VARIABILITY ANALYSIS IN CALLICLONES OF BLACK PEPPER (*Piper nigrum* L.)

By
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THESIS

Submitted in partial fulfilment of the requirements for the degree of

Master of Science in Horticulture

Faculty of Agriculture
Kerala Agricultural University

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2000

DECLARATION

I hereby declare that this thesis entitled "**Variability analysis in calliclones of black pepper (*Piper nigrum* L.)**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
13.11.2000

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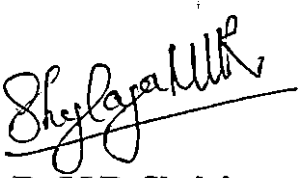
CERTIFICATE

Certified that this thesis, entitled "**Variability analysis in calliclones of black pepper (*Piper nigrum* L.)**" is a record of research work done independently by **Miss.Sanchu, C.R.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.



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Sanchu, C.R.

Dedicated To My
Achan And Amma

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INTRODUCTION

INTRODUCTION

Black pepper (*Piper nigrum* L.), popularly known as the 'king of spices' and 'black gold' is an important spice crop of India. It fetched 635 crores of rupees as foreign exchange in the year 1998-99 contributing 38 per cent of the total export earning (1650.02 crores) from spices (Peter, 2000).

India is the largest producer of black pepper occupying an area of 2.38 lakh ha with production of 75,000 t (Nambiar and Menon, 2000). However, the productivity of black pepper in India is the lowest (315 kg ha^{-1}) when compared to other pepper producing countries. The global demand of black pepper is estimated to be 120-125,000 t and that of white pepper is 25-32,000 t (Nambiar and Menon, 2000). To achieve the increasing global demand and to meet the growing internal consumption, the productivity of the crop has to be improved. The most important production constraint in black pepper is still the *Phytophthora* foot rot disease caused by *Phytophthora capsici*. The annual crop loss due to *Phytophthora* foot rot estimated by Sarma *et al.* (1994) on a global scale was to the tune of 4.5-7.5 million dollars. There is no effective control measure to tackle the disease and hence integrated management practices involving cultural, chemical and biological methods were being practised to combat the disease.

The conventional breeding programmes so far carried out in black pepper could not isolate any genotypes resistant to this serious malady, since there is no resistance in the cultivated varieties and the available resistance in wild species is difficult to transfer. Efforts are now underway on the utilisation of non conventional breeding techniques to tackle the problem of *Phytophthora* foot rot in black pepper. In an attempt to exploit the somaclonal variation for *Phytophthora* foot rot disease tolerance/resistance in black pepper, Shylaja and Nair (1996) reported the occurrence of high amount of somaclonal variation in black pepper for *Phytophthora* foot rot disease reaction. They had also reported that the calliclones of Cheriakanyakkadan showed more tolerance to the disease when

compared to other black pepper cultivars like Kalluvally, Balankotta, Karimunda and Panniyur 1.

With the increasing concern about food contamination and health hazards world over, the isolation of resistant/highly tolerant varieties is very important in an export oriented commodity like black pepper to reduce the pesticide residues.

The present investigations on variability analysis in calliclones of black pepper were carried out to make an indepth analysis of the extent of variability in field planted calliclones of black pepper (cultivar Cheriakanyakkadan) for morphological, yield, quality and reaction to *Phytophthora* foot rot disease, so as to exploit the variability in further breeding programmes and to select calliclones with desirable attributes.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Genetic variation is the basis of breeding new cultivars. In many crops, genetic variation can be obtained through sexual recombination of genes from which desired genotypes are selected. Para sexual techniques of induced mutagenesis, tissue culture and molecular genetics can be used to complement recombination to generate variation. Cell and tissue culture techniques and molecular methods are more recently utilized to generate variation for plant improvement. *In vitro* culture induced genetic variation (somaclonal variation) has been exploited in many crop plants for isolation of desirable plant types showing improvement in yield, quality and resistance/tolerance to biotic and abiotic stresses.

Somaclonal variation has been observed in many monocots and dicots, sexually as well as asexually reproducing species (Larkin and Scowcroft, 1981). Somaclonal variants were found to occur at high frequencies, the frequency of variation has been estimated to be as high as 30-40 per cent for the number of plants showing some type of variation and from 0.2 to almost 3 per cent for variation in a particular trait (Evans *et al.*, 1984).

The causes of somaclonal variation are thought to be due to combination of factors. Some of the variability is due to pre-existing mutations in cells of the explant material (Lorz, 1984 and Orton, 1984). A large part of the variation is induced during the culture cycle and this variation is attributed to chromosomal abnormalities commonly observed in cultured cells. Ploidy changes and changes including translocations, deletions, amplifications and point mutations occur in culture cycle (Larkin *et al.*, 1984). Also changes occur both in single gene and in polygenic traits (Evans and Sharp, 1983; Evans *et al.*, 1984; Larkin *et al.*, 1984) and in both organelle and nuclear genomes (Mc Nay *et al.*,1984).

Many factors are found to influence the rate of somaclonal variation. They include growth regulators (Evans, 1988; Griesbach *et al.*, 1988; Shoemaker *et al.*, 1991), cultivar (Kurtz and Lineberger, 1983; Hwang and Ko, 1986); cultivar age (Shepard *et al.*, 1980), ploidy level (Heinz and Mee, 1969; Bingham and McCoy, 1986), explant source (Shepard *et al.*, 1980; Gui *et al.*, 1993; Tsai *et al.*, 1992), length of time *in vitro* (Skirvin *et al.*, 1994), proliferation rate (Smith and Drew, 1990) and cultural conditions (Skirvin *et al.*, 1994).

Somaclonal variation was effectively utilized for producing useful phenotypic variants in carnation (Buiatti *et al.*, 1986) for improving yield in sugarcane (Dhumale *et al.*, 1994) and Mustard (Katiyar, 1997), for upgrading quality in mint (CIMAP, 1992) and datepalm (Booij *et al.*, 1993) and for disease tolerance in tomato (Shahin and Spivey, 1986) and banana (Trujillo and Garcia, 1996).

The present investigations on 'Variability analysis in calliclones of black pepper (*P. nigrum* L.) are aimed at measuring the extent of variability in the field planted calliclones of black pepper (cv. Cheriakanyakkadan) for yield, quality and reaction to *Phytopthora* foot rot disease. As there are only limited research works pertaining to the field performance of tissue culture derived black pepper plants, similar works on other crops are included in this review. The review focuses on the field performance and extent of variation in phenotypic and yield characters, quality attributes and resistance/tolerance to diseases in tissue culture derived plants.

2.1 Variation in phenotypic and yield characters in tissue culture derived plants

Field crops

Wheat

Galiba *et al.* (1984) examined 3100 second generation (Sc_2) somaclones regenerated from calluses of seven winter wheat cultivars. When compared to the

original cultivars, 16 Sc_2 families showed differences in height, heading date, spike morphology, awns, shoot colour, leaf necrosis, waxiness and chromosome number. Similar study conducted by Maddock and Semple (1986) to assess the range of variation in regenerated R_2 lines of wheat observed noticeable changes in height and plant morphology. They reported that changes were stable in R_3 regeneration also.

Hashim *et al.* (1988) utilized somaclonal variation for wheat germplasm improvement. They evaluated Sc_1 and Sc_2 regenerants in green house condition and observed differences in flag leaf size, plant height, number of tillers, ear length, awn length and number of grains per head. Microscopic studies revealed that these changes were the result of gross chromosomal changes in some somaclonal plants.

Mohmand and Nabors (1990) evaluated variation in the somaclones regenerated from callus cultures of wheat cultivars, Glennson, Pavon and PAK 16171 for variation in agronomic and morphological characters. They reported significant differences between somaclones and their parents for plant height, spike length, grains/spike and 100 grain weight.

Ivanov *et al.* (1998) evaluated somaclones (R_3 and R_4 generation) regenerated from five winter wheat genotypes for plant height, top internode length, spike length, number of seeds per spike and 100 seed weight. They concluded that plant height and top internode length of all somaclones derived from cv. Charodeika and Pliska decreased while spike length of Moulina increased compared to their parents.

Villareal *et al.* (1999) compared tissue culture derived lines (TCDL) of spring wheat cv. Pavon with the parent cultivar and reported that TCDL possessed greater number of grains/m^2, spikes/m^2 and grains/spike, early flowering and dwarf habit.

Rice

Zhao *et al.* (1984) noticed variation in maturity, plant height, panicle number, grain number and 1000 grain weight in the 5500 somaclones derived from 25 varieties of rice and promising somaclones were identified. Similar study conducted by Fan *et al.* (1991) reported variations in plant height, panicle emergence date, fertility, number of grains/panicle and weight of panicle in rice somaclones.

High frequency somaclonal variations for shooting ability, husk colour, length and width of grain, panicle length and percentage of husk were reported in rice by Marassi and Rapela (1992).

Shen *et al.* (1993) isolated desirable somaclonal variants from IR-26 showing increased 1000 grain weight, grains per panicle and grain weight per plant.

Zhao *et al.* (1993) obtained a black kernel rice "Heizhenmi" from white kerneled Basmati 370 by exploiting somaclonal variation. Heizhenmi recorded higher yield and shorter duration of 125 days compared to that of Basmati 370.

In a field experiment conducted to examine the variability in eight rice somaclones, Abbasi *et al.* (1999) reported a significant reduction in plant height and days to flowering and increase in kernel length and recovery percentage. They selected somaclone TF_4 having stiff stems, earliness in flowering, semidwarf habit with high fertility and greater yield potential than parent. They noted that grains of all somaclones were fine, slender and long.

Sorghum

Bhaskaran *et al.* (1987) reported stable somaclonal variation for several traits in sorghum, including reduced height, increased tiller number, increased grain yield and increased seed number. Seed size was reduced in all of the

somaclones. Smith and Bhaskaran (1988) further reported that the increased tiller number and reduced height characteristics were expressed even after three generations of selfing.

Cai *et al.* (1990) evaluated 48,000 R_2 progenies of sorghum in a field experiment to assess the variation. They observed 43 variant phenotypes that included chlorophyll deficiencies, dwarfing, narrow leaves, ragged leaves and a developmental variation which produced large number of panicles.

Maralappanavan *et al.* (1995) evaluated somaclonal variation for quantitative characters in plants regenerated from well established callus of two popular rabi sorghum var. M-35-1 and A-1. They observed nine families out of 76 showed morphological variation for characters like chlorophyll, leaf arrangement and mid rib structure in M-35-1 and three out of 30 showed variation in branching pattern and male sterility in A-1 lines.

Barley

Ahloowalia *et al.* (1987) observed variation in the plants regenerated from embryo callus cultures in morphological characters such as chlorophyll deficiency, plant height, stem thickness, spike shape and in pollen fertility and ploidy level.

Somaclones of leger barley differed in awn characters, grain yield, test weight, 1000 grain weight, height, lodging resistance and heading date (Choo *et al.*, 1992).

Oat

Omel-Yan-Chuk *et al.* (1989) reported somaclonal variation in regenerants obtained from callus cultures of oat cvs. Olimpiiskii, Taezhnik and Poheda. They observed significantly higher frequency of sterile and dwarf plants

and plants with twin panicles in regenerants of the three cultivars than source varieties.

Dahleen *et al.* (1991) reported significant stable changes in oat somaclones in height, heading date, seed protein, flag leaf area, seed weight, seed number and grain yield.

Fan and Cui (1995) reported heritable somaclonal variation in agronomically important traits in naked oat (*Avena nuda* L.). They reported that longer the duration in culture, higher was the extend of somaclonal variation, recording 42.4 per cent variation in regenerants from 80 weeks culture period.

Foxtail millet

Min *et al.* (1999) reported somaclonal variation in plantlets regenerated from adventitious buds and calluses derived from immature young inflorescence of cv. Zheng 407. They reported 10 per cent variation in R_2 for plant height, days from sowing to heading, spike length, flag leaf length, grains per spike and sterility.

Sugarcane

Dhumale *et al.* (1994) reported variation in yield and in number of internodes per plant in the callus regenerants of cv. COC 671 of sugarcane.

Taghian and Fabmy (1998) evaluated 90 somaclones regenerated from tissue culture of sugarcane variety C-310 and their donor parent. They found significant differences among the somalcones than the donor parent and higher heritability was found for stalk length (98%), stalk number (73%), yield (73%) and stalk volume (72%).

Fruit crops

Banana

Stover (1987) observed the regenerants from Grand Naine and Saba bananas under field conditions. He observed no variation in Saba but 25 per cent of the population showed variation for flowering and fruit maturation in Grand Naine. Other variant traits noted include finger and flower abnormalities, variegation, tetraploidy and aneuploidy.

Ventura *et al.* (1988) observed somaclonal variation in micropropagated bananas. Clones of genomic group ABB were evaluated in the field. Among the plants, 10.4 per cent showed somaclonal variation in bunch characters (number of hands per bunch, shorter fruits, increased fruits per bunch and atrophied fruits) and 19.6 per cent in plant height.

Robinson and Nel (1989) found the highest percentage of mutation in Dwarf Cavendish (3.33 per cent) and the lowest in Grand Naine (0.63 per cent). The smallest plants (50-100 mm in height) had the highest mutation rate (9.61 per cent) and those measuring 200-500 mm in height had the lowest (0.83 per cent). The survival rate on transplanting was 100 per cent.

Vuylsteke and Swennen (1990) assessed the levels of somaclonal variation in seven shoot tip micropropagated plantains (AAB group). They reported that the levels of somaclonal variation ranged from 0 to 69.1 per cent. The plants displayed variation in inflorescence type, female fertility, pseudostem characters, petiole and bract colour, leaf and growth habit.

Israeli *et al.* (1991) studied somaclonal variants of seven *in vitro* propagated banana cultivars. Variation were expressed in the somaclones in plant stature, abnormal leaves, pseudostem pigmentation, persistence of flowers and split fingers. Dwarfism was the most common variant character observed in the

population. Variation for leaf characters observed in subgroups Cavendish and red were suspected to be due to aneuploidy.

Vuylsteke *et al.* (1996) identified four types of morphologically distinct somaclonal variants in a population of False Horn plantain (*Musa* spp. AAB group) produced by shoot tip culture. Field evaluation of these variants showed that three of the four variants were horticulturally inferior due to inflorescence degeneration and abnormal foliage. In contrast, the variations reported in the Cavendish bananas propagated by apical meristem was low as reported by DeGomez and DeGarcia (1997).

Grillo *et al.* (1999) reported the use of banana somaclonal variants for ornamental purpose. Variation has been found for plant height, leaf shape and colour, pseudostem morphology and colour, reproductive organ morphology and sucker emission rate.

Pineapple

Martin *et al.* (1994) observed somaclonal variation in field planted *in vitro* produced pineapple clones. They categorized the clones as dwarf, variegated, thorny leaved and pigmented as compared to the control plants.

Date palm

Clones of two cultivars Thoory and Zahdi produced through *in vitro* culture was field analysed for two successive seasons to evaluate the flowering behaviour and fruit set by Al-Ghamdi (1996). He observed significant difference between the cultivars in flowering date, flowering duration and number of flowers produced per tree per week, number of bunches, spikes, fruit drop, total fruits per tree and percentage fruit set.

Grape

Liuni *et al.* (1998) reported no significant variation between the micropropagated plants of table grape and source cultivars for phenological phases, flower differentiation and berry development. But during the vegetative development stage the micropropagated plants grew more vigorously than source cultivars.

Black berry

McPheeters and Skirvin (1989) compared the regenerants produced from shoot tip culture of black berry for different vegetative and reproductive traits and observed variation in growth habit, vigour, flower number and fertility.

Norton *et al.* (1997) evaluated the stability of the thornless character in thornless evergreen somaclones of black berry after seven years of field planting. They observed thornlessness as a stable variation and thornless character was directly related to dwarfism.

Spices and aromatic plants

Cardamom

Sudharshan *et al.* (1997) evaluated micropropagated cardamom plants for growth and yield. Tissue culture derived clones showed variations in the type of panicle, capsule shape and size. The overall variability in tissue cultured plants was observed as 4.5 per cent as against 3 per cent in open pollinated seedling progenies.

Chandrappa *et al.* (1997) evaluated tissue cultured promising cardamom selections for their yield performance for three years. The lines TC_5, TC_6 and TC_7 were found promising as compared to the other lines. These three selections also differed among themselves for yield and yield attributes. Clonal crop of the two

ruling varieties viz., Mudigere 1 and Mudigere 2 yielded significantly low compared to TC_5 but was on par with other seven tissue cultured selections.

Ginger

Nazeem *et al.* (1998) tried to induce variability in ginger through indirect organogenesis and *in vitro* mutagenesis. Calli were induced from different explants of ginger var. Maran. The regenerants from irradiated culture were hardened and planted out. The plantlets were maintained and evaluated in the field for two seasons. They observed considerable variations for growth parameters and abiotic stress tolerance.

Pepper mint

CIMAP (1992) released two improved somaclones of *Mentha arvensis* SC-93 and SC-179 from multiple shoot cultures of CIMAP hybrid-77. These somaclones were characterised by higher herbage yield, better regeneration of foliage after the first cut, better harvesting index in the first cut, lower distillation costs and increased tolerance to high moisture regimes.

Han *et al.* (1998) reported greater genetic variation in regenerants from young stem segments of pepper mint var. 73-8 for fresh weight per plant, flowering date, stem and branch characters.

Cymbopogon

Mathur *et al.* (1988) evaluated the plants regenerated from leaf sheath derived callus cultures of (*Cymbopogon winterianus* Jowitt) variety Jorhat. Out of 500 plants 250 showed extensive somaclonal variation for six or seven agronomic traits viz., herbage yield, tiller number, diameter of the tiller, area of longest leaf and fresh and dry weight ratio.

Patnaik *et al.* (1999) reported somaclonal variation in cell suspension culture regenerats of *Cymbopogan martinii* (Roxb.) Wats. var. motia for plant height, yield and tiller number.

Pulses and oil seeds

Soyabean

Hua *et al.* (1998) identified four promising strain of soyabean somaclonal variants with respect to plant height, leaf shape, number of branches and 100 grain weight in regenerated plants of SC_1, SC_2 and SC_3 generations.

Peanut

Eapen *et al.* (1998) reported a decrease in plant height, leaflet size, number of pegs, seeds and seed weight and an increase in the number of primary branches in the regenerated R_1 plants of peanut in comparison with seed derived control plants. In the R_2 generation low percentage of variants (<1%) was noticed, one with shorter and smaller pods and the other with taller and constricted pod.

Mustard

Jain *et al.* (1987) isolated a somaclonal dwarf variant named `Prakash' having better yield and yield contributing characters when compared to the parent cultivar.

Katiyar (1997) evaluated somaclones of exotic mustard variety Varuna and yellow seeded *Brassica campestris* for desirable traits. He released a somaclone 'Pusa Jaikisan' from Varuna with higher yield and seed weight.

Sunflower

Barotti *et al.* (1995) evaluated the R_2 generation of sunflower from *in vitro* regenerated plants for quantitative traits. They observed a broad spectrum of

phenotypic variations for chlorophyll and carotenoid deficiencies, chimaerical variegation, fasciated stem, capitulum and abnormal shoot development.

Pigeon pea

Prasannalatha *et al.* (1994) reported somaclonal variation in R_1 plants regenerated from cotyledon. They observed floral variations such as petaloid sepal, 2 standard petals, 2-4 wing petals, split keel, 12-14 anthers and 2-3 gynoceia. The R_2 regeneration plants showed segregation with respect to flower colour, plant height, leaf shape and growth habit and one plant failed to set pod inspite of profuse flowering.

Similarly Chintapalli *et al.* (1997) exploited somaclonal variation for varietal improvement of pigeon pea. They reported range of alterations in floral morphology like anthers protruding from buds, petalloid sepals and petalloid stamen in R_1 population.

Pea

Griga *et al.* (1995) analysed phenotypic, isozyme and protein variations in pea somaclones and no characteristic changes in qualitative and quantitative traits were observed.

Safflower

Seeta and Anwar (1992) evaluated somaclones from cotyledon explants of safflower genotype 'Mangira'. Variations for plant height, leaf shape, plant type, capitulum, flower colour, seed shape, days to flowering and maturity, number of primary and secondary branches per plant, number of capitula per plant and seed yield per plant were observed in the SC1 generation. These characters were found to be heritable.

Solanaceous vegetables

Potato

Thomas (1981) reported large amount of variation in growth and leaf morphology in plants regenerated from stem tip culture derived protoplast of the tetraploid British potato cultivar Maris Bard.

Thomson (1987) observed large difference in total harvested yield among the somaclones derived from protoplast cultures of cultivars Feltwell and Marispiper. Six out of 197 of the cultivar Feltwell and two out of 229 of Marispiper somaclones out yielded their parents.

Carrasco *et al.* (1998) reported significant variation for seven of the eight morphological traits studied in potato protoclones evaluated in a field experiment.

Tomato

Zagorska *et al.* (1986) reported variability in morphological and cytological traits and pollen fertility in regenerants obtained from tissue cultures of tomato leaves and flower buds. Self compatible forms from initially completely self incompatible plants of *Lycopersicon peruvianum* and *L. chilense* were obtained. A recessive jointless pedicel mutation was observed in plants regenerated from tissue cultures of *L. peruvianum*. Segregation of fruit carotenoid composition occurred in R_1 progenies of regenerants from *L. cheesmani.*

High frequency somaclonal variation for agronomically important traits such as early flowering and orange fruit colour was reported by Somasundar and Gostimsky (1992) in tomato.

Ornamental plants

Carnation

Buiatti *et al.* (1986) assessed the field performance of plantlets derived from petals, mericlones and cuttings of carnation (cv. Corrida) and obtained higher heritability for plant height, flower number and flowering date.

Chrysanthemum

Ohishi and Sakurai (1988) observed morphological changes in chrysanthemum somaclones derived from petal tissue. High incidence of mutations like increase in disc florets or decrease in ray florets were observed in the somaclones and increase in disc florets was linked to male sterility. Other variations noted in the population were in petel colour, flower shape and size and leaf shape.

Cymbidium

Laneri (1990) reported somaclonal variation in plantlets derived from *in vitro* cultured immature flower buds of cymbidium. The somaclones produced racemes with a slightly longer labellum and more pointed, yellow brown tepals.

Cyclamen

In *Cyclamen persicum*, the frequency of variation ranged from 4.5 to 36.3 per cent. The variation observed were in growth habit, flower shape and colour, chlorophyll pattern and shape of leaves (Schwenkel and Grunewaldt, 1990).

Begonia

Begonia x elator plantlets regenerated from leaf disc callus showed differences in flower morphology, flower size, plant height, plant morphology and number of flowers per plant (Jain, 1993).

Similar somaclonal variations were reported in *Saintpaulia ionantha* (Jain, 1993, El-Mardi *et al.*, 1993) and *Zinnia marylandica* (Stieve *et al.*, 1992).

Rose

Arene *et al.* (1993) reported that plants regenerated from callus in *Rosa hybrida* (cv. Meirutral) exhibited variation in number, colour and shape of petals, growth habit and height.

Rudbeckia

In Rudbeckia, plants regenerated through callus cultures exhibited variation in terms of flower shape, number of ray florets/flower, flower colour, polyploidy and aneuploidy (Khilbas, 1995).

2.2 Variation in quality attributes in tissue culture derived plants

Field crops

Wheat

Galiba *et al.* (1984) reported variability in chemical composition such as gliadin and ß amylase content in the SC_2 somaclones regenerated from calluses of winter wheat cultivars.

Zheng *et al.* (1989) reported variation with high α amylase and prolanine content in the regenerants obtained from young spikes of winter wheat.

A high frequency variation in flour protein content was reported in the tissue culture derived lines of spring wheat (Villareal *et al.*, 1999).

Sorghum

Cai *et al.* (1995) reported significant stable increase in polyphenol levels in somaclones of sorghum lines.

Sugarcane

Dhumale *et al.* (1994) compared the callus derived plants of sugarcane cultivar COC 671 with control for quality parameters. They obtained two clones with more than 20 per cent sucrose than control.

Fruit crops

Date palm

Booij *et al.* (1993) studied the sugar and free aminoacid variation in five field planted dates which were propagated by *in vitro* method. The fruits from the cultivar Deglet Nour contained virtually no sucrose and more glutamic acid, glutamine, γ-amino butyric acid and arginine and less alanine than fruits from conventionally propagated plants.

Mulberry

Zaman *et al.* (1997) observed biochemical variation in the micropropagated mulberry plants but failed to get significant nutritional differences between micropropagated and normal plants.

Spices, medicinal and aromatic plants

Cardamom

Sudharshan and Bhat (1998) reported variability in quality parameters in tissue cultured plants (TC) of cardamom. The essential oil content was better in TC plants (7.2%) than in open plllinated seedlings (6.9%).

Cymbopogon

Mathur *et al.* (1988) recorded extensive variability for 6 major constituents of essential oil viz., citronellal, citronellol, geraniol, citronellyl acetate, geranyl acetate and elemol in the regenerated somaclones of *Cymbopogon winterianus* var. Jorhat. They selected plants with higher herbage yield (>3 fold)

and oil content (>1.5-2 fold) with improved oil quality with desirable constituents such as citronellal, citronellol, geraniol and geranyl acetate and reduced level of elemol.

Patnaik *et al.* (1999) isolated three superior lines of *Cymbopogon martinii* (Roxb.) with high oil and high geraniol content.

Pepper mint

Okuyama *et al.* (1995) reported protoclonal variation in menthol and menthone content in regenerants of pepper mint.

Han *et al.* (1998) also described somaclones of pepper mint with high oil and menthol content of 27.77 per cent and 8.16 per cent respectively.

Hyoscyamus muticus

Giri and Ahuja (1994) observed wide range of variations for total alkaloid content and relative concentration of hyoscyamine and scopalamine or hyoscine in somaclones of Hyoscyamus.

Hypericum perforatum cv. Topes

Cellarova *et al.* (1994) recorded significant variability in hypericin content in *in vitro* regenerated plants.

Datura

Ghiorghite *et al.* (1987) studied the biochemical aspects of datura somaclones and found that the alkaloid content ranged from 0.23 to 0.69 per cent.

Oil seeds

Sunflower

Roseland *et al.* (1991) isolated somaclonal variants of sunflower with modified coumarin content. A higher concentration of scopoletin (12.75 µg/g fresh

weight) was observed in S_1 progenies when compared to the parental inbred line (5.66 µg/g).

2.3 Tissue culture derived variation for screening for disease resistance/tolerance

Field crops

Wheat

Chawla and Wenzel (1987) reported increased resistance to *Helminthosporium sativum* in wheat somaclones.

Shen *et al.* (1996) isolated a wheat somaclone line tolerant to *Fusarium graminea.*

Rice

In rice Ling *et al.* (1985) identified one plant among the regenerants derived by somatic cell culture of var. IR 54 resistant to the brown spot disease caused by *Helminthosporium oryzae.* The progeny of this plant segregated for resistance and susceptibility in the ratio 5:4. The resistance was suggested to be conferred by a dominant mutation.

Sugarcane

Krishnamurthi and Tslaskal (1974) and Heinz *et al.* (1977) regenerated plantlets from callus cultures of shoot meristem. Plants regenerated were found to be resistant to Fiji virus. The increased resistance was found to be transmitted to the vegetative propagules also.

Larkin and Scowcroft (1983) reported resistance to *Helminthosporium sacchari* in sugarcane calliclones. Liu (1981) observed increased resistance to *Ustilago scitaminae* in regenerants from calli.

Field resistance to *Puccinia melanocephala* in callus regenerants of sugarcane was reported by Sreenivasan *et al.* (1987).

Leal *et al.* (1996) identified sugarcane calli clone resistant to eye spot disease (*Dreschslera bipolaris*) under field condition.

Tobacco

Calliclones of tobacco were found to be tolerant to *Pernospora tabacina* and tobacco mosaic virus. The resistance was found to be transmitted to the progeny (Zagorska and Atanassov, 1985).

Daub and Jenns (1989) analysed a total of 854 protoclones of two flue-cured tobacco cultivars and their progeny in green house and field for yield, leaf chemistry and resistance to black shank, bacterial wilt, tobacco mosaic virus and root knot nematode (*Meloidogyne incognita*). Progeny of the somaclones had normal phenotype and did not differ significantly from the parent cultivars in yield and leaf chemistry. Significant variation was found in resistance to black shank and bacterial wilt, two diseases for which the parental cultivars had low level of resistance. No somaclones were identified with resistance to tobacco mosaic virus and *Meloidogyne incognita*. They concluded that genetic variation occurred in the somaclones, that the magnitude of variation was slight and the variation depended both on the genotype of the parent cultivar and the trait.

Solanaceous vegetables

Potato

Plants regenerated from mesophyll protoplast of potato showed resistance to *Alternaria solani*. The resistance observed in protoclones was found to be transmitted to the vegetatively propagated progeny (Matern *et al.*, 1978).

Shepard *et al.* (1980) reported increased resistance to *Phytophthora infestans* in protoclones of potato.

Meulemans and Fouarge (1986) also reported increased resistance to *Phytophthora infestans* in protoclones, calliclones and explant derived plantlets of potato.

Increased field resistance to *Streptomyces scabies* in protoclones was reported by Thomson *et al.* (1986).

Field resistance to potato virus X and potato virus Y was observed in calliclones and explant derived plantlets by Cassels *et al.* (1987).

Sebastiani *et al.* (1994) isolated a calliclone of potato resistant to *Verticillium dahliae*.

Tomato

Bardan *et al.* (1986); Smit and Murakishi (1987) reported resistance to tobacco mosaic virus in tissue culture regenerants. The resistance was found to be transmitted to progenies.

Calliclones showing monogenic resistance to *Fusarium oxysporum* f.s.p. *lycopersici* race 2 was observed by Miller *et al.* (1985) and Evans (1987).

Shahin and Spivey (1986) regenerated protoclones from cotyledonary tissue of UC-82 which was susceptible to *Fusarium oxysporum* f.s.p. *lycopersici* race 2. Analysis of the R_2 progenies showed that *Fusarium* resistant plants were either homozygous or heterozygous dominant for the gene confirming resistance.

Van-den-Bulk *et al.* (1991); Van-den-Bulk and Don (1993) evaluated somaclone progenies produced from tomato canker (*Clavibactor michiganensis* sub sp. *michiganensis*) susceptible cultivar Money maker. No differences in disease reaction were detected between the somaclones and control.

Spices

Shylaja and Nair (1996) exploited somaclonal variation in black pepper (*P. nigrum* L.) cultivars for *Phytophthora* foot rot disease reaction. Out of the callus regenerants evaluated among the four cultivars viz., Kalluvally, Balankotta, Karimunda and Cheriakanyakkadan, the cultivar Kalluvally exhibited higher

variation for the character studied. They also reported that the longer the duration in culture, the higher was the variability in lesion development and the rate of somaclonal variation in black pepper is dependent on genotype. Shylaja *et al.* (1996) also reported that some of the regenerants derived from unscreened calli exhibited higher degree of tolerance to the disease revealing the possibility of exploiting somaclonal variation for *Phytophthora* foot rot disease screening in black pepper. The calliclones of Cheriakanyakkadan recorded greater degree of tolerance to the disease as compared to others.

Babu *et al.* (1998) reported genetic variability in micropropagated plants of Zingiberaceous spice crops like ginger (*Zingiber officinale* Rosc.), cardamom (*Elettaria cardamomum* Maton) and turmeric (*Curcuma longa* L.). They observed considerable variation in the micropropagated plants after 2-5 years of field evaluation. The extent of variation was more in callus regenerated plants. Useful variations were observed in important characters like plant type, quality, yield, boldness of rhizomes and reactions to diseases.

Fruit crops

Banana

Hwang and Ko (1988); Hwang and Ko (1992) observed increased resistance to *Fusarium oxysporum* f.s.p. *cubense* in meristem culture derived plantlets. They isolated a wilt resistant somaclone named GC TCV 215-1 for commercial planting.

Drew *et al.* (1992) reported that micropropagated bananas from banana bunchy top virus infected plants displayed no symptoms of bunchy top disease.

Trujillo and Garcia (1996) developed a banana somaclonal variant (CIEN-BTA-03) resistant to yellow sigatoka disease. This plant displayed satisfactory yield and all the characteristics of resistance to yellow sigatoka.

Matsumoto *et al.* (1999) evaluated the regenerants produced from shoot meristems of fusarium wilt susceptible banana var. Maca after mutagenesis. Disease resistance was evaluated by artificial inoculation with the pathogen under green house condition. Regenerants showed tolerance were transplanted to field and different levels of field resistance to *Fusarium oxysporum* f.s.p. *cubense* were observed.

Strawberry

Toyodo *et al.* (1991) isolated two resistant lines of strawberry to *Fusarium oxysporum* f.s.p. *fragariae* from calliclones.

Peach

Hammerschlag (1996) reported increased levels of disease resistance to bacterial leaf spot (*Xanthomonas campestris* pv. *pruni*) in toxin selected and unselected peach regenerants under green house and field conditions.

Hammerschlag (2000) isolated peach somaclone 122-1 progeny with high level of field tolerance to bacterial spot (*X. campestris* pv. *pruni*) and bacterial canker (*Pseudomonas syringae* pv. *syringae*).

Apple

Chevreau *et al.* (1998) evaluated four somaclonal variant of apple var. Greensleeves for fire blight disease (*Erwinia amylovora*) under field and green house condition and the clone R 46/3 was less susceptible to the disease.

Oil seeds

Sesame

Abd-El-Moneem *et al.* (1997) evaluated the plants derived from epicotyl segments of sesame for charcoal root rot (*Sclerotium bataticola*),

Macrophomina phaseolina and wilt disease (*Fusarium oxysporum* f.s.p. *sisesami*) and found that the somaclones super exceeded the donor plant for resistance.

Mustard

Katiyar and chopra (1990) isolated somaclones of mustard tolerant to *Albugo candida*, *Pernospora parasitica*, *Alternaria brassicae* and *A. brassicola*.

Sharma and Singh (1995) field tested the plants derived from callus cultures of irradiated and non irradiated seeds of mustard and observed resistance to *Albugo candida* and *Alternaria brassicae*.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigations were carried out in the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur during October 1998 to July 2000.

Calliclones of black pepper regenerated from axenic seedling of the cultivar Cheriakanyakkadan, planted in Pepper Research Scheme of the Department during June 1995 were utilised for the study (Plate 1). The objective of the study was to assess the variability among the calliclones of black pepper based on morphological, yield and quality attributes and degree of incidence to *Phytophthora* foot rot. Thirty calliclones flowered during 1997 were evaluated for morphological and floral characters, yield and quality attributes as per the IBPGR descriptor. The calliclones were given uniform management practices as suggested in the Package of Practices Recommendations of the Kerala Agricultural University (KAU, 1993).

3.1 Morphological characters

Morphological characters were recorded in the thirty calliclones based on IBPGR descriptor. Characters of the vegetative bud, young leaf, mature leaf, stem, branch, stolon, flower and fruit were observed. Five observations were made for each character in the different calliclones and the mean was worked out.

3.1.1 Vegetative bud

In each calliclone, the colour of the vegetative bud, shape and length-width ratio of the bud were observed. Based on the presence or absence of anthocyanin pigmentation, the colour of the bud was described as light purple or green. The shape of the vegetative bud was described as conical curved or conical straight. The length and width of the vegetative bud were measured and length-width ratio calculated.

Plate 1a. General view of the field planted calliclones of black pepper (cv. Cheriakanyakkadan)

Plate 1b. A calliclone in flowering stage


CC-33

3.1.2 Young leaf

Colour of the emerging leaves on upper and lower side, leaf shape and length-width ratio of young leaf were observed. Shape of leaf tip, leaf base and leaf lamina were observed as per Lawrence (1973).

3.1.3 Mature leaf

3.1.3.1 Petiole

Characters like length, shape, thickness and colour of the petiole, colour of the leaf sheath, presence or absence of hairiness on the petiole were observed. The length and thickness were measured for petioles of leaves produced on plageotropes selected at random and the mean was worked out. The colour of the petiole was observed either light green or yellowish green. The colour of the leaf sheath was judged based on presence or absence of anthocyanin pigmentation.

3.1.3.2 Leaf blade

The shape of the leaf, length-width ratio, type of venation, colour of the leaf on upper and lower surface, shape of leaf tip and base, arrangement of leaf, leaf area, texture of the leaf, type of leaf margin and presence or absence of hairiness on leaves were observed and recorded. Shape of the leaf was described as per Lawrence, 1973. Length-width ratio and leaf area were measured from 10 leaves selected at random and the mean was worked out. The leaf area was estimated as reported by Ibrahim *et al.* (1985), for black pepper.

The position of the leaf blade (phyllotaxy) in each calliclone was observed in orthotropes and plageotropes.

The texture of the leaf was described as coriaceous or crustaceous. The leaf surface was described as either glabrous or pubescent and venation of the leaf as acrodromous or campylodromous.

3.1.4 Stem

Observations on the shape and colour of the stem, length of the internode, thickness at node and internode were recorded for each calliclone.

3.1.5 Branch

Observations on internodal length, thickness at node and internode, angle of laterals, direction of branch, hairiness and rate of lateral production were recorded. The angle subtended by the lateral with the main stem was measured in degrees and if it was more than 80°, it was designated as drooping and if less than 80°, it was designated as semi erect. The number of lateral branches produced per unit area was determined by placing a wooden frame of 1 m^2 on the vine at chest height and counting the branches inside the frame.

3.1.6 Stolon

Amount of adventitious root, its holding ability and rate of runner production were recorded. The total number of runners produced per vine was also observed.

3.1.7 Flower

Observations on number of spikes per branch, length of spike, nature of flowering were recorded. Length of 10 spikes were taken at the time of flowering and mean value calculated. Flowering behaviour was recorded as profuse, medium and low based on the number of spikes per 0.5 m^2 area. The spike number upto 25 was graded as low, 25 to 39 as medium and 40 and above as profuse.

3.1.8 Flowering habit

The number of hermaphrodite, pistillate and staminate flowers in a spike were counted with the help of a hand lens. Number of stigmatic lobes for each calliclone was observed. The receptivity of the stigma was also observed. Creamy

white shiny stigma was considered as receptive while dried and brownish one was considered as non receptive.

Fertility of pollen grains was determined by noting the number of well filled, oval, well stained pollen grains. Microscopic slides of pollen grains of each type were prepared after staining them with acetocarmine dye (Zirkle, 1937). Observations were recorded from five microscopic fields. Percentage of fertile pollen was determined as

$$\text{Pollen fertility \%} = \frac{\text{Number of fertile pollen}}{\text{Total number of pollen}} \times 100$$

3.1.9 Fruit/spike

The following fruit/spike characters were observed independently in the thirty calliclones.

3.1.9.1 Spike length

After harvest, 10 spikes were randomly selected from each calliclone and the length was measured and mean calculated.

3.1.9.2 Berry colour and shape

Colour of young and mature berries and shape of the mature berries were observed and recorded for each calliclone.

3.1.9.3 Time taken to maturity

Time taken to maturity was observed by counting the days taken from berry set to berry maturity.

3.1.9.4 Number of berries per spike

The total number of berries, number of well developed and under developed berries per spike were counted for each calliclone and the percentages worked out.

3.1.9.5 Berry weight and volume

Weight of 100 well developed green and dry berries were determined for each calliclone. The volume of 100 green and dry berries were determined by water displacement method. From this, the ratio of fresh/dry berry weight was worked out.

3.1.9.6 Mean spike/berry ratio from 25 spikes

The mean spike/berry ratio of 25 spikes was determined by noting the fresh weight of 25 spikes and berries separated from the spikes.

3.1.9.7 Seed weight and volume

The weight and volume of 100 well developed seeds (deskinned) were recorded for each calliclone.

3.1.9.8 Pericarp thickness

Transverse hand sections of mature berries were taken, stained with safranin and temporary mounts were prepared and examined under microscope. Microscopic observations on pericarp thickness were made with ocular and stage micrometer.

3.2 Yield per calliclone

Total fresh spike weight, berry weight, dry berry weight and dry recovery percentage (driage) were recorded for individual calliclones.

3.3 Quality attributes

The major quality parameters like essential oil, oleoresin and piperine were evaluated in each calliclone for two seasons viz. 1998 and 1999. Two replications were maintained for different parameters for the two seasons.

3.3.1 Essential oil

The apparatus used for the distillation of essential oil was clevenger apparatus. Twenty gram of ground pepper powder was taken in the round bottomed flask and water distilled with 200 ml of distilled water for 2 h. The volatile oil being lighter than water condensed and collected on the top of the clevenger trap. The percentage of essential oil in the sample was worked out.

3.3.2 Oleoresin

The content of oleoresin in the sample was estimated using the soxhlet method of extraction as per Horwitz (1980). After grinding the pepper berries, five gram of the powder was wrapped in filter paper and placed in the extraction chamber of the apparatus. Extraction was carried out with 100 per cent acetone till the solvent become colourless. The acetone extract of the sample was transferred to a pre-weighed beaker and the solvent evaporated and the weight of the beaker recorded. The percentage of oleoresin was calculated as

$$\frac{\text{Weight of oleoresin}}{\text{Weight of spice powder}} \times 100$$

3.3.3 Piperine

The piperine content in dried berries was determined by Spectrophotometric method described by Sowbhagya *et al.* (1990). Freshly powdered pepper (100 mg) was transferred to a 100 ml volumetric flask. The volume was made up with 100 per cent acetone. The flask was shaken well and allowed to settle for 2 h. Then 0.5 ml of the solution was pipetted out from the

volumetric flask and made upto 5 ml with acetone. The absorbance of the solution was read at 337 nm. Standard values for pure piperine at different concentrations were also found out following the same procedure and standard curve for piperine was plotted.

3.3.4 Moisture content

Moisture content was estimated by gravimetric method. Twenty gram of dried pepper was oven dried at 70°C till constant weight was obtained. From the difference in weight the percentage of moisture content was calculated.

3.4 Isolation of *Phytophthora capsici* and evaluation of *Phytophthora* foot rot disease reaction in calliclones

The pathogen, *Phytophthora capsici* was isolated from the infected leaves of black pepper. Small bits of infected portion of leaves were surface sterilised with 0.1 per cent mercuric chloride for 1 minute and washed free off the sterilant with sterile distilled water and inoculated to Potato Dextrose Agar (PDA) medium. The culture was purified and pathogenecity of the isolated culture was tested. The stock isolate was maintained by periodic transfer to PDA medium.

Artificial inoculation of *Phytophthora capsici* on leaves was conducted as per the procedure reported by Kueh and Khew (1980). From the calliclones, leaves of same maturity were taken and washed thoroughly and wiped with 70 per cent ethyl alcohol. With sterile needle, minute pin pricks were made on the lower side of the leaf and 5 mm culture discs of the pathogen were inoculated. The inoculated leaves were kept in a bell jar, provided with high humidity. The lesion development was measured after 48 and 72 h of inoculation. Based on the average diameter of the lesions formed, calliclones were grouped into four classes as shown below;

Class	Lesion diameter in cm
1a	Up to 0.25
1	
1b	0.26-0.50
2	0.51-1.00
3	1.01-1.50
4	1.51-2.00

3.5 Statistical analysis

The phenotypic variability was measured in the calliclones as per the procedure described by Deklerk (1990). Frequency distribution of each character was fitted using M STAT C package. Path coefficient analysis was done as per Singh and Chaudhary (1979). Clustering of calliclones based on yield and quality attributes was done using principal component analysis (Chatfield and Collins, 1986). The divergence between the clusters was measured using matrix of dissimilarity.

RESULTS

Thirty calliclones of black pepper (cv. Cheriakanyakkadan) planted during 1995 and started flowering during 1997 were analysed for variations in morphological, yield, quality attributes and disease reaction to *Phytophthora* foot rot. The variation at the phenotypic level was measured using the procedure described by Deklerk (1990) where values of standard deviations of quantitative phenotypic traits in the population were used as accurate measures of the extent of variation. The calliclones observed were grouped into different groups for each character by fitting a frequency distribution using MSTATC package.

4.1 Morphological characters

4.1.1 Vegetative bud

The vegetative bud characters of different calliclones are presented in Table 1. The results show that the colour of the vegetative bud was light purple and shape was conical curved in all calliclones.

Length of the bud ranged from 1.32-3.10 cm and width from 0.10 to 0.36 cm.

Length-width ratio ranged from 5.00 to 15.50. The length-width ratio exhibited a high coefficient of variation of 32.84 and a standard deviation of 3.12 (Fig.1). The different calliclones studied could be fitted in four frequency classes as presented in Table 1c.

4.1.2 Young leaf

The colour of young leaf was light green on upper surface and pale purple on lower surface (Table 2). Leaf tip was accuminate. Leaf base was either round or cordate-round. Shape of the young leaf was either cordate or ovate. The length of young leaf ranged from 2.45 to 7.13 cm and width from 1.50 to 4.13 cm. Length-width ratio of young leaf ranged from 1.19 to 1.91. Wide variation was

Table 1. Vegetative bud characters in different calliclones of black pepper (*P. nigrum* L.)

Sl. No.	Calliclone No.	Colour	Shape	Mean length (cm)	Mean width (cm)	Mean length width ratio
1	CC 4	Light purple	Conical curved	1.66	0.26	6.38
2	CC 8	Light purple	Conical curved	1.33	0.27	5.00
3	CC 9	Light purple	Conical curved	1.50	0.10	15.00
4	CC 10	Light purple	Conical curved	2.00	0.36	5.55
5	CC 12	Light purple	Conical curved	1.63	0.32	5.00
6	CC 23	Light purple	Conical curved	2.10	0.24	8.75
7	CC 25	Light purple	Conical curved	2.06	0.20	10.30
8.	CC 27	Light purple	Conical curved	2.78	0.18	15.44
9	CC 28	Light purple	Conical curved	1.50	0.30	5.00
10	CC 31	Light purple	Conical curved	1.66	0.26	6.38
11	CC 33	Light purple	Conical curved	2.30	0.23	10.22
12	CC 35	Light purple	Conical curved	1.32	0.20	6.60
13	CC 36	Light purple	Conical curved	2.60	0.24	10.83
14	CC 37	Light purple	Conical curved	2.42	0.22	11.00
15	CC 38	Light purple	Conical curved	2.45	0.20	12.25
16	CC 40	Light purple	Conical curved	1.62	0.32	5.00
17	CC 41	Light purple	Conical curved	2.10	0.22	9.55
18	CC 43	Light purple	Conical curved	2.34	0.28	8.35
19	CC 45	Light purple	Conical curved	2.10	0.20	10.50
20	CC 46	Light purple	Conical curved	2.35	0.27	8.70
21	CC 55	Light purple	Conical curved	2.00	0.28	7.14
22	CC 56	Light purple	Conical curved	1.70	0.13	13.07
23	CC 57	Light purple	Conical curved	2.00	0.23	8.69
24	CC 58	Light purple	Conical curved	2.50	0.22	11.36
25	CC 60	Light purple	Conical curved	3.10	0.20	15.50
26	CC 61	Light purple	Conical curved	2.40	0.18	13.33
27	CC 62	Light purple	Conical curved	2.10	0.20	10.50
28	CC 64	Light purple	Conical curved	1.80	0.20	9.00
29	CC 65	Light purple	Conical curved	2.24	0.22	10.18
30	CC 69	Light purple	Conical curved	1.92	0.18	10.66
	SD			0.429	0.055	3.12
	CV			20.91	23.91	32.84

Frequency distribution for vegetative bud characters in calliclones of black pepper (*P. nigrum* L.)

Table 1a. Length of the vegetative bud

Group No.	Length (cm)	Frequency (%)
1	1.00-1.49	6.66
2	1.50-1.99	30.00
3	2.00-2.49	50.00
4	2.50-2.99	10.00
5	3.00-3.49	3.33

Table 1b. Width of the vegetative bud

Group No.	Width (cm)	Frequency (%)
1	0.10-0.14	6.66
2	0.15-0.19	10.00
3	0.20-0.24	50.00
4	0.25-0.29	20.00
5	0.30-0.34	10.00
6	0.35-0.39	3.33

Table 1c. Length width ratio of the vegetative bud

Group No.	Length width ratio	Frequency (%)
1	5.00-7.99	30.00
2	8.00-10.99	43.33
3	11.00-13.99	16.66
4	14.00-16.99	10.00

Fig.1. Variability in length - width ratio of the vegetative bud in different calliclones of black pepper (*P. nigrum* L.)

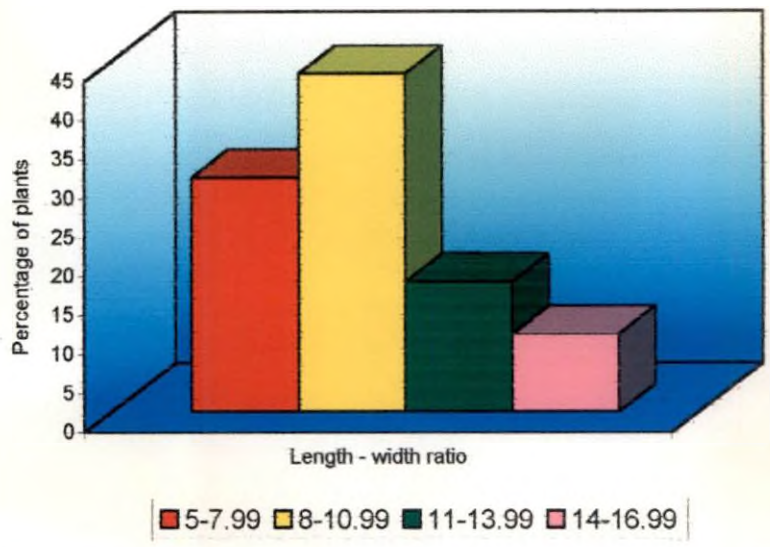


Fig.2. Variability in leaf area in different calliclones of black pepper (*P. nigrum* L.)

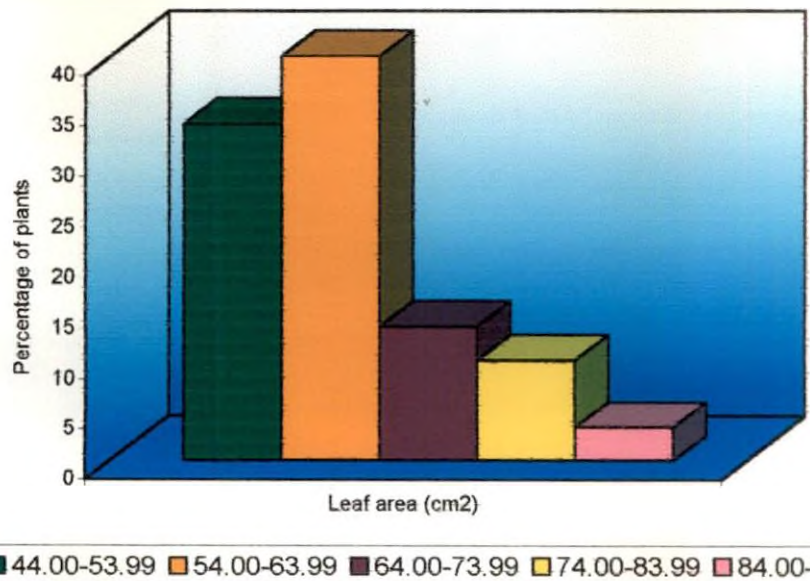


Table 2. Characters of young leaf in different calliclones of black pepper (*P. nigrum* L.)

Sl. No.	Calliclone No.	Colour of emerging leaves on		Shape			Mean length (cm)	Mean width (cm)	Mean length width ratio
		Upper side	Lower side	Leaf lamina	Tip	Base			
1	CC 4	Light green	Pale purple	Cordate - Ovate	Acuminate	Cordate - Round	2.93	1.60	1.75
2	CC 8	Light green	Pale purple	Ovate	Acuminate	Round	3.50	2.70	1.34
3	CC 9	Light green	Pale purple	Ovate	Acuminate	Round	5.50	3.00	1.83
4	CC 10	Light green	Pale purple	Cordate - Ovate	Acuminate	Cordate - Round	3.00	2.00	1.50
5	CC 12	Light green	Pale purple	Ovate	Acuminate	Round	2.45	1.50	1.70
6	CC 23	Light green	Pale purple	Ovate	Acuminate	Round	4.00	2.30	1.78
7	CC 25	Light green	Pale purple	Ovate	Acuminate	Round	3.54	2.40	1.53
8	CC 27	Light green	Pale purple	Ovate	Acuminate	Round	7.13	4.13	1.65
9	CC 28	Light green	Pale purple	Ovate	Acuminate	Round	5.25	3.00	1.70
10	CC 31	Light green	Pale purple	Ovate	Acuminate	Round	2.68	1.56	1.82
11	CC 33	Light green	Pale purple	Ovate	Acuminate	Round	4.33	2.68	1.55
12	CC 35	Light green	Pale purple	Ovate	Acuminate	Round	3.62	1.90	1.91
13	CC 36	Light green	Pale purple	Ovate	Acuminate	Round	4.76	2.74	1.68
14	CC 37	Light green	Pale purple	Cordate - Ovate	Acuminate	Cordate - Round	3.54	2.02	1.78
15	CC 38	Light green	Pale purple	Ovate	Acuminate	Round	3.08	1.68	1.90
16	CC 40	Light green	Pale purple	Ovate	Acuminate	Round	2.45	1.50	1.70
17	CC 41	Light green	Pale purple	Ovate	Acuminate	Round	3.78	2.08	1.81
18	CC 43	Light green	Pale purple	Ovate	Acuminate	Round	4.44	2.72	1.27
19	CC 45	Light green	Pale purple	Ovate	Acuminate	Round	2.50	1.65	1.45
20	CC 46	Light green	Pale purple	Ovate	Acuminate	Round	3.50	2.20	1.59
21	CC 55	Light green	Pale purple	Ovate	Acuminate	Round	2.80	1.60	1.79
22	CC 56	Light green	Pale purple	Ovate	Acuminate	Round	4.53	2.63	1.65
23	CC 57	Light green	Pale purple	Ovate	Acuminate	Round	4.87	2.76	1.75
24	CC 58	Light green	Pale purple	Ovate	Acuminate	Round	2.95	2.43	1.19
25	CC 60	Light green	Pale purple	Ovate	Acuminate	Round	4.47	2.80	1.45
26	CC 61	Light green	Pale purple	Ovate	Acuminate	Round	6.35	3.78	1.66
27	CC 62	Light green	Pale purple	Ovate	Acuminate	Round	4.60	2.85	1.75
28	CC 64	Light green	Pale purple	Ovate	Acuminate	Round	4.70	2.90	1.61
29	CC 65	Light green	Pale purple	Ovate	Acuminate	Round	3.20	1.68	1.67
30	CC 69	Light green	Pale purple	Cordate - Ovate	Acuminate	Cordate - Round	4.30	3.02	1.83
	SD						1.346	0.671	0.18
	CV						34.87	28.08	10.83

Frequency distribution for young leaf characters in calliclones of black pepper.

Table 2a. Length of the young leaf

Group No.	Length (cm)	Frequency (%)
1	2.00-2.99	23.33
2	3.00-3.99	30.00
3	4.00-4.99	33.33
4	5.00-5.99	6.66
5	6.00-6.99	3.33
6	7.00-7.99	3.33

Table 2b. Width of the young leaf

Group No.	Width (cm)	Frequency (%)
1	1.50-1.99	30.00
2	2.00-2.49	23.33
3	2.50-2.99	30.00
4	3.00-3.49	10.00
5	3.50-3.99	3.33
6	4.00-4.49	3.33

Table 2c. Length width ratio of the young leaf

Group No.	Length width ratio	Frequency (%)
1	1.00-1.19	3.33
2	1.20-1.39	6.66
3	1.40-1.59	20.00
4	1.60-1.79	50.00
5	1.80-1.99	20.00

observed in the length of the leaf in the different calliclones studied. The coefficient of variation recorded for the character was 34.87 and standard deviation was 1.346. The calliclones could be grouped into six frequency classes as shown in Table 2a.

4.1.3 Mature leaf

4.1.3.1 Petiole

The calliclones were found to vary for petiole length and thickness which ranged from 1.24-3.94 cm and 0.72-1.86 cm respectively. Colour of the petiole was light green in 76.66 per cent of calliclones and light green to yellow in 23.33 per cent of calliclones. All the calliclones exhibited similar characters for colour of leaf sheath, shape of petiole and hairiness (Table 3). The colour of the leaf sheath was purple, petiole was grooved and without hairs in all the calliclones studied.

4.1.3.2 Leaf blade

The results presented in Table 4 show that calliclones exhibited wide variations for leaf shape. The different leaf shapes noted were ovate, cordate-ovate, elliptic-ovate and ovate lanceolate (Plate 2). Ovate leaf shape was observed in 46.66 per cent of calliclones, ovate lanceolate in 30 per cent, cordate-ovate in 13.33 per cent and elliptic-ovate in 10 per cent of the clones. The leaf base was either round, cordate or cuneate. The colour of the mature leaves both on the upper and lower sides varied widely in different calliclones. The colour on the upper surface was green in 56.66 per cent of calliclones and dark green in 43.33 per cent of calliclones whereas the colour on the lower side was light green in 56.66 per cent and green in 43.33 per cent. The leaves were alternate, glabrous, coriaceous with acrodromous venation and with entire leaf margin in all the calliclones studied. Leaf tip was acuminate and hairiness was absent in the leaves of all calliclones. The length and width of leaf blade varied in between 10.93 to 13.64 cm

Plate 2. Variability in leaf shape in calliclones of black pepper

1) Cordate
2) Ovate
3) Ovate lanceolate
4) Elliptic


CC.4
CC.41
CC.43
CC.27

Table 3. Petiole characters of mature leaf in different calliclones of black pepper (*P. nigrum L.*)

Sl. No.	Calliclone No.	Mean length (cm)	Shape	Mean thickness (cm)	Colour	Colour of leaf sheath	Hairiness
1	CC 4	1.84	Grooved	1.10	Light green	Purple	Absent
2	CC 8	3.16	Grooved	1.86	Light green	Purple	Absent
3	CC 9	1.92	Grooved	1.12	Light green to yellow	Purple	Absent
4	CC 10	1.47	Grooved	1.16	Light green	Purple	Absent
5	CC 12	2.30	Grooved	1.50	Light green	Purple	Absent
6	CC 23	2.58	Grooved	1.16	Light green	Purple	Absent
7	CC 25	2.14	Grooved	1.10	Light green	Purple	Absent
8	CC 27	2.14	Grooved	1.12	Light green	Purple	Absent
9	CC 28	2.10	Grooved	1.40	Light green	Purple	Absent
10	CC 31	2.38	Grooved	1.26	Light green	Purple	Absent
11	CC 33	2.64	Grooved	0.96	Light green to yellow	Purple	Absent
12	CC 35	1.24	Grooved	0.80	Light green	Purple	Absent
13	CC 36	2.52	Grooved	1.16	Light green	Purple	Absent
14	CC 37	2.32	Grooved	1.08	Light green to yellow	Purple	Absent
15	CC 38	2.30	Grooved	1.06	Light green to yellow	Purple	Absent
16	CC 40	2.30	Grooved	1.06	Light green to yellow	Purple	Absent
17	CC 41	3.60	Grooved	0.96	Light green	Purple	Absent
18	CC 43	3.94	Grooved	0.72	Light green to yellow	Purple	Absent
19	CC 45	2.17	Grooved	0.78	Light green	Purple	Absent
20	CC 46	3.90	Grooved	0.86	Light green	Purple	Absent
21	CC 55	2.60	Grooved	0.96	Light green	Purple	Absent
22	CC 56	2.10	Grooved	0.88	Light green	Purple	Absent
23	CC 57	2.04	Grooved	0.92	Light green	Purple	Absent
24	CC 58	2.16	Grooved	0.86	Light green	Purple	Absent
25	CC 60	1.86	Grooved	1.00	Light green to yellow	Purple	Absent
26	CC 61	2.10	Grooved	1.02	Light green	Purple	Absent
27	CC 62	1.82	Grooved	0.96	Light green	Purple	Absent
28	CC 64	1.92	Grooved	1.02	Light green	Purple	Absent
29	CC 65	2.18	Grooved	1.14	Light green	Purple	Absent
30	CC 69	2.60	Grooved	1.32	Light green	Purple	Absent
	SD	0.618		0.230			
	CV	26.35		21.38			

Frequency distribution for petiole characters of the mature leaf in calliclones of black pepper (*P. nigrum* L.)

Table 3a. Petiole length

Group No.	Length (cm)	Frequency (%)
1	1.00-1.99	23.33
2	2.00-2.99	63.33
3	3.00-3.99	13.33

Table 3b. Petiole Thickness

Group No.	Thickness (cm)	Frequency (%)
1	0.70-0.89	20.00
2	0.90-1.09	36.66
3	1.10-1.29	30.00
4	1.30-1.49	6.66
5	1.60-1.69	3.33
6	1.70-1.89	3.33

Table 4. Mature leaf blade characters in different calliclones of black pepper (*P. nigrum* L.)

Calli-clone No.	Leaf arrang-ement	Upper leaf surface	Shape			Venation	Mature leaf colour		Texture of mature leaf	Leaf margin	Hairi-ness	Mean length of leaf blade (cm)	Mean width of leaf blade (cm)	L/W ratio	Leaf area (cm^2)
			Leaf lamina	Leaf tip	Leaf base		Upper side	Lower side							
CC 4	Alternate	Glabrous	Cordate - Ovate	Acuminate	Cordate - Round	Acrodro-mous	Dark green	Green	Coriaceous	Entire	Absent	13.11	7.57	1.74	69.58
CC 8	,,	,,	Ovate	,,	Round	,,	Dark green	Green	,,	,,	,,	12.10	8.50	1.59	62.66
CC 9	,,	,,	Ovate	,,	Round	,,	Dark green	Green	,,	,,	,,	12.20	6.08	2.00	50.52
CC 10	,,	,,	Ovate	,,	Round	,,	Green	Light green	,,	,,	,,	11.10	6.60	1.67	49.35
CC 12	,,	,,	Ovate	,,	Round	,,	Green	Light green	,,	,,	,,	12.40	7.85	1.59	66.45
CC 23	,,	,,	Ovate	,,	Round	,,	Dark green	Green	,,	,,	,,	11.24	6.38	1.91	54.47
CC 25	,,	,,	Elliptic - Ovate	,,	Cuneate - Round	,,	Dark green	Green	,,	,,	,,	11.80	6.40	1.88	51.45
CC 27	,,	,,	Elliptic - Ovate	,,	Cuneate - Round	,,	Green	Light green	,,	,,	,,	11.30	6.08	1.76	60.59
CC 28	,,	,,	Ovate	,,	Round	,,	Green	Light green	,,	,,	,,	12.05	6.69	1.78	54.68
CC 31	,,	,,	Ovate	,,	Round	,,	Green	Light green	,,	,,	,,	12.02	7.40	1.60	60.23
CC 33	,,	,,	Ovate lanceolate	,,	Round	,,	Green	Light green	,,	,,	,,	13.39	8.05	1.66	74.16
CC 35	,,	,,	Ovate	,,	Round	,,	Green	Light green	,,	,,	,,	11.25	7.19	1.55	56.75
CC 36	,,	,,	Ovate	,,	Round	,,	Green	Light green	,,	,,	,,	12.63	6.79	1.90	58.51
CC 37	,,	,,	Elliptic - Ovate	,,	Cuneate - Round	,,	Dark green	Green	,,	,,	,,	13.57	6.72	1.93	62.15
CC 38	,,	,,	Ovate lanceolate	,,	Round	,,	Green	Light green	,,	,,	,,	11.61	6.68	1.70	52.61
CC 40	,,	,,	Ovate	,,	Round	,,	Green	Light green	,,	,,	,,	11.74	6.41	1.77	51.13
CC 41	,,	,,	Ovate	,,	Round	,,	Dark green	Green	,,	,,	,,	13.64	8.14	1.69	75.11
CC 43	,,	,,	Ovate lanceolate	,,	Round	,,	Green	Light green	,,	,,	,,	13.22	8.16	1.63	77.02

Contd.

Table 4. Mature leaf blade characters in different calliclones of black pepper (*P. nigrum* L.) continued.

Calli-clone No.	Leaf arrang-ement	Upper leaf surface	Shape			Venation	Mature leaf colour		Texture of mature leaf	Leaf margin	Hairi-ness	Mean length of leaf blade (cm)	Mean width of leaf blade (cm)	L/W ratio	Leaf area (cm^2)
			Leaf lamina	Leaf tip	Leaf base		Upper side	Lower side							
CC 45	,,	,,	Ovate	,,	Round	,,	Green	Light green	,,	,,	,,	13.47	7.97	1.68	73.80
CC 46	,,	,,	Ovate lanceolate	,,	Round	,,	Dark green	Green	,,	,,	,,	13.44	9.24	1.45	84.54
CC 55	,,	,,	Cordate - Ovate	,,	Cordate - Round	,,	Dark green	Green	,,	,,	,,	13.05	7.72	1.70	72.34
CC 56	,,	,,	Ovate lanceolate	,,	Round	,,	Green	Light green	,,	,,	,,	11.70	7.00	1.69	56.13
CC 57	Alter-nate	Glabrous	Ovate lanceolate	Acuminate	Round	Acrodro-mous	Dark green	Green	Coriaceous	Entire	Absent	12.45	6.98	1.77	59.07
CC 58	,,	,,	Cordate - Ovate	,,	Cordate - round	,,	,,	,,	,,	,,	,,	11.04	6.56	1.68	49.45
CC 60	,,	,,	Ovate lanceolate	,,	Round	,,	,,	,,	,,	,,	,,	11.47	6.13	1.84	47.91
CC 61	,,	,,	Ovate lanceolate	,,	,,	,,	Green	Light green	,,	,,	,,	11.59	7.05	1.83	61.84
CC 62	,,	,,	,,	,,	,,	,,	Dark green	Green	,,	,,	,,	11.95	6.29	1.89	51.38
CC 64	,,	,,	Ovate	,,	,,	,,	Green	Light green	,,	,,	,,	11.10	5.77	1.89	44.49
CC 65	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	12.10	7.02	1.72	57.65
CC 69	,,	,,	Cordate - ovate	,,	Cordate - round	,,	Green	,,	,,	,,	,,	10.93	6.08	1.83	44.99
SD												0.858	0.847	0.13	10.36
CV												7.05	12.04	7.34	17.34

Frequency distribution for the mature leaf characters in calliclones of black pepper (*P. nigrum* L.)

Table 4a. Length of the mature leaf

Group No.	Length (cm)	Frequency (%)
1	10.00-10.99	3.33
2	11.00-11.99	43.33
3	12.00-12.99	26.66
4	13.00-13.99	26.66

Table 4b. Width of the mature leaf

Group No.	Width (cm)	Frequency (%)
1	5.00-5.99	3.33
2	6.00-6.99	53.33
3	7.00-7.99	26.66
4	8.00-8.99	13.33
5	9.00-9.99	3.33

Table 4c. Length width ratio of the mature leaf

Group No.	Length width ratio	Frequency (%)
1	1.40-1.59	13.33
2	1.60-1.79	53.33
3	1.80-1.99	33.33

Table 4d. Area of the mature leaf

Group No.	Area (cm^2)	Frequency (%)
1	44.00-53.99	33.33
2	54.00-63.99	40.00
3	64.00-73.99	13.33
4	74.00-83.99	10.00
5	84.00-93.99	3.33

and 5.77 to 9.24 cm respectively. Length-width ratio of leaf lamina ranged between 1.45 to 2.00.

The leaf area was found to vary widely in the calliclones studied (Fig.2). It ranged from 44.49 to 84.54 cm^2 in the different clones studied and recorded a coefficient of variation of 17.34 and standard deviation of 10.36. In 40 per cent of calliclones, the leaf area ranged between 54.47 to 62.66 cm^2 while in 33.33 per cent it ranged between 44.49 to 52.61 cm^2. The highest leaf area of 74.16 to 84.54 cm^2 was observed in 13.33 per cent of calliclones and the clones could be grouped into five frequency classes as shown in Table 4d.

4.1.4 Stem

The data presented in Table 5 show that the stem was cylindrical and the colour of the stem varied from grey to dark green in all calliclones studied. Length of the internode ranged from 4.78 to 8.70 cm. In majority of the calliclones (66.66 per cent) the internodal length ranged in between 6.18 to 7.84 cm. Closely spaced internodes (4.78-5.50 cm) were observed in 9.99 per cent of calliclones whereas in 23.33 per cent of calliclones, the internodes were widely spaced (8.00-8.7 cm).

The thickness at node and internode varied in between 1.90 to 4.10 cm and 1.06 to 3.38 cm respectively.

4.1.5 Lateral branch

The length of internode and the thickness at node and internode varied considerably in the lateral branches of different calliclones. As presented in Table 6 the length of internode ranged between 4.70 to 11.90 cm, thickness at node 1.60 to 4.02 cm and thickness at internode 1.32 to 2.58 cm. Closely spaced internodes (4.70-6.80 cm) were observed in 6.66 per cent of calliclones while in 23.32 per cent of calliclones internodes were widely spaced (10.00-11.90 cm).

Table 5. Stem characters of orthotropes in different calliclones of black pepper (*P. nigrum* L.)

Sl. No.	Calli-clone No.	Shape	Colour	Mean length of internode (cm)	Mean thickness at	
					Node (cm)	Internode (cm)
1	CC 4	Cylindrical	Dark green to grey	7.50	2.44	1.82
2	CC 8	,,	,,	7.70	2.34	1.96
3	CC 9	,,	,,	7.40	2.98	2.14
4	CC 10	,,	,,	5.50	1.90	1.58
5	CC 12	,,	,,	6.40	2.50	2.20
6	CC 23	,,	,,	7.04	3.60	2.40
7	CC 25	,,	,,	8.70	3.20	1.98
8	CC 27	,,	,,	8.16	4.10	3.38
9	CC 28	,,	,,	7.84	3.70	2.72
10	CC 31	,,	,,	5.10	2.34	2.08
11	CC 33	,,	,,	6.18	2.54	2.04
12	CC 35	,,	,,	6.20	2.16	1.80
13	CC 36	,,	,,	6.94	2.42	1.06
14	CC 37	,,	,,	6.84	3.02	2.34
15	CC 38	,,	,,	6.60	2.90	2.12
16	CC 40	,,	,,	8.10	2.98	1.74
17	CC 41	,,	,,	7.44	2.24	1.92
18	CC 43	,,	,,	8.50	2.30	1.88
19	CC 45	,,	,,	6.50	2.56	2.20
20	CC 46	,,	,,	7.20	2.86	2.14
21	CC 55	,,	,,	7.20	2.40	1.88
22	CC 56	,,	,,	7.80	3.08	2.50
23	CC 57	,,	,,	7.40	3.42	1.96
24	CC 58	,,	,,	8.30	3.66	2.28
25	CC 60	,,	,,	8.60	3.58	2.52
26	CC 61	,,	,,	6.96	3.18	2.26
27	CC 62	,,	,,	8.00	3.32	2.50
28	CC 64	,,	,,	6.20	3.24	2.38
29	CC 65	,,	,,	6.70	3.36	2.46
30	CC 69	,,	,,	4.78	2.48	2.20
	SD			0.996	0.553	0.404
	CV			13.98	19.12	18.81

Frequency distribution for stem characters in orthotropes of calliclones of black pepper (*P. nigrum* L.)

Table 5a. Length of internode

Group No.	Length (cm)	Frequency (%)
1	4.00-4.99	3.33
2	5.00-5.99	6.66
3	6.00-6.99	33.33
4	7.00-7.99	33.33
5	8.00-8.99	23.33

Table 5b. Thickness at node

Group No.	Thickness (cm)	Frequency (%)
1	1.00-1.99	3.33
2	2.00-2.99	53.33
3	3.00-3.99	40.00
4	4.00-4.99	3.33

Table 5c. Thickness at internode

Group No.	Thickness (cm)	Frequency (%)
1	1.00-1.49	3.33
2	1.50-1.99	33.33
3	2.00-2.49	46.66
4	2.50-2.99	13.33
5	3.00-3.49	3.33

Table 6. Lateral branch characters in different calliclones of black pepper (*P. nigrum* L.)

Sl. No.	Calli-clone No.	Internodal length of laterals (cm)	Thickness at		Angle of laterals (°)	Direction of lateral	Lateral branches per 1 m² area (No.)	Hairiness
			Node (cm)	Internode (cm)				
1	CC 4	7.40	2.76	2.28	72.00	Semi erect	17	Absent
2	CC 8	6.80	1.68	1.32	63.00	,,	8	,,
3	CC 9	11.90	3.14	1.66	79.00	,,	11	,,
4	CC 10	4.70	1.77	1.48	70.00	,,	9	,,
5	CC 12	8.80	2.20	1.36	78.00	,,	23	,,
6	CC 23	10.80	3.40	2.00	84.00	Drooping	16	,,
7	CC 25	7.30	3.26	2.40	84.00	,,	10	,,
8	CC 27	7.80	4.02	2.58	82.00	,,	11	,,
9	CC 28	10.20	3.24	2.28	79.00	Semi erect	11	,,
10	CC 31	7.30	1.60	1.40	77.50	,,	11	,,
11	CC 33	7.00	2.16	1.52	76.00	,,	13	,,
12	CC 35	8.50	2.28	1.48	86.00	Drooping	6	,,
13	CC 36	9.56	3.20	1.62	83.00	,,	21	,,
14	CC 37	10.00	3.88	2.08	86.00	,,	24	,,
15	CC 38	9.70	3.28	1.46	83.75	,,	17	,,
16	CC 40	8.50	3.16	1.66	83.00	,,	15	,,
17	CC 41	7.14	1.98	1.46	81.00	,,	11	,,
18	CC 43	9.00	2.20	1.42	75.00	Semi erect	9	,,
19	CC 45	9.90	2.34	1.86	85.00	Drooping	16	,,
20	CC 46	11.25	2.27	1.80	82.50	,,	5	,,
21	CC 55	9.50	2.07	1.65	87.00	,,	8	,,
22	CC 56	7.87	1.85	1.50	76.00	Semi erect	10	,,
23	CC 57	11.24	3.30	1.84	88.00	Drooping	10	,,
24	CC 58	7.70	3.40	1.70	80.00	Semi erect	15	,,
25	CC 60	9.00	3.64	2.00	93.00	Drooping	13	,,
26	CC 61	8.60	3.74	1.82	78.00	Semi erect	20	,,
27	CC 62	8.80	3.30	1.84	88.00	Drooping	12	,,
28	CC 64	9.50	3.25	1.97	81.25	,,	11	,,
29	CC 65	11.60	3.62	1.94	89.00	,,	14	,,
30	CC 69	7.40	3.07	1.88	69.00	Semi erect	7	,,
	SD	1.65	0.723	0.325	6.529		4.824	
	CV	18.70	25.50	18.31	8.10		37.69	

Frquency distribution of lateral branch characters in calliclones of black pepper (*P.nigrum* L.)

Table 6a. Internodal length of laterals

Group No.	Internodal length (cm)	Frequency (%)
1	4.00-5.49	3.33
2	5.50-6.99	3.33
3	7.00-8.49	30.00
4	8.50-9.99	40.00
5	10.00-11.49	16.66
6	11.50-12.99	6.66

Table 6b. Thickness at node

Group No.	Thickness (cm)	Frequency (%)
1	1.00-1.99	16.66
2	2.00-2.99	26.66
3	3.00-3.99	53.33
4	4.00-4.99	3.33

Table 6c. Thickness at internode

Group No.	Thickness (cm)	Frequency (%)
1	1.00-1.49	26.66
2	1.50-1.99	50.00
3	2.00-2.49	20.00
4	2.50-2.99	3.33

Table 6d. Angle of laterals

Group No.	Angle (°)	Frequency (%)
1	60.00-64.99	3.33
2	65.00-69.99	3.33
3	70.00-74.99	6.66
4	75.00-79.99	26.66
5	80.00-84.99	33.33
6	85.00-89.99	23.33
7	90.00-94.99	3.33

Table 6e. Lateral production per 1 m^2 area

Group No.	Lateral production (No.)	Frequency (%)
1	5.00-9.99	23.33
2	10.00-14.99	43.33
3	15.00-19.99	20.00
4	20.00-24.99	13.33

Angle of laterals ranged in between 63.00 to 93.00° and accordingly the laterals were grouped as semierect and drooping. The rate of lateral branch production per 1 m^2 area varied in between 5 to 24 (Fig.3). The highest number of laterals (15-24) was observed in 33.33 per cent of calliclones while in 43.33 per cent of calliclones, it ranged between 10 to 14 and in 23.33 per cent it ranged between 5 to 9. Hairiness was absent in lateral branches of all calliclones.

4.1.6 Stolon

The number of clinging roots was found to be in between 3.0 to 8.8 (Table 7). Fifty per cent of calliclones showed adventitious roots in the range of 5.0 to 6.4 while in 43.33 per cent of calliclones it ranged from 3.0 to 4.8 and in 6.66 per cent from 7.1 to 8.8. Holding ability of adventitious roots was found to be good in all the calliclones studied. The rate of runner shoot production showed wide variations in the calliclones, the number ranged in between 3 to 17. High rate of runner shoot production (16-17) was observed in 6.66 per cent of calliclones while in 30 per cent of the clones the rate observed was low (3-5). Rate of runner production showed a high coefficient of variation of 43.29 and high standard deviation of 8.43 (Fig.4).

4.1.7 Spike/flower

From the results presented in Table 8, it could be seen that the calliclones varied widely in the number of spikes produced. The number of spikes per lateral varied between 4.00 to 13.40. The spike production was found to be high (13.40) in 3.33 per cent of calliclones while it ranged from 7.00 to 11.40 in 36.66 per cent of calliclones (Fig.5).

The length of spike at flowering ranged from 3.22 to 6.80 cm. Thirty per cent of calliclones exhibited the maximum length of 6.18 to 6.80 cm while 16.66 per cent of calliclones exhibited the minimum length of 3.22 to 3.84 cm.

Fig.3. Variability in lateral branch production in different calliclones of black pepper (*P. nigrum* L.)

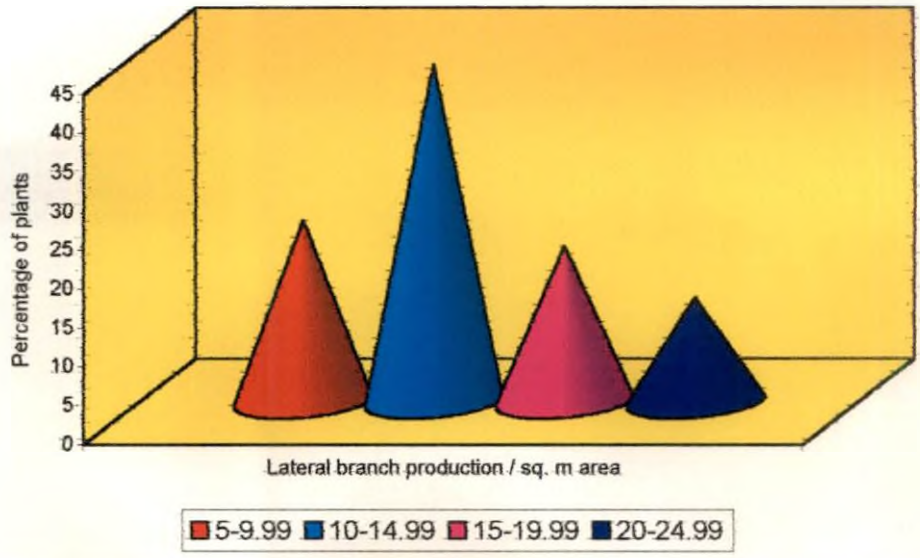


Fig.4. Variability in runner production in different calliclones of black pepper (*P. nigrum* L.)

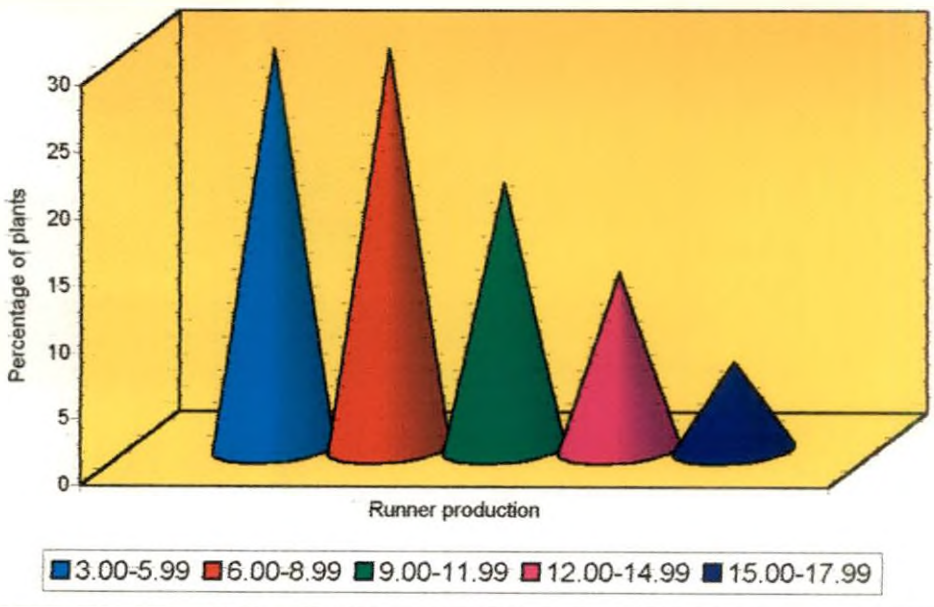


Table 7. Stolon characters in different calliclones of black pepper (*P. nigrum.* L.)

Sl. No.	Calliclone No.	No. of adventitious roots	Holding ability	No. of runner shoots
1	CC 4	3.8	Good	13
2	CC 8	3.2	,,	9
3	CC 9	3.2	,,	11
4	CC 10	4.4	,,	5
5	CC 12	4.8	,,	13
6	CC 23	3.4	,,	16
7	CC 25	4.4	,,	10
8	CC 27	4.4	,,	8
9	CC 28	4.8	,,	10
10	CC 31	6.2	,,	5
11	CC 33	3.2	,,	8
12	CC 35	5.0	,,	3
13	CC 36	6.0	,,	17
14	CC 37	7.1	,,	11
15	CC 38	6.0	,,	5
16	CC 40	5.4	,,	5
17	CC 41	4.8	,,	8
18	CC 43	5.8	,,	4
19	CC 45	6.4	,,	6
20	CC 46	6.0	,,	13
21	CC 55	6.0	,,	5
22	CC 56	5.6	,,	8
23	CC 57	5.8	,,	6
24	CC 58	8.8	,,	5
25	CC 60	6.2	,,	7
26	CC 61	5.8	,,	13
27	CC 62	5.4	,,	6
28	CC 64	5.8	,,	5
29	CC 65	4.4	,,	10
30	CC 69	3.0	,,	8
	SD	1.304		8.43
	CV	25.22		43.29

Frequency distribution for stolon characters in calliclones of black pepper (*P.nigrum* L.)

Table 7a. Number of adventitious root

Group No.	Adventitious root (No.)	Frequency (%)
1	3.00-4.99	43.33
2	5.00-6.99	50.00
3	7.00-8.99	6.66

Table 7b. Rate of runner production

Group No.	Runner production (No.)	Frequency (%)
1	3.00-5.99	30.00
2	6.00-8.99	30.00
3	9.00-11.99	20.00
4	12.00-14.99	13.33
5	15.00-17.99	6.66

The nature of flowering in calliclones was graded as profuse, medium and low (Plate 6). Majority of calliclones (66.66%) exhibited profuse flowering while 26.66 per cent and 6.66 per cent of clones exhibited medium and low flowering respectively.

4.1.8 Flowering habit

All the calliclones studied produced both hermaphrodite and pistillate flowers (Table 8). The variation observed for the percentage of hermaphrodite flowers was found to be very low and all calliclones produced hermaphrodite flowers in the range of 93.51 to 97.72. Very high percentage of hermaphrodite flowers (96.03-97.72%) was observed in 73.33 per cent of calliclones. In the different calliclones studied, the pistillate flowers ranged between 2.28 to 6.50 per cent. The number of stigmatic lobes was four in the flowers of all calliclones and stigma was receptive for 5 to 7 days (Plate 3). The pollen fertility percentage ranged in between 83.00 to 100.00 in the calliclones studied and 80 per cent of the clones exhibited a high fertility percentage of 95.00 to 99.50 (Plate 4 and 5).

4.1.9 Spike/fruit characters

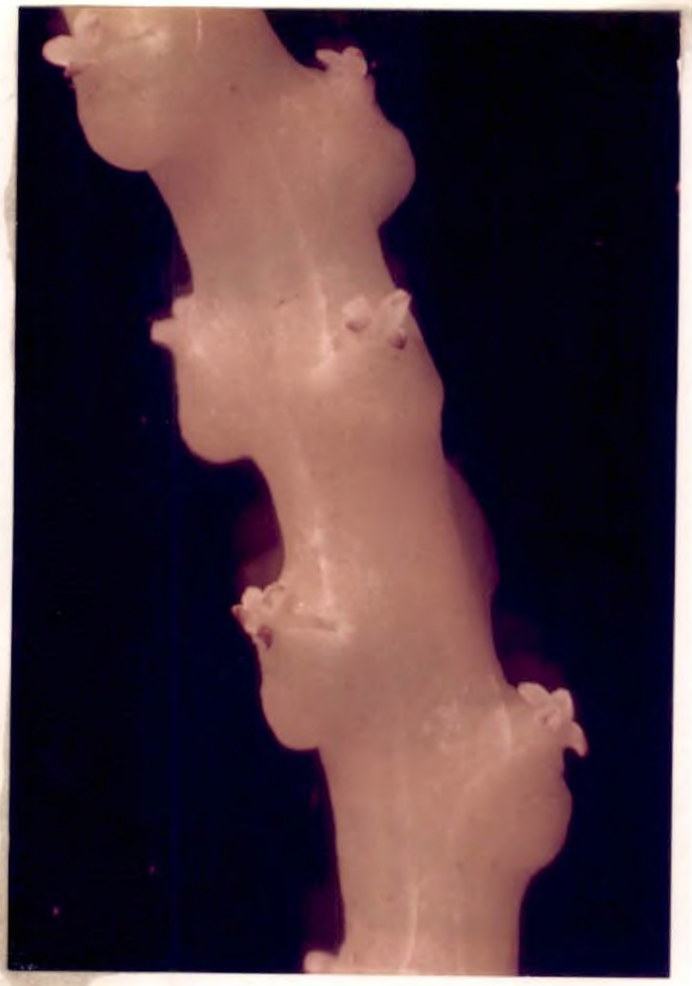
The different spike characters such as spike length, berry colour, berry shape, time taken to maturity, number of berries per spike, berry weight and volume, mean spike/berry ratio from 25 spikes and seed weight and volume are presented in Table 9.

4.1.9.1 Spike length

The average spike length ranged between 4.00 to 7.60 cm (Plate 7). The maximum spike length of 7.10 to 7.60 cm was observed in 6.66 per cent of calliclones, and the spike length ranged from 4.00 to 6.96 cm in the rest of the calliclones.

Plate 3. Stigma at the receptive stage (10 x)

Plate 4. Anthers at the dehising stage (10 x)



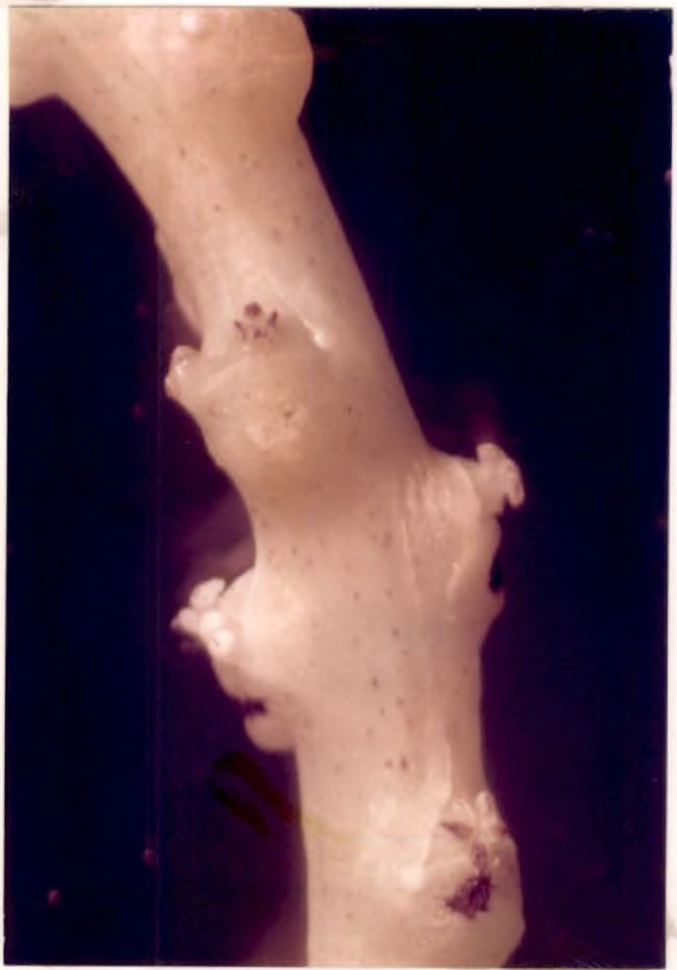


Plate 5. Viable pollen grains of calliclones of black pepper (50 x)

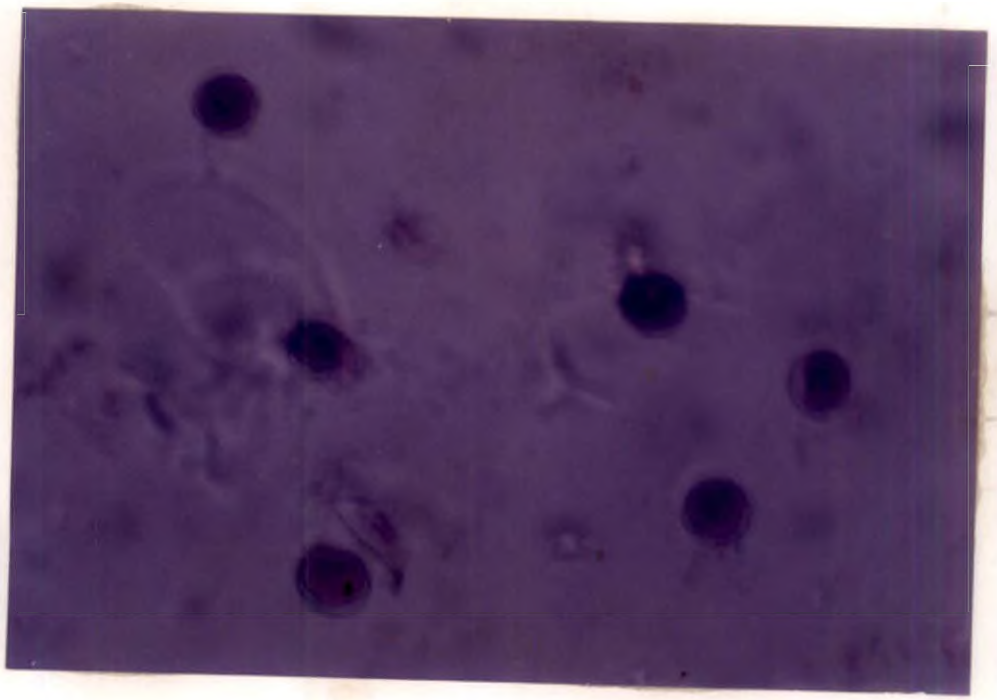


Plate 6. Variability in number of spikes /unit area in different calliclones of black pepper


CC 60


CC 57


CC 27

Table 8. Spike and flower characters in different calliclones of black pepper (*P. nigrum* L.)

Sl. No.	Calliclone No.	Mean number of spikes per lateral	Mean length of spike (cm)	No. of spikes / 50 cm^2	Nature of flowering	Hermaphrodite flowers (%)	Pistillate flowers (%)	No. of stigmatic lobes	Receptivity of stigma (days)	Fertile pollen (%)
1	CC 4	8.20	6.60	108	Profuse	97.54	2.46	4	5-7	83.00
2	CC 8	11.40	5.96	32	Medium	93.90	6.10	,,	,,	98.23
3	CC 9	9.80	5.50	46	Profuse	94.51	5.50	,,	,,	96.85
4	CC 10	4.20	5.18	29	Medium	95.68	4.32	,,	,,	97.00
5	CC 12	7.40	4.54	34	,,	93.94	6.06	,,	,,	95.00
6	CC 23	6.40	6.70	76	Profuse	96.58	3.42	,,	,,	96.00
7	CC 25	6.80	5.60	68	,,	97.62	2.38	,,	,,	96.00
8	CC 27	5.20	5.32	56	,,	96.92	3.07	,,	,,	98.00
9	CC 28	5.60	5.18	38	Medium	96.28	3.72	,,	,,	97.50
10	CC 31	6.00	3.80	38	,,	96.03	3.97	,,	,,	98.00
11	CC 33	7.60	6.74	58	Profuse	96.43	3.57	,,	,,	97.00
12	CC 35	4.00	5.28	6	Low	96.91	3.09	,,	,,	96.80
13	CC 36	6.40	5.64	55	Profuse	96.75	3.25	,,	,,	97.50
14	CC 37	5.80	5.00	71	,,	97.16	2.84	,,	,,	98.00
15	CC 38	6.80	4.76	76	,,	96.48	3.52	,,	,,	98.00
16	CC 40	5.40	3.84	60	,,	96.48	3.52	,,	,,	95.50
17	CC 41	6.52	4.78	48	,,	94.61	5.38	,,	,,	96.30
18	CC 43	5.60	6.58	29	Medium	97.10	2.90	,,	,,	92.25
19	CC 45	6.00	6.18	39	Profuse	97.02	2.98	,,	,,	94.50
20	CC 46	13.40	4.08	90	,,	95.33	4.66	,,	,,	99.50
21	CC 55	6.60	4.14	37	Medium	97.38	2.62	,,	,,	100.00
22	CC 56	7.20	6.74	104	Profuse	97.22	2.78	,,	,,	98.25
23	CC 57	10.20	6.42	98	,,	97.58	2.42	,,	,,	97.00
24	CC 58	5.40	3.58	98	,,	93.51	6.50	,,	,,	99.00
25	CC 60	8.60	6.72	74	,,	97.70	2.30	,,	,,	96.40
26	CC 61	7.20	6.80	62	,,	97.25	2.75	,,	,,	95.23
27	CC 62	6.80	3.22	68	,,	97.16	2.84	,,	,,	93.25
28	CC 64	6.60	3.36	38	Medium	95.24	4.76	,,	,,	98.00
29	CC 65	7.80	5.24	74	Profuse	97.72	2.28	,,	,,	92.50
30	CC 69	7.00	5.32	12	Low	93.51	2.75	,,	,,	97.50
	SD	2.013	1.11			1.232	1.232			3.126
	CV	28.50	21.03			1.278	34.03			3.25

Frequency distribution for spike and flower characters in calliclones of black pepper (*P.nigrum* L.)

Table 8a. Number of spikes per branch

Group No.	Spikes per branch (No.)	Frequency (%)
1	4.00-6.99	60.00
2	7.00-9.99	30.00
3	10.00-12.99	6.66
4	13.00-15.99	3.33

Table 8b. Length of spike

Group No.	Length (cm)	Frequency (%)
1	3.00-3.99	16.66
2	4.00-4.99	16.66
3	5.00-5.99	36.66
4	6.00-6.99	30.00

Table 8c. Percentage of hermaphrodite flowers in a spike

Group No.	Hermaphrodite flowers (%)	Frequency (%)
1	93.00-93.99	10.00
2	94.00-94.99	6.66
3	95.00-95.99	10.00
4	96.00-96.99	30.00
5	97.00-97.99	43.33

Table 8d. Percentage of pistillate flowers in a spike

Group No.	Pistillate flowers (%)	Frequency (%)
1	2.00-2.99	43.33
2	3.00-3.99	30.00
3	4.00-4.99	10.00
4	5.00-5.99	6.66
5	6.00-6.99	10.00

Table 8e. Percentage of fertile pollen

Group No.	Fertile pollen (%)	Frequency (%)
1	80.00-84.99	3.33
2	85.00-89.99	0.00
3	90.00-94.99	13.33
4	95.00-99.99	80.00
5	100-104.99	3.33

Table. 9. Spike/fruit characters in different calliclones of black pepper (*P. nigrum* L.)

Calli-clone No.	Shape of berry	Colour of berries		Time taken to maturity (months)	Spike length (cm)			Number of berries / spike			Well developed berries (%)			Under developed berries (%)			Mean spike/berry of 25 spikes		
		Young	Mature		1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean
CC 4	Globose	Green	Dark green	6	3.02	6.41	4.72	15.4	42.8	29.10	82.80	93.00	87.90	17.58	7.82	12.70	1.20	1.08	1.14
CC 8	,,	,,	,,	6	2.97	7.62	5.30	24.80	35.40	30.10	89.70	88.22	88.96	10.40	11.68	11.04	1.25	1.15	1.20
CC 9	,,	,,	,,	5½	5.38	8.23	6.81	30.20	37.20	33.70	91.74	84.20	87.97	7.50	15.72	11.61	1.06	1.60	1.11
CC 10	,,	,,	,,	5½	2.56	5.75	4.16	17.80	33.60	25.70	83.20	93.07	88.13	16.60	8.24	12.42	-	1.15	1.15
CC 12	,,	,,	,,	5½	3.75	6.14	4.95	21.80	27.00	24.40	79.80	92.50	86.15	20.10	7.32	13.71	1.10	1.14	1.12
CC 23	,,	,,	,,	6	5.37	6.40	5.89	21.60	30.00	25.80	85.68	97.24	91.46	14.26	2.72	8.49	1.16	1.18	1.17
CC 25	,,	,,	,,	6	5.23	5.66	5.45	31.20	3.160	31.40	86.40	95.60	91.00	13.50	4.34	8.92	1.18	1.62	1.40
CC 27	,,	,,	,,	6	5.41	7.10	6.23	27.40	33.00	30.20	89.40	92.04	90.72	10.80	7.94	9.37	1.20	1.11	1.15
CC 28	,,	,,	,,	6	5.28	4.50	4.89	31.20	31.40	31.30	91.30	98.56	94.93	8.60	1.54	5.06	1.20	1.22	1.21
CC 31	,,	,,	,,	5½	3.50	4.50	4.00	21.20	37.33	29.26	89.80	90.00	89.90	10.10	9.90	10.00	1.33	1.13	1.23
CC 33	,,	,,	,,	5½	8.00	6.20	7.10	47.00	41.20	44.10	89.96	94.96	92.46	10.05	4.78	7.41	1.14	1.18	1.16
CC 35	,,	,,	,,	5½	4.00	4.70	4.35	17.00	27.60	22.30	93.50	97.80	95.65	6.50	2.14	4.32	-	1.25	1.25
CC 36	,,	,,	,,	5½	6.33	8.86	7.60	38.00	42.80	40.40	92.18	83.28	87.73	7.82	16.72	12.27	1.14	1.18	1.16
CC 37	,,	,,	,,	5½	6.73	7.19	6.96	37.40	39.40	38.40	88.60	89.65	89.13	11.44	10.41	10.93	1.06	1.14	1.10
CC 38	,,	,,	,,	5½	5.45	7.33	6.39	34.00	34.00	34.00	94.10	87.47	90.79	5.90	9.76	7.83	1.13	1.12	1.13
CC 40	,,	,,	,,	6	6.51	4.25	5.38	34.80	21.00	27.90	86.04	99.00	92.52	13.92	1.00	7.46	1.17	1.10	1.14
CC 41	,,	,,	,,	6	6.38	6.35	6.37	41.20	36.40	38.80	89.60	97.16	93.38	10.40	2.78	6.59	1.08	1.12	1.10
CC 43	,,	,,	,,	6	5.12	5.80	5.46	28.20	34.20	31.20	88.40	98.38	93.38	11.54	1.66	6.60	1.10	1.15	1.13
CC 45	,,	,,	,,	5½	5.54	6.10	5.82	27.20	38.75	30.48	93.02	83.61	88.32	6.94	16.33	11.63	1.07	1.15	1.11
CC 46	,,	,,	,,	6	4.89	5.70	5.30	31.8	41.60	36.70	92.34	89.02	90.68	7.50	9.36	8.43	1.08	1.09	1.09
CC 55	,,	,,	,,	6	4.83	4.50	4.66	24.00	28.00	26.00	83.14	93.47	88.30	16.80	6.49	11.65	1.12	1.11	1.12
CC 56	,,	,,	,,	6	5.17	7.17	6.17	28.20	43.00	35.60	86.80	86.37	86.59	13.30	13.55	13.43	1.20	1.11	1.15
CC 57	,,	,,	,,	5½	5.81	4.05	4.93	28.60	20.60	24.60	93.60	91.80	92.70	6.98	8.20	7.59	1.09	1.10	1.10
CC 58	,,	,,	,,	6	4.50	6.11	5.31	34.8	37.00	35.90	87.96	87.60	87.78	12.00	12.47	12.23	1.08	1.03	1.06
CC 60	,,	,,	,,	5½	6.38	7.01	6.70	33.40	42.60	38.00	90.30	86.76	88.53	9.80	13.16	11.48	1.15	1.09	1.12
CC 61	,,	,,	,,	5½	6.10	6.53	6.31	36.00	35.60	35.80	92.50	96.77	94.63	7.50	3.21	5.36	1.15	1.14	1.15
CC 62	,,	,,	,,	5½	6.80	7.09	6.95	34.00	35.60	34.80	92.90	95.37	94.14	7.06	4.57	5.82	1.12	1.14	1.13
CC 64	,,	,,	,,	5½	5.28	4.07	4.68	28.80	25.20	27.00	94.80	97.84	96.32	5.14	2.14	3.64	1.07	1.15	1.11
CC 65	,,	,,	,,	5½	5.40	6.10	5.75	35.8	27.00	31.40	94.12	97.32	95.72	5.82	2.66	4.24	1.14	1.16	1.15
CC 69	,,	,,	,,	5½	4.11	6.76	5.43	18.60	41.20	28.90	81.90	91.66	86.78	18.10	8.28	13.19	1.25	1.06	1.16
SD							0.938			5.44			3.030			19.09			0.064
CV							16.55			16.76			3.378			144.67			5.57

Contd.

Table 9. Spike/fruit characters in different calliclones of black pepper (*P. nigrum* L.) continued

Calli-clone No.	Weight of 100 fresh berries (g)			Weight of 100 dried berries (g)			Volume of 100 fresh berries (cc)			Volume of 100 dried berries (cc)			Mean of FB/DB ratio of 100 berries			Peri-carp thick-ness (mm)	100 Seed	
	1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean		Wt. (g)	Vol. (cc)
CC 4	9.00	12.75	10.88	6.50	6.10	6.30	9	10	9.50	3	4.0	3.50	1.38	2.09	1.74	0.760	4.94	3.0
CC 8	9.00	11.77	10.39	5.80	5.60	5.70	7	10	8.50	3	4.0	3.50	1.55	2.10	1.82	0.674	5.23	4.0
CC 9	12.00	11.54	11.77	7.00	5.10	6.05	9	10	9.50	4	4.5	4.25	1.71	2.26	1.99	0.683	4.60	3.0
CC 10	10.20	12.27	11.23	6.20	6.30	6.25	7	10	8.50	3	4.0	3.50	1.64	1.95	1.80	0.651	5.50	4.0
CC 12	11.00	10.84	10.92	6.10	4.80	5.45	9	8	8.50	4	3.0	3.50	1.80	2.26	2.03	0.766	4.47	2.0
CC 23	9.90	11.27	10.59	5.40	5.30	5.35	6	9	7.50	3	2.5	2.75	1.83	2.13	1.98	0.663	4.70	3.0
CC 25	10.90	11.86	11.38	5.60	6.00	5.80	8	10	9.00	3	4.0	3.50	1.95	1.98	1.96	0.649	5.10	2.5
CC 27	11.90	12.19	12.05	6.50	6.30	6.40	9	8	8.50	4	4.0	4.00	1.83	1.93	1.88	0.836	4.80	2.5
CC 28	10.50	12.17	11.34	5.80	6.10	5.95	8	9	8.50	4	3.5	3.75	1.81	1.99	1.90	0.711	4.90	3.0
CC 31	11.90	10.96	11.43	6.80	5.10	5.95	9	10	9.50	4	4.0	4.00	1.75	2.15	1.95	0.754	4.70	4.0
CC 33	13.00	11.89	12.45	6.10	5.30	5.70	10	10	10.0	4	4.0	4.00	2.13	2.24	2.19	0.643	4.70	2.5
CC 35	11.10	11.81	11.46	6.00	6.30	6.15	8	8	8.00	4	4.0	4.00	1.85	1.87	1.86	0.669	5.05	3.5
CC 36	12.00	12.00	12.00	5.40	6.30	5.85	10	10	10.00	4	4.5	4.25	2.22	1.90	2.06	0.750	4.40	2.5
CC 37	13.30	10.43	11.87	6.40	5.20	5.80	11	10	10.50	5	3.5	4.25	2.08	2.00	2.04	0.806	4.60	3.0
CC 38	12.10	11.97	12.04	5.70	6.00	5.85	9	10	9.50	3	4.0	3.50	2.12	1.99	2.06	0.648	4.70	3.0
CC 40	11.63	13.63	12.63	5.70	6.90	6.30	8	11	9.50	4	5.0	4.50	2.04	1.97	2.00	0.709	5.50	3.5
CC 41	10.66	11.17	10.91	6.30	6.10	6.20	8	8	8.00	3	4.0	3.50	1.69	1.83	1.76	0.669	5.20	3.0
CC 43	11.03	12.31	11.67	6.40	6.30	6.35	8	9	8.50	2	4.5	3.25	1.72	1.95	1.84	0.778	4.60	2.0
CC 45	11.70	10.20	10.95	5.50	4.70	5.10	9	10	9.50	4	3.0	3.50	2.12	2.17	2.14	0.852	4.80	3.0
CC 46	11.60	8.98	10.29	5.90	4.30	5.10	9	9	9.00	4	4.0	4.00	1.96	2.09	2.03	0.729	4.95	4.0
CC 55	9.30	11.20	10.25	4.00	6.20	5.10	8	10	9.00	3	4.0	3.50	2.32	1.80	2.07	0.717	4.60	3.0
CC 56	11.00	9.83	10.41	5.70	4.60	5.15	8	10	9.00	4	4.0	4.00	1.93	2.16	2.04	0.739	5.12	3.0
CC 57	12.00	12.35	12.18	4.60	5.40	5.00	9	10	9.50	4	3.5	3.25	2.60	2.20	2.4	0.679	4.60	3.0
CC 58	10.30	11.54	10.92	5.40	5.50	5.45	7	12	9.50	2	5.5	3.75	1.91	2.09	2.00	0.649	4.92	3.0
CC 60	11.15	13.17	12.16	5.90	6.00	5.95	9	14	11.50	4	5.0	4.50	1.89	2.19	2.04	0.663	4.90	2.0
CC 61	11.00	12.28	11.64	5.50	6.10	5.80	8	10	9.00	2	4.0	4.00	2.00	2.01	2.00	0.750	5.10	2.0
CC 62	9.79	9.72	9.75	5.30	4.80	5.05	7	8	7.50	4	3.5	3.25	1.85	2.02	1.93	0.667	4.99	2.0
CC 64	12.60	11.86	12.23	6.30	5.60	5.95	10	9	9.50	4	4.0	4.00	1.07	1.15	1.11	0.688	4.90	2.50
CC 65	13.50	11.87	12.68	6.90	5.80	6.35	11	10	10.50	5	3.5	4.25	1.96	2.05	2.00	0.670	5.21	3.0
CC 69	11.00	11.77	11.39	5.70	5.50	5.60	9	8	8.50	3	4.5	3.75	1.9	2.14	2.02	0.659	4.60	2.5
SD			0.765			0.442			1.46			0.432			0.207	0.069	0.282	0.607
CV			6.71			7.66			15.53			11.58			10.56	9.6	5.78	20.93

Frequency distribution for fruit/spike characters in calliclones of black pepper (*P.nigrum* L.)

Table 9a. Spike length

Group No.	Length (cm)	Frequency (%)
1	4.00-4.99	30.00
2	5.00-5.99	33.33
3	6.00-6.99	30.00
4	7.00-7.99	6.66

Table 9b. Total number of berries per spike

Group No.	Number of berries	Frequency (%)
1	22.00-26.99	20.00
2	27.00-31.99	36.66
3	32.00-36.99	23.33
4	37.00-41.99	16.66
5	42.00-46.99	3.33

Table 9c. Percentage of well developed berries per spike

Group No.	Well developed berries (%)	Frequency (%)
1	85.00-89.99	46.66
2	90.00-94.99	43.33
3	95.00-99.99	10.00

Table 9d. Percentage of under developed berries per spike

Group No.	Under developed berries (%)	Frequency (%)
1	3.00-5.99	16.66
2	6.00-8.99	26.66
3	9.00-11.99	23.33
4	12.00-14.99	30.00

Table 9e. Spike berry ratio

Group No.	Spike berry ratio	Frequency (%)
1	1.00-1.09	6.66
2	1.10-1.19	76.66
3	1.20-1.29	13.33
4	1.30-1.39	0.00
5	1.40-1.49	3.33

Table 9f. Weight of 100 fresh berries

Group No.	Weight (g)	Frequency (%)
1	9.50-10.49	16.66
2	10.50-11.49	40.00
3	11.50-12.49	36.66
4	12.50-13.49	6.66

Table 9g. Weight of 100 dry berries

Group No.	Weight (g)	Frequency (%)
1	5.00-5.49	30.00
2	5.50-5.99	40.00
3	6.00-6.49	30.00

Table 9h. Volume of 100 fresh berries

Group No.	Volume (cc)	Frequency (%)
1	7.00-7.99	6.66
2	8.00-8.99	30.00
3	9.00-9.99	46.66
4	10.00-10.99	13.33
5	11.00-11.99	3.33

Table 9i. Volume of 100 dried berries

Group No.	Volume (cc)	Frequency (%)
1	2.50-2.99	3.33
2	3.00-3.49	13.33
3	3.50-3.99	40.00
4	4.00-4.49	36.66
5	4.50-4.99	6.66

Table 9j. Mean fresh/dry berry ratio

Group No.	Mean fresh/dry berry ratio	Frequency (%)
1	1.00-1.49	3.33
2	1.50-1.99	43.33
3	2.00-2.49	53.33

Table 9k. Pericarp thickness

Group No.	Pericarp thickness (mm)	Frequency (%)
1	0.600-0.699	53.33
2	0.700-0.799	36.66
3	0.800-0.899	10.00

Table 9l. 100 seed weight

Group No.	Weight (g)	Frequency (%)
1	4.00-4.99	6.66
2	4.50-4.99	63.33
3	5.00-5.49	23.33
4	5.50-5.99	6.66

Table 9m. 100 seed volume

Group No.	Volume (cc)	Frequency (%)
1	2.00-2.49	16.66
2	2.50-2.99	20.00
3	3.00-3.49	43.33
4	3.50-3.99	6.66
5	4.00-4.49	13.33

4.1.9.2 Berry colour

The colour of the young berries was green, mature berries was dark green and that of ripe berries was orange red in all the calliclones. Shape of the berry was globose in all the clones.

4.1.9.3 Time taken to maturity

Time taken to maturity was 5½ to 6 months from fruit set to maturity in all the calliclones studied.

4.1.9.4 Number of berries per spike

The total number of berries per spike varied between 22.30 to 44.10 (Plate 8). The highest number (44.10) was observed in 3.33 per cent of calliclones while in 36.66 per cent of clones the number ranged from 27.00 to 31.40. Percentage of well developed berries ranged from 86.15 to 96.32 in the different calliclones studied. Ten per cent of calliclones showed a very high percentage of well developed berries (95.65-96.32) while in the rest of the clones it ranged from 86.15 to 94.93 per cent. The percentage of under developed berries ranged from 3.64 to 13.71 in the different calliclones studied.

4.1.9.5 Berry weight and volume

Weight of 100 fresh berries varied in between 9.75 to 12.68 g. The highest berry weight of 12.63 to 12.68 was observed in 6.66 per cent of calliclones while in 76.66 per cent of the clones it ranged from 10.59 to 12.45.

Weight of 100 dry berries varied from 5.00 to 6.40 g. The maximum dry berry weight of 6.05 to 6.40 g was observed in 30 per cent calliclones and it ranged from 5.00 to 5.95 g in the rest of the clones.

Volume of 100 fresh berries varied from 7.50 to 11.50 cc and that of dry berries from 2.75 to 4.50 cc. In 16.66 per cent of calliclones studied, the volume

Plate 7. Variability in spike length

Plate 8. Variability in fruit set (%)


CC-57
CC-33


CC-64
CC-31
CC-33

was found to be high (10.00-11.50) and it ranged from 8.00 to 9.50 cc in 76.66 per cent of the clones. The volume of 100 dry berries was found to be low (2.75-3.25) in 16.66 per cent of calliclones and it ranged from 3.50 to 4.25 in 76.66 per cent of the clones studied.

The ratio of mean 100 fresh to dry berry ranged in between 1.11 to 2.40.

4.1.9.6 Mean spike/berry ratio from 25 spikes

The mean spike/berry ratio of 25 spikes varied from 1.06 to 1.40. Majority of calliclones (76.66 per cent) showed a spike-berry ratio of 1.10 to 1.17.

4.1.9.7 Seed weight and volume

The weight and volume of 100 seeds ranged from 4.40 to 5.50 g and 2.00 to 4.00 cc respectively. The seed weight ranged from 4.60 to 5.23 g in 86.66 per cent of calliclones. Variability was found to be high for seed volume with coefficient of variation 20.93 and standard deviation 0.607 and the calliclones were distributed in five frequency classes as shown in Table 9l.

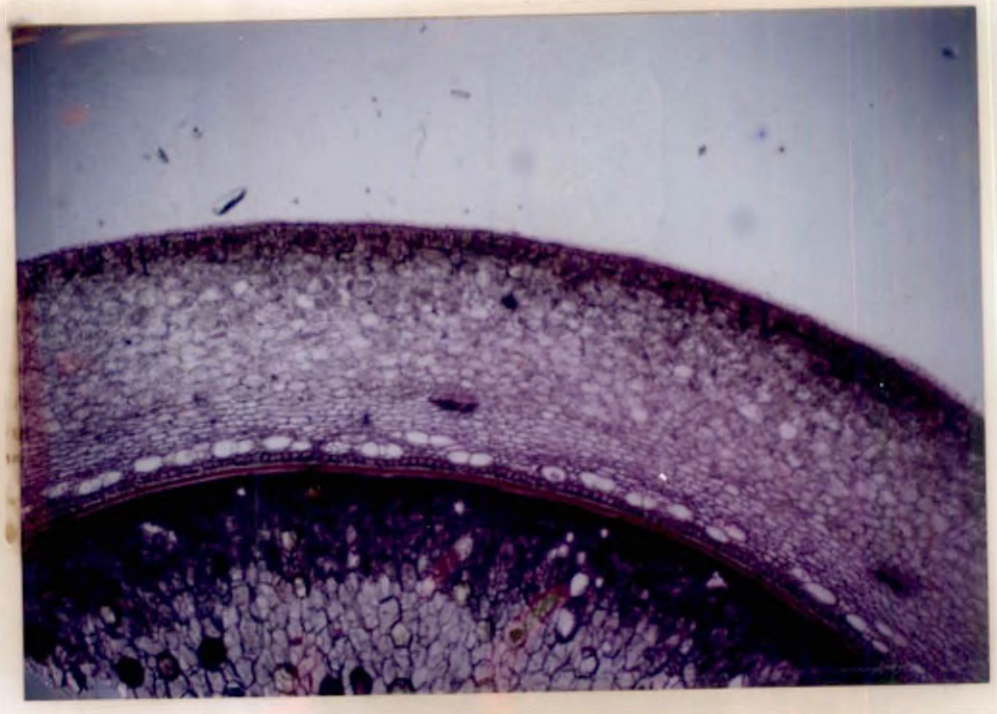
4.1.9.8 Pericarp thickness

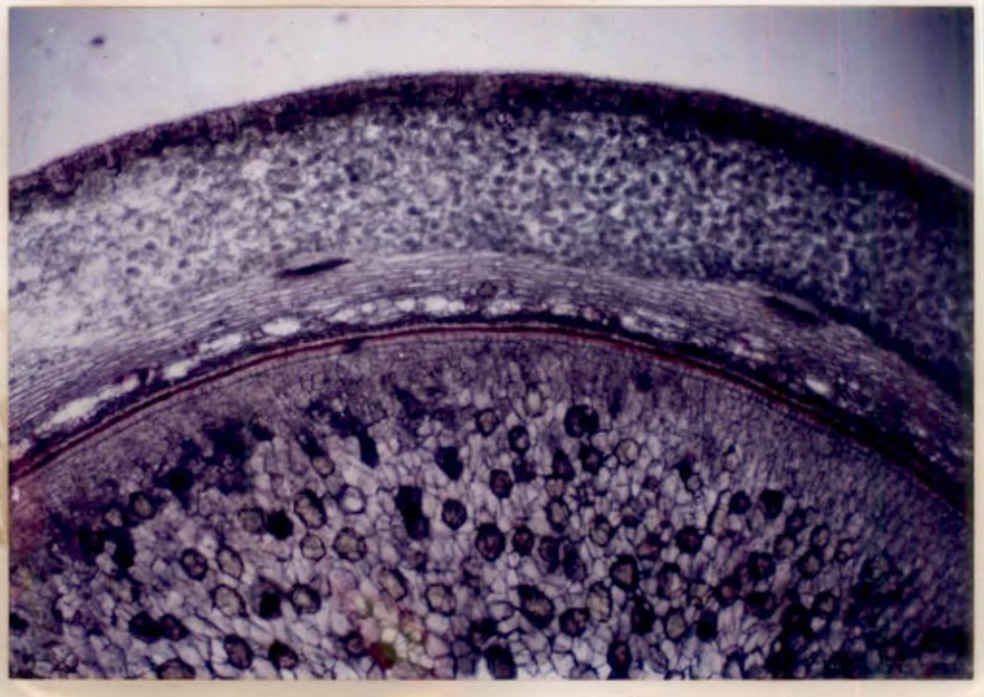
The thickness of pericarp ranged from 0.643 to 0.852 mm. The variation observed for this character was low with a standard deviation of 0.069 and coefficient of variation of 9.6 (Plate 9). Of the clones studied, 53.33 per cent recorded pericarp thickness in the range of 0.643 to 0.688 mm, showing the uniformity of the character studied.

4.2 Yield

The different yield characters such as total spike weight, fresh berry weight, dry berry weight and dry recovery percentage are presented in Table 10.

Plate 9. Uniform pericarp thickness observed in calliclones of black pepper (50 x)





4.2.1 Total spike weight

Total spike weight was found to be in between 75.00 to 1660.00 g. The maximum spike weight of 1595.00 to 1660.00 g was observed in 6.66 per cent of calliclones and the spike yield ranged from 75.00 to 992.50 g in 69.99 per cent of calliclones. The variation was found to be high with a coefficient of variation 58.94 and standard deviation 475.4 (Fig.6).

4.2.2 Total fresh berry weight

Total fresh berry weight varied between 47.50 to 1515.00 g. The maximum berry weight of 1222.50 to 1515.00 g was observed in 16.66 per cent of calliclones and the berry weight ranged from 47.50 to 725.00 in 63.32 per cent of calliclones.

4.2.3 Total dry berry weight

Total dry berry weight showed considerable variation in the different calliclones studied and it ranged from 21.90 to 609.50 g. The coefficient of variation for the character studied was 57.18 per cent and standard deviation was 164.97. The plants could be grouped into five frequency classes as shown in Table 10c.

Maximum dry berry weight (600.00-609.50 g) was recorded by 6.66 per cent of calliclones while 20 per cent of calliclones recorded low dry berry weight of 21.90 to 123.45 g.

4.2.4 Dry recovery

The dry recovery percentage varied in between 38.21 to 54.40 in the different calliclones studied. In 60 per cent of the calliclones, the driage recorded varied in between 40.17 to 44.42 per cent. The highest driage (52.41-54.40) was found in 6.66 per cent of calliclones while 16.66 per cent of clones recorded the lowest driage of 38.21 to 39.84.

Table 10. Yield and dry recovery (%) in different calliclones of black pepper (*P. nigrum* L.)

Sl. No.	Calli-clone No.	Total spike weight (g)			Total berry weight (g)			Total dry berry weight (g)			Dry recovery (%)		
		1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean
1	CC 4	60.00	1085.00	572.50	45.00	950.00	497.50	-*	385.00	205.00	-*	40.50	40.50
2	CC 8	35.00	810.00	422.50	25.00	620.00	322.50	-	305.00	160.65	-	49.19	49.19
3	CC 9	755.00	1230.00	992.50	650.00	980.00	815.00	295.00	425.00	360.00	45.30	43.36	44.33
4	CC 10	25.00	295.00	160.00	20.00	240.00	130.00	-	115.00	63.35	-	49.72	49.72
5	CC 12	155.00	350.00	252.50	130.00	290.00	210.00	-	157.00	107.30	-	54.40	54.40
6	CC 23	65.00	1005.00	535.00	45.00	770.00	407.50	-	320.00	170.00	-	41.35	41.35
7	CC 25	380.00	1090.00	735.00	315.00	925.00	620.00	125.00	390.00	257.50	39.60	42.16	40.88
8	CC 27	735.00	1765.00	1250.00	575.00	1475.00	1025.00	250.00	665.00	457.50	43.00	45.08	44.04
9	CC 28	285.00	680.00	482.50	225.00	555.00	390.00	95.00	245.00	170.00	42.20	44.14	43.17
10	CC 31	55.00	600.00	327.50	35.00	460.00	247.50	-	226.10	123.45	-	49.15	49.15
11	CC 33	720.00	2210.00	1465.50	605.00	1915.00	1260.00	230.00	760.00	495.00	38.80	39.68	39.24
12	CC 35	20.00	130.00	75.00	5.00	90.00	47.50	-	40.00	21.90	-	44.40	44.40
13	CC 36	150.00	1925.00	1037.50	125.00	1600.00	862.50	50.00	685.00	367.50	40.00	42.81	41.41
14	CC 37	660.00	1025.00	842.50	560.00	840.00	700.00	210.00	385.00	297.50	37.50	45.83	41.66
15	CC 38	190.00	1140.00	665.00	155.00	905.00	530.00	57.70	385.00	221.35	37.20	39.22	38.21
16	CC 40	800.00	605.00	702.50	680.00	515.00	597.50	275.00	235.00	255.00	40.44	45.63	43.04
17	CC 41	525.00	1310.00	917.50	450.00	1175.00	812.50	210.00	575.00	392.50	46.60	48.94	47.77
18	CC 43	145.00	775.00	460.00	110.00	650.00	380.00	50.00	285.00	167.50	45.00	43.84	44.42
19	CC 45	295.00	900.00	597.50	245.00	620.00	432.50	-	325.00	207.50	-	52.41	52.41
20	CC 46	130.00	1400.00	765.00	110.00	1140.00	625.00	45.00	510.00	277.50	40.90	44.73	42.82
21	CC 55	120.00	490.00	305.00	90.00	415.00	252.50	41.20	190.00	115.60	45.77	45.78	45.78
22	CC 56	405.00	2550.00	1477.50	345.00	2100.00	1222.50	132.00	870.00	501.00	38.26	41.42	39.84
23	CC 57	355.00	2600.00	1477.50	300.00	2500.00	1400.00	119.00	1100.00	609.50	-	44.00	44.00
24	CC 58	580.00	1200.00	890.00	490.00	960.00	725.00	200.00	435.00	317.50	40.80	45.31	43.06
25	CC 60	590.00	2600.00	1595.00	505.00	2260.00	1382.50	195.00	905.00	550.00	38.60	40.04	39.32
26	CC 61	775.00	1405.00	1090.00	650.00	1330.00	990.00	255.00	565.00	410.00	39.00	42.48	40.74
27	CC 62	1074.00	1485.00	1279.50	905.00	1255.00	1080.00	395.00	555.00	475.00	43.60	41.00	42.30
28	CC 64	360.00	570.00	465.00	310.00	495.00	402.50	125.00	235.00	180.00	40.30	47.00	43.65
29	CC 65	1390.00	1930.00	1660.00	1225.00	1805.00	1515.00	470.00	730.00	600.00	38.30	40.44	39.37
30	CC 69	40.00	640.00	340.00	20.00	560.00	290.00	11.40	225.00	118.20	-	40.17	40.17
	SD			475.40			407.42			164.97			4.025
	CV			58.94			60.59			57.18			9.214

*Total dry berry weight and dry recovery (%) not recorded because of less threshed berry weight

Frequency distribution for yield and dry recovery (%) in calliclones of black pepper (*P.nigrum* L.)

Table 10a. Total spike weight

Group No.	Spike weight (g)	Frequency (%)
1	0-500	33.33
2	500-1000	36.66
3	1000-1500	23.33
4	1500-2000	6.66

Table 10b. Total fresh berry weight

Group No.	Berry weight (g)	Frequency (%)
1	10.00-409.99	36.66
2	410.00-809.99	26.66
3	810.00-1209.99	20.00
4	1210.00-1609.99	16.66

Table 10c. Total dry berry weight

Group No.	Dry berry weight (g)	Frequency (%)
1	0-150	20.00
2	150-300	40.00
3	300-450	16.66
4	450-600	16.66
5	600-750	6.66

Table 10d. Dry recovery (%)

Group No.	Dry recovery (%)	Frequency (%)
1	35.00-39.99	16.66
2	40.00-44.99	60.00
3	45.00-49.99	16.66
4	50.00-54.99	6.66

Fig.5. Variability in number of spikes per lateral in different calliclones of black pepper (*P. nigrum* L.)

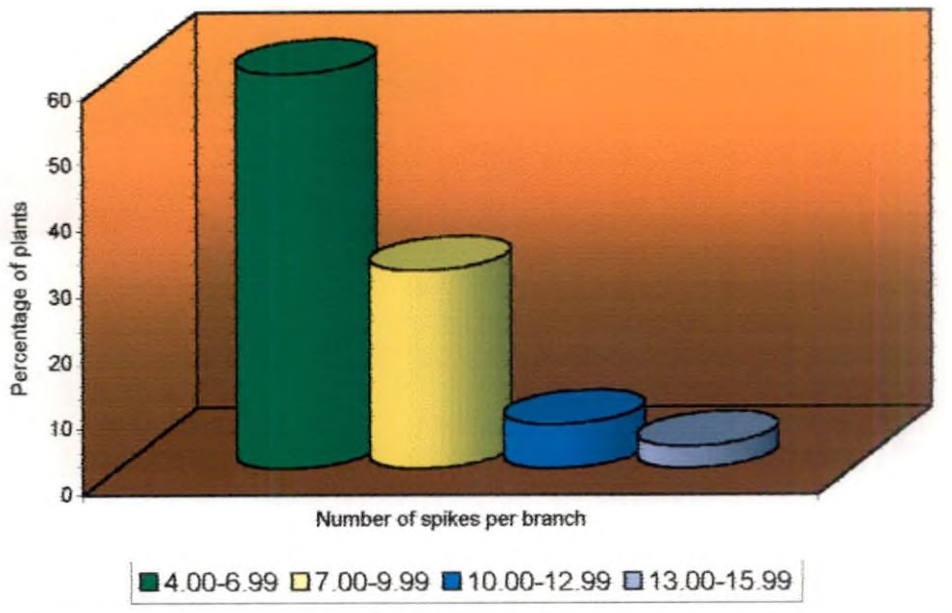
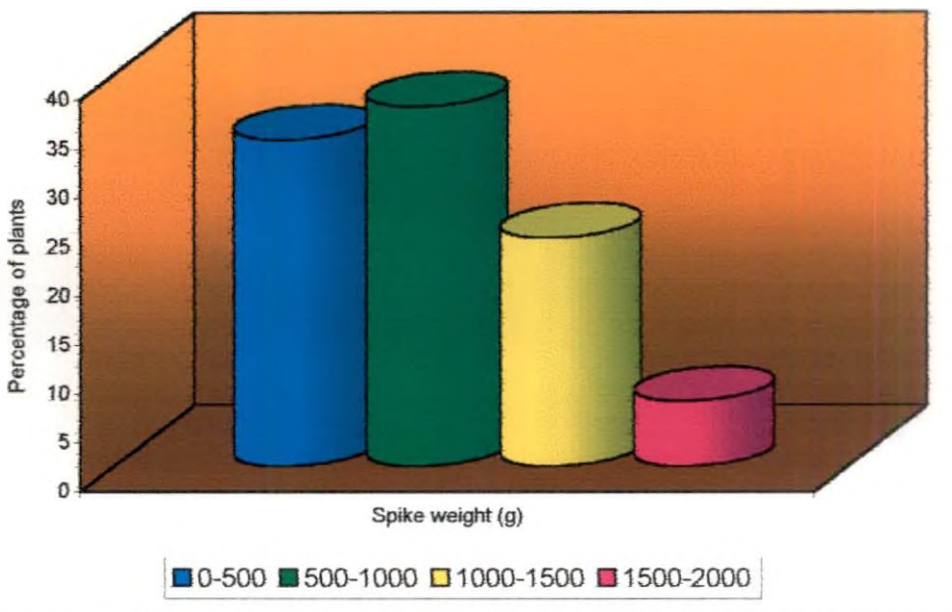


Fig.6. Variability in yield in different calliclones of black pepper (*P. nigrum* L.)



4.3 Quality attributes

Quality parameters like essential oil, oleoresin, piperine and moisture content were studied in different calliclones and the results are presented in Table 11.

4.3.1 Essential oil

The essential oil content varied in between 2.19 to 3.63 per cent in the calliclones studied (Fig.7a). The highest recovery of 3.50 to 3.63 per cent was observed in 6.66 per cent of calliclones while the lowest (2.19-2.46) was observed in 16.66 per cent of calliclones. In the rest of the clones studied the essential oil ranged in between 2.50 to 3.42 per cent.

4.3.2 Oleoresin

Oleoresin content varied in between 8.63 to 12.50 per cent in the calliclones studied. The highest content of 11.02 to 12.50 observed in 16.66 per cent of calliclones while in majority of the clones (56.66%) the content ranged from 9.01 to 9.97 per cent.

4.3.3 Piperine

The content of piperine varied from 3.67 to 6.36 per cent in the calliclones (Fig.7b). The highest content of 6.36 per cent was observed in 3.33 per cent of calliclones while in majority of the clones (60%) the content ranged from 4.12 to 4.99 per cent.

4.3.4 Moisture content

Moisture content ranged from 6.50 to 9.48 per cent in the clones studied. In 13.33 per cent of the clones the content was from 9.19 to 9.48 and in 79.99 per cent it ranged from 7.22 to 8.84.

Table 11. Essential oil, oleoresin, piperine and moisture content in different calliclones of black pepper (*P. nigrum* L.)

Sl. No.	Calli-clone No.	Essential oil (%)			Oleoresin (%)			Piperine (%)			Moisture content (%)		
		1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean	1998-1999	1999-2000	Mean
1	CC 4	2.34	4.50	3.42	11.00	11.78	11.39	3.36	5.61	4.49	8.89	7.12	8.01
2	CC 8	3.00	4.00	3.50	7.00	10.25	8.63	5.87	3.90	4.88	7.77	8.49	8.13
3	CC 9	2.00	3.40	2.70	10.20	10.41	10.31	4.57	5.79	5.18	8.20	7.68	7.94
4	CC 10	2.00	2.58	2.29	9.20	8.81	9.01	4.88	5.03	4.95	6.82	9.61	8.22
5	CC 12	2.80	3.00	2.90	11.13	9.31	10.22	4.60	5.49	5.05	7.55	8.01	7.79
6	CC 23	2.66	3.50	3.08	7.50	11.67	9.59	4.21	4.67	4.44	8.74	8.57	8.66
7	CC 25	2.50	3.75	3.13	7.90	9.30	9.27	5.46	5.30	5.38	8.61	8.97	8.79
8	CC 27	3.60	2.38	2.99	9.20	9.46	9.46	5.04	4.50	4.77	7.53	8.00	7.77
9	CC 28	2.00	2.88	2.44	9.80	9.83	9.82	5.52	3.80	4.66	6.08	8.36	7.22
10	CC 31	1.60	3.25	2.43	8.40	11.00	9.70	4.67	3.69	4.18	9.77	8.79	9.29
11	CC 33	3.60	2.54	3.07	10.20	9.02	9.61	3.52	4.20	3.86	6.49	8.93	7.71
12	CC 35	2.50	3.50	3.00	8.27	9.80	9.04	3.97	3.72	3.85	6.2	8.48	7.34
13	CC 36	1.50	3.50	2.50	11.50	9.20	10.35	5.05	4.23	4.64	8.02	8.13	8.08
14	CC 37	2.80	2.38	2.59	12.60	10.13	11.37	5.69	5.16	5.43	9.95	8.78	9.38
15	CC 38	2.25	4.50	3.38	10.61	10.40	10.51	5.78	4.19	4.99	6.86	8.41	7.64
16	CC 40	3.60	3.13	3.36	10.33	9.60	9.97	3.85	4.73	4.29	6.28	8.63	7.61
17	CC 41	3.00	2.83	2.92	9.70	9.33	9.52	5.23	3.43	4.33	9.31	7.91	8.61
18	CC 43	1.50	4.60	3.05	10.00	10.52	10.26	3.27	4.07	3.67	6.98	8.49	7.74
19	CC 45	1.60	4.00	2.80	10.50	13.00	11.75	5.96	4.45	5.21	6.98	8.76	7.87
20	CC 46	2.00	2.38	2.19	9.46	11.86	10.66	4.41	5.24	4.82	9.33	8.04	8.69
21	CC 55	2.50	3.00	2.75	9.01	9.39	9.20	3.08	5.26	4.17	5.50	9.09	9.19
22	CC 56	3.60	3.00	3.33	11.00	8.82	9.91	6.94	5.78	6.36	8.87	7.85	8.37
23	CC 57	3.00	4.25	3.63	10.53	11.51	11.02	5.54	4.68	5.11	7.86	7.12	7.49
24	CC 58	2.00	3.75	2.88	12.40	8.22	10.31	4.75	4.33	4.54	7.92	11.04	9.48
25	CC 60	2.40	3.90	3.15	11.70	13.29	12.50	4.28	4.43	4.36	6.40	6.60	6.50
26	CC 61	3.20	3.50	3.35	11.19	7.61	9.40	4.33	3.53	3.93	5.77	7.95	6.86
27	CC 62	1.60	3.75	2.68	9.90	8.70	9.30	5.45	4.23	4.84	9.31	8.04	8.68
28	CC 64	1.55	3.38	2.46	10.30	9.50	9.90	4.45	3.78	4.12	8.35	8.98	8.67
29	CC 65	2.70	3.00	2.85	9.50	10.41	9.96	6.02	4.51	5.27	9.00	7.11	8.06
30	CC 69	2.66	3.50	3.08	8.80	9.61	9.21	5.02	4.66	4.84	8.62	9.06	8.84
	SD			0.375			0.888			0.576			0.733
	CV			12.803			8.835			12.28			8.99

Frequency distribution for essential oil, oleoresin, piperine and water content in calliclones of black pepper (*P.nigrum* L.)

Table 11a. Essential oil (%)

Group No.	Essential oil (%)	Frequency (%)
1	2.00-2.49	16.66
2	2.50-2.99	36.66
3	3.00-3.49	40.00
4	3.50-3.99	6.66

Table 11b. Oleoresin (%)

Group No.	Oleoresin (%)	Frequency (%)
1	8.00-8.99	3.33
2	9.00-9.99	56.66
3	10.00-10.99	23.33
4	11.00-11.99	13.33
5	12.00-12.99	3.33

Table 11c. Piperine (%)

Group No.	Piperine (%)	Frequency (%)
1	3.00-3.99	13.33
2	4.00-4.99	60.00
3	5.00-5.99	23.33
4	6.00-6.99	3.33

Table 11d. Moisture content (%)

Group No.	Moisture content (%)	Frequency (%)
1	6.00-6.99	6.66
2	7.00-7.99	36.66
3	8.00-8.99	43.33
4	9.00-9.99	13.33

Fig.7a. Variability in essential oil content in different calliclones of black pepper (*P. nigrum* L.)

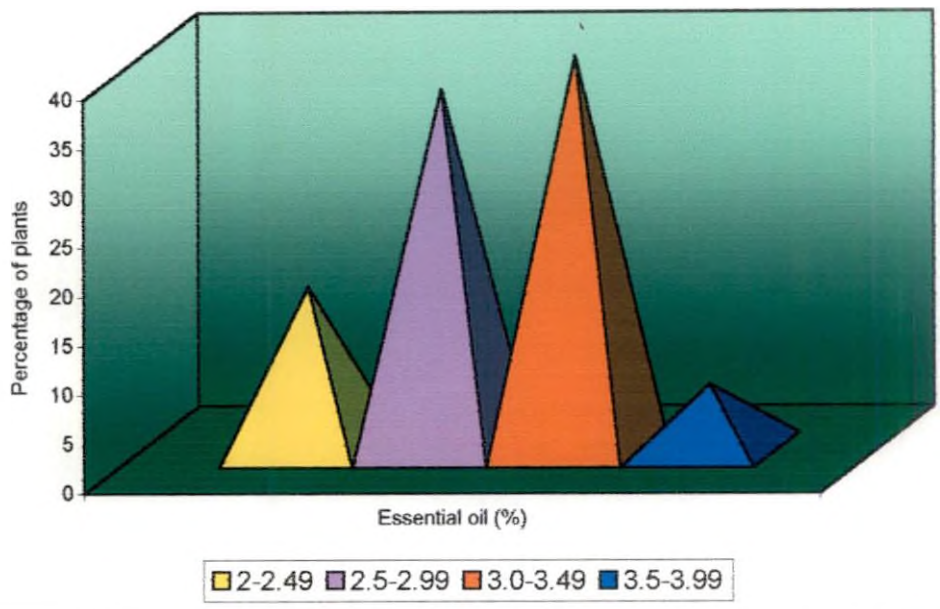
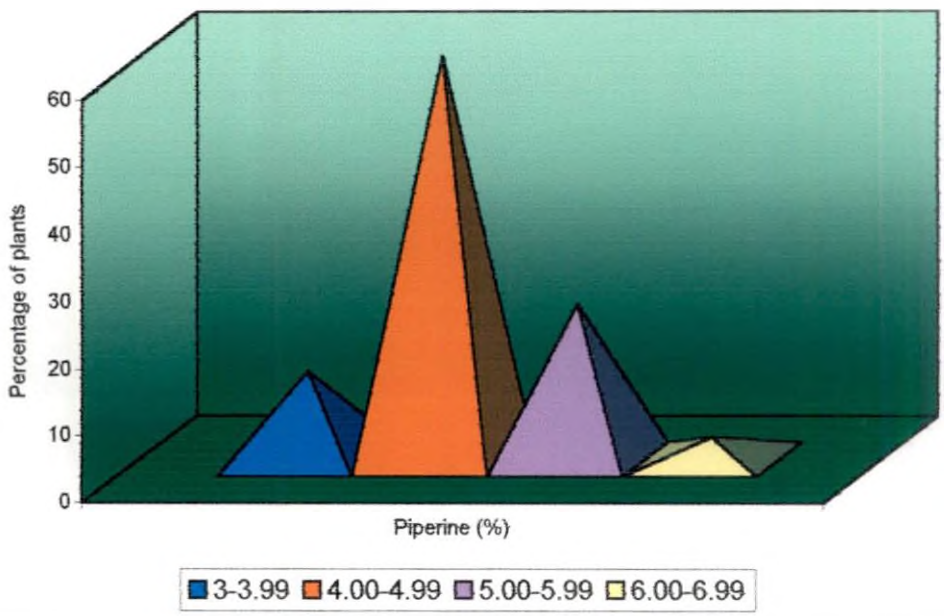


Fig.7b. Variability in piperine content in different calliclones of black pepper (*P. nigrum* L.)



4.4 Clustering of calliclones based on yield and quality attributes

The technique of principal component analysis was carried out based on the economic characters like spike weight, essential oil, oleoresin, piperine and dry recovery percentage to group the calliclones. The first principal component explained 99.99 per cent of the variation. Hence the clustering of genotypes was carried out based on the first principal component scores (Fig.8). The number of clusters based on the scores was found to be ten. The cluster members and the group mean for the characters are presented in Table 12. The calliclones coming in each cluster are uniform for the characters studied.

The path coefficient analysis was carried out based on 20 characters namely total spike weight, berry weight, dry weight, dry recovery, weight of 100 dry berry, essential oil, oleoresin, piperine, number of hermaphrodite flowers, internodal length of orthotrope, L/W ratio of mature leaf, internodal length of laterals, angle of laterals, rate of lateral production, number of spikes per branch, percentage of fertile pollen, percentage of well developed berries, number of berries per spike, spike length and leaf area. The yield or total spike weight was taken as the dependent variable and the rest of the parameters as explanatory variables. The matrix of direct and indirect effects are presented in Table 13, along with the rate/scales of path coefficients (Table 13a). From the results it was found that the berry weight was the only character which has high direct effect on spike weight. Hence the total berry weight was taken into account for computing the numerical distance between the clusters.

The numerical distance could be regarded as a measure of divergence of the clusters. Based on the numerical distance, the matrix of dissimilarity was computed and presented in Table 14. From the matrix of dissimilarity it was evident that the clusters 1 and 10 were more divergent followed by 1 & 9, 2 & 10, 2 & 9 and 3 & 10.

Fig. 8. Clustering of calliclones of black pepper (*P. nigrum* L.) based on yield and quality parameters

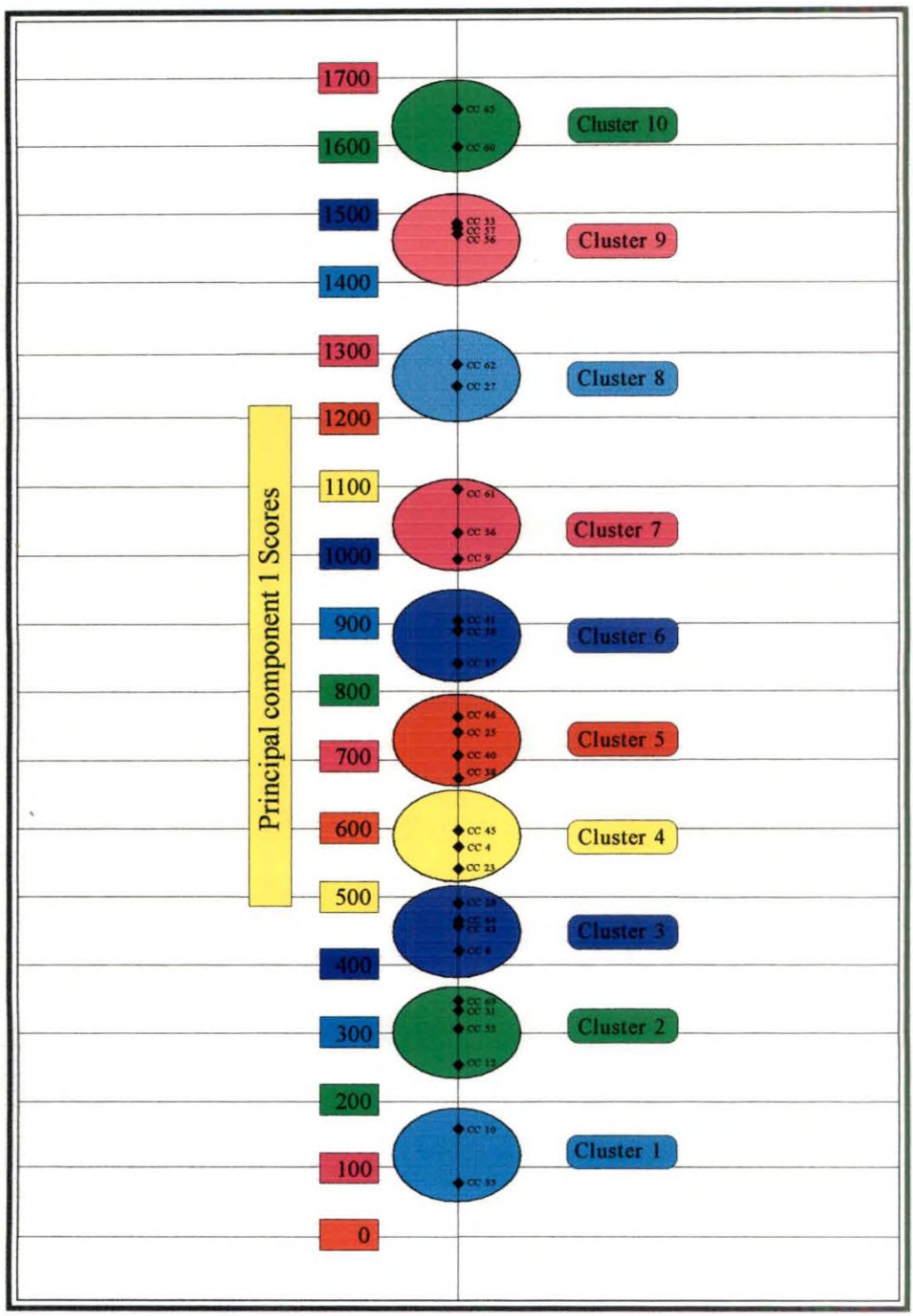


Table 12. Grouping of different calliclones of black pepper (*P. nigrum* L.) based on yield and quality attributes

Cluster No.	Calliclone Nos.	Group mean				
		Spike weight (g)	Essential oil (%)	Oleoresin (%)	Piperine (%)	Dry recovery (%)
I	10, 35	117.50	2.64	9.03	4.40	47.06
II	12, 55, 31, 69	306.25	2.79	9.58	4.56	47.38
III	8, 43, 28, 64	457.50	2.86	9.65	4.33	45.12
IV	23, 4, 45	568.33	3.10	10.91	4.71	44.75
V	38, 40, 25, 46	716.87	3.02	10.10	4.87	41.24
VI	37, 58, 41	883.20	2.80	10.40	4.77	44.16
VII	9, 36, 61	1040.00	2.85	10.02	4.58	42.16
VIII	27, 62	1264.75	2.84	9.38	4.81	43.17
IX	56, 57, 33	1473.33	3.34	10.18	5.11	41.03
X	60, 65	1627.50	3.00	11.23	4.82	39.35

Table 13. Direct and indirect effect of yield components on yield in calliclones of black pepper (*P. nigrum* L.)

	Weight of 100 dry berry	Total berry weight	Total dry weight	Dry recovery	Essential oil	Oleoresin	Piperine	No. of female flowers	Length of internode	L/W of mature leaf
Weight of 100 dry berry	-0.0316	-0.0911	0.0054	0.0052	0.0003	-0.0035	0.0076	-0.0076	0.0000	-0.0028
Total berry weight	0.0029	0.9965	-0.0513	0.0289	-0.0161	0.0135	-0.0061	0.0261	-0.0008	-0.0126
Total dry weight	0.0033	0.9908	-0.0516	0.0264	-0.0153	0.0130	-0.0062	0.0232	-0.0008	-0.0127
Dry recovery	0.0029	-0.5097	0.0241	-0.0566	0.0155	-0.0053	0.0003	-0.0191	0.0005	0.0181
Essential oil	0.0001	0.2774	-0.0137	0.0152	-0.0578	-0.0021	-0.0007	0.0185	-0.0007	-0.0011
Oleoresin	0.0026	0.3093	-0.0155	0.0069	-0.0028	0.0435	-0.0034	0.0173	-0.0005	-0.0054
Piperine	0.0105	0.2645	-0.0140	0.0008	-0.0019	0.0064	-0.0230	-0.0018	-0.0002	-0.0071
No. of female flowers	0.0033	0.3599	-0.0165	0.0149	-0.0148	0.0104	-0.0006	0.0724	-0.0003	-0.0060
Length of internode stem	-0.0005	0.3761	-0.0206	0.0133	-0.0182	0.0098	-0.0019	0.0103	-0.0021	-0.0076
L/W of mature leaf	-0.0020	0.2852	-0.0148	0.0232	-0.0015	0.0053	-0.0037	0.0098	-0.0004	-0.0442
Internodal length of latervals	0.0083	0.2711	-0.0141	0.0118	0.0066	0.0182	-0.0034	0.0128	-0.0003	-0.0118
Angle of laterals	0.0069	0.4199	-0.0217	0.0141	0.0058	0.0179	-0.0005	0.0156	-0.0006	-0.0119
Rate of lateral production	-0.0012	0.1572	-0.0070	0.0018	-0.0047	0.0178	-0.0025	0.0053	0.0000	-0.0156
No. of spike per branch	0.0115	0.3073	-0.0164	0.0049	-0.0086	0.0092	-0.0052	-0.0007	-0.0003	0.0063
Percentage of fertile pollen	0.0101	-0.0624	0.0022	-0.0051	0.0168	-0.0124	-0.0023	-0.0211	0.0003	0.0032
Percentage of well developed berry	-0.0086	0.1554	-0.0083	0.0130	0.0007	-0.0107	0.0102	0.0020	-0.0002	-0.0014
No. of berries per spike	0.0014	0.4759	-0.0244	0.0271	0.0043	0.0082	-0.0017	0.0103	-0.0002	-0.0089
Spike length	0.0020	0.6152	-0.0320	0.0253	-0.0059	0.0105	-0.0042	0.0239	-0.0006	-0.0218
Leaf area	0.0047	-0.0393	0.0010	-0.0162	0.0036	0.0067	0.0046	0.0166	-0.0001	0.0252

Residual effect = 0.0101

Contd.

Table 13. Direct and indirect effect of yield components on yield in calliclones of black pepper (*P. nigrum* L.) continued

	Internodal length of laterals	Angle of laterals	Rate of lateral product-ion	No. of spike per branch	% of fertile pollen	% of well developed berry	No. of berries per spike	Spike length	Leaf area	Correlation coefficient
Weight of 100 dry berry	0.0188	0.0136	-0.0004	0.0025	-0.0166	0.0068	0.0024	-0.0086	-0.0008	-0.1006
Total berry weight	-0.0193	-0.0310	-0.0017	-0.0021	-0.0032	0.0039	-0.0258	0.0837	-0.0002	0.9852
Total dry weight	-0.0194	-0.039	-0.0015	-0.0021	-0.0022	0.0040	-0.0254	0.0840	-0.0001	0.9763
Dry recovery	0.0148	0.0184	0.0004	0.0006	0.0047	-0.0057	0.0258	-0.0606	0.0016	-0.5292
Essential oil	0.0081	0.0074	-0.0009	-0.0010	-0.0150	-0.0003	0.0040	0.0139	-0.0004	0.0252
Oleoresin	-0.0298	-0.0302	-0.0044	-0.0014	-0.0148	-0.0061	-0.0101	0.0327	0.0009	0.2887
Piperine	-0.0106	-0.0016	-0.0012	-0.0015	0.0052	-0.0110	-0.0039	0.0249	-0.0011	0.2334
No. of female flowers	-0.0126	-0.0159	-0.0008	0.0001	-0.0151	0.0007	-0.0077	0.0447	0.0013	0.4185
Length of internode stem	-0.0102	-0.0202	0.0001	-0.0010	-0.0075	0.0021	-0.0039	0.0384	0.0003	0.3566
L/W of mature leaf	-0.0190	-0.0197	0.0039	0.0010	-0.0037	0.0008	-0.0108	0.0670	-0.0032	0.2653
Internodal length of latervals	-0.0710	-0.0433	-0.0017	-0.0024	0.0027	0.0059	0.0033	0.0313	0.0004	0.2245
Angle of laterals	-0.0418	-0.0735	-0.0020	0.0006	0.0052	0.0078	0.0021	0.0367	-0.0004	0.3794
Rate of lateral production	-0.0111	-0.0135	-0.0110	0.0014	-0.0135	-0.0046	-0.0092	0.0571	-0.0001	0.1463
No. of spike per branch	-0.0256	0.0066	0.0022	-0.0068	0.0012	-0.0036	-0.0105	0.0162	0.0017	0.2896
Percentage of fertile pollen	-0.0037	-0.0074	0.0024	-0.0002	0.0518	-0.0021	-0.0074	0.0070	-0.0009	-0.0309
Percentage of well developed berry	-0.0168	-0.0231	0.0020	0.0010	-0.0043	0.0248	0.0081	-0.0089	-0.0001	0.1350
No. of berries per spike	0.0043	0.0029	-0.0019	-0.0013	0.0071	-0.0037	-0.0539	0.1012	-0.0006	0.5474
Spike length	-0.0164	-0.0199	0.0046	-0.0008	0.0027	-0.0016	-0.0403	0.1356	0.0001	0.6669
Leaf area	-0.0055	0.0051	0.0002	-0.0020	-0.0087	-0.0003	-0.0059	0.0017	0.0056	-0.0030

Residual effect = 0.0101

Table 13a. Scales for path coefficient

Values for direct/ indirect effect	Rate scale
0.00 – 0.09	Negligible
0.10 – 0.19	Low
0.2 – 0.29	Moderate
0.3 – 0.99	High
>1	Very high

Table 14. Matrix of dissimilarity (Distant Matrix of the clusters)

Clusters	1	2	3	4	5	6	7	8	9	10
1	0	161.25	285	357.08	504.375	657.08	800.416	963.75	1205.45	1350
2	161.25	0	123.75	195.83	343.125	495.83	639.166	802.50	1044.20	1198.75
3	285	123.75	0	72.08	219.375	372.08	515.416	678.75	920.45	1075.00
4	357.08	195.83	72.08	0	147.295	300	443.336	606.68	848.37	1002.92
5	504.375	343.125	219.375	147.295	0	152.705	296.04	459.37	701.07	855.62
6	657.08	495.83	372.08	300	152.705	0	143.336	306.60	548.37	702.92
7	800.416	639.166	515.416	443.336	296.04	143.336	0	163.33	405.04	559.58
8	963.75	820.5	678.5	606.68	459.37	306.60	163.33	0	241.7	396.25
9	1205.45	1044.2	920.45	848.37	701.07	548.37	405.04	241.7	0	137.5
10	1360	1198.75	1075	1002.92	855.62	702.92	559.58	396.25	137.5	0

4.5 *Phytophthora* foot rot disease reaction in calliclones

The *Phytophthora* foot rot disease reaction of different calliclones of black pepper was assessed using leaf symptom bioassay by artificially inoculating 5 mm culture disc of *P. capsici*. Based on the extent of lesion development for two successive seasons viz. 1998-99 and 1999-2000 the calliclones were grouped into different classes (Plate 10). The lesion diameter ranged from 0.119 to 0.866cm and 0.322 to 1.867 cm after 48 and 72 h of inoculation respectively (Table 15). The results showed that 6.66 per cent of calliclones had the lowest lesion diameter of ≤0.25 cm and 36.66 per cent had the highest lesion diameter of 0.51 to 1.00 cm after 48 h of inoculation (Table 16 and Fig.9). After 72 h of inoculation, increment in lesion diameter was observed in all the calliclones (32.45 to 252.77%). The calliclones of the 1st class showing lesion diameter <0.50 cm even after 72 h could be designated as highly tolerant and clones in the 2nd class as tolerant to *Phytophthora* foot rot disease. Since majority of the calliclones (40%) came in the same class of 0.51 to 1.00 cm after 72 h, it can be concluded that the clones exhibited less variability with respect to the character studied.

Plate 10. Variability in leaf lesion diameter observed in calliclones of black pepper (artificial inoculation done with culture disc of *Phytophthora capsici*)


CC 23
CC 4
CC61
CC 43


CC36
CC33
CC41

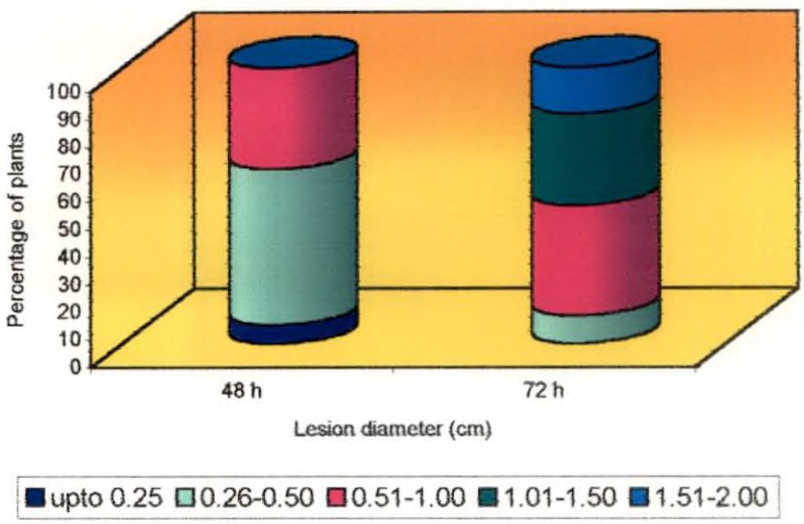
Table 15. Lesion development in leaves of different calliclones of black pepper (*P. nigrum* L.) after 48 and 72 h of inoculation with *P. capsici*.

Sl. No.	Calli-clone No.	Lesion diameter (cm)						Mean lesion diameter (cm)		
		Seasons								
		1998-1999			1999-2000					
		48 h	72 h	Increment in lesion diameter (%)	48 h	72 h	Increment in lesion diameter (%)	48 h	72 h	Increment in lesion diameter over 48 h (%)
1	CC 4	0.490	1.455	196.94	0.262	0.900	243.51	0.376	1.178	213.30
2	CC 8	0.236	0.772	227.11	0.225	0.625	177.78	0.243	0.699	187.65
3	CC 9	0.349	0.834	138.97	0.262	0.763	191.22	0.306	0.799	161.11
4	CC 10	0.324	0.475	46.60	0.350	0.600	71.43	0.337	0.538	59.64
5	CC 12	0.464	1.005	116.59	0.463	1.525	229.37	0.464	1.265	172.62
6	CC 23	0.252	0.780	209.52	0.375	0.988	163.46	0.314	0.884	181.53
7	CC 25	0.522	1.005	92.53	0.844	1.038	22.99	0.683	1.022	49.63
8	CC 27	0.444	1.623	265.54	0.788	1.40	77.66	0.616	1.515	145.94
9	CC 28	0.793	1.815	128.88	0.938	1.919	104.58	0.866	1.867	115.59
10	CC 31	0.673	1.303	93.61	0.633	1.476	122.62	0.668	1.389	107.93
11	CC 33	0.965	1.842	90.88	0.650	1.600	146.15	0.808	1.721	112.99
12	CC 35	0.281	0.753	167.97	0.532	1.219	129.14	0.401	0.986	145.89
13	CC 36	0.742	1.555	109.57	0.507	1.232	142.99	0.624	1.314	110.58
14	CC 37	0.612	1.135	85.46	0.682	1.494	119.06	0.647	1.538	137.71
15	CC 38	0.651	1.972	202.92	0.625	1.175	88.00	0.638	1.574	146.71
16	CC 40	0.464	1.122	141.81	0.557	0.988	77.88	0.599	1.055	76.13
17	CC 41	0.460	0.990	115.26	0.413	1.338	223.97	0.437	1.164	166.36
18	CC 43	0.075	0.106	41.33	0.162	0.538	232.09	0.119	0.322	170.59
19	CC 45	0.324	0.778	140.12	0.450	1.325	194.44	0.345	1.014	193.91
20	CC 46	0.474	1.090	129.96	0.232	0.850	266.38	0.353	0.970	174.79
21	CC 55	0.240	0.386	60.83	0.510	0.830	62.75	0.375	0.608	62.13
22	CC 56	0.585	0.815	40.03	0.451	1.119	148.12	0.517	0.967	87.04
23	CC 57	0.079	0.306	287.34	0.550	0.713	29.64	0.314	0.509	62.10
24	CC 58	0.106	0.331	212.26	0.412	0.618	50.00	0.259	0.469	81.08
25	CC 60	0.336	0.468	27.87	0.238	0.332	39.49	0.302	0.400	32.45
26	CC 61	0.366	0.536	46.45	0.294	0.819	178.57	0.330	0.678	105.45
27	CC 62	0.442	0.885	100.23	0.613	1.594	160.03	0.528	1.240	124.85
28	CC 64	0.271	0.298	9.96	0.463	1.07	131.10	0.366	0.684	86.89
29	CC 65	0.395	1.630	312.66	0.363	1.044	187.60	0.379	1.337	252.77
30	CC 69	0.346	0.354	2.310	0.537	1.088	102.60	0.442	0.719	62.67

Table 16. Extent of lesion development in different calliclones of black pepper (*P. nigrum* L.)

Class based on lesion diameter (cm)	1998-99		1999-2000		Mean	
	Percentage of plants (48 h)	Percentage of plants (72 h)	Percentage of Plants (48 h)	Percentage of plants (72 h)	Percentage of plants (48 h)	Percentage of plants (72 h)
1. Upto 0.50						
1a. Upto 0.25	16.66	3.33	13.33	-	6.66	-
1b. 0.26-0.50	56.66	23.33	40.00	3.33	56.66	10.00
2. 0.51-1.00	26.66	30.00	46.66	40.00	36.66	40.00
3. 1.01-1.50	-	23.33	-	4333	-	33.33
4. 1.51-2.00	-	20.00	-	13.33	-	16.66

Fig. 9. Variability in Phytophthora foot rot disease reaction in different calliclones of black pepper (*P. nigrum* L.)



DISCUSSION

DISCUSSION

Phytophthora foot rot disease incited by *Phytophthora capsici* is the most devastating disease affecting black pepper and there is no effective control measure to combat this serious malady. The conventional breeding programmes so far carried out could hardly trace out or evolve any genotype resistant to this disease. Therefore *in vitro* culture induced genetic variation (somaclonal variation) constitute an important source of variability for the improvement of black pepper.

Shylaja and Nair (1996) reported the occurrence of high amount of somaclonal variation in black pepper for *Phytophthora* foot rot disease reaction. They reported that the rate of somaclonal variation in black pepper is dependent on genotype and longer the duration in culture, higher was the variability observed.

The present investigations on variability analysis in the calliclones of black pepper are taken up to assess the extent of variability in the calliclones of cv. Cheriakanyakkadan for phenotypic characters, yield and quality attributes and tolerance/resistance to *Phytophthora* foot rot disease.

The results of the various aspects of the study are discussed in this chapter.

5.1 Morphological characters

Wide variations in morphological characters were observed in the different calliclones studied.

The vegetative bud was light purple and conical curved in all calliclones. The length-width ratio of the vegetative bud showed wide variations with a coefficient of variation of 32.84 in the different calliclones (Table 1 and Fig.1).

The various young leaf characters studied were more or less uniform in all the clones except for the length and width of the leaf. Wide variations were observed for the length of the young leaf with a coefficient of variation 34.87 and width with a coefficient of variation 28.08. The colour of the young leaf was light green above and pale purple below. The leaf tip was accuminate, leaf base was round or cordate-round and the shape of the young leaf was ovate or cordate (Table 2).

The calliclones were found to vary for length, thickness and colour of the petiole (Table 3). The length of the petiole ranged from 1.24 to 3.94 cm and thickness from 0.72 to 1.86 cm with high coefficients of variation. The colour of the petiole was either light green or light green to yellow. The colour of the leaf sheath was purple, petiole was grooved and without hairs in all the calliclones.

The thirty calliclones studied exhibited variation for shape and colour of the mature leaf and area of the leaf (Table 4). The different leaf shapes recorded were ovate, ovate-cordate, elliptic-ovate and ovate lanceolate (Plate 2). In 46.66 per cent of the calliclones, the leaves were ovate. The colour of the mature leaf on upper surface was either dark green or green and that of lower surface was either green or light green. The leaf area was found to vary widely in the calliclones (Fig.2) and ranged from 44.49 to 84.54 with a coefficient of variation of 17.34. In all the calliclones studied, the leaves were alternate, glabrous, coriaceous with acrodromous venation and with entire leaf margin. The leaf tip was acuminate and there was no hairiness on leaves in all the clones.

As observed in the present study, variation for leaf morphology was reported by various workers in micropropagated plants of different crop species. Mathur *et al.* (1988) reported extensive somaclonal variation in callus derived plants of Java citronella for length and area of the longest leaf. Similarly, Israeli *et al.* (1991) observed variations in leaf morphology of micropropagated field planted bananas of Cavendish and Red sub groups. Cai *et al.* (1990) studied the

genetics of somaclonal variation in sorghum wherein variations such as short culm and narrow leaf were described as simple recessive gene mutations.

In the present study, 20 per cent of calliclones exhibited high leaf area. The high leaf area observed in the calliclones may help for high rate of photosynthesis as reported by Zaman *et al.* (1997) in micropropagated mulberry plants.

Similar variations on morphological characters were also reported in *in vitro* regenerants of wheat (Hashim *et al.*, 1988), sorghum (Maralappanavan *et al.*, 1995), oat (Dahleen *et al.*, 1995), foxtail millet (Min *et al.*, 1999), pigeon pea (Prasannalatha *et al.*, 1994) and safflower (Seeta and Anwar, 1992).

Qualitative characters like colour and shape of the orthotrope showed no variation while quantitative characters like internodal length, thickness at node and internode showed variation in the different calliclones (Table 5). Stem was cylindrical and grey to dark green in all the calliclones. Closely spaced internodes were observed in 43.22 per cent of calliclones. In black pepper the internodal length of the orthotrope is an important character which decides the rate of lateral branch production. The thickness at node and internode varied in between 1.90 to 4.10 cm and 1.06 to 3.38 cm respectively with high coefficients of variation.

Lateral branch characters exhibited high variation in the 30 calliclones studied. The length of the internode, thickness at node and internode and rate of lateral branch production varied considerably with high coefficients of variation (Fig.3) while angle of laterals showed less variation (Table 6).

The internodal length of laterals in pepper is of economic interest as the shorter internodes increase the number of fruiting branches. In 70 per cent of calliclones closely spaced internodes of 7.00 to 9.90 cm were observed in the laterals.

The number of runner shoots produced and the number of adventitious roots on nodes exhibited variation in the calliclones (Table 7 and Fig.4)). The holding ability of the adventitious roots was found good in all calliclones. The high rate of runner shoot production observed in the calliclones is an indication of the growth and vigour of the plants.

In the present investigation the calliclones showed wide variability in the number of spikes emerged per lateral, length of spike at the time of flowering and percentage of pistillate flowers with high coefficients of variation while percentage of hermaphrodite flowers, number of stigmatic lobes, receptivity of stigma and pollen fertility showed less variation (Table 8 and Fig.5). In all the calliclones, both hermaphrodite and pistillate flowers were observed.

The percentage of hermaphrodite flowers was high in all the clones and ranged from 93.51 to 97.72. The percentage of pistillate flowers ranged from 2.28 to 6.50 and the spike length from 3.20 to 6.80 cm. The nature of flowering in the calliclones was graded as profuse, medium and low (Plate 6). Majority of the calliclones (66.66%) registered profuse flowering.

The spike length, number of berries per spike and percentage of under developed berries exhibited wide variations among the clones with high coefficients of variation (Table 9 and Plates 7 & 8). The spike length ranged from 4.00 to 7.60 cm, number of berries per spike from 22.30 to 44.10 and percentage of under developed berries from 3.64 to 13.71 in different clones. The colour of the young berry was green, mature berry was dark green and ripe berry was orange red and shape was globose in all callicones. In all the clones high percentage of well developed berries was observed and ranged from 86.15 to 96.32. The time taken from flowering to maturity of the berries was 5½ to 6 months in all the clones. The spike length, number of berries per spike and percentage of well developed berries were high in CC 27, CC 33, CC 36, CC 56, CC 57, CC 60, CC 61, CC 62 and CC 65.

The weight and volume of 100 fresh and dry berries, weight of 100 seeds and pericarp thickness showed less variation.

Variability in flowering characters was observed in tissue culture regenerants of many crop species. In cardamom, Sudharshan *et al.* (1997) evaluated the micropropagated plants for growth characters and reported that tissue culture derived clones showed variations in the type of panicle, capsule shape and size. The overall variability in tissue cultured plant was 4.5 per cent as against 3.0 per cent in open pollinated seedling progenies. Al-Ghamdi (1996) reported significant variation in flowering date, flowering duration and number of spikes produced in the field planted oil palm regenerants.

Similar variations were reported in wheat by Galiba *et al.* (1984); Mohmand and Nabors (1990) and Ivanov *et al.* (1998), in barley by Ahloowalia *et al.* (1987), in grape by Liuni *et al.* (1998), in pigeon pea by Prasannalatha *et al.* (1994) and Chintapalli *et al.* (1997), in banana by Vuylsteke and Swennen (1990) and in black berry by McPheeters and Skirvin (1989).

5.2 Yield characters

The thirty calliclones studied differed significantly with respect to yield and fresh/dry berry weight (Table 10 and Fig.6). Based on the yield, the clones could be grouped into low, medium and high yielders. High yields were recorded in CC 27, CC 33, CC 36, CC 56, CC 57, CC 60, CC 61, CC 62 and CC 65. The high yielding nature observed in these clones may be due to the inherent genetic potential. Also, these clones were characterised by more number of laterals, spikes per branch, high percentage of hermaphrodite flowers, lengthy spikes and more number of well developed berries. The low yields were recorded in clones like CC 35, CC 10, CC 12, CC 55, CC 31, CC 69, CC 8, CC 43, CC 28 and CC 64 and these clones were characterised by the less number of laterals, spikes per lateral and poor set.

In the present study, the dry recovery percentage observed ranged from 38.21 to 54.40 and exhibited less variation as shown in Table 10. Majority of the clones (60%) recorded high driage in between 40.17 to 44.42 per cent. The pericarp thickness recorded in this investigation also showed less variation and supported this observation (Plate 9). As compared to other black pepper cultivars, the dry recovery percentage observed in the calliclones of Cheriakanyakkadan was high. This is in conformity with the report by Pruthi (1993) wherein the cultivar Cheriakanyakkadan recorded a high dry recovery percentage of 42 while other cultivars of black pepper recorded dry recovery percentage in between 37 and 39. So the high dry recovery recorded in the calliclones of Cheriakanyakkadan may be due to the effect of the genotype. The high relative humidity recorded during the crop maturation period in both the seasons also cannot be ruled out in this regard (Appendix-I).

Chandrappa *et al.* (1997) observed variability for yield and yield attributes, in micropropagated field planted cardamom clones. Yield performance of the tissue culture derived cardamom plants for three years revealed that clone TC 5, TC 6 and TC 7 were promising compared to other five lines. These three clones differed among themselves for yield and yield attributes. Besides higher yields, TC 5 recorded higher values for number of panicles per clump, panicle length, flowers per node, weight and volume of dry capsules.

Variability in yield and yield contributing characters were also reported in cymbopogon (Mathur *et al.*, 1988), mustard (Katiyar, 1997), sugarcane (Dhumale *et al.*, 1994), wheat (Mohmand and Nabors, 1990; Villareel *et al.*, 1999), pepper mint (CIMAP, 1992; Han *et al.*, 1998) and cardamom (Sudharshan *et al.*, 1997).

5.3 Quality attributes

From the results it is clear that the quality parameters like the content of essential oil and piperine showed more variation as compared to the contents of oleoresin and moisture (Table 11).

The essential oil varied in between 2.19 to 3.63 per cent in the clones studied (Fig.7a). In majority of the clones (76.66) the essential oil ranged in between 2.50 to 3.42 per cent. The highest recovery of essential oil was noticed in CC 8 and CC 57.

The oleoresin content ranged from 8.63 to 12.5 per cent in the calliclones studied. In 56.66 per cent of the clones the content ranged from 9.01 to 9.97 per cent. The highest oleoresin content of 11.02 to 12.5 per cent was observed in CC 4, CC 37, CC 45, CC 57 and CC 60.

The piperine content ranged from 3.67 to 6.36 per cent in the clones and in 60 per cent of the clones the content ranged from 4.12 to 4.99 per cent (Fig.7b). The highest piperine (6.36%) content was recorded in CC 56.

Variations in quality attributes of tissue culture derived plants were reported by Sudharshan and Bhat (1998) in cardamom. They recorded high oil content of 7.8 per cent in tissue cultured cardamom 'SKP 14 TC' while the content in open pollinated seedling was 7.4 per cent. Similar report in pepper mint by Han *et al.* (1998) showed that the recovery of oil and menthol was high in the somaclones.

In cymbopogon, Mathur *et al.* (1988) isolated two elite somaclones with better oil quality with high citronellal, geranoil, geranyl acetate and low elemol content from 49 somaclones. They observed that one clone had two fold increase in oil content, 33 maintained same or slightly improved oil content while remaining 15 plants contained significantly less oil than the parent. Since 67 per cent of clones recorded more or less same oil yield, the variability for oil yield was low in the somaclones of cymbopogon. Similar observations on oil yield was recorded in the present study also.

5.4 Grouping of calliclones based on yield and quality attributes

In general, phenotype is an interaction of genotype and environment. Since the calliclones under investigation were given uniform management practices the extent of contribution of environment to the observed variability is uniform. Therefore the observed variability can be considered as a rough estimate of the relative magnitude of genetic variability. In order to assess the extent of variability and to group the calliclones, the techniques PCA and matrix of dissimilarity were applied.

The technique of principal component analysis was done based on five economic characters like total spike weight, essential oil, oleoresin, piperine and dry recovery (%). Since the first principal component explained 99.99 per cent of variation, the scores of first principal component was taken for grouping the genotypes. The technique of principal component analysis was reported by Griga *et al.* (1995) for variability assessment in seed progenies of pea somaclones of T_1 to T_3 generation produced via organogenesis/somatic embryogenesis. In the present study, 30 calliclones could be grouped into ten clusters as shown in Table 12 and Fig.8. According to the yield, the clusters could be further grouped as high yielders (Clusters VII, VIII, IX and X), medium yielders (cluster IV, V and VI) and low yielders (Cluster I, II and III). The clones with high content of essential oil (>3%) were present in clusters of IV, V, IX, X and clones with oleoresin above 10 per cent were in clusters of IV, V, VI, VII, IX and X. Clones with high piperine content (>4.5%) were distributed in majority of the clusters.

Path coefficient analysis with yield as the dependent variable and 19 yield contributing characters as the explanatory variable revealed that the total fresh berry weight was the only character which has high direct effect on spike weight (Table 13). The dry weight, the percentage of oleoresin, number of hermaphrodite flowers, internodal length of orthotrope, angle of laterals, number of spikes per lateral, number of berries per spike and spike length were found to have

high influence on yield through total berry weight. The positive direct effects of the number of hermaphrodite flowers, number of spikes per branch, number of berries per spike and spike length on yield was also reported by Kanakamony (1982) and Sujatha (1991) in black pepper. The essential oil, piperine, L/W of mature leaf and internodal length of laterals had moderate indirect effect and rate of lateral production, and percentage of well developed berries have low indirect effect through berry weight. The dry recovery percentage had high negative indirect effect on berry weight. Since the total fresh berry weight was the only character having significant and positive direct effect on total spike weight, total fresh berry weight was taken as the marker character for all the other characters analysed.

The numerical distance worked out based on the marker character gave an indication of the divergence of the clusters. The matrix of dissimilarity presented in Table 14 revealed that the clusters I and X were most divergent followed by I and IX, II and X, II and IX and III and X. So the calliclone CC 10 and CC 35 were more divergent to calliclones CC 60 and CC 65 for all the characters analysed. Similarly CC 10 and CC 35 were more distant to CC 33, CC 56 and CC 57. The divergence observed in the calliclones can be exploited in further crop improvement programmes.

5.5 Assessment of *Phytophthora* foot rot disease reaction in calliclones

The *Phytophthora* foot rot disease reaction in different calliclones of black pepper (*P. nigrum* L.) was assessed by detached leaf symptom bioassay (Table 15 and Plate 10). Based on the intensity of lesion development the calliclones were grouped into different classes as shown in Table 16 and Fig.9. Observations were recorded at 48 and 72 h after inoculation. In all the calliclones, increment in lesion diameter was noticed after 72 h of inoculation. The average increment in lesion diameter after 72 h of inoculation over 48 h ranged from 32.45 to 252.77 per cent. After 48 h of inoculation, the clones CC 8 and CC 43 were

found to be highly tolerant recording lesion diameter 0.243 and 0.119 respectively. But after 72 h of inoculation, CC 8 recorded a high increment in lesion diameter (187.65%). Based on the average lesion diameter 72 h of inoculation it could be seen that three calliclones out of 30 viz. CC 43, CC 58 and CC 60 were highly tolerant, recording lesion diameter of 0.322 cm, 0.469 cm and 0.400 cm respectively. Hence the three clones CC 43, CC 58 and CC 60 could be designated as highly tolerant. It was also observed that 40 per cent of the calliclones recorded a lesion diameter less than 1.00 cm even after 72 h of inoculation and hence they could be designated as tolerant. Since most of the plants (40%) belong to the tolerant class (Class II) as evident in Table 16, it could be concluded that the variability for the disease reaction was low in calliclones of Cheriakanyakkadan.

The tolerant nature of calliclones of Cheriakanyakkadan to *Phytophthora* foot rot disease was also reported by Shylaja and Nair (1996) when they assessed the extent of somaclonal variation in four black pepper cultivars viz. Kalluvally, Balankotta, Karimunda and Cheriakanyakkadan by three different methods like natural screening, screening by electrolyte leakage and artificial inoculation of leaves with culture disc of *P. capsici*. As observed in the present study, they also observed less variability in disease reaction in calliclones of Cheriakanyakkadan as compared to the clones of Kalluvally, Balankotta and Karimunda. In the elaborate *in vivo* screening studies conducted by Shylaja *et al.* (1996) also, the tolerance of Cheriakanyakkadan to *Phytophthora* foot rot and less variability in disease reaction in calliclones of Cheriakanyakkadan as compared to other black pepper cultivars like Kalluvally, Balankotta, Karimunda and Panniyur 1 was observed. This shows the stability of the tolerance level of the calliclones of Cheriakanyakkadan to *Phytophthora* foot rot disease.

The effect of genotype on the expression of disease, as observed in the present investigation was also reported by Daub and Jenns (1989) in tobacco when they evaluated the somaclones for black shank, bacterial wilt and tobacco mosaic disease.

In a recent evaluation of peach somaclone by detached leaf bioassay for *Xanthomonas campestris pv. Pruni*, Hammerschlag (2000) observed higher levels of resistance to the pathogen in all the progenies of the resistant somaclone '122-1'.

Similar reports on high level of resistance/tolerance to various diseases in somaclones were made by Shahin and Spivey (1986) in tomato to *Fusarium oxysporum* f.sp. *lycopersici*, Matsumoto *et al.* (1999) in banana to *Fusarium oxysporum* f.sp. *cubense*, Ling *et al.* (1985) in rice to *Helminthosporium oryzae*, Larkin and Scowcroft (1983) in sugarcane to *Helminthosporium sacchari*, Shepard *et al.* (1980) in potato to *Phytophthora infestans*, Toyodo *et al.* (1991) in strawberry to *Fusarium oxysporum* f.sp. *fragariae* and Chevreau *et al.* (1998) in apple to *Erwinia anylovora*.

From this study it can be concluded that wide variation is present in calliclones of Cheriakanyakkadan for phenotypic characters, yield and quality attributes.

The phenotypic characters showing wide variations were the length-width ratio of vegetative bud, length and width of young leaf, petiole length, thickness and colour, shape and colour of the mature leaf, leaf area, thickness at node and internode of orthotrope, internodal length of laterals, thickness at node and internode of laterals, rate of lateral branch production, number of adventitious roots and number of runner shoots.

The yield and yield contributing characters showing variations were the number of spikes per lateral, length of spike, percentage of under developed berries and yield.

Quality parameters like the content of essential oil and piperine showed more variation as compared to the content of oleoresin.

In the evaluation of *Phytophthora* foot rot disease reaction, majority of the calliclones (40%) were found to be tolerant to *Phytophthora capsici* and variability for the disease reaction was low as compared to the phenotypic characters studied in the present investigation. Three calliclones, namely CC 43, CC 58 and CC 60 were found to be highly tolerant to *Phytophthora* foot rot disease recording lesion diameter <0.50 cm even after 72 h of inoculation.

Some of the clones identified for the desirable traits are as shown below:

I. Yield contributing characters

1. Lengthy spike	CC 9, CC 27, CC 33, CC 36, CC 37, CC 38, CC 41, CC 56, CC 60, CC, 61, CC 62
2. More no. of spikes/lateral	CC 57, CC 46, CC 8, CC 4, CC 9, CC 60
3. More no. of berries/spike	CC 33, CC 36, CC 37, CC 41, CC 46, CC 56, CC 58, CC 60, CC 61
4. High yield	CC 27, CC 23, CC 56, CC 47, CC 36, CC 60, CC 61, CC 62, CC 65

II. Quality attributes

1. High essential oil	CC 4, CC 8, CC 23, CC 25, CC 33, CC 35, CC 38, CC 40, CC 43, CC 56, CC 57, CC 60, CC 61, CC 69
2. High oleoresin	CC 4, CC 9, CC 12, CC 36, CC 37, CC 38, CC 60, CC 43, CC 45, CC 46, CC 57, CC 58
3. Higher piperine	CC 9, CC 12, CC 25, CC 37, CC 45, CC 56, CC 57, CC 65

III. Tolerance to *Phytophthora* foot rot disease

1. Highly tolerant	CC 60, CC 43, CC 58
2. Tolerant	CC 8, CC 9, CC 10, CC 23, CC 35, CC 46, CC 55, CC 56, CC 57, CC 61, CC 64, CC 69

From the study elite clones with desirable attributes (Plate 11) could be selected and are listed below:

1. Clones with high tolerance to *P. capsici* - CC 43, CC 58, CC 60
2. Clones with high yield and quality - CC 56, CC 57, CC 60
3. Clones with high yield, quality and tolerance to *P. capsici* - CC 56, CC 57
4. Clones with high yield, quality and highly tolerant to *P. capsici* - CC 60

Based on overall attributes the calliclone "CC 60" can be selected as the superior clone showing high yield, quality and high tolerance to *P. capsici* (Plate 12). The characters of the superior clone are listed below:

General appearance of the clone	Healthy, vigorous
Lateral branches per $1m^2$ area	13
Number of spikes per lateral	8.6
Spike length (cm)	6.7
Percentage of hermaphrodite flowers	97.7
Pollen fertility (%)	96.4
Mean number of berries/spike	38
Yield (g)	1595
Dry recovery (%)	39.32
Essential oil (%)	3.15
Oleoresin (%)	12.5
Piperine (%)	4.36
Reaction to *Phytophthora* foot rot	Highly tolerant [Lesion diameter 0.302 cm (48 h) and 0.400 cm (72 h)]

Plate 11: Selected elite calliclones

Plate. 12. The Superior calliclone


CC – 60

The superior and elite clones with desirable attributes can be further exploited in crop improvement/production programmes. The clones can also be multiplied in large scale by resorting to *in vitro* techniques or by vegetative propagation methods.

SUMMARY

SUMMARY

Investigations on "Variability analysis in calliclones of black pepper (*P. nigrum* L.)" was carried out in the Department of Plantation crops and Spices, College of Horticulture, Vellanikkara from October 1998 to July 2000. The study was aimed at assessing the variability in calliclones of black pepper (cv. Cheriakanyakkadan) based on morphological, yield, quality and reaction to *Phytophthora* foot rot disease. The salient findings of the investigations are listed below:

1. The calliclones showed wide variations in morphological characters.
   * The length-width ratio of the vegetative bud exhibited wide variations.
   * The various young leaf characters studied exhibited more or less uniform trend in all the clones except for the length and width of the leaf.
   * The mature leaf showed variations for length, thickness and colour of the petiole. The leaf shape, colour and leaf area also varied widely. The different leaf shapes recorded were ovate, ovate-cordate, elliptic-ovate and ovate lanceolate. The leaf area ranged from 44.49 to 84.54cm^2 in different calliclones.
   * Qualitative characters like colour and shape of the orthotrope showed no variation while quantitative characters like internodal length, thickness at node and internode showed variations in different calliclones.
   * Lateral branch characters like length of the internode, thickness at node and internode and rate of lateral branch production exhibited variations.
   * The number of runner shoots produced and number of adventitious roots on nodes exhibited variations in calliclones.
   * The calliclones showed wide variability in the number of spikes emerged per lateral, length of spikes at flowering and percentage of pistillate flowers while percentage of hermaphrodite flowers, number of stigmatic lobes, receptivity of stigma and pollen fertility exhibited less variation.

* Spike characters like length, total number of berries per spike and percentage of under developed berries exhibited wide variations in the calliclones studied.
* The weight and volume of 100 fresh and dry berry, weight of 100 seeds and pericarp thickness showed less variations.

2. The calliclones exhibited wide variations in yield and quality attributes.
   * The 30 calliclones differed significantly with respect to yield, fresh and dry berry weight. The average yield for the two seasons studied ranged from 75.00 to 1660.00 g in different calliclones. The dry recovery percentage recorded was generally high in calliclones and the calliclones exhibited less variation for the character.
   * Quality parameters like the content of essential oil and piperine showed more variation as compared to the content of oleoresin and moisture. The essential oil content varied from 2.19 to 3.63 per cent and oleoresin from 8.63 to 12.5 per cent and piperine from 3.67 to 6.36 per cent in different calliclones.
3. The calliclones were grouped into ten clusters based on principal component analysis. In order to study the divergence between the clusters, matrix of dissimilarity was worked out. The matrix showed that the clusters 1 and 10 were more divergent followed by clusters 1 and 9.
4. Investigations on the *Phytophthora* foot rot disease reaction of calliclones revealed that the clones CC 43, CC 58 and CC 60 were "highly tolerant" with lesion diameter <0.50 cm even after 72 h of inoculation. Clones with lesion diameter <1.00 cm after 72 h of inoculation were designated as 'tolerant clones'. Fourty per cent of the clones studied fell in the tolerant class.
5. Five elite clones viz. CC 43, CC 58, CC 60, CC 56 and CC 57 were identified with desirable traits such as high yield, quality and tolerance to *Phytophthora capsici*.
6. The calliclone "CC 60" was selected as the superior clone with yield, quality and high tolerance to *Phytophthora capsici*.

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APPENDIX-I

1998

Monthly weather data during crop period at Vellanikkara, Thrissur

Month	Max. Temp (°C)	Min. Temp(°C)	Mean RH (%)	Rainfall (mm)	Rainydays	Sunshine hours
Jan	33.1	22.8	64	0.0	0	288.5
Feb	34.4	23.6	64	0.0	0	269.2
Mar	36.2	23.6	67	11.0	1	310.4
Apr	36.5	25.6	68	61.4	4	270.2
May	34.1	25.2	77	203.0	9	235.4
Jun	30.2	23.3	87	809.3	24	103.1
Jul	29.2	21.8	88	752.9	28	101.6
Aug	29.8	22.8	86	433.6	18	112.5
Sep	30.2	23.3	87	571.3	24	123.4
Oct	28.0	22.8	85	452.8	18	148.5
Nov	31.5	23.1	78	109.4	9	214.6
Dec	30.1	22.9	69	33.0	4	20.0

1999

Month	Max. Temp (°C)	Min. Temp(°C)	Mean RH (%)	Rainfall (mm)	Rainydays	Sunshine hours
Jan	32.4	21.5	58	0.0	0	288.3
Feb	34.5	23.3	56	22.8	1	255.6
Mar	35.5	25.6	68	0.0	0	272.8
Apr	33.4	24.5	73	39.0	4	308.0
May	30.7	24.7	82	430.5	18	152.2
Jun	29.4	23.0	85	500.2	23	150.8
Jul	28.4	23.0	89	823.3	28	75.8
Aug	29.8	22.9	84	260.1	12	169.5
Sep	31.6	23.4	76	28.4	3	213.7
Oct	30.5	23.2	85	506.2	15	147.6
Nov	31.4	22.7	69	9.1	1	204.1
Dec	30.7	22.7	60	0.0	0	272.5

VARIABILITY ANALYSIS IN CALLICLONES OF BLACK PEPPER (*Piper nigrum* L.)

By
SANCHU, C. R.

ABSTRACT OF THE THESIS

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requirements for the degree of

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Faculty of Agriculture
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2000

ABSTRACT

The investigations on 'Variability analysis in calliclones of black pepper' were carried out in the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara from October, 1998 to July, 2000. Thirty calliclones of black pepper regenerated from axenic seedling of the cultivar Cheriakanyakkadan planted in Pepper Research Scheme of the Department during June, 1995 were utilised for the study.

The objective of the study was to assess the variability among the calliclones of black pepper based on morphological, yield and quality attributes and reaction to *Phytophthora* foot rot disease.

Morphological descriptions of the thirty clones were made based on the IBPGR descriptor. The variation at the phenotypic level was measured using the procedure described by Deklerk (1990). Screening of calliclones for *Phytophthora* foot rot resistance/tolerance was carried out as per the procedure reported by Kueh and Khew (1980).

Wide variability was observed for morphological characters like leaf shape, leaf area, internodal length of laterals, number of runner shoots and lateral branches in the different calliclones studied. The calliclones also exhibited variations for yield and yield contributing characters like the number of spikes per lateral, spike length and number of berries per spike. The dry recovery percentage recorded was generally high in all the calliclones and the clones exhibited less variation for the character.

The content of essential oil and piperine showed more variation than the content of oleoresin in the different calliclones studied.

The assessment of *Phytophthora* foot rot disease reaction of the calliclones revealed that the clones CC 43, CC 58 and CC 60 were highly tolerant.

Five elite clones viz. CC 58, CC 43, CC 60, CC 56 and CC 57 with desirable traits like high yield, quality and tolerance to *Phytophthora capsici* were identified. Based on overall attributes, the calliclone `CC 60' was selected as the superior clone with high yield, quality and high tolerance to *Phytophthora* foot rot.

The superior and elite clones with desirable attributes can be further exploited in crop improvement/production programmes.