules per raceme. Even though they are independent traits they can be expected to behave as a cluster in their inheritance. The second factor was found to be associated with seeds per capsule, recovery percentage and volatile oil content. The third factor was associated with internodal length and panicle length. The fourth factor was associated with racemes per panicle, fifth factor with leaf breadth, vegetative buds per clump and leaves per tiller and the sixth factor with dry capsules per kilogram. The maximum factor loading was shown by internodal length of panicle and it could be identified as a marker character.

Analysis of genetic divergence was carried out so as to group the genotypes under study into different clusters based on the genetic distance between them. Principal component analysis was carried out for the purpose and six principal components were identified which could contribute 76.44 per

cent variability. The main contributors towards the majority of variations observed in the data related to eleven growth and yield attributes such as leaf breadth, bearing tillers per clump, total tillers per clump, capsules per raceme, racemes per panicle, yield per clump, panicles per clump, internodal length, seeds per capsule, recovery percentage and number of capsules per kilogram. The ninety genotypes were grouped into eight clusters based on minimum generalized distance. Genotypes belonging to the most distant clusters can be utilized for hybridization programmes so as to bring out the combinations of superior alleles belonging to distant gene pools since such combinations may result in the production of better and promising hybrids.

Performance evaluation of seventeen elite clones has been carried out presently so as to identify the most promising types among them. Total tillers per clump, tiller height, vegetative buds per clump, bearing tillers per clump, leaf length, leaf breadth, panicles per clump, panicle length, racemes per panicle, capsules per raceme, seeds per capsule, number of capsules per kilogram and yield per hectare showed significant variation among the clones. The seventeen clones were classified into three groups based on yield. The clones MHC-26, MCC-73 and MHC-24 have been found to be high yielders and among them MHC-26, which is a hybrid recorded 127.04 per cent increase over ICRI-1 and 106.94 per cent increase over ICRI-2. Large scale cultivation of these high yielding clones adopting high production technology and modern technologies of propagation would substantially

enhance production and productivity of cardamom. The high yielding clones identified above have been found to be superior in terms of quality also.

Molecular characterization of fourteen genotypes has been attempted presently by RAPD analysis to find out their molecular relationship. High molecular weight genomic DNA was isolated from fresh leaves of the cardamom genotypes and quantified in a standard agarose gel. Eight operon primers were found to be successful in the amplification of the DNA samples extracted.

Among the fourteen genotypes studied, high similarity was observed between ICRI-1 and ICRI-2 and MHC-26 did not show similarity with any other genotype thus indicating the identity of ICRI-1 and ICRI-2 at molecular level and the unique nature of MHC-26. Four major clusters of genotypes were formed in the present study. ICRI-1 and ICRI-2 formed the first cluster, MCC-12 and MCC-40 formed the second cluster, MCC-16, MCC-73, MHC-24, MHC-18, MCC-260 and MCC-346 formed the third cluster and MCC-26 formed the fourth cluster. Interestingly, morphological and molecular types of characterization yielded almost similar results.

The present experiments have generated considerable amount of information on the phenology of tillers and panicles of cardamom, the genetic control of its characters and variability, heritability, divergence and genetic advance of characters in cardamom. It has further helped to identify

new genotypes that are promising and suited for distribution to farmers and also for use as parents in hybridization programmes. It is hoped that further investigations carried out in future may enhance the information level and contribute novel things.

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