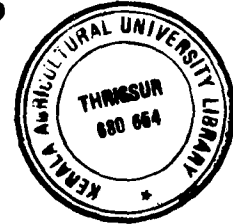


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**ASSESSMENT AND INDUCTION OF
VARIABILITY FOR HIGHER YIELD AND
PHOTOINSENSITIVITY IN COLEUS
(*Coleus parviflorus* BENTH)**



**By
MAREEN ABRAHAM**

THESIS

**Submitted in partial fulfilment of the
requirement for the degree of**

Doctor of Philosophy in Agriculture

**Faculty of Agriculture
Kerala Agricultural University**

**Department of Plant Breeding and Genetics
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA
2002**

DECLARATION

I hereby declare that the thesis entitled “**Assessment and induction of variability for higher yield and photoin sensitivity in coleus (*Coleus parviflorus* Benth)**” 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.

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Certified that this thesis, entitled “**Assessment and induction of variability for higher yield and photoinsensitivity in coleus (*Coleus parviflorus* Benth)**” is a record of research work done independently by **Smt.Mareen Abraham** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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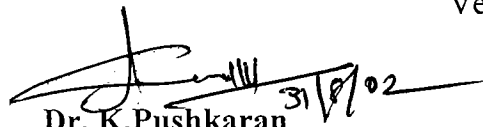
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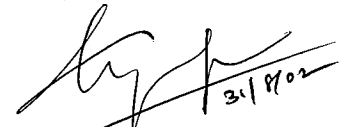
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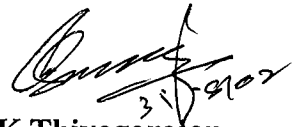
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Mareen Abraham

*Dedicated
to the
Memories of my Father*

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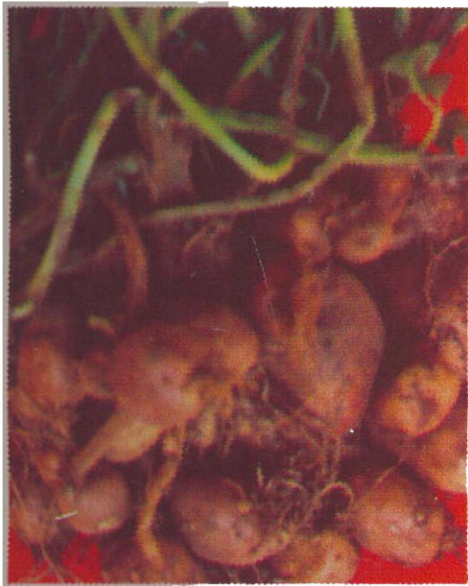
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Introduction

INTRODUCTION

Tuber crops represent a group of crops which accumulate carbohydrates in the enlarged under ground stems or roots. They are either the staple food or the important subsidiary food for one-fifth of the world population. They have a high potential for yielding large amount of food and are efficient producers of calories. Area and production of tuber crops in India according to Nair and Nair (1983) is over 1.3 million hectares and 16.4 million tonnes respectively. Area under tuber crops in Kerala is about 0.1 million hectares and the estimated annual production is 3 million tonnes (Farm Guide, 2002). Tropical tuber crops including cassava and sweet potato account for about half of this area and production. Other tuber crops grown in India include the Yams (*Dioscorea* spp.) *Amorphophallus*, *Colocasia*, *Xanthosoma*, Arrow root and *Coleus*.

Coleus (*Solonostemon rotundifolius* (Poir) J.K.Morton) (syn. *Coleus tuberosus* or *Coleus parviflorus* Benth) is popularly known as Chinese Potato or poor man's potato. It is grown extensively as a vegetable in the homestead gardens of Kerala, Karnataka and Tamil Nadu. *Coleus* is an environmentally friendly crop which requires less amount of fertilizers and other chemicals and it is fairly tolerant to stressful environmental conditions.

This aromatic vegetable-cum-tuber possesses an elite flavour and taste and it enjoys a covetable consumer preference. The nutritive status of this crop compares favourably with many of the major tuber crops. *Coleus* tuber contains 20.1 to 30.0 per cent dry matter, 14.7 to 20.8 per cent starch, 0.04 to 0.31 per cent protein and 0.57 to 0.96 per cent sugar (Sreekumari and Abraham, 1985). Since

ancient times *Coleus spp.* have been used in Ayurvedic medical practice. The major uses are in heart diseases, abdominal colic, respiratory disorders, insomnia and convulsions. They are also used for the treatment of dysentery and certain eye disorders (Ammon and Muller, 1985). According to Sandhya (1996) the flavanoids present in the tuber help to lower cholesterol level of blood.

The plant is a bushy annual herb belonging to the family Lamiaceae. The plant produces small dark brown aromatic tubers in clusters at the base of the stem. The crop prefers a hot humid climate for a luxuriant vegetative growth. Further, low night temperature is essential for proper tuberization. Due to the same reasons, in Kerala, it is cultivated in the rice fallows during summer and in the uplands during rainy season (CTCRI, 1987). Due to photosensitivity for tuberization, the availability of the tubers in the market is seasonal. The normal season is confined to the months of July to November when the market will be flooded with the tubers. However, the produce if available during the off-season (December to September) will fetch premium price. This is possible only if photo-insensitive variety is available for cultivation. In this context, it will be highly rewarding to produce a photo insensitive type as such a genotype is not available.

The yield of minor tuber crops in Kerala is very low ranging from 20-80 quintals per hectare. This is attributed to the poor yielding capacity of the existing varieties (Hrishi and Nair, 1972). In the case of *Coleus*, the per hectare yield of the crop is very low. Despite this, no systematic works have been undertaken to enhance the productivity of this crop (Prakash, 1996).

It is well known that the genetic improvement of a crop in terms of productivity, resistance to various stress conditions and adaptability to

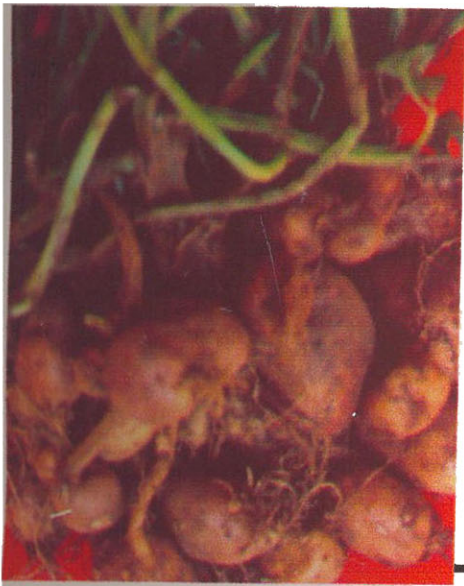
environment is possible if variability in respect of a particular trait is available in the considered population or species. Programmes of crop breeding have been successful for a long time because, the genetic variability already available was efficiently utilized in addition to making available variation by crossing plants from different populations, varieties, genera and species. However, in some cases, significant progress through classical methods of breeding becomes difficult or impossible. *Coleus* is a crop where natural variability in the population is meagre. This is because of poor seed set resulting from pollen sterility. The plant is in a polyploid state with $2n = 6x = 72$, meiotic abnormality may be the cause for pollen sterility (Sreekumar and Abraham, 1985).

The possibility offered by mutation breeding in such a situation is of considerable interest. In this context induced mutation is used to produce genetic variation artificially. As a thumb rule, larger the variation induced, greater is the possibility of getting positive responses to selection.

The present investigation was taken up as an innovative approach to the “Assessment and induction of variability for higher yield and photo insensitivity in *Coleus* (*Coleus parviflorus* Benth.)”.

The objectives of the present investigation are as follows

- To evaluate *Coleus* genotypes for photo insensitivity, tuberization, tuber yield and its attributes
- Clustering of genotypes based on the desirable attributes
- Induction of variability through *in vivo* and *in vitro* mutagenesis for photo insensitivity and higher yield.



Review of Literature

2. REVIEW OF LITERATURE

The pertinent literature on *Coleus parviflorus* is reviewed under the various heads in this chapter. The study of minor crops is fraught with linguistic pitfalls; these species are no more minor to the people who use them. The fact is that they have been neglected by western based research or that world production statistics are either not published or indicate low volumes compared to better known crops. The pattern of large scale research tend to draw attention away from crops of no perceived economic value out side their immediate area. Characterizing the minor tuber crop with greater clarity contributes to food security in periods of nutritional stress and they merit more public sector attention than they have received (Blench, 1997). No organized research has so far been done in *Solenostemon rotundifolius* which is classified under minor tuber crop.

The genus *Coleus* belonging to the family Lamiaceae syn. Labiatae was first described by Loureiro in 1720 and has over 3000 spp. which includes both annuals and perennials. The generic name is derived from the Greek word *Koleos* meaning sheath. Its members have four didynamous dedinate stamens whose filaments unite as a sheath at the base (Morton, 1962 and Codd, 1971). *Solenostemon rotundifolius* is a small herbaceous annual, 1-2 feet high ,prostate or ascending with succulent stem and aromatic thick leaves. It produces small dark brown aromatic tubers in clusters at the base of the stem (CSIR, 1950). *Coleus* is supposed to be a native of central or east Africa and it has come to India at an early period. It has adapted well in South East Asia including India and Sri Lanka

(Purseglove, 1974). The plant is grown for its small edible tubers, which are used as a substitute for potato. Though *Coleus* is a crop of lesser importance, its tubers sell in the market at a high premium. The aromatic flavour of the tuber makes it a delicacy among vegetables. Despite the high market price and consumer preference, it is not available all the year round. (Sreekumari and Abraham, 1985). Karyomorphometrical data has revealed the symmetrical and primitive Karyotype, which seems to be correlated with the essential oil composition and chemical characterization. Major essential oil components found in *Coleus parviflorus* are beta thujone and alpha farnescene (Thoppil and Jose, 1995). Jacob (1986) reported that the tuber contained 0.05 to 0.12 per cent essential oil. This oil has 65.2 per cent phenolics; predominant among them are Ethyl Salicylate, Thymol, Curvacol, Eugenol and Chavicol. *Coleus* is a season bound crop (Vimala, 1994). Vasudevan and Jos (1990a) reported that *Coleus* is a strictly seasonal crop and tuber production is found in July to September planting. Singh and Mandal (1976) reported that the optimum time for planting *Coleus* for getting maximum yield is during the first week of October.

2.1 Studies on Variability

For any crop improvement programme, a detailed knowledge of genetic variability of various quantitative characters and their contribution to yield is an essential prerequisite. Thus a basic step to the planning of any breeding programme is to determine how much proportion of the total variability in a character would be caused by the differences in the genetic make up of the individuals of the crop species. A quantitative measure of this is provided by the coefficient of heritability.

The variability available in a population can be partitioned into heritable and non heritable components with the aid of genetic parameters such as phenotypic and genotypic variation, heritability, genetic advance and genetic gain which serve as the basis for selection. The extent of variability in tuber crops has been studied by estimating genetic parameters. Lush (1940) and Johnson *et al.* (1955a) devised an accurate procedure for calculation of genetic advance under specified intensities of selection. Burton (1952) introduced a convenient procedure for the calculation of genotypic and phenotypic coefficient of variation. Hanson *et al.* (1956) proposed the mathematical relationship of various estimates on computation of heritability. In asexually reproducing plants like cassava, any combination of genetic factors that yield a superior genotype can be used through clonal propagation. In such circumstances, all genetic variability can be used and heritability estimates have meaning (Hanson, 1963). Six diverse cassava populations grown during 1979 to 80 and 1980 to 1981 at IITA were investigated to estimate genetic parameters for 22 traits of Cassava. The data on analysis revealed that considerable variation existed both within and between populations for most of the characters. The coefficient of variation for phenotype and genotype were largest for root yield and roots per plant, moderate for harvest index and total number of branches and low for stem girth, canopy width and plant height at harvest. Heritability estimates as well as expected genetic gain also varied considerably. On an average root yield and number of roots showed moderately high heritability and high response to selection. Relatively high heritability estimates were obtained for harvest index and dry matter content, but they were associated with expected genetic gain of only

50% and 29% respectively. Agronomic traits such as stem girth, canopy width and plant height at harvest showed moderate to low heritability values associated with low expected genetic gain (Mahangu *et al.*, 1983). In a study to estimate the variability among ten varieties of cassava by Rai *et al.* (1986), characters like height of plant, average weight of tubers number of marketable tubers, girth of tuber, length of tuber and weight of tuber per plant showed higher phenotypic variants than genotypic variants. Height of the plant had maximum variation due to environment. Variability studies in cassava showed that genotypic coefficient of variations were high for all the characters studied except stem diameter (Naskar *et al.* 1991). In cassava Suthanthirapandian *et al.* (1994) reported that the genotypic and phenotypic coefficient of variation for the different traits did not vary much revealing that they were not influenced much by environment and selection can be based on phenotypic value themselves.

According to Jones (1986) in sweet potato, additive component of genetic variance was relatively more important than non-additive for total root weight, average weight per root and number of roots. For total weight of roots and stem, the main components of genetic variance were non-additive type. Total weight, average weight and number of roots showed comparatively high heritability of 44.5 per cent, 50 per cent and 43.5 per cent respectively. With a selection intensity of 10 per cent, the predicted and observed genetic advance closely coincided. Singh and Mishra (1975) studied genetic variability in 41 varieties of sweet potato in 1975 for six characters. A wide range of variability was observed in all the quantitative characters. Difference among the varieties was

highly significant. Phenotypic, genotypic and environmental variances were higher for all the characters studied except number of branches per plant. Total leaf area gave highest genotypic coefficient of variation followed by length of vine, number of leaves and yield of tubers. Estimates of heritability percentage in broad sense were of high magnitude for total leaf area, length of vine, number of leaves and yield of tubers. Total leaf area per plant, length of vine per plant and yield of tuber per plant had high values for genotypic coefficient of variation, heritability percentage and genetic advance indicating the scope for selection of these characters on the basis of their phenotype.

Investigation carried out by Thamburaj and Muthukrishnan (1976) in sweet potato indicate that in general, genotypic coefficient of variation was lower than phenotypic coefficient of variation indicating larger measure of environmental influence. Weight of tuber per vine, number of leaves per vine and the weight of foliage, exhibited a high degree of both phenotypic and genotypic coefficient of variation while girth of stem had low coefficient of variation. High heritability estimates were obtained for length of petiole and number of leaves per vine. High heritability and low genetic advance were observed for all the characters except girth of tubers and number of tubers per vine in which the genetic advance was very high. Kamalam *et al.* (1977) reported high variability and breeding potential together with scope for selecting early desirable genotypes in open pollinated seedling progenies of sweet potato for characters like vine length, stem thickness, petiole length, skin colour, flesh colour and weight of tuber. In a study with 12 varieties of cassava to estimate genetic variability, for seven quantitative characters

the phenotypic coefficient of variation was higher than genotypic coefficient of variation for all characters. Phenotypic and genotypic coefficient of variation was high for number of nodes and tuber yield per plant (Biradar *et al.*, 1978). Maluf *et al.* (1983) observed high heritability values in sweet potato for number of marketable roots and vine length. When 11 characters were evaluated, in 15 cultivars of sweet potato, weight of dry matter, length of main stem, stem/tuber weight, internodes length and yield showed high heritability values. Stem/tuber weight and number of tubers had high genotypic coefficient of variation and genetic advance followed by yield per plant, length of main stem and branch number (Lin, 1983).

Teratological variations in *Coleus parviflorus* was reported by Amalraj *et al.* (1989). Evaluation of 40 collections at NBPGR, Vellanikkara showed that difoliately compound leaves, three whorled phyllotaxy, stem fasciations and rectangular stem were observed in place of simple decussate leaf on quadrangular stem. Variability within *Coleus* was reported by Sreekumari and Abraham (1985). This variation in morphology especially three whorled phyllotaxy may be considered physiologically significant since they possess more bio mass than ordinary plants. Vimala (1994) reported that there was no significant difference in yield between the different accessions of *Coleus* maintained in the germplasm at CTCRI. However, one accession CP-58 gave a comparatively higher yield and was released as 'Sreedhara'. Analysis of hybrid progenies of sweet potato, by Vimala and Nair (1988) showed that yield variability was highly significant between family variants or means indicating superiority of some parental

combinations. Variability in the hybrid progenies of sweet potato was noted by Pillai *et al.* (1990). Goswami (1991) reported variation in growth attributes and quality parameters in some sweet potato genotypes in Assam. Kamalam (1991) reported high variability for quantitative characters like vine length, vine thickness number of branches number of tubers and tuber yield in clonal population of sweet potato indicating scope for selection for desirable types. A field trial of sweet potato cultivars showed significant variations for characters like tuber length, tuber yield and weevil infestation (Sarkar *et al.*, 1992). Evaluation of chemical composition and starch content for different cultivars of sweet potato showed significant difference in protein, starch, fibre and ascorbic acid content (Batistute *et al.* 1992). Contrary to this finding Prakash (1996) reported that tuber characters like tuber yield per plant, tuber length, tuber girth and tuber length to girth ratio did not show any significant difference among genotypes of *Coleus*. The low genetic gain of economically useful characters such as protein and starch content of tubers, suggest that selection can bring less improvement over the mean value. Genotypic coefficient of variation was significantly different for vine and root yield characters of two commercial Egyptian cultivars of sweet potato (Shalaby *et al.* 1993).

Lakshmi and Amma (1980) reported that the phenotypic coefficient of variation was higher than genotypic coefficient of variation for all characters in Asian Greater Yam. But genotypic correlations were higher than phenotypic correlations.

2.2 Heritability and genetic advance

Heritability specifies the proportion of total variability that is due to genetic causes or it is the ratio of the genetic variance to the total variance (Allard, 1960). It indicates the effectiveness with which the selection of genotypes can be based on phenotypic performance (Johnson *et al.*, 1955b). The heritability estimates also provide a clear picture of the average effect of the genes transmitted from parents to the offspring or the extent to which the variability of the quantitative character is transferable to the progeny. However heritability estimates along with genetic gain were more useful and reliable than heritability estimate alone in predicting the selection response.

Reports on heritability and genetic advance are numerous for various quantitative characters in a number of cultivated plants especially in seed propagated crops; but its application in vegetatively propagated crops are quite meagre. Investigations with 15 clones of sweet potato indicated that characters like weight of tubers per plant, number of leaves per vine and weight of foliage exhibited high heritability and low genetic advance, but girth of tubers and number of tubers per vine exhibited high heritability and high genetic advance (Thamburaj and Muthukrishnan, 1976). High heritability estimates and genetic advance were reported for length of vine and number of tubers in sweet potato (Kamalam *et al.*, 1977). In an investigation with 12 varieties of cassava, heritability and genetic advance estimates were high for number of nodes and tuber yield per plant (Biradar *et al.*, 1978).

Naskar *et al.* (1991) by studying the performance of F_1 populations of cassava reported high heritability estimates and genetic advance for plant height, stem diameter, number of tubers and tuber yield indicating their efficiency in selection. By genetic analysis of 12 characters in 25 accessions of taro, Pillai and Unnikrishnan (1991) reported high heritability and genetic advance estimates for characters like weight of cormels per plant and number of cormels per plant. These results show the scope of individual plant selection based on these characters for the genetic improvement of taro. Evaluation of sweet potato lines for yield and its parameters in West Bengal revealed high heritability for vine length (Sen and Goswami, 1991). Vimala and Lakshmi (1991) reported high heritability estimates for tuber length, tuber weight, number of branches, tuber girth and vine weight indicating additive genetic variance in sweet potato. Low heritability estimates were observed for vine length, vine girth, number of leaves per plant, petiole length and number of tubers.

In a study involving 76 genotypes of cassava, Suthanthirapandian *et al.* (1994) reported that highest genetic advance was noticed for number of leaves. Among the economic characters, highest genetic advance was noticed for tuber yield per plant followed by number of tubers, single tuber weight and tuber length. The highest heritability was for number of leaves. In taro, estimate of genotypic and phenotypic variance coupled with high heritability was noticed for length and diameter of cormel. All the characters showed heritability exceeding 60 per cent. This indicates the scope for attaining high yielding clones from local population of

taro (Apte *et al.*, 1994). Harvest index did not become an important feature of crop assessment until after the publication of the review on harvest index by Donald and Hamblin in 1976 (Hay, 1995). Harvest index is closely associated with yield (Sinclair, 1998). Kawano *et al.* (1978) had reported that there was enormous genetic variation for harvest index in seedling populations of cassava and by virtue of its high heritability could be effectively used as an indicator for seedling selection. In cassava, heritability of harvest index was much higher than that of root yield and total plant weight under both very high yielding and low yielding environments. This indicated that harvest index was a highly stable character over a wide range of environment whereas yield and total plant weight were not as stable. Holmes and Wilson (1974) recommended that harvest indices could be made use of as a measure for assessment of the yield potential in a collection of cultivars grown under similar conditions. According to Cock (1976), high yielding variety should have high harvest index. Kawano *et al.* (1977) stated that varietal evaluation and yield and harvest index were sufficient for making efficient visual selection in cassava.

2.3 Correlation studies

One of the important objectives in a breeding programme is the incorporation of the genetic potential from high yield in a variety. Since yield is a complex character, it is worthwhile to estimate the influence of the association existing between the variable characters and yield. Correlation studies were conducted to determine the interrelationship among various traits which are useful

in making selection. Generally estimates of genetic correlations are of very low precision. A knowledge of the magnitude and sign helps to understand how the improvement in one character will cause simultaneous change in other characters. A comparison of phenotypic and genotypic correlations would give an indication of the effect of environment on the genetic performance of individuals of a population.

Galton (1889) conceived the idea of correlation of variables for the first time. Williams and Gazali (1979) has reported that harvest index of high yielding cassava was very high and that high yield was not necessarily due to the production of large total biomass. They also suggested that differences in yield might be associated with an improved canopy structure with narrow leaves that becomes more vertically oriented at mid day. The mean components of yield in tuber crops were the number and mean weight of tubers which were directly related to the process of tuber initiation and tuber growth (Wilson 1973).

Enyi (1973) reported negative relationship between leaf area and tuber yield in dwarf plants of tapioca. Bulking rate increased with increase in leaf area index, reaching a maximum at a leaf area index of 3.5. In tapioca high yields could be achieved with low leaf production cultivars provided that high harvest index were realized. An investigation with M_4 variety of tapioca revealed that the yield of tubers had a positive correlation with length of tuber, girth of tuber, number of nodes per plant and height of plants. Leaf area and tuber yield were negatively correlated while leaf length and breadth were positively correlated. The node

number had a positive correlation with characters other than leaf area. The height of plants recorded a positive relationship with length, girth and yield of tubers while leaf area exhibited negative relationship (Muthukrishnan *et al.*, 1974). Magron and Krishnan (1973) reported that in tapioca tuber length, circumference and number per plant were positively correlated with tuber yield but tuber number per plant was negatively correlated with tuber length and circumference. High leaf number was positively correlated with high yield.

The traits such as starch, dry matter, crude protein and cyanide content were independently inherited. Holmes and Wilson (1974) reported that in cassava, there was significant negative correlation between tuber number and mean tuber weight in high yielding and low yielding cultivars. Works at IITA Nigeria, indicated significant positive correlation between number and size of tubers with yield in tapioca (Anon, 1987). Determination of correlation in sweet potato revealed that an increase in the length of vine causes significant increase in tuber yield, but leaf area had negative correlation with yield (Pushkaran *et al.*, 1976). An investigation with 65 clones of sweet potato indicated the number of tuber per vine, girth of tuber, weight of foliage, number of branches per vine, number of leaves per vine, length of petiole had high degree of positive association with tuber yield both at phenotypic and genotypic levels while the characters like the girth of stem and length of vine had negative correlation (Thamburaj and Muthukrishnan, 1976).

In a study to estimate correlation in sweet potato, Kamalam *et al.* (1977), found that number of tubers had positive significant correlation with yield.

But length as well as the weight of vine showed significant negative correlation with yield. In cassava, the harvest index, number of tubers per plant and mean tuber weight showed positive correlation with the yield (Biradar *et al.*, 1978). Kawano *et al.* (1978), reported significant positive correlation between harvest index and yield based on population studies in cassava. Lakshmi and Amma (1980) recorded positive significant correlation of number of shoots, number of branches and leaves with tuber yield in Asian greater yam. Naskar *et al.* (1986) worked out correlation for seven characters in sweet potato and showed that number of branches, girth and length of tubers had positive correlation with yield. Correlation studies by Sreekumari and Abraham (1985) showed that in *Coleus* tuber yield was positively and significantly correlated with shoot length and number of branches. The harvest index was positively correlated with number of tubers and tuber girth.

Ibrahim (1987) found new significant correlation between tuber yield in sweet potato. Mohankumar *et al.* (1990) indicated that mean weight of cormel; number of cormels per plant and leaf area index was positively and significantly correlated with yield. Correlation studies for 7 characters in cassava, showed that tuber yield was positively and significantly correlated with all the characters except petiole length (Naskar *et al.*, 1991). Sreekumari and Abraham (1985) reported negative correlation between shading and tuber development and that it was less for shoot and leaf formation. Girth of stem and tuber showed significant positive

correlation with tuber yield under shade. Nanda (1994) reported that in sweet potato, marketable tuber yield was positively and significantly correlated with number of tubers per plant but neck length was negatively correlated with yield. In sweet potato vine length, number of branches number of leaves and tuber yield showed high genotypic and phenotypic coefficient of variation from data on eight quantitative characters in 25 genotypes of sweet potato (Kumar *et al.*, 1996).

2.4 Path Coefficient Analysis

Path coefficient analysis devised by Wright (1921) is a standardized partial regression coefficient and as such measures the direct influence of one variable upon another and permits the separation of correlation coefficients into components of direct and indirect effects. Working on Crested weed grass Dewey and Lu (1959) demonstrated the method of path coefficient on the analysis of correlation in a system of correlated variables which was widely employed by animal breeders but only rarely by plant breeders. After their pioneering work, many research workers in many crop plants including tuber plants extensively undertook the path coefficient analysis. Analysis of 17 varieties of sweet potato by Pushkaran *et al.* (1976) showed that yield was influenced by the first order components like girth of tubers, number of tubers and length of tubers; the direct effect of the last two being more pronounced. An increase in the second order component of vine, length of vine, caused a significant increase in tuber yield but was associated with an increase in leaf area which had negative correlation with yield. It is concluded that attempt to increase vine length should be coupled with selection of reduced leaf area.

Thamburaj and Muthukrishnan (1976) on sweet potato indicated that weight of foliage, girth of tuber and number of tubers per vine contributed maximum direct effect on tuber yield indicating the importance of these traits as selection indices for sweet potato. Characters like number of leaves length of petiole and length of tuber had negative direct effect on tuber yield. The study to estimate path coefficients using 6 characters in 10 varieties of sweet potato (Kamalam *et al.*, 1977) found that number of tubers showed maximum positive direct as well as indirect effects on yield. Path analysis in turmeric by Palhania *et al.* (1981) revealed that plant height had maximum direct contribution towards yield followed by number of secondary fingers and number of leaves. Path coefficient analysis in sweet potato for seven characters with 22 genotypes showed that length of tuber had maximum direct effect on yield (Naskar *et al.*, 1986).

Ibrahim (1987) reported that root characters like tuber girth, number of tubers and tuber length showed high path values than shoot characters indicating that in a breeding programme for yield based on component character shoot character will be of little importance. Maximum direct effect was observed for mean weight of cormels with yield in taro by Mohankumar *et al.* (1990). Rekha *et al.* (1991) reported that the path analysis of yield components of eight cassava accessions revealed that single tuber weight contributed maximum direct effect to tuber yield. Single tuber weight, girth of tuber and length of tuber were found to be the three factors exerting considerable influence directly and indirectly on tuber yield in cassava. Path coefficient analysis revealed that the maximum direct effect on tuber yield was through tuber weight in sweet potato (Kumar *et al.*, 1996).

2.5 Genetic Diversity - Cluster Analysis

The choice of parents is of paramount importance in any breeding programme. Selection of parents based on the extent of genetic divergence has been successfully utilized in different crop plants by Moll *et al.* (1969), Miller and Marani, (1963), Murthy and Anand (1966) and Bhatt (1970). Assessment of large number of genotypes through cluster analysis and the importance of cluster formation based on genetic and geographic diversity using a common set of environmental conditions (Stevens and Reid, 1980 in soybean, Beard and Williams, 1982 in sunflower). Knowledge of the genetic diversity among the different entries of the germplasm is necessary for the proper choice of the parents in any breeding programme. The genetic heterogeneity of the population was positively associated with heterogeneity in space and time. (Thoday, 1953) and geographic diversity (Beardmore and Shami, 1976). But several authors viewed this association as not always true (Arunachalam and Jowar, 1967; Bhatt, 1970; Malhotra *et al.*, 1974). The geographic diversity alone cannot be considered while selecting the parents for breeding nor can it be used as a measure to judge the genetic diversity.

Assessment of genetic diversity that exist in large number of genotypes of diverse origin can be carried out through cluster analysis. Anitha and Dorairaj (1990) reported in sesame that among the plant attributes number of seeds per capsule, fruiting nodes, total capsules per plant, days to flowering were found to be important in the study of genetic divergence based on D^2 analysis. Vivekanandan and Subramanian (1993) assessed the nature and magnitude of genetic divergence

in 28 genotypes in rainfed rice using Mahalanobis D^2 statistics. The population was grouped in to five clusters. The geographical diversity was not related to genetic diversity. Such unparallelism between genetic diversity and geographic diversity might be due to forces other than geographic distance such as ancestral relationship, genetic drift, exchange of breeding material from one place to another and varied nature of selection in different regions. The D^2 analysis of 43 accessions of *Coleus* by Muralidharan *et al.* (1985) indicated some varietal difference between the accessions. However yield trials conducted at Central Tuber Crops Research Institute and at different Districts of Kerala showed no significant difference between the accessions (CTCRI, 1987,1988). There was no variation with regard to frequency of different size of tubers between the accessions (CTCRI, 1989). Divergence analysis of Panikachu (*Colacasia esculenta* (L) Schott.) was attempted by Mannan *et al.* (1993) and information on genetic diversity was derived from multivariate analysis of divergence among 39 accessions for eight yield components. The accessions were grouped into four clusters using Mahalanobis D^2 method. There was no relationship between genetic divergence and geographic distribution of the accessions. Plant height, number of stolons per plant and length of stolons contributed most towards total diversity. Information on genetic divergence of sweet potato was derived from data on eight quantitative characters in 18 genotypes using Mahalanobis D^2 method. Naskar and Kurup (1996) grouped sweet potato genotypes into seven different clusters.

2.6 Mutagenesis

Plant breeding is controlled evolution. Breeders have been exploiting the two major factors of evolution- recombination and selection to their best

advantage for quite sometime. The extensive use of these factors, in breeding demanded the use of many refined techniques in the first half of the century. Research in the last 40 years have elucidated the importance of the third, the most important basic factor in evolutions - mutation as potential tool for modifying plants more or less in the same manner as by the conventional breeding methods. The seventies witnessed the practical utilization of induced mutations in a wide range of crops (Gregory, 1956). Physical mutagenesis are being tried right from the beginning of third century while chemical mutagenesis are recent introductions (Broertjes and Vanharten, 1978). Mutation induced by gamma rays for creating desirable character combinations have been reported by Goud (1967) and Singh (1970). In spite of many investigations the maximum effectiveness and efficiency of radiation for inducing change in plant characters especially those of economic importance have not yet been realized (Nilan *et al.*, 1965). Large number of varieties developed by mutation breeding has arisen from materials irradiated with ionizing radiations (Micke, 1962).

Even before the discovery of the mutagenic effects of X-rays research for chemicals capable of causing mutations began (Auerbach, 1967). Induction of mutation by chemical treatments was amply demonstrated by Auerbach in England with mustard gas (Auerbach and Robson, 1947) and by Oehlkers (1943) in Germany with methane. With this discovery workers all over the world started using different chemicals for their mutagenic activity. Among the various chemical mutagenesis known, the alkylating agents have been found to be most efficient in inducing mutations in a wide range of organisms (Auerbach, 1961). Within this

group, mono functional agents in general and Ethyl Methane Sulphonate in particular, appeared to be more efficient in producing mutations in several organisms including higher plants (Swaminathan *et al.*, 1962). The mutagenic efficiency of Ethyl Methane Sulphonate was first demonstrated in Barley in Heslot *et al.* (1959). For inducing mutations in vegetatively propagated crops chemical mutagenesis have been less frequently used due to the poor uptake and penetration of the chemicals in the vegetative parts (Bowen, 1965; Broertjes and Vanharten, 1978). Further they established that in bulky plant materials like bulbs rhizomes and tubers reproducible results from chemical mutagenesis are particularly difficult to obtain. The treatment duration must be long enough to prevent hydration and through infusion (Konzak *et al.*, 1965). In USSR it is reported that breeders concentrate on, even use exclusively, chemicals for the induction of mutations (Broertjes and Vanharten, 1978). Amirov (1974) claimed that chemical mutagenesis had higher efficiency and output of mutation if the duration of the treatment and the concentrations are well adjusted (IAEA, 1973). Swaminathan (1966b) observed that alkylating agents are more efficient than radiations for inducing point mutations, but less efficient for inducing chemical aberrations. EMS has been successfully used in vegetatively propagated crops such as Chrysanthemum, Rose, Apple, Mango, Ginger, Sweet Potato, Banana, Mint, etc.

2.7 Induced Mutations in tuber crops

Genetic improvement of tuber crops through conventional breeding techniques (Introduction, Selection and hybridization) is possible when sexual reproduction is available, in order to create a broad genetic base. In some tuber

crops clonal selection of some spontaneous mutations has been practiced for a long time and produced many of the varieties which are cultivated at present (Gustafsson and Gadd, 1965). The tuber crops which are vegetatively propagated are a very suitable group of plants for the application of mutation breeding methods. Generally the high degree of heterozygosity which cause complex inheritance of genetic factors as well as frequent polyploidy both serious handicaps in conventional breeding are advantageous in mutation breeding as large variations can often be observed in the irradiated plants. Mutations are the only source of variability in sterile plants and in obligate apomicts (Nybom, 1961). The main advantage of inducing mutations in vegetatively propagated crops is the ability to change one or a few characters in an otherwise outstanding cultivars without altering the remaining and often unique part of the genotype (Broertjes and Vanharten, 1978). Physical mutagen such as gamma rays, X-rays and neutrons are known to be more effective in vegetatively propagated crops because they reach the target easily and can be applied precisely as compared to the chemical mutagen (Konzak, 1984). Irradiation has been more useful than chemical mutagen in producing mutations in asexually propagated crops (Nybom and Koch, 1965). This may be due to insufficient penetration of the chemicals and generally poor chromosome breaking ability. For asexually propagated crops chemical mutagen that produce chromosomal mutations may be more useful than that produced gene mutations. Moreover, EMS was apparently effective as a mutagen in polyploids but relatively ineffective on diploids. This has been suggested by Krishnaswami (1968). They expressed the view that EMS is capable of inducing intragenic

alterations and in the reduplicated genomes of a polyploid many of those genes are non essential that are available for drastic changes that may alter their basic function. Such changes in gene function are not tolerated in diploids. According to many studies polyploids exhibit lower mutation frequencies than diploids. In practice, it appears that in polyploids mutagenic treatments lead to gross chromosomal damage with a dominant expression for certain traits (Broertjes, 1976).

Swaminathan *et al.* (1962) found that EMS induced chromosome and chromatid breaks at mitosis. The relatively low toxic and high genetic effects of EMS (Gaul, 1961) and its high mutagenic effectiveness as well as efficiency in higher plants (Konzak *et al.*, 1961) led to its enhanced practical applications. The effect of alkylating agents and their mechanism of action in biological test systems has been reviewed by Loveless (1966) and Lawley (1973). Information about frequencies of spontaneous and induced mutation in vegetatively propagated crops is rather scarce. Mutations are said to occur at random and there are differences in mutability between different loci and regions of chromosome. Ramachandran (1967) reported that *Coleus* is an amphidiploids and contained $2n=68$ chromosomes. Thoppil and Jose (1995) analyzed the constitution of *Coleus parviflorus* and reported that it is a polyploid with $2n=6X=72$. *Coleus* is reported to be more radio resistant than other tuber crops (Vasudevan and Jos, 1988). Generally a delay in sprouting and reduction in germination percentage was noticed consequent to irradiation at higher doses as reported by Sparrow and Christenson (1950) in potato tubers, Jalaja (1971) in sugar cane, Vasudevan *et al.* (1967) and

Thamburaj *et al.* (1985) in cassava, Vasudevan *et al.* (1968) in *Colocasia*, Natarajan (1975) in turmeric and Gupta *et al.* (1982) in *Costus*. Abraham (1970) reported in cassava that the maximum sprouting of buds from irradiated stem cuttings were obtained at doses of less than 1.5 kR whereas no sprouts were produced at doses of 5.0 kR and more. The percentage of sprouting decreased as the dose of gamma rays increased in tuberose (Sambandamurthi, 1983). The doses for physical mutagen should be as low as possible comparable with efficient mutation induction since in vegetatively propagated crops it is not possible to eliminate undesirable mutations induced simultaneously with desirable ones. The dose rate recommended is between one and 100 Gy per minute (Vasudevan 1994). Vasudevan and Jos (1992) showed that in *Dioscorea alata* and *Dioscorea esculenta*, LD 50 for gamma ray treatment was between 2-3 kR. In tapioca, the percentage of sprouting decreased with increase in the dose of EMS (Thamburaj *et al.*, 1985).

In vegetatively propagated crops, large number of useful types with high yield was obtained as a result of mutation. In potato similar to *Coleus* it is reported that useful mutants could be induced following mutagenic treatments. A genotype having a day neutral response with a low photorespiration rate and high photosynthetic efficiency would be suitable for warmer tropical and subtropical regions (Upadhyya and Purohit, 1973). In cassava (*Manihot esculenta*), Vasudevan *et al.* (1967) could observe mutants with high starch content and decreased HCN content which could enhance the industrial value of cassava. Abraham (1970) and Nair (1991) obtained mutants with high yield in cassava. Higher exposure of

gamma rays was effective in increasing the tuber yield and tuber number in sweet potato (Sumabai and Nayar, 1990). In *costus*, diosgenin content increased as a result of 2 kR gamma ray treatment whereas it decreased at 30 kR (Gupta *et al.*, 1982). Rangaswamy (1986) obtained mutants in *Curcuma longa* by X-ray irradiation which had orange yellow rhizomes with a high curing percentage and curcumin content. In banana, significant variations in the quality of fruit were noticed with increase in dose of gamma rays in MV₂ and MV₃ generations (Radhadevi and Nayar, 1996). The mutagenic treatments reduced the crop durations which is directly proportional to an increase in the dose/concentration of the mutagens.

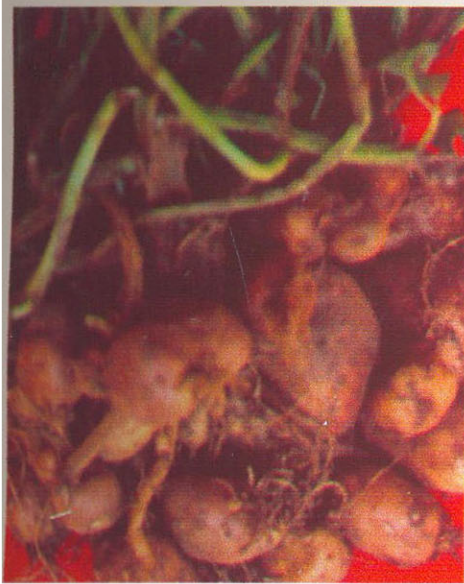
Early maturing mutagens in cassava, could be produced due to mutagenic treatment with gamma rays and EMS (Moh, 1962). Vasudevan and Jos (1988) reported gamma ray induced mutants in *Thamarakannan a*, triploid variety of *Colocasia* with better consumer quality and improved tuber storability. In *Dioscorea alata* and *Dioscorea esculenta*, few clones were identified as dwarf plant types and early maturing types (8 months) as the traditional crop takes 10 months for maturity (Vasudevan and Jos, 1990b). Screening and isolation of mutants with different photosensitivity and thermo sensitivity response has not been conducted to a great extent. The heading time for rice plant is governed by the basic vegetative growth period, which is influenced by photosensitivity and thermo sensitivity. The former two characters have been altered by mutations (Kudo, 1966, 1967). Studies with arabidopsis by Devi *et al.* (1963), gave evidence for the occurrence of mutations that had lost the light requirements for

germination. Photo insensitive mutants from M₃ populations of cotton variety MCU-5 was obtained using different doses of gamma rays. This data suggest that selection in mutagen treated populations may also prove valuable for breaking the barriers to wide adaptations based on photo and thermal sensitivity (Swaminathan, 1968). Vasudevan and Jos (1989) could produce photo-insensitive mutants in *Coleus* by gamma ray irradiation. But these mutants are under various stages of evaluation (Vimala, 1994). Early maturing mutants were successfully induced in *Coleus* and taro using gamma ray treatments Vasudevan and Jos (1990b). They also reported that 12 mutants were induced in cultivars CP-11 of *Coleus* by treatment with 1-4 kR gamma rays. Compared to untreated plants, the mutants showed reduced canopy spread; top yield and tubers/ plant whereas higher values were noted for harvest index, yield per plant and tuber size. Mutants with higher tuber yields and better quality were obtained when genetically pure seeds of yam bean were treated with various combinations of EMS (Nair and Abraham, 1992). Gamma ray induced mutants were obtained in *Xanthosoma* that differed with respect to yield (Vasudevan and Jos, 1990b). Mutants for tuber colour were obtained in sweet potato by gamma irradiation (Vasudevan and Jos, 1996).

2.8 In vitro mutagenesis in tuber crops

Vegetatively propagated crops do not produce seed and often the size of the propagule is too big to treat larger populations with mutagen. *In vitro* techniques allow mutagenic treatments of large numbers and multiplication of the selected genotypes in a small space and duration under disease free conditions. After treatments with mutagen, the chimeral tissues can be separated out into

mutated and non-mutated sectors without loss of plants. *In vitro* culture techniques allow selection of the desired variants from large populations of cells. Even though the occurrence of desired mutation is empirical and random, the combination of *in vitro* and mutation techniques can speed up the breeding of vegetatively propagated plants (Ahloowalia, 1995). Mutation breeding including *in vitro* mutagenesis may be resorted for inducing variability. Exploitation of somaclonal variation is another possibility (Rajmohan, 1988). Somaclonal variations are considered as mutational hot spots (Larkin and Scowcroft, 1978). Berljak (1991) reported a wide range of somaclonal variations in potato plants regenerated from shoot culture and tuber derived callus culture. Many of the regenerants differed from the donor plants and from each other. Successful mutagenesis have been reported in *Cymbidium* (Wimber and Vancott, 1967), *Dendrobium* (Sanguthai and Kammoto, 1973) and *Vanda* (Sanguthai and Sagawa, 1973) using Colchicine. According to Vajrabhaya (1977) treatment with chemical or physical mutagens can cause chromosome or gene mutations spontaneously *in vitro*. Any of the affected cells may divide and form Chimeras which subsequently become complete plantlets with new characteristics. Shoot tips, nodal segments with axillary meristems, inter nodal segments, petiole and leaf lamina have been successfully used for the regeneration of the whole plants in tubers (Nair, 1991). Very little work has been done on the tissue culture of *Coleus* (Vimala, 1994). Bejoy *et al.* (1990) and Asokan *et al.* (1984) could produce adventitious shoots from callus regenerated from internodes and leaf explants of *Coleus*.



Materials and Methods

3. MATERIALS AND METHODS

In order to cover the objectives envisaged in this project, three major field experiments were laid out under the Department of Plant Breeding and Genetics during 1999 to 2002. The initial studies of this project were carried out at the Agricultural Research Station, Mannuthy (15 m above MSL) and the later studies at the College of Horticulture, Vellanikkara (22.5 m above MSL) situated between 10° 32' N latitude and 76° 10' E longitude.

3.1 Experiment I

3.1.1 Survey and Collection of *Coleus* genotypes

Eco-geographical survey of the cultivation of *Coleus* was conducted in three southern states of India viz., Kerala, Tamil Nadu and Karnataka. Tuber samples were collected from both the farmers' fields and from the main markets of the area. The wild progenitors were not noticed in the area of high domestication of this crop. Details of the sixty accessions collected are given in Table 1. These 60 accessions formed the basis of this study.

State and district wise distribution of *Coleus* genotypes collected from the survey is given in Table 2.

3.1.2 Evaluation of *Coleus* genotypes

Before starting the field experiment, the seed tubers collected from various sources were multiplied in a primary nursery and 45 day old cuttings from the top having 15 cm length with four internodes were used for planting in the main field for evaluation at the Agricultural Research Station, Mannuthy during

Table 1. List of *Coleus* accessions collected from South India

Sl. No.	Name of the accession	Sl. No.	Name of the accession
1	Edathara Local 2	31	Pothanmala Local
2	IC 65724	32	IC 85704
3	CP-79	33	IC 85708
4	Thachanatukara Local	34	Madurai Local
5	Kodamala Local	35	Meloor Local
6	Alipparambu local	36	IC 85700
7	Sreedhara	37	IC 65737
8	Edathara Local 3	38	IC 65709
9	Pattambi Local	39	IC 85688
10	CP-74	40	Mullurkara Local 2
11	Edathara Local 1	41	IC 85679
12	Thalassery Local	42	IC 65735
13	Karnataka Local 1	43	Akamala Local
14	Manjeri Local 2	44	Chalakkudy Local
15	Perigalloor Local	45	IC 85703
16	KanjangadLocal	46	IC 85689
17	Kasaragod Local	47	Mullurkkara Local 3
18	Manjeri Local 1	48	Parlikad Local
19	Kapoor Local 2	49	Mullurkkara Local 1
20	Mundathicode Local	50	IC 85682
21	Kapoor Local 1	51	Mudikkodu Local
22	Madakkathara Local	52	Payipra Local
23	Kolazhy Local	53	Mannamangalam Local 2
24	IC 65725	54	Asamannur Local
25	Kottapparambil Local	55	Mannamangalam Local 1
26	Edathara Local 4	56	Vilanganoor Local
27	Trivandrum Local	57	IC 65730
28	IC 85707	58	Karnataka Local 2
29	IC 85697	59	Kuzhipulli Local 1
30	Thirunelveli Local	60	Kuzhipulli Local 2

Table 2. State and districtwise collection of *Coleus* accessions

Sl. No.	Name of the accession	Place of Collection (State/District)	No. of accessions Collected
1	Kasaragod Local 1	Kasaragod	2
2	Kanjangad Local		
3	Thalassery local	Kannur	1
4	IC 85707	Kozhikode	1
5	Edathara Local 1	Malappuram	11
6	Edathara Local 2		
7	Edathara Local 3		
8	Edathara Local 4		
9	Manjeri local 1		
10	Manjeri Local 2		
11	Peringalloor Local		
12	Kapoor local 1		
13	Kapoor local 2		
14	IC 65725		
15	IC 65724		
16	CP 79	Palghat	8
17	Pattambi Local		
18	CP 74		
19	IC 85704		
20	IC 65737		
21	IC 65735		
22	IC 85703		
23	IC 65730		
24	Thachanattukara Local	Thrissur	15
25	Kodamala Local		
26	Alipparambu local		
27	Mundathicodu local		
28	Madakkathara Local		
29	Kolazhy Local		
30	IC 85708		
31	IC 65709		
32	Chalakkudy Local		
33	Mullurkkara Local 1		
34	Mullurkkara Local 2		
35	Mullurkkara Local 3		
36	Parlikkadu Local		
37	Mudikkodu Local		
38	Vilangannoor Local		

Contd.

Table 2. Continued

Sl. No.	Name of the accession	Place of Collection	No. of accessions Collected
39	IC 85682	Ernakulam	8
40	Akamala Local		
41	Paipra Local		
42	Mannamangalam Local 1		
43	Mannamangalam Local 2		
44	Asamannur Local		
45	Kuzhipulli Local 1		
46	Kuzhipulli Local 2		
47	Kottapparambil Local	Kottayam	3
48	Potthanmala Local		
49	Meloor Local		
50	IC 85689	Pathanamthitta	2
51	IC 85688		
52	Trivandrum Local	Trivandrum	2
53	Sreedhara		
54	Karnataka Local 1	Karnataka	3
55	Karnataka Local 2		
56	IC 85679		
57	Madurai local	Tamil Nadu	4
58	Thirunelveli Local		
59	IC 85697		
60	IC 85700		

April- October 1999. The experiment was laid out in RBD with two replications each having 60 accessions of *Coleus* as treatments. Uniform care and management were given to the crop following the Package of Practice Recommendation (KAU, 1993). Each plot was a raised bed with 10 plants planted in two rows with 60 cm between rows and 30 cm between plants in a row. Five plants from each treatment were randomly selected leaving the border plants and were labelled for the study.

3.1.2.1 Recording of Biometric observations

Observations of the following biometric characters were recorded at 120 days after planting in the main field.

1	Plant height
2	Days to flowering
3	Days to tuberization
4	Tuber yield
5	Point of tuberization
6	Number of tubers per plant
7	Tuber girth
8	Tuber volume
9	Average weight of tubers
10	Tuber density
11	Susceptibility to nematode infestation
12	Biological yield
13	Harvest Index

1. Plant height at harvest

The length of the shoot was measured at harvest as the height from the ground level to the tip of the top most leaf. The average of five plants were computed and expressed in cm.

2. Days to flowering

The number of days taken from planting till the appearance of the first flower opening was recorded as the days to flowering. This was recorded at 15 days interval till 50 per cent of the plants in the plot flowered and scores were given as follows.

< 100 days	-	4
100-115 days	-	3
115-130 days	-	2
> 130 days	-	1

3. Days to Tuberization

Initiation of tuberization was observed at 15 days interval, after 90 days of planting and scores were given as

< 100 days	-	4
100-115 days	-	3
115-130 days	-	2
> 130 days	-	1

4. Point of tuberization

This parameter was employed to denote the point of tuber formation on the plant. Tuberisation was at the base of the stem or at leaf nodes or both scores were assigned as follows to denote this character.

Base of stem alone	-	3
Base of stem + Leaf nodes	-	2
Leaf nodes only	-	1

5. Tuber yield per plant

Fresh weight of tubers from five randomly selected plants were recorded using a top pan weighing balance after removing under developed tubers and soil particles. The average yield was expressed in grams

6. Tubers per plant

The average number of tubers obtained from the five selected plants excluding aerial tubers was recorded as number of tubers per plant.

7. Girth of tuber

It was recorded as the average girth of 25 tubers randomly selected from each treatment and expressed as centimeters. The length was measured using a cord and a meter scale.

8. Mean volume of tuber

The volume of 25 randomly selected tubers from each treatment was recorded by water displacement using measuring jar. Mean volume was expressed in cubic centimeter (cc).

9. Mean tuber weight

The mean weight of 25 tubers selected from each treatment was taken and expressed in grams.

10. Tuber Density

Density of tubers was calculated as the ratio of tuber weight in grams to the tuber volume in cc.

11. Susceptibility to infestation by nematodes

Severity of infection on tubers by nematodes was scored visually and score of 0, 1, 2, 3 and 4 were assigned depending on the severity of infection.

Nil – 0; <25% - 1; 26-50% - 2; 51-75% - 3; >100% - 4

12. Biological yield

The weight of plant tops was recorded from five selected plants at random and average was worked out.

13. Harvest index

It is computed from five selected plants as the ratio of economic yield to total biomass.

3.1.2.2 Statistical analysis

Measures like mean, variance, standard deviation (SD), standard error and coefficient of variation were analysed in SPAR 1 (Statistical Package for Agricultural Workers developed at IARI). Genetic advance, genetic gain, genotypic and phenotypic correlation, path analysis and D^2 analysis were carried out following the methods compiled by Singh and Choudhary (1985). Computations were carried out using the software GENSTAT (developed at the College of Horticulture, KAU).

3.2 Experiment II

3.2.1 Clustering of accessions

The sixty accessions as in 3.1.1 were grouped in clusters based on 13 characters by performing Mahalanobis D^2 statistics. The accessions fell into 10

clusters. From each of these 10 clusters, one superior genotype was selected as the representative genotype, based on superiority for characters viz. plant height, tuber yield, tuber girth and harvest index.

3.2.2 Mutation treatment

Two mutagens were tried. Details of the mutagens used are given in Table 3.

Table 3. Source, dose rate and mode of action of mutagens

Sl. No.	Mutagens	Source	Dose rate	Mode of action
1.	^{60}Co (gamma ray source)	Gamma chamber 900 of BARC, Mumbai with a dose rate of 830 Gy per hour situated at College of Horticulture, Vellanikkara	5000 rads/minute	Ionisation
2.	Ethyl methane sulphonate (EMS) $\text{CH}_3\text{SO}_2\text{OC}_2\text{H}_5$	Sisco Research Laboratories, Bombay	30 hrs.	Alkylolation

3.2.3 Estimation of LD_{50} of EMS

Aqueous solutions of 0.05, 0.10, 0.15, 0.20, 0.30, 0.40, 0.50, 1.00, 1.50, 2.00 and 2.50 per cent EMS were prepared for the estimation of LD_{50} of EMS.

The seed tubers of the variety Sreedhara were sown in the primary nursery and 45 day old single noded cuttings were prepared. Distal part of the leaf was removed from these cuttings and 10 cuttings were immersed in each of the solutions for 30 minutes. A treatment time of 60 minutes was tried in another set of cuttings. The treatment duration of 30 and 60 minutes was selected based on the observations in a preliminary study involving four durations viz., 15, 30, 60, and

90 minutes. The cuttings were then taken out and washed thoroughly under running water and planted in potting mixture taken in pots. Survival was recorded at 15 days interval and on the basis of survival the LD_{50} value was estimated. The LD_{50} value for EMS was found to be 0.4 per cent. Based on this, the doses of EMS were selected as 0.2, 0.4, 0.6, 0.8 and 1.0 per cent.

3.2.4 Estimation of LD_{50} of gamma rays

The seed tubers of the variety Sreedhara were sown in the primary nursery and 45 day old single noded cuttings were selected. The distal part of the leaf was removed from these cuttings and 10 cuttings each were irradiated at gamma ray doses of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 50, 75 and 100 Gy. The cuttings were planted in pots and survival was recorded at ten days interval and the LD_{50} was calculated. LD_{50} for gamma rays was found to be 40 Gy. Based on this, the doses of gamma ray irradiation were selected as 10, 20, 30, 40 and 50 Gy.

3.2.5 Mutagenesis

The seed tubers from selected genotypes were sown in the primary nursery and 45 day old single noded cuttings were subjected to mutagenic treatments. The single noded cutting from 10 genotypes selected were then treated with doses fixed for chemical and physical mutagen. The treated cuttings were then planted in the main field in raised beds. Spacing and after care were given as per Package of Practice recommendations 'Crops' (KAU, 1993). The observations on 13 characters were taken as in Experiment I. At maturity, the plants were harvested individually and observations on the individual plants were recorded separately.

3.2.5.1 Statistical Analysis

The data was analysed statistically in a 10 x 3 factorial RBD for gamma irradiation and 10 x 6 factorial RBD for EMS treatment with two replications. In this analysis genotypes were taken as main effects and control and doses employed were kept as sub effects. Superior genotypes were identified based on morphological and yield characters and advanced to further generation for testing yield and photo insensitivity.

3.2.6 *In vitro* mutagenesis

In vitro mutagenesis was tried as an alternative method to produce superior somaclonal variants. Several reports indicate that the tuber crop plants are amenable to *in vitro* multiplication (Arditti, 1979; Chin, 1982; Bajaj, 1987).

3.2.6.1 Culture media

The medium suggested by Murashige and Skoog (1962) was reported to be the best basal medium for *in vitro* culture of *Coleus parviflorus* by Bejoy *et al.* (1990) and hence was used as the basal medium in the present study. The composition is given in Appendix-I.

All the chemicals of AR grade used as ingredients and additives in the MS medium were procured from M/s Merck India Limited, Sisco Research Laboratories Private Limited, British Drug house and Sigma Limited. Borosilicate glassware of Corning, Vensil and Borosil brands were used. Medium was prepared by following the standard procedure adopted by Gamborg and Shyluk (1981). Stock solutions of major and minor elements were prepared and stored in cleaned

amber coloured bottles in refrigerated conditions. The composition of the stock solution of major and minor elements is given in Appendix-II. Aliquots from all stock solutions were pipetted in proportionate volumes into a cleaned steel vessel. Required quantities of sucrose and inositol were added and dissolved. The desired volume was made up by adding distilled water. The pH of the medium was adjusted in between 5.5 to 5.8 using NaOH or HCl. Agar was added at 0.75 per cent (weight by volume) concentration and the medium was heated to melt the agar. About 15 ml medium was poured to test tubes (15 x 2.5 cm), which were then plugged with cotton. Test tubes containing media were sterilized in an autoclave by applying a pressure of 15 psi at 121°C for 20 minutes.

3.2.6.1 Growth regulators

Different plant growth regulators were added to the basal MS medium. The stock solutions of growth regulators at 1000 mg/L were prepared and stored under refrigeration and aliquots were taken from them for use after dilution. These aliquots were added to the medium before the pH was adjusted.

The growth regulators used were auxin (NAA and IBA) and cytokinin (BA). The media combinations used in this study are given in Table 4.

3.2.6.2 Collection and preparation of explants

Explants for *in vitro* culture were collected from potted plants (Acc. Paipra local) maintained in the glass house of College of Horticulture, Vellanikkara. From these potted plants leafy shoots were collected and put in a sterile glass container and taken to the Plant Tissue Culture Laboratory. In the

Table 4. Media composition used in this study

Treatments	Basal	BAP (mg l ⁻¹)	NAA (mg l ⁻¹)
T ₁	MS	1	3
T ₂	MS	1	4
T ₃	MS	1	5
T ₄	MS	1	6
T ₅	MS	2	3
T ₆	MS	2	4
T ₇	MS	2	5
T ₈	MS	2	6
T ₉	MS	3	3
T ₁₀	MS	3	4
T ₁₁	MS	3	5
T ₁₂	MS	3	6
T ₁₃	MS	4	3
T ₁₄	MS	4	4
T ₁₅	MS	4	5
T ₁₆	MS	4	6

laboratory, leaves were removed from the shoots and then the shoots were wiped with 70 per cent ethanol. From the shoots, internodal segments of 0.5-0.7 mm were excised and subjected to surface sterilization.

3.2.6.2a Surface sterilization and inoculation

The explants were surface sterilized under aseptic conditions maintained inside a laminar airflow cabinet. The inter nodal segments were dipped in 0.1 per cent mercuric chloride solution for five minutes with intermittent shaking. Later on they were thoroughly washed 5-6 times with sterile distilled water and were spread on sterile filter paper to drain inside the laminar airflow chamber. Explants were then trimmed at edges to remove all dried up tissues.

Inoculation was carried out under strict aseptic conditions inside laminar airflow chamber. Surface sterilized explants were inoculated into the culture tubes. One explant per test tube was placed horizontally on the surface of the media. Fifteen tubes were inoculated per treatment.

3.2.6.2b Culture conditions

The cultures were incubated in a close room in which temperature was maintained at $26^{\circ} \pm 2^{\circ}\text{C}$ and humidity between 60 and 80 per cent. For callus induction the cultures were maintained in dark conditions. After callus induction the culture were transferred to light providing 3000 lux fluorescent light for a period of 16 hours followed by eight hours dark period daily.

3.2.6.3 Induction of *in vitro* mutation

In vitro mutation was attempted using gamma rays from a ^{60}Co source (Gamma chamber 900 of BARC, Mumbai) with a dose rate of 830 Gy per hour.

The doses tried were 0.5, 1.0, 1.5 and 2.0 Gy after fixing the LD₅₀. Callus of 9-12 weeks old induced from the internodal segments were made into uniform pieces and subjected to gamma rays at doses fixed. After irradiation the callus pieces were immediately transferred to fresh MS medium with BAP 4 mg l⁻¹ and NAA 2 mg l⁻¹. This is to avoid formation of toxic compounds and enhance radiation efficiency (Ahloowalia, 1998). The growth of the calli and the regeneration of shoots from the calli were noted.

3.2.6.4 Rooting of callus regenerants

The shoots regenerated from calli were excised and transferred to MS basal medium with IBA 1.0 mg l⁻¹.

3.2.6.5 Observations of growth of cultures

a) Percentage callus induction

Of all the inoculated cultures, those which showed signs of callusing were counted and expressed as percentage of total number of inoculated tubes.

b) Percentage of callus regeneration after irradiation

The number of calli cultures with regenerants to the total number of calli surviving after irradiation and expressed as percentage.

c) Percentage recovery of hardened plants

Number of plants survived to the total number of plants regenerated and expressed as percentage.

3.3 Experiment III

3.3.1 Screening of mutants obtained from experiment II

Fourteen superior mutants isolated from mutagenic treatments in experiment II were tested for photo insensitivity in 3 seasons. Three plantings were done in December 1999, February 2000 and April 2000. Crops were harvested in May, July and October 2001, respectively. Chemical and organoleptic quality parameters were determined.

3.3.1.1 Statistical analysis

Analysis of variance for different factors and its interactions were worked out as per Singh and Choudhary (1985) in MSTAT C.

3.3.1.2 Estimation of starch

It was estimated by acid hydrolysis of starch to simple sugars like glucose which was then estimated by heating with Anthrone in the presence of sulphuric acid (Hedge and Hofreiter, 1962).

3.3.1.3 Estimation of protein

It was estimated by preparing protein suspension and mixing with sodium potassium tartarate. After adding the phenol reagent, absorbence was measured at 750 nm. The protein content was calculated by referring to the calibration curve prepared with standard protein (Lowry *et al.*, 1974).

3.3.1.4 Estimation of organoleptic qualities

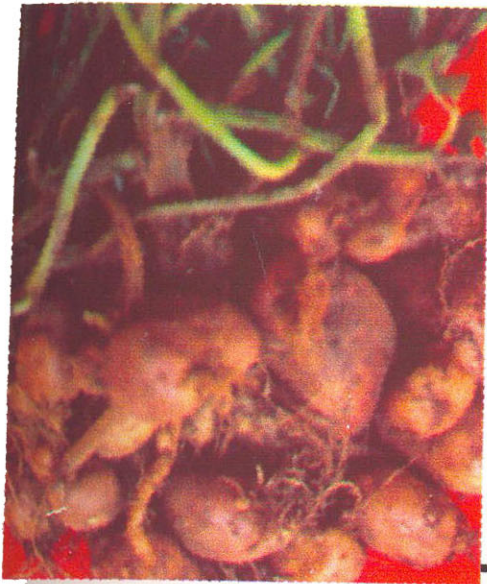
Ten tubers from each of the 14 mutants were washed clean with water and outer skin was peeled off using a stainless steel knife. The peeled tubers were then chopped into small pieces and five table spoon full of the plant part was cooked with 250 ml water for 20 minutes. The cooked tubers was sauted for 10 minutes to remove excess water. The cooked tubers were then kept in plates and

acceptability tests for five quality parameters was done by a select panel of ten judges from a group of twenty five healthy women between the age of 18 and 40 years (Jellinek, 1985). The five organoleptic parameters namely, colour, appearance, texture, flavour and taste of the cooked tubers were estimated by scoring method (Swaminathan, 1974). Scores of 1, 2, 3 and 4 were assigned respectively for grades like very poor, poor, good and very good respectively using a hedonic scale. The score card developed is given in Appendix-III. The cumulative scores were worked out and analyzed statistically.

3.3.1.5 Estimation of essential oils (Alpha thujone and Beta farnescene)

This work was done at the Aromatic and Medicinal Plant Research Station at Odakkali. The essential oil in the tubers was extracted by steam distillation in Clevenger's apparatus (Guenther, 1948). Weighed quantities (800-1000 g) of sliced tubers were put in the 3:1 capacity distillation flask of the apparatus and about 1500 mL water was added. The flask was kept on a 750 W heating mantle and the contents distilled for about 3 to 5 hours. At the end of the period, the distillate flask was replaced with another containing another lot of 800-1000 g of sliced tubers, from the same experimental sample. Thus, after 4 distillations, about 4 kg of the experimental material was distilled and the oil obtained from all the samples were collected in the same condenser/receiver. The volume of oil collected in the receiver was recorded. From the volume of essential oil collected (Vml) and the quantity of the tubers distilled (Wg) the essential oil content of the sample was calculated as

$$\frac{V}{W} \times 100 = x \text{ ml } 100 \text{ g}^{-1}.$$



Results

4. RESULTS

4.1 Experiment I - Field evaluation of 60 genotypes of *Coleus* for variability in yield and yield contributing traits during July 1999 to November 1999

4.1.1 Genetic variability

Development of an effective plant breeding programme is dependent upon the existence as well as magnitude of genetic variability. The efficiency of selection largely depends on the extent to which the desirable characters are heritable. Therefore the estimates of variability in respect of yield and its attributes are prerequisites of success of any crop improvement programme. For the systematic assessment of variability, certain genetic parameters such as genotypic and phenotypic coefficients of variability, heritability, genetic advance and genetic gain have to be studied. In the present study, the extent of genetic variability with respect to thirteen quantitative characters in a set of sixty diverse accessions of *Coleus* were estimated. The analysis of variance revealed significant differences among the genotypes for all the thirteen characters studied (Table 5).

Mean performance of the sixty genotypes in terms of the thirteen different qualitative characters are given in Table 6.

The data on range, mean and estimates of genetic parameters for various quantitative characters are presented in Table 7.

Tuber yield of the accessions ranged from 47.5 g to 500 g with an average of 224.71 g, whereas the tuber girth ranged from 3.30 cm to 6.65 cm with a mean of 4.90 cm. Point of tuberization had a value which ranged from 2.0 to 3.0

Table 5. Analysis of variance of 60 genotypes for 13 characters

Source of variation	df	Tuber yield	Tuber girth	Point of tuberization	Volume per tuber	Weight per tuber	Density per tuber	Tubers per plant	Days to tuberization	Nematode susceptibility	Days to flowering	Plant height	Biological yield	Harvest index
Replication	1	107102.00	218.2187	0.408325	20.50146	24.1127	0.0037	2855.718	421.875	0.1333	26.133	898.5	293040.0	393.828
Genotype	59	21467.839	374.545	1.19949	11.5816	13.6097	0.01006	2533.91	47.383	10.8927	7.8228	903.419	188590.3	346.498
Error	59	3746.788	53.845	0.2218	1.37601	1.46249	0.00027	320.87	4.052	1.0316	1.59	135.555	23804.407	47.288
F. value		5.729*	6.956**	5.408*	8.417**	9.306**	37.259**	7.897**	11.694**	10.559**	4.920*	6.665*	7.922**	7.320**

* Significant at 5 per cent level

** Significant at 1 per cent level

Table 6. Means of characters for different genotypes

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tuber yield (g)	355	360	340	312.5	242.5	302.5	187.5	147.5	142.5	272.5	207.5	65	225	57.5	267.5
Tuber girth (cm)	4.73	4.95	5.75	4.00	4.30	5.18	4.85	5.40	4.45	4.75	5.15	4.28	5.90	4.35	4.35
Point of tuberization	2.5	2.0	2.5	2.0	2.0	2.0	2.0	2.3	2.0	2.0	3.0	2.0	2.0	3.0	2.5
Volume/tuber (cc)	8.2	4.4	3.3	8.4	8.4	8.0	4.8	3.0	4.8	4.1	6.4	4.1	5.8	4.0	8.2
Weight/tuber (g)	8.3	8.7	3.5	8.5	8.5	8.3	4.9	3.2	5.0	4.2	6.5	4.1	6.3	4.1	8.5
Density/tuber (g/cc)	1.0	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tubers/plant	75.7	61.2	61.8	84.2	84.2	36.2	46.6	42.9	71.8	61.5	89.2	84.4	114.3	59.8	32.7
Days to tuberization	122.5	115.0	122.5	122.5	122.5	122.5	122.5	115.0	130.0	122.5	130.0	130.0	122.5	130.0	122.5
Nematode susceptibility	4.0	4.0	4.0	4.0	4.0	3.0	4.0	3.5	1.0	2.0	3.0	2.0	2.5	1.0	2.0
Days to flowering	4.0	4.0	2.5	2.5	2.5	3.5	3.0	3.5	2.0	1.5	2.5	3.5	1.5	2.5	4.0
Plant height (cm)	99.4	77.8	77.3	77.3	77.3	83.5	125	113.5	129.5	74.8	75.2	134.2	123.6	140.9	102.1
Biol. yield (g)	423.8	722.5	746.3	746.3	746.3	663.8	1391.3	1036.3	1443.8	731.8	263.8	1057.5	868.8	978.8	476.3
Harvest index	0.39	0.40	0.30	0.30	0.30	0.31	0.23	0.13	0.10	0.36	0.44	0.60	0.18	0.07	0.30
Characters	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Tuber yield (g)	152.5	322.5	65	297.5	92.5	330	500	395	260	420	347.5	167.5	305	90	102.5
Tuber girth (cm)	5.03	4.00	5.50	5.28	4.95	5.60	6.65	6.43	6.00	5.90	4.15	5.60	5.05	6.15	3.70
Point of tuberization	3.0	2.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.5	2.5	2.0	2.0	1.5	2.0
T. volume (cc)	6.3	8.1	2.5	6.1	4.2	6.9	6.2	6.6	4.9	8.3	6.9	2.9	4.3	3.1	3.9
T. weight (g)	6.6	8.8	2.5	6.4	4.3	7.6	6.7	6.8	5.0	8.9	7.3	3.0	4.5	3.2	4.0
T. density (g/cc)	1.0	1.1	1.0	1.0	1.0	1.2	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.0
Tubers/plant	42.4	57	58.8	79.4	66.6	57.5	78.8	51.9	53.6	52.8	61.7	41.6	66.9	35.7	53.9
Date of Tuber	130.0	130.0	130.0	122.5	130.0	122.5	122.5	122.5	130.0	122.5	122.5	107.5	130.0	130.0	130.0
Nematode susceptibility	2.5	2.5	1.5	2.5	2.0	0.5	2.0	0.0	2.0	2.0	2.0	2.0	2.5	3.0	2.0
Days to flowering	2.5	3.5	1.0	2.5	1.0	3.0	25	4.0	2.5	4.0	4.0	3.5	2.5	1.0	2.5
Plant height (cm)	113.0	83.8	131.8	98.9	150.2	102.8	93.5	75.3	98.3	90.6	90.6	136.8	71.3	120.5	119.8
Biol. yield (g)	1097	756.3	1075	450	1372.5	542.5	576.3	275	610	525	525	882.5	397.5	902.5	900
Harvest index	0.11	0.31	0.06	0.38	0.07	0.37	0.30	0.57	0.30	0.40	0.40	0.14	0.40	0.09	0.11

Contd.

Table 6. Continued.

Characters	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Tuber yield (g)	172.5	110	152.5	122.5	100	302.5	185	362.5	247.5	272.5	317.5	267.5	267.5	240	275
Tuber girth (cm)	5.12	5.00	5.28	4.00	4.45	3.97	4.78	4.95	4.33	4.19	5.03	3.52	5.88	3.98	4.41
Point of tuberization	2.5	3.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.5	2.5	2.5	2.0	2.0	2.0
Volume/tuber (cc)	6.4	3.7	5.6	2.7	4.5	8.2	4.6	5.6	6.1	5.5	6.2	5.7	5.7	13.2	8.5
Weight/tuber (g)	6.5	3.7	6.4	3.2	4.7	8.6	5	5.9	6.3	5.8	6.7	5.9	7.2	14.1	9.2
Density/tuber (g/cc)	1.0	1.0	1.2	1.2	1.1	1.0	1.1	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0
Tubers/plant	51.2	38	47	47.5	39.5	32.8	35.2	34.1	58.7	408	60.1	47.8	87.3	55.5	25.3
Days to tuberization	130	130	130	130	130	130	122.5	130	130	130	130	130	130	122.5	130
Nematode susceptibility	2.0	2.5	2.0	3.0	4.0	2.5	1.0	2.0	2.5	1.5	3.0	2.5	2.5	3.5	2.5
Days to flowering	2.5	1.5	1.0	2.5	1.5	2.0	2.5	3.0	2.0	3.0	2.5	2.0	2.5	2.0	1.0
Plant height (cm)	110.3	98.3	118.8	1222.3	103.3	69.5	88.5	83.5	81.1	70.8	83.5	82.6	104.1	82.7	77.2
Biol. yield (g)	882.5	1353.8	936.3	704.8	990.8	340.8	406.3	631	444.8	392.5	715.3	292.3	650	480	281.3
Harvest index	0.19	0.07	0.14	0.16	0.11	0.45	0.32	0.42	0.38	0.41	0.32	0.48	0.31	0.32	0.45
Characters	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Tuber yield (g)	230	145	287.5	232.5	167.5	212.5	82.5	347.5	60	287.5	220	155	132.5	147.5	47.5
Tuber girth (cm)	4.98	4.53	5.68	4.62	4.73	5.05	5.42	5.67	5.88	4.48	4.19	4.78	4.85	5.37	3.30
Point of tuberization	2.0	2.0	2.0	3.0	3.0	2.0	2.5	2.0	2.5	3.0	3.0	3.0	3.0	2.5	2.5
Volume/tuber (cc)	10.3	4.6	10.7	6.5	6.3	10.3	2.1	7.7	4.9	5.8	4.2	7.1	7.7	4.4	2.6
Weight/tuber (g)	10.8	5.1	13.3	6.8	6.5	10.8	3.0	8.1	5.1	6.6	5.1	7.6	8.2	4.6	2.7
Density/tuber (g/cc)	1.0	1.1	1.2	1.0	1.0	1.0	1.4	1.1	1.0	1.1	1.3	1.1	1.1	1.1	1.1
Tubers/plant	34.7	55.6	51.2	56.5	65.4	62.4	70.1	56.9	59.5	31.1	40.6	36.8	46.9	27.6	35.3
Days to tuberization	130	130	122.5	122.5	130	130	130	130	130	130	130	122.5	130	130	130
Nematode susceptibility	2.5	2.0	4.0	2.0	4.0	2.5	2.0	3.0	2.0	1.0	2.5	2.5	2.5	2.0	2.0
Days to flowering	2.5	1.5	2.5	2.5	2.5	1.0	1.5	2.5	1.5	2.0	2.5	3.0	1.5	1.0	1.5
Plant height (cm)	94.2	111	75.2	91.2	75.4	120.4	104.3	108.3	105.9	98.6	84.9	71.6	78.9	120.8	116.7
Biol. yield (g)	592	922.5	276	472.5	237.5	873.8	503.8	535	758.8	436.3	530	361.3	288	596.3	407.5
Harvest index	0.30	0.17	0.50	0.30	0.35	0.18	0.17	0.39	0.08	0.39	0.27	0.29	0.33	0.18	0.11

Table 7. Range, mean and estimates of genetic parameters for thirteen characters in *Coleus*

Sl. No.	Character	Mean	S.E	Range	PV	GV	P.C.V	G.C.V	h^2 (%)	G.A	G.G
1	T. Y [g]	224.71	6.21	47.50 - 500.00	12609.04	8860.46	49.97	41.89	70.70	162.56	72.34
2	T. G [mm]	49.11	7.34	33.00 - 66.50	79.74	25.81	18.18	10.35	33.16	5.96	12.14
3	PT	2.28	0.47	2.0 - 3.0	0.27	0.04	22.72	9.34	17.74	0.18	7.89
4	V/T [cc]	5.96	1.17	2.45 - 10.70	6.50	5.11	42.71	37.90	79.08	4.13	69.30
5	W/T [g]	6.44	1.21	2.70 - 14.07	7.56	7.51	42.63	38.27	80.89	4.56	70.81
6	TD [g/cc]	1.06	5.22	1.0 - 1.25	0.01	0.01	7.53	5.70	57.92	0.09	8.49
7	T/P	54.31	17.91	25.30 - 89.20	464.83	464.83	39.69	22.09	31.72	13.75	25.32
8	DT	126.63	6.37	107.5 - 130.0	44.09	44.09	5.24	1.46	8.64	1.07	0.85
9	NS	2.37	1.02	0 - 4.0	1.32	0.29	48.54	22.68	22.63	0.52	21.94
10	DF	2.38	1.26	1.0 - 4.0	1.59	0.01	52.94	1.33	0.01	0.0004	0.16
11	PH [cm]	98.54	11.64	56.65 - 140.90	519.38	384.16	23.13	19.89	74.28	34.70	35.21
12	BY	667.83	154.29	275.0 - 1443.75	106210.81	82386.22	48.80	42.98	77.92	520.84	77.99
13	HI	0.27	0.07	0.06 - 0.57	1.97	1.49	51.81	45.15	76.31	0.22	81.09

with an average of 2.28. Volume of one tuber ranged from 2.45 cc to 10.70 cc with an average of 5.96 cc. Average weight of tubers ranged from 2.70 g to 14.07 g with an average of 6.44 g. Tuber density ranged from 1.00 g/cc to 1.25 g/cc with an average of 1.06 g/cc. Number of tubers per plant ranged from 25.30 to 89.20 with an average of 54.31. Days to tuberization ranged from 107.5 to 130.0 with an average of 126.63. Nematode susceptibility scores ranged from 0 to 4.0 with an average of 2.37. Days to flowering ranged from 1.00 to 4.00 with a mean of 2.38. Plant height ranged from 56.65 cm to 140.90 cm with mean of 98.54 cm. Biological yield ranged from 275.00 g to 1443.75 g with an average of 667.83 g. Harvest index ranged from 0.06 to 0.57 with an average of 0.28.

4.1.2 Genotypic coefficient of variation (GCV) and Phenotypic coefficient of variation (PCV)

Generally the magnitude of PCV was greater than that of GCV. Maximum PCV was observed in the case of character, days to flowering (52.94%). This character had a GCV of 1.33 per cent. Tuber yield, mean volume of tuber, mean weight of tuber and biological yield showed high values of PCV (49.97%, 42.71%, 42.63% and 48.80%). Similarly GCV for these characters were also high (41.89%, 37.90%, 38.27% and 42.98%). Days to flowering had a PCV of 52.94 per cent, but this character showed the lowest GCV of 1.33 per cent. Tuber density and days to tuberization showed lower values for PCV (7.53%, 5.24%) as well as GCV (5.70%, 1.46%). Moderate PCV and GCV were observed in case of tuber girth (18.18% and 10.35%), point of tuberization (22.72% and 9.34%), plant height (23.13% and 19.89%) and number of tubers per plant (39.69% and 22.09%).

4.1.3 Heritability

Among the quantitative characters heritability estimates (broad sense) ranged from 0.01 per cent in case of days to flowering to 80.89 per cent in case of mean weight per tuber. High heritability estimates were obtained for mean weight of tuber (80.89%), tuber yield (70.70%), tuber density (57.92%), plant height (74.28%), biological yield (77.92%) and harvest index (76.31%). On the contrary, low heritability estimates were obtained for point of tuberization (17.74%), days to tuberization (8.64%), nematode susceptibility (22.63%) and days to flowering (0.01%). Moderate heritability was seen in case of tuber girth (33.16%) and number of tubers per plant (31.72%).

4.1.4 Genetic advance and Genetic gain

Genetic advance, expressed as percentage of mean varied from 0.16 per cent in case of days to flowering to 81.09 per cent in case of harvest index. High genetic gain was shown by the characters such as harvest index (81.09%), biological yield (77.99%), tuber weight (70.81%), tuber yield (72.34%) and tuber volume (69.30%). Moderate genetic gain was observed in the case of plant height (35.21%) where as low genetic gain was observed for nematode susceptibility (21.94%), tuber girth (12.14%), point of tuberization (7.89%), tuber density (8.49%), days to tuberization (0.85%) and days to flowering (0.16%).

The characters such as tuber yield plant⁻¹, mean volume of tuber, mean weight of tuber and biological yield plant⁻¹ had high heritability, high genetic gain, moderate PCV and GCV. Harvest Index had high values for PCV, heritability, genetic gain and moderate value of GCV.

Tubers per plant had high heritability, moderate values for PCV and low values for both GCV and genetic gain. Tuber density plant⁻¹ had high heritability and low values for PCV, GCV and genetic gain. Length plant⁻¹ had high heritability, moderate values for genetic gain and low values for PCV and GCV. Point of tuberization had low PCV, GCV, heritability and genetic gain. Tuber girth plant⁻¹ had moderate heritability, low GCV, PCV and genetic gain whereas nematode susceptibility had moderate PCV, low GCV, heritability and genetic gain. Days to flowering had high PCV, low GCV, low heritability and genetic gain.

4.1.5 Correlations

The genotypic and phenotypic correlation coefficients between tuber yield plant⁻¹ and its 12 contributing traits are presented in Table 8. The genotypic correlations were found to higher than phenotypic correlation. At genotypic level tuber yield had highly significant positive association with volume per tuber (0.477), weight per tuber (0.507), days to flowering (1.727) and harvest index (0.831). Significant positive association was noticed for tuber girth (0.329) and point of tuberization (0.301). Highly significant negative association was noticed for days to tuberization (-0.614) and plant height (-0.639) and significant negative association was noticed with biological yield (-0.520) and point of tuberization (-0.251). With respect to phenotypic correlation coefficient highly significant positive correlation with yield were noticed for characters - volume per tuber, weight per tuber, biological yield and harvest index. Days to flowering had

Table 8. Genotypic correlations (upper diagonal) and phenotypic correlation (lower diagonal) of 13 characters of *Coleus*

	TY	TG	PT	V/T	W/T	TD	TP	DT	NS	DF	PH	BY	HI
TY		0.329*	-0.251*	0.477**	0.507**	0.030	0.301*	-0.614**	-0.006	1.727**	-0.639**	-0.520**	0.831**
TG	0.192		-0.478**	-0.116	-0.057	0.304*	-0.105	-0.849**	-0.197	0.217	0.090	0.066	0.081
PT	-0.099	-0.155		-0.040	-0.091	0.057	-0.551**	1.388**	-0.025	1.050**	-0.382**	-0.388**	0.048
V/T	0.437**	-0.095	-0.035		0.970**	-0.123	0.046	-0.075	0.366**	0.197	-0.373**	-0.342*	0.509**
W/T	0.477**	-0.079	-0.048	0.971**		0.005	0.063	-0.253*	0.433**	0.331*	-0.407**	-0.360**	0.528**
TD	0.034	0.098	0.097	-0.082	0.042		-0.003	-0.026	-0.184	0.707**	-0.083	-0.289*	0.092
TP	0.052	0.338*	0.003	-0.053	-0.047	0.005		-0.177	0.212	1.098**	0.095	0.119	-0.001
DT	-0.310*	-0.145	0.058	-0.117	-0.150	0.104	-0.053		-0.844**	1.995*8	0.122	0.076	-0.106
NS	0.001	-0.165	-0.112	0.246*	0.291*	-0.019	0.059	-0.027		0.892**	-0.198	-0.002	0.186
DF	0.355*	-0.041	-0.005	0.167	0.189	-0.051	-0.051	-0.433**	-0.022		-0.240*	-0.189	0.373**
PH	-0.554**	0.066	-0.169	-0.389**	-0.419**	-0.117	0.115	0.085	-0.134	-0.168		0.772**	-0.840**
BY	0.437**	0.025	-0.191	-0.311*	0.329*	-0.223	0.086	0.051	0.047	-0.121	0.711**		-0.807**
HI	0.770**	0.008	0.044	0.473**	0.498**	0.092	-0.037	-0.182	-0.042	0.287*	0.734**	-0.749**	

significant positive phenotypic correlation with yield. There was significant negative correlation for the character - days to tuberization and highly negative phenotypic association for plant height. With respect to the inter correlations of the different yield contributing traits at genotypic level, tuber girth had significant positive correlation with tuber density (0.304) and highly significant negative association with point of tuberization and days to tuberization. Point of tuberization had highly significant positive association with days to flowering (1.050), days to tuberization (1.388) and highly significant negative association with point of tuberization (-0.551), plant height (-0.382) and biological yield (-0.388). Volume per tuber had a highly significant positive association with weight per tuber and nematode susceptibility whereas it had a highly significant negative correlation with plant height (-0.373) and biological yield (-0.342). Weight per tuber had a highly significant positive association with nematode susceptibility significant positive association with days to flowering and highly significant negative association with plant height (-0.407) and biological yield (-0.360). It had a significant negative association with days to tuberization. Tuber density had a highly significant positive association with days to flowering and significant negative association with biological yield. Tubers per plant had a highly significant positive correlation with days to flowering. Days to tuberization had a significant positive association with days to flowering and highly significant negative association with nematode susceptibility. Nematode susceptibility and days to flowering exhibited a highly significant positive inter correlation (0.892).

Days to flowering had a negative significant association with plant height and plant height had a significant positive association with biological yield. Biological yield and harvest index had a highly significant negative association (-0.807). Phenotypic inter correlations revealed that weight per tuber had a highly significant positive association with volume per tuber. Significant positive associations were noticed between tuber per plant and tuber girth (0.338) and nematode susceptibility and volume per tuber (0.246). Days to flowering exhibited a highly significant negative association with days to tuberization. Plant height and biological yield exhibited a highly significant negative association with volume per tuber and weight per tuber (-0.389, -0.419). Biological yield exhibited a highly significant positive association with plant height (0.711). Harvest index exhibited highly significant positive associations with volume per tuber, weight per tuber and plant height, a significant positive association with days to flowering and a highly significant negative association with biological yield.

4.1.6 Path Analysis

The character, number of days to flowering, was excluded while estimating path coefficients since this character was not uniform among the genotypes. The estimates of direct and indirect effects of these selected 11 yield contributing characters, on yield are presented in Table 9. The residual effect of path analysis was found to be 0.0803. It was observed that the characters viz., tuber weight, tuber girth and harvest index exerted positive direct influence in that order of magnitude on yield. Tuber volume (-0.605), plant height (-0.402) tuber density

Table 9. Direct and indirect effects of yield and yield attributes of *Coleus*

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	r
X1	0.419	0.104	0.070	-0.047	-0.086	-0.034	-0.126	0.041	-0.036	-0.004	0.031	0.332*
X2	-0.200	-0.217	0.024	-0.075	-0.016	-0.177	0.206	0.005	0.153	-0.022	0.018	-0.256*
X3	-0.048	0.009	-0.605	0.798	0.035	0.015	-0.011	-0.076	0.150	0.020	0.193	0.477**
X4	-0.024	0.020	-0.587	0.823	-0.002	0.020	-0.038	-0.090	0.163	0.021	0.200	0.507**
X5	0.127	-0.012	0.075	0.004	-0.282	-0.001	-0.004	0.038	0.033	0.017	0.035	0.030
X6	-0.044	0.120	-0.028	0.052	0.001	0.321	-0.026	-0.044	-0.038	-0.007	0.000	0.306*
X7	-0.332	-0.302	0.046	-0.208	0.007	-0.057	0.149	0.176	-0.049	-0.004	-0.040	-0.614**
X8	-0.082	0.005	-0.222	0.356	0.052	0.068	-0.125	-0.208	0.080	0.000	0.070	-0.006
X9	0.038	0.083	0.226	-0.335	0.023	0.031	0.018	0.041	-0.402	-0.044	-0.318	-0.639**
X10	0.027	0.084	0.207	-0.297	0.082	0.038	0.011	0.000	-0.310	-0.057	-0.305	-0.520**
X11	0.034	-0.010	-0.308	0.434	-0.026	0.000	-0.016	-0.039	0.337	0.046	0.378	0.831**

Residual=0.0803

X1 – Tuber girth

X2 – Point of tuberization

X3 – Tuber volume

X4 – Tuber weight

X5 – Tuber density

X6 – Tubers per plant

X7 – Days to tuberization

X8 – Nematode susceptibility

X9 – Plant height

X10 – Biological yield

X11 – Harvest index

(-0.282), point of tuberization (-0.217) and nematode susceptibility (-0.208), exerted negative influence on yield. Highest indirect positive effect was shown by tuber volume through tuber weight (0.798). Point of tuberization exerted indirect positive effects on yield through tuber volume (0.024), days to tuberization (0.206) and plant height (0.153). Tuber volume exerted indirect positive effect on yield through tuber density (0.035), plant height (0.150), biological yield (0.020) and harvest index (0.193). Nematode susceptibility exerted indirect positive effect on yield through tuber weight (0.356) whereas plant height exerted its indirect positive influence through tuber volume (0.226). Biological yield exerted positive indirect effect on yield through tuber volume (0.207). Tuber girth exerted negative indirect effect on yield through tuber weight (-0.047), tuber density (-0.086), plant height (-0.036) and days to tuberization (-0.126). Tuber weight exerted negative indirect effect on yield through tuber volume (-0.587), nematode susceptibility (-0.090) and days to tuberization (-0.038). Number of tubers per plant exerted negative effect on yield through tuber girth (-0.044), tuber volume (-0.028), days to tuberization (-0.026), nematode susceptibility (-0.044), plant height (-0.038) and biological yield (-0.007). Harvest index exerted indirect negative effect on yield through tuber volume (-0.308), tuber density (-0.026) and nematode susceptibility (-0.039).

4.1.7 Genetic diversity

Genetic diversity arises due to geographical separation or due to genetic barriers to crossability. This potent variability in the indigenous cultivars is also

due to the result of prolonged natural and artificial selection. The D^2 statistics permit the size assessment of genetic diversity in the population. The present investigation has been carried out with 60 indigenous genotypes collected from different eco- geographical regions.

4.1.7.1 Clustering

The D^2 and D values computed for the 60 *Coleus* genotypes are presented in Table 10. Eventhough the character, number of days to flowering was not uniform among all the genotypes, it has been included for clustering.

Table 10. D^2 and D values of 60 accessions of *Coleus* in 10 clusters

	I	II	III	IV	V	VI	VII	VIII	IX	X
I	4.618 (2.149)									
II	16.112 (4.014)	4.735 (2.176)								
III	17.381 (4.169)	31.461 (5.609)	3.561 (1.887)							
IV	29.866 (5.465)	15.555 (3.944)	30.162 (5.492)	4.072 (2.018)						
V	6.508 (2.551)	14.003 (3.742)	18.714 (4.326)	28.891 (5.375)	2.839 (1.685)					
VI	23.629 (4.861)	13.440 (3.660)	36.542 (6.045)	20.775 (4.558)	27.374 (5.232)	6.333 (2.517)				
VII	8.151 (2.855)	29.052 (5.390)	18.241 (4.271)	33.235 (5.765)	8.474 (2.911)	35.307 (5.942)	4.999 (2.236)			
VIII	23.339 (4.831)	9.517 (3.085)	19.749 (4.444)	13.090 (3.618)	22.477 (4.741)	9.816 (3.133)	29.594 (5.440)	4.301 (2.074)		
IX	26.822 (5.179)	35.490 (5.995)	54.494 (7.382)	44.036 (6.636)	37.369 (6.113)	57.244 (7.566)	37.577 (6.130)	54.76 (7.400)	0 (0)	
X	11.785 (3.433)	6.938 (2.634)	20.603 (4.539)	7.377 (2.716)	14.715 (3.836)	11.276 (3.358)	17.800 (4.219)	6.906 (2.628)	37.516 (6.125)	3.546 (1.883)

Based on the relative magnitude of D^2 values 60 *Coleus* genotypes were grouped into ten clusters. The clustering pattern of 60 genotypes were given in Table 11. The computed D^2 value varied from 2.839 to 57.244 showing high divergence among different genotypes. Clustering pattern revealed that cluster X was the largest group consisting of 10 genotypes, which were indigenous to varied ecogeographical regions. The size of the clusters then decreased in the order of cluster II with 9 genotypes followed by cluster I with eight genotypes, cluster VIII and VI with seven genotypes, Cluster IV, V and VII with five genotypes each, cluster III with three genotypes and cluster IX with a single genotype. The inter-cluster distance was found to be more than intra-cluster distances. The maximum inter-cluster distance was between cluster VI and IX ($D^2 = 57.244$) and the lowest inter-cluster distance is between cluster V and I ($D^2 = 6.51$). The maximum intra-cluster distance was in cluster VI ($D=2.517$) indicating that variability exists between the genotype of the cluster.

It was noticed that genotypes habitating the same location fell into different clusters. In cluster I, Thirunelveli local and Madurai local two genotypes from Tamil Nadu were grouped together with Meloor local from Kerala. At the same time, IC-85700, another genotype which belongs to Thirunelveli occupied position in cluster VI. In general genotypes belonging to different regions got grouped in the same cluster and genotypes belonging to same region got grouped in different clusters. The maximum inter-cluster distance existed between clusters VI and IX ($D^2 = 57.244$) and the minimum between clusters V and I ($D^2 = 6.51$).

Table 11. Clustering of *Coleus* genotypes based on D values

Cluster	Genotypes	Number included in each cluster
I	IC-85697, Thirunalveli local, IC-85708, Madurai local, Mullurkara local-III, Meloor local, Kuzhipulli local-I, Kuzhipulli local-II	8
II	Edathara local-I, IC-85682, IC-65735, Mullurkara local-I, Mullurkara local-II, Mannamangalam local-I, Vilangannor local, IC-65730, Karnataka local-II	9
III	Sreedhara, Edathara local-III, Trivandrum local	3
IV	CP-79, Kapoor local-I, Madakkathara local, Kolazhy local, Kottaparambil local	5
V	Manjeri local-II, Kanjangad local, Pothanmala local, IC-85704, Asamanoor local	5
VI	Kodamala local, IC-85700, Chalakudy local, IC-85703, IC-85689, Parlikad local, Mudicode local	7
VII	Pattambi local, Thalasseri local, Mundathicode local, Manjeri local-I, Karnataka local-I	5
VIII	Edathara local-II, Edathara local-IV, Thachanattukara local, Aliparambu local, Peringalloor local, Kasargode local, IC-65724	7
IX	Paipra local-I	1
X	CP-74, Kapoor local-II, IC-65725, IC-85707, IC-65737, IC-65709, IC-85688, IC-85679, Akamala local, Mannamangalam local-II	10

4.1.7.2 Ranking of genotypes in clusters

The accessions in each cluster were evaluated on the basis of major yield characters viz., tuber yield per plant, girth tuber⁻¹, harvest index plant⁻¹ and height plant⁻¹ (Table 12).

Table 12. Intra-cluster ranking of *Coleus* genotypes based on yield and yield attributes

Accession	Tuber yield per plant (g)		Tuber girth / tuber (mm)		Harvest Index		Plant height (cm)		Overall rank
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
CLUSTER I									
IC-85697	90	7	61.5	1	0.09	8	120.5	6	5.5
Thirunelveli local	102.5	5	37.0	7	0.11	6	119.8	5	5.8
IC-85708	152.5	1	52.8	3	0.14	4	118.8	4	3.0
Madurai local	122.5	4	40.0	6	0.16	3	122.3	8	5.3
Meloor local	100.0	6	44.5	5	0.11	6	103.3	1	4.5
Mullurkkara local-	145.0	3	45.3	4	0.17	2	111.0	2	2.8
Kuzhipulli local-I	147.5	2	53.7	2	0.18	1	120.8	7	3.0
Kuzhipulli local-II	47.5	8	33.0	8	0.11	5	116.7	3	6.0
CLUSTER II									
Edathara local-I	207.5	6	51.5	1	0.44	2	75.2	4	3.3
Mullurkkara local-	272.5	2	41.9	7	0.41	3	70.8	1	3.3
IC-65735	267.5	3	35.2	9	0.48	1	82.6	6	4.8
Mullurkkara local-I	232.5	4	46.2	5	0.30	7	91.2	8	6.0
IC-85682	167.5	7	47.0	4	0.35	5	75.4	5	5.3
Mannamangalam local-I	287.5	1	44.8	6	0.39	4	98.6	9	5.0
Vilangannor local	220.0	5	41.9	7	0.27	9	84.9	7	7.0
IC-65730	155.0	8	47.8	3	0.29	8	71.6	3	5.5
Karnataka local-II	132.5	9	48.5	2	0.33	6	70.9	2	4.8
CLUSTER III									
Sreedhara	187.5	1	48.5	3	0.23	1	125.0	2	1.8
Edathara local-III	147.5	3	54.0	2	0.13	3	113.5	1	2.3
Trivandrum local	167.5	2	56.0	1	0.14	2	136.8	3	2.0
CLUSTER IV									
CP-79	340.0	3	57.5	4	0.31	4	56.7	1	3.0
Kapoor local-I	330.0	4	56.0	5	0.37	3	102.8	5	4.3
Madakkathara local	300.0	5	66.5	1	0.30	5	93.5	3	3.5
Kolazhy local	395.0	2	64.3	2	0.57	1	75.3	2	1.8
Kottaparambil local	420.0	1	59.0	3	0.42	2	94.3	4	2.5
CLUSTER V									
Manjeri local-II	57.5	5	43.5	5	0.07	5	140.9	5	5.0
Kanjangad local	152.5	2	50.3	3	0.11	2	113.0	4	2.8
Pothemala local	172.5	1	51.2	2	0.19	1	110.3	3	1.8
IC-85704	110.0	3	50.0	4	0.07	4	98.3	1	4.3
Asamannur local	60.0	4	58.8	1	0.08	3	105.9	2	2.5

Contd.

Table 12. Continued

Accession	Tuber yield per plant (g)		Tuber girth / tuber (mm)		Harvest Index		Plant height (cm)		Overall rank
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
CLUSTER VI									
Kodamala local	242.5	4	43.0	5	0.26	6	93.5	5	5.0
IC-85700	302.5	1	39.7	7	0.45	3	69.5	1	3.0
Chalakkudy local	240.0	5	39.8	6	0.32	4	82.7	4	4.8
IC-85703	275.0	3	44.1	4	0.45	2	77.2	3	3.0
IC-85689	230.0	6	49.8	3	0.30	5	94.2	6	5.0
Parlikad local	287.5	2	56.8	1	0.50	1	75.2	2	1.5
Mudicode local	212.5	7	50.5	2	0.18	7	120.4	7	5.8
CLUSTER VII									
Pattambi local	142.5	2	44.5	4	0.10	2	129.5	2	2.5
Thalasseri local	65.0	4	42.8	5	0.51	4	134.2	4	4.3
Karnataka local-I	225.0	1	59.0	1	0.18	1	123.6	1	1.0
Manjeri local-I	65.0	4	55.0	2	0.51	5	131.8	3	3.5
Mundathicode local-I	92.5	3	49.5	3	0.07	3	150.2	5	3.5
CLUSTER VIII									
Edathara local-II	355.0	2	47.3	3	0.39	2	99.4	6	3.3
IC-65724	360.0	1	49.5	2	0.31	5	77.8	2	2.5
Thachanattukara local	312.5	5	40.0	6	0.30	6	77.3	1	4.5
Aliparambu local	302.5	6	51.8	1	0.31	4	83.5	3	3.5
Peringalloor local	267.5	7	43.5	4	0.30	6	102.1	7	6.0
Kasargode local	322.5	4	40.0	6	0.31	3	83.8	4	4.3
Edathara local-IV	347.5	3	41.5	5	0.40	1	90.6	5	3.5
CLUSTER IX									
Paipra local	82.5	1	54.2	1	0.17	1	104.3	1	1
CLUSTER X									
CP-74	272.5	6	47.5	9	0.36	6	74.8	2	5.8
Kapoor local-II	297.5	5	52.8	4	0.38	4	98.9	8	5.3
IC-65725	260.0	8	60.0	1	0.30	10	98.3	7	6.5
IC-85707	305.0	4	50.5	5	0.40	2	71.3	1	3.0
IC-65737	185.0	10	47.8	8	0.32	8	88.5	6	8.0
IC-65709	362.5	1	49.5	7	0.42	1	83.5	4	3.3
IC-85688	247.5	9	43.3	10	0.38	5	81.1	3	6.8
IC-85679	317.5	3	50.3	6	0.32	7	83.5	5	5.3
Akamala local	267.5	7	58.8	2	0.31	9	104.1	9	6.8
Mannamangalam local-II	347.5	2	56.7	3	0.39	3	108.3	10	4.5

The accessions were sequenced on the basis of a character and assigned ranks in that order. This was done in case of every character. The mean rank obtained by each accession was calculated. This helped to identify the overall best yielder in each cluster. In cluster I, the accession Mullurkkara local-III ranked first. In cluster II, it was Edathara local-I which rank first based on its overall mean values for the yield attributes. In cluster III, it was Sreedhara which ranked first whereas in cluster IV it was Kolazhy local. In cluster V, Pothemala local and in cluster VI Parlikad local rank first. In cluster VII it was Karnataka local and in cluster VIII Edathara local-III. In cluster IX there was only one genotype Paipra local and cluster X it was IC-85707 which ranked the highest.

4.2 Experiment II - Field evaluation of Gamma irradiated and EMS treated mutants of *Coleus* during July 2000 to November 2000

Ten genetically divergent genotypes having the maximum scores for various yield attributes and representing each cluster identified in experiment No.I were selected for experiment II. These selected genotypes were subjected to physical and chemical mutagenic treatments.

Single noded cuttings of the selected genotypes were treated with different doses of the physical mutagen - gamma rays and different concentrations of the chemical mutagen - ethyl methane sulphonate. The variability induced by the mutagens was evaluated for three generations. The results of the studies are presented below.

4.2.1 Sensitivity study

The effect of various doses of mutagens in causing mutagenic changes in *Coleus* was studied. The survival rate (sprouting percentage) was used to calculate the LD50 of the mutagen under the experimental condition.

4.2.1.1 Gamma rays

Different doses of gamma rays were tried under laboratory conditions, based on the percentage of variation of sprouting of the cuttings over the control subjected to irradiation (Table 13).

Table 13. Effect of varying doses of gamma rays on the sprouting of single noded cuttings of *Coleus* (Laboratory conditions)

Dose of gamma irradiation (Gy)	Sprouting (%)	Depression of sprouting over control (%)
Control	80	0
1	50	62.5
2	40	50.0
3	20	25.0
4	40	50.0
5	50	62.5
6	20	25.0
7	50	62.5
8	20	25
9	30	37.5
10	30	37.5
15	70	87.5
20	40	50.0
25	20	25.0
30	40	50.0
35	30	37.5
40	50	62.5
50	20	25.0
75	20	25.0
100	10	12.5

As the dose increased up to 40 Gy the percentage of variation of sprouting over the control was 62.5 per cent and thereafter there was a sharp

decline of 25 per cent and less than that. Hence LD₅₀ of the gamma rays was fixed as 40 Gy. Therefore four doses of mutagen at regular intervals namely, 10, 20, 30, 40 and 50 Gy were applied on the cuttings and were tried in the field trials.

4.2.1.2 Ethyl methane sulphonate (EMS)

The data on the effect of different concentrations of EMS and treatment duration on the development of sprouts under lab conditions are presented in Table 14.

Table 14. Effect of varying doses of EMS and duration of treatment on sprouting of *Coleus* (Laboratory conditions)

Concentration of EMS (%)	Sprouting (%) at treatment duration		Depression of sprouting over control for treatment duration (%)	
	30 minutes	60 minutes	30 minutes	60 minutes
Control	100	80	0	0
0.05	80	80	80	0
0.1	90	70	90	87.5
0.15	40	40	40	50.0
0.2	100	80	100	0
0.3	90	80	90	0
0.4	50	40	50	50.0
0.5	60	30	60	37.5
1.0	40	30	40	37.5
1.5	00	00	0	0
2.0	10	00	10	0
2.5	00	00	0	0

Sixty per cent sprouting was seen upto 0.5 per cent and thereafter there was a decline in sprouting when the duration of treatment was 30 minutes. When the duration was extended upto 60 minutes 50 per cent sprouting was observed

upto the dose 0.4 per cent concentration, while the percentage of sprouting decreased to 37.5 per cent at 0.5 per cent and 1 per cent concentration. So LD₅₀ was fixed as 0.4 per cent. Hence five doses of EMS at 0.2, 0.4, 0.6, 0.8 and 1 per cent and two durations namely 30 minutes and 60 minutes were tried.

4.2.2 Field studies

Morphological and yield attributes of plants that received various doses of mutagens in MV₁ generation were compared statistically and the results are presented below.

4.2.2.1 Gamma irradiation

Field trials were conducted with single noded cuttings treated with five selected doses of gamma rays. Maximum plant recovery was noticed for two doses viz., 10 and 20 Gy. Hence these two doses were selected for studies on survival, tuber yield and other characters.

Analysis of variance for gamma irradiation (Table 15) revealed that there was significant variation between the genotypes for all the characters except tubers per plant and tuber density.

Significant effect of dose on genotypes were noticed for characters viz., survival and plant height. Genotype x dose interaction was significant for tuber girth and harvest index.

4.2.2.1.1 Survival

The effect of gamma rays on survival of 10 accessions after 30 days of planting is presented in Table 16.

Table 15. Analysis of variance for survival, yield and yield contributing characters due to gamma irradiation

		Source		
		Genotype (df = 9)	Dose (df = 2)	Genotype x Dose Interaction (df = 18)
Survival	MSS	3.261	8.750	1.194
	F value	2.6804*	7.1918**	0.9817
Tuber yield (g plant ⁻¹)	MSS	60642.370	56622.444	32380.870
	F value	3.4471**	3.2186*	1.8406
Tuber girth	MSS	0.982	0.241	0.950
	F value	5.8316**	1.4311	5.6410**
Tubers/plant	MSS	1119.289	2753.841	433.221
	F value	1.9244	3.7031*	0.7448
Harvest index	MSS	0.090	0.011	0.028
	F value	21.0012**	2.5830	6.4334**
Plant height	MSS	261.768	632.527	68.987
	F value	3.1472**	7.6048**	0.8294
Volume/tuber	MSS	3.454	2.655	1.121
	F value	3.5682**	2.7423	1.1578
Weight/tuber	MSS	3.327	3.340	1.017
	F value	2.9883**	3.0010	0.9132
Density/tuber	MSS	0.066	0.018	0.042
	F value	1.7133	0.4773	1.0929
Biological yield	MSS	1396669.492	713002.731	144632.396
	F value	6.4606**	3.2981*	0.6690

* Significant at 5% level

** Significant at 1% level

Table 16. Survival of cuttings of *Coleus* genotypes treated with different doses of gamma irradiation (Gy)

Genotype	Control	Dose		Mean
		10	20	
Mullurkara L3	1.50	3.50	1.50	2.17
Mullurkara L2	3.50	3.00	3.00	3.17
Sreedhara	4.50	3.00	3.00	3.50
Kolazhy local	3.00	2.50	1.50	2.33
Pothenmala local	3.00	2.50	2.00	2.50
Parlikad local	4.50	1.50	2.00	2.67
Karnataka L1	4.00	2.50	3.50	3.33
IC 65724	3.00	3.00	2.50	2.83
Paipra local	3.50	1.00	1.50	2.00
IC 85707	2.50	0.50	0.00	1.00
Mean	3.30	2.30	2.05	

CD for genotypes 0.51

CD for doses 0.936

When gamma rays at 4 levels were given (10, 20, 40 and 60 Gy) to all the accessions studied it was seen that survival percentage was very less in the doses 40 and 60 Gy. Hence these doses were eliminated from further studies. So the doses studied was limited to 10 and 20 Gy. The effect of gamma rays on survival of 10 accessions after 30 days of planting is presented in Table 16. When gamma rays at 2 levels were given to all the accessions studied it was seen that survival percentage was affected. Maximum survival was observed in control (3.30). On comparison of the effect of radiation on the accessions it is seen from the survival figures that 20 Gy had significantly lower survival rate (2.05) than 10 Gy (2.30). While comparing the dose x genotype interaction control plants showed the maximum survival indicating that the doses employed reduced survival

rate. Maximum survival was noticed for Sreedhara (3.50) followed by Kolazhy local (3.30) and Mullurkkara L2 (3.17). Lowest value was recorded for IC 85707 (1.00).

4.2.2.1.2 Tuber yield

Table 17 depicts the tuber yield of 10 accessions of *Coleus* after subjecting to two doses of gamma irradiation.

Table 17. Tuber yield (g) of *Coleus* genotypes treated with different doses of gamma irradiation (Gy)

Genotype	Control	Dose		Mean
		10	20	
Mullurkara L3	529.17	376.25	306.25	403.89
Mullurkara L2	387.50	425.00	258.33	356.94
Sreedhara	400.63	482.50	329.17	404.10
Kolazhy local	475.00	519.58	423.67	472.75
Pothenmala local	200.00	241.67	162.50	201.39
Parlikad local	120.00	837.50	456.25	471.25
Karnataka L1	363.33	193.75	212.50	256.53
IC 65724	233.33	178.13	243.75	218.40
Paipra local	269.79	300.00	211.24	260.35
IC 85707	311.25	375.26	269.62	318.71
Mean	329.00	392.96	287.33	

CD for genotypes 61.68

CD for doses 112.62

Maximum tuber yield was shown by Kolazhy local (472.75) followed by Parlikad local (471.25). The lowest tuber yield was shown by IC 65724 (218.40). Mean tuber yield was highest for 10 Gy (392.96) followed by control (329.00) and the lowest was for 20 Gy (287.33). With respect to the interaction of

genotypes with dose the Parlikad local when treated with 10 Gy gave the maximum yield of 837.50 g per plant whereas the influence of mutagen over the Kolazhy local was not significant in increasing the tuber yield (519.58 g per plant).

4.2.2.1.3 Tuber girth

Table 18 depicts the tuber girth of 10 accessions after subjecting to 2 doses of gamma irradiation.

Table 18. Tuber girth (cm) of *Coleus* genotypes treated with different doses of gamma irradiation (Gy)

Genotype	Control	Dose		Mean
		10	20	
Mullurkara L3	4.97	5.18	4.30	4.81
Mullurkara L2	5.37	5.03	4.93	5.11
Sreedhara	4.76	5.43	5.04	5.08
Kolazhy local	5.33	5.03	6.71	5.69
Pothenmala local	5.08	4.56	4.55	4.73
Parlikad local	4.00	5.31	4.91	4.74
Karnataka L1	5.00	4.74	4.94	4.89
IC 65724	4.50	4.81	2.88	4.06
Paipra local	4.57	4.25	5.94	4.92
IC 85707	4.23	4.31	5.80	4.78
Mean	4.78	4.86	5.00	

CD for genotype 0.19

CD for genotype x dose interaction

Among the genotypes Kolazhy local recorded maximum tuber girth of 5.69 cm. Maximum tuber girth was recorded at 20 Gy (5.0 cm). Kolazhy local at 20 Gy gave maximum tuber girth of 6.71 cm while IC 65724 recorded the least value (2.88 cm). The effects of radiation for 2 doses for other accessions remain in

between. In general it is seen that average tuber girth increased with 20 Gy irradiation (5.00 cm).

4.2.2.1.4 Tubers/plant

Table 19 provides the data on the number of tubers produced per plant by different accessions at varying levels of gamma radiation.

Table 19. Number of tubers/plant of *Coleus* genotypes treated with different doses of gamma irradiation (Gy)

Genotype	Control	Dose		Mean
		10 Gy	20 Gy	
Mullurkara L3	78.17	54.70	48.00	60.29
Mullurkara L2	60.25	66.67	47.17	58.03
Sreedhara	61.55	90.10	50.33	67.33
Kolazhy local	66.68	88.33	62.84	72.61
Pothenmala local	38.50	55.17	28.25	40.64
Parlikad local	38.17	104.00	51.25	64.47
Karnataka L1	63.53	33.13	36.46	44.37
IC 65724	31.00	27.25	37.00	31.75
Paipra local	39.13	67.00	38.40	48.18
IC 85707	38.75	50.10	30.17	39.67
Mean	51.57	63.64	42.99	

CD for doses 20.47

While comparing the individual effects of 2 doses of gamma irradiation on the accessions it is seen that Parlikad local recorded a mean of 104 tubers at 10 Gy dose. The accession IC-65724 recorded the lowest number of tubers per plant (27.25) at 10 Gy. Accession Kolazhy local responded with an average of 62.84 tubers while Pothenmala local recorded the least number of tubers (28.25), on application of 20 Gy dose. On comparing the mean effect of doses, it is seen that

increase in gamma ray doses significantly reduced the number of tubers per plant (63.64 at 10 Gy and 42.99 at 20 Gy respectively). On comparison of accession means it seen that Kolazhy local recorded the maximum value of 72.61 followed by Sreedhara (67.33). Accession IC-65724 recorded the least value (31.75).

4.2.2.1.5 Harvest Index

The mean effect of two doses of gamma irradiation on the harvest index of ten accessions of *Coleus* is presented in Table 20.

Table 20. Harvest index of *Coleus* genotypes treated with different doses of gamma irradiation (Gy)

Genotype	Control	Dose		Mean
		10	20	
Mullurkara L3	0.51	0.49	0.27	0.42
Mullurkara L2	0.41	0.32	0.29	0.34
Sreedhara	0.40	0.37	0.36	0.37
Kolazhy local	0.42	0.44	0.43	0.43
Pothenmala local	0.14	0.08	0.11	0.11
Parlikad local	0.05	0.44	0.53	0.34
Karnataka L1	0.18	0.13	0.18	0.16
IC 65724	0.12	0.14	0.21	0.16
Paipra local	0.20	0.11	0.16	0.15
IC 85707	0.21	0.22	0.55	0.33
Mean	0.26	0.27	0.31	

CD for genotypes 0.03

CD for genotype x dose

The harvest index of accessions were found to be marginally influenced by higher doses of gamma radiation (0.27 at 10 Gy and 0.31 at 20 Gy). Genotype x dose interaction revealed that Mullurkkara L3 at 10 Gy (0.49) and Paipra local and

Pothenmala local at 10 and 20 Gy (0.11) recorded the maximum and minimum values respectively. Among the genotypes Kolazhy local exhibited high harvest index of 0.43 while Pothenmala local with a harvest index of 0.11 was ranked the minimum.

4.2.2.1.6 Mean plant height

Plant height of 10 accessions as influenced by two doses of gamma irradiation is given in Table 21.

Table 21. Mean plant height of *Coleus* genotypes treated with different doses of gamma irradiation (Gy)

Genotype	Control	Dose		Mean
		10	20	
Mullurkara L3	54.00	51.65	53.75	53.13
Mullurkara L2	52.33	60.00	46.33	52.89
Sreedhara	62.78	49.30	51.00	54.36
Kolazhy local	55.50	59.75	48.67	54.64
Pothenmala local	80.33	75.42	59.25	71.67
Parlikad local	76.13	52.50	43.75	57.46
Karnataka L1	70.97	67.63	62.04	66.88
IC 65724	70.33	64.25	65.00	66.53
Paipra local	62.96	65.00	55.03	60.99
IC 85707	66.17	61.74	55.00	60.97
Mean	65.15	60.72	53.98	

CD for genotypes 4.24

CD for doses 7.73

The observed values revealed that neither 10 Gy (60.72 cm) nor 20 Gy (53.98 cm) were significantly effective in increasing plant length (control - 65.15 cm). Of the 10 genotypes Pothenmala local registered a maximum plant

height of 71.67 cm followed by Karnataka L1 (66.88 cm). Mullurkkara L2 recorded minimum plant height of 52.89 cm. On comparing genotype with doses it was observed that Pothemala local at 10 Gy had maximum plant height of 75.42 cm while minimum plant height was observed by Paralikad local at 20 Gy (43.75 cm).

4.2.2.1.7 Mean biological yield

The biological yield obtained for 10 accessions of *Coleus* under the influence of two levels of gamma irradiation is provided in Table 22.

Table 22. Mean biological yield of *Coleus* genotypes treated with different doses of gamma irradiation (Gy)

Genotype	Control	Dose		Mean
		10	20	
Mullurkara L3	500.00	425.00	512.50	479.17
Mullurkara L2	597.92	891.67	491.67	660.42
Sreedhara	665.00	775.00	608.33	682.78
Kolazhy local	658.33	650.00	357.98	555.44
Pothemala local	1387.50	1912.50	1500.00	1600.00
Parlikad local	1136.68	1037.50	400.00	858.06
Karnataka L1	1731.67	1556.25	1070.83	1452.92
IC 65724	1875.00	1446.88	1312.50	1544.79
Paipra local	1202.08	2500.00	1554.86	1752.31
IC 85707	1100.00	1260.20	883.96	1081.39
Mean	1085.42	1245.50	869.26	

CD for genotypes 216.10

CD for doses 394.70

Among the genotypes Paipra local had highest biological yield of 1752.31 g followed by Pothemala local (1600.0 g). Genotype Mullurkkara L3

recorded lowest value of 479.17 g. The dose 10 Gy had a positive influence in increasing the biological yield (1245.50 g). Genotype x dose interaction revealed that Paipra local at 10 Gy at maximum biological yield of 2500 g while Kolazhy local at 20 Gy recorded the minimum biological yield of 375.98 g.

4.2.2.1.8 Mean volume of tubers

Table 23 provides the details of volume per tuber of different accessions of *Coleus* at 2 levels of gamma irradiation.

Table 23. Mean volume of tubers of *Coleus* genotypes treated with different doses of gamma irradiation (Gy)

Genotype	Control	Dose		Mean
		10	20	
Mullurkara L3	6.00	5.20	4.13	5.11
Mullurkara L2	6.44	5.55	5.12	5.70
Sreedhara	4.85	5.85	4.50	5.07
Kolazhy local	5.80	6.29	5.42	5.84
Pothenmala local	5.00	3.13	4.25	4.13
Parlikad local	3.92	7.25	4.88	5.35
Karnataka L1	4.97	4.25	4.06	4.43
IC 65724	4.17	4.06	4.00	4.08
Paipra local	4.69	5.00	4.22	4.64
IC 85707	3.67	3.75	3.08	3.50
Mean	4.95	5.03	4.37	

CD for genotypes 0.83

It is seen from the data that mean volume per tuber marginally increased with 10 Gy dose (5.03 cc). Within the accessions Parlikad local maintained the higher tuber volume while accession IC-85707 recorded the least value of 3.75 cc under the influence of 10 Gy dose. Among the accessions Kolazhy local

maintained higher volume per tuber (5.84 cc) while accession IC-85707 recorded the least value (3.50 cc). Paralikkad local at 10 Gy recorded maximum volume per tuber (7.25 cc) followed by Kolazhy local at 10 Gy (6.29 cc). Accession IC 85707 at 20 Gy recorded the least volume per tuber (3.08 cc).

4.2.2.1.9 Mean weight of tuber

Weight of tubers of 10 accessions of *Coleus* as influenced by 2 levels of gamma radiation is presented in Table 24.

Table 24. Mean weight of tubers of *Coleus* genotypes treated with different doses of gamma irradiation (Gy)

Genotype	Control	Dose		Mean
		10	20	
Mullurkara L3	6.87	6.73	5.20	6.27
Mullurkara L2	7.01	6.17	5.33	6.17
Sreedhara	5.34	5.12	5.10	5.19
Kolazhy local	6.67	6.40	5.83	6.30
Pothenmala local	5.57	4.22	4.90	4.89
Parlikad local	4.76	8.15	5.58	6.16
Karnataka L1	5.63	4.83	4.35	4.94
IC 65724	5.07	4.78	4.70	4.85
Paipra local	4.95	5.60	4.57	5.04
IC 85707	4.48	4.50	3.78	4.26
Mean	5.63	6.65	4.93	

CD for genotypes 0.48

CD for doses

The dose 10 Gy was found to be promising as it had increased weight per tuber (6.65 g) over control (5.63 g). Among the genotypes Kolazhy local recorded maximum weight per tuber of 6.30 g followed by Mullurkkara L3

(6.27 g). The accession IC-85707 recorded the minimum value for weight per tuber (4.26 g). Paralikkad local at 10 Gy (8.15 g) followed by Mullurkkara L3 at 10 Gy (6.73 g) recorded higher values for weight per tuber while Paipra local at 20 Gy recorded the least value of 3.78 g.

4.2.2.1.10 Mean tuber density

Tuber density of *Coleus* accessions as influenced by 10 Gy and 20 Gy gamma irradiation is presented in Table 25.

Table 25. Mean density of tubers of *Coleus* genotypes treated with different doses of gamma irradiation (Gy)

Genotype	Control	Dose		Mean
		10	20	
Mullurkara L3	1.14	1.34	1.33	1.27
Mullurkara L2	1.10	1.12	1.05	1.10
Sreedhara	1.11	0.90	1.14	1.05
Kolazhy local	1.14	1.05	1.07	1.09
Pothenmala local	1.12	1.83	1.16	1.37
Parlikad local	1.22	1.13	1.15	1.17
Karnataka L1	1.14	1.15	1.07	1.12
IC 65724	1.24	1.17	1.26	1.22
Paipra local	1.10	1.11	1.07	1.09
IC 85707	1.26	1.31	1.26	1.27
Mean	1.16	1.21	1.16	

The genotype Paralikkad local recorded maximum tuber density of 1.66 g per cc while Sreedhara recorded the lowest value of 1.05 g per cc. The dose 10 Gy (1.21 g/cc) was found to increase the tuber density over control (1.16 g/cc). Sreedhara at 10 Gy recorded the lowest tuber density of 0.90 g/cc while Pothenmala local recorded the highest value (1.83 g/cc).

4.2.2.2 Ethyl Methane Sulphonate (EMS)

Analysis of variance (Table 26) for EMS treatment revealed significant variation between the genotypes for all the characters studied. Significant effect of dose on genotypes were noticed for survival, tuber yield, tuber girth, tubers per plant, volume per tuber and weight per tuber. Genotype x dose interaction was significant only for volume per tuber.

4.2.2.2.1 Survival

The effect of EMS on survival after 30 days of planting is presented in Table 27.

Table 27 provides the survival percentage of 10 accessions of *Coleus* on subjecting to 5 incremental doses of chemical mutagen EMS.

Table 27. Survival of *Coleus* genotypes treated with different concentrations of EMS (%)

Genotypes	Control	Concentration					Mean
		0.20	0.40	0.60	0.80	1.00	
Mullurkara L3	3.50	1.50	3.25	2.75	0.50	0.00	1.92
Mullurkara L2	5.00	3.50	2.75	2.50	1.25	1.00	2.20
Sreedhara	5.00	4.75	3.75	4.25	2.25	2.50	3.50
Kolazhy local	4.00	2.75	3.00	3.50	2.75	1.75	2.75
Pothenmala local	5.00	3.00	3.00	1.75	1.00	1.58	2.56
Parlikad local	4.50	0.25	2.00	0.50	1.25	2.00	1.75
Karnataka L1	4.50	1.25	1.50	1.00	0.25	0.75	1.54
IC 65724	3.00	2.00	2.00	1.50	2.00	1.25	1.96
Paipra local	3.00	2.25	2.00	0.75	1.50	0.25	1.63
IC 85707	2.00	0.50	0.75	0.75	1.00	1.00	1.00
Mean	3.95	2.18	2.40	1.93	1.38	1.21	

CD for genotypes 0.393

CD for concentrations 0.507

Table 26. Analysis of variance for survival, yield and yield contributing characters due to EMS treatment

		Source		
		Genotype (df = 9)	Dose (df = 5)	Genotype x Dose Interaction (df = 45)
Survival	MSS	7.812	19.353	1.102
	F value	10.9292**	27.0737**	1.5416
Tuber yield (g plant ⁻¹)	MSS	207960.052	92663.316	23372.572
	F value	6.2874**	3.8016*	0.7066
Tuber girth	MSS	0.516	0.390	0.135
	F value	3.5321*	2.6730*	0.9271
Tubers/plant	MSS	18932.153	12690.919	4153.161
	F value	4.1981**	2.8141*	0.9209
Harvest index	MSS	0.186	0.004	0.012
	F value	19.6405**	0.3964	1.2806
Volume/tuber	MSS	3.599	2.315	0.810
	F value	7.9407**	5.1073**	1.7874*
Weight/tuber	MSS	5.945	2.321	0.845
	F value	9.7690**	3.8137**	1.3885
Density/tuber	MSS	0.052	0.023	0.017
	F value	3.6191**	1.5652	1.1537
Plant height	MSS	1172.372	174.893	113.311
	F value	5.0748**	0.7571	0.4905
Biological yield	MSS	2420867.436	1007976.006	477070.435
	F value	4.7167**	1.9639	0.9295

* Significant at 5% level

** Significant at 1% level

In general it is seen that increasing doses of EMS has significantly reduced that survival percentage of the treated accessions. Among the genotypes Sreedhara recorded maximum survival of 3.75 per cent while accession IC-85707 recorded the minimum survival percentage of one per cent. Sreedhara when treated with 0.2 per cent concentration of EMS recorded highest survival percentage of 4.75 per cent. Paralikkad local at 0.2 per cent, Karnataka L1 at 0.8 per cent and Paipra local at 1.0 per cent recorded the lowest survival percentage of 0.25 per cent.

4.2.2.2.2 Mean tuber yield

Table 28 depicts tuber yield of 10 accessions of *Coleus* as influenced by 5 incremental doses of EMS treatment.

Table 28. Mean tuber yield of *Coleus* genotypes treated with different concentrations of EMS (%)

Genotypes	Control	Concentration					Mean
		0.20	0.40	0.60	0.80	1.00	
Mullurkara L3	367.29	764.58	522.92	445.83	504.81	616.28	536.95
Mullurkara L2	442.50	390.83	382.67	296.25	241.67	425.00	363.15
Sreedhara	275.20	375.00	450.00	290.00	357.50	452.50	366.70
Kolazhy local	337.50	391.67	801.04	422.00	510.00	687.50	524.95
Pothenmala local	200.00	306.25	156.25	177.08	191.67	283.33	219.10
Parlikad local	202.85	523.49	687.50	414.09	422.40	476.88	454.54
Karnataka L1	216.88	441.67	300.00	500.00	344.29	455.76	376.43
IC 65724	115.00	158.75	220.83	204.17	290.63	293.07	213.74
Paipra local	237.50	715.63	229.17	351.30	345.83	471.08	391.75
IC 85707	720.83	689.35	512.50	562.50	537.50	699.73	620.40
Mean	311.56	475.72	426.29	366.32	374.63	486.11	

CD for genotypes 84.58

CD for doses 109.20

Highest tuber yield of 620.4 g was recorded by Paipra local and the lowest tuber yield was recorded by IC-65724 (213.74 g). One per cent concentration recorded maximum tuber yield of 486.11 g while 0.6 per cent recorded maximum tuber yield of 366.32 g. Genotype x dose interaction revealed that Kolazhy local at 0.4 per cent concentration recorded the highest tuber yield (801.04 g) while Pothenmala local at the same concentration recorded the minimum yield of 156.25 g.

4.2.2.2.3 Mean tuber girth

Tuber girth of *Coleus* accessions consequent to treatment with EMS at 5 different doses is presented in Table 29.

Table 29. Mean tuber girth of *Coleus* genotypes treated with different concentrations of EMS (%)

Genotypes	Control	Concentration					Mean
		0.20	0.40	0.60	0.80	1.00	
Mullurkara L3	5.22	4.92	5.11	5.32	5.21	5.33	5.18
Mullurkara L2	4.66	5.21	5.22	5.28	5.28	5.49	5.19
Sreedhara	4.78	5.26	4.65	4.81	4.76	5.01	4.88
Kolazhy local	4.89	5.31	5.78	5.06	5.59	5.19	5.30
Pothenmala local	4.76	4.62	4.65	4.96	4.98	4.98	4.83
Parlikad local	4.54	4.88	5.95	4.95	4.92	5.15	5.07
Karnataka L1	5.08	5.42	5.25	5.39	5.35	5.47	5.33
IC 65724	4.61	5.01	5.77	5.14	5.13	5.31	5.16
Paipra local	4.91	4.96	5.20	5.03	4.63	5.12	4.97
IC 85707	4.63	4.70	4.20	5.05	4.85	4.86	4.71
Mean	4.81	5.03	5.18	5.10	5.07	5.19	

CD for genotypes 0.177

CD for doses 0.229

The genotype Karnataka L1 had the highest tuber girth of 5.33 cm followed by Kolazhy local (5.30 cm). The accession IC-85707 recorded the minimum tuber girth (4.70 cm). The accession IC-85707 recorded minimum tuber girth (4.7 cm). Among the different doses 1.0 per cent (5.19 cm) and 0.4 per cent (5.18 cm) yielded mutants will higher tuber girth. Genotype x dose interaction revealed that Parlikkad local at 0.4 per cent concentration recorded maximum tuber girth (5.95 cm), while accession IC-85707 at the same concentration recorded minimum tuber girth of 4.20 cm.

4.2.2.2.4 Mean tubers/plant

The mean effect of EMS on the genotypes for tubers per plant is depicted in Table 30.

Table 30. Mean number of tubers per plant of *Coleus* genotypes treated with different concentrations of EMS (%)

Genotypes	Control	Concentration					Mean
		0.20	0.40	0.60	0.80	1.00	
Mullurkara L3	97.29	102.83	91.21	113.17	69.35	124.99	99.80
Mullurkara L2	102.90	91.38	66.17	65.10	86.67	115.25	87.91
Sreedhara	53.40	84.50	79.38	66.60	47.80	100.88	72.09
Kolazhy local	94.38	62.50	119.92	140.00	61.50	135.25	102.26
Pothenmala	57.40	83.40	36.63	64.08	70.33	84.83	66.11
Parlikkad local	62.38	107.66	68.00	91.56	48.85	97.38	79.30
Karnataka L1	62.13	59.50	46.50	88.00	32.25	87.89	62.71
IC 65724	35.70	55.38	76.33	78.50	97.25	98.84	73.67
Paipra local	110.88	415.38	42.67	175.76	47.67	188.68	163.50
IC 85707	170.00	203.23	138.00	222.00	116.00	200.06	174.88
Mean	84.64	126.57	76.48	110.48	67.77	123.40	

CD for genotypes 31.22

CD for doses 40.31

The lowest dose 0.2 per cent concentration recorded the maximum number of tubers per plant (126.57), while 0.8 per cent concentration recorded the minimum value (67.77). Paipra local at 0.6 per cent concentration recorded the highest number of tubers per plant (222). Pothenmala local at 0.4 per cent concentration recorded the least value 36.63. Among the genotypes accession IC-85707 recorded the maximum value of 174.88 while Karnataka L1 recorded the minimum value of 62.71.

4.2.2.2.5 Harvest Index

Harvest index as influenced by different doses of EMS in 10 accessions is presented in Table 31.

Table 31. Harvest index of *Coleus* genotypes treated with different concentrations of EMS (%)

Genotypes	Control	Concentration					Mean
		0.20	0.40	0.60	0.80	1.00	
Mullurkara L3	0.39	0.66	0.56	0.43	0.50	0.48	0.50
Mullurkara L2	0.59	0.37	0.41	0.35	0.30	0.43	0.41
Sreedhara	0.35	0.42	0.58	0.43	0.44	0.40	0.44
Kolazhy local	0.41	0.47	0.52	0.44	0.47	0.40	0.45
Pothenmala local	0.24	0.08	0.14	0.18	0.22	0.15	0.17
Parlikad local	0.33	0.40	0.47	0.41	0.39	0.36	0.39
Karnataka L1	0.37	0.16	0.17	0.44	0.28	0.25	0.28
IC 65724	0.20	0.30	0.10	0.11	0.15	0.14	0.16
Paipra local	0.19	0.20	0.16	0.22	0.26	0.18	0.20
IC 85707	0.25	0.30	0.24	0.44	0.26	0.27	0.29
Mean	0.33	0.34	0.33	0.34	0.33	0.30	

CD for genotypes 0.045

The highest harvest index was recorded by the doses 0.2 per cent and 0.6 per cent respectively (0.34), while the lowest harvest index was shown by 1.0

per cent (0.30). Among the genotypes the highest harvest index was shown by Mullurkkara L3 (0.50) and the lowest by IC-65724 (0.16). Regarding the dose x genotype interaction the maximum harvest index was shown by Mullurkkara L3 when treated with 0.2 per cent dose (0.66) and minimum harvest index was shown by Pothenmala local when treated with the same dose (0.08).

4.2.2.2.6 Mean volume per tuber

Effect of incremental levels of EMS on 10 accessions of *Coleus* in influencing the tuber volume has been depicted in Table 32.

Table 32. Mean volume of tubers of *Coleus* genotypes treated with different concentrations of EMS (%)

Genotypes	Control	Concentration					Mean
		0.20	0.40	0.60	0.80	1.00	
Mullurkara L3	5.26	5.26	6.00	5.68	5.55	5.79	5.59
Mullurkara L2	5.00	4.97	6.32	6.18	5.86	6.83	5.86
Sreedhara	4.76	5.32	6.28	6.25	4.45	5.02	5.35
Kolazhy local	4.31	5.72	7.06	4.91	6.88	5.88	5.79
Pothenmala local	4.10	4.13	3.88	4.38	4.23	5.13	4.31
Parlikad local	4.74	4.86	6.25	5.25	5.09	4.57	5.13
Karnataka L1	5.46	5.41	5.41	5.40	5.43	5.66	5.46
IC 65724	3.86	4.01	6.42	3.50	4.44	4.68	5.49
Paipra local	4.54	4.79	4.54	5.01	4.96	5.08	4.82
IC 85707	5.67	5.42	5.00	7.25	4.90	5.89	5.69
Mean	4.77	4.99	5.72	5.38	5.18	5.45	

CD for genotypes 0.31

CD for doses 0.40

The highest volume per tuber was shown by Mullurkkara L2 (5.86 cc) and the lowest value was recorded by Pothenmala local (4.31 cc). When the doses

were compared the highest volume was shown by EMS concentrations 0.4 per cent (5.72 cc) and lowest by control (4.77 cc). When the dose x genotype interaction was considered the highest value of 7.06 cc was obtained when 0.4 per cent concentration interacted with genotype Kolazhy local and lowest value of 3.50 cc was obtained when 0.6 per cent concentration interacted with IC-65724.

4.2.2.2.7 Mean weight per tuber

Weight of 10 accessions of *Coleus* tuber as influenced by 5 incremental doses of EMS is given in Table 33.

Table 33. Mean weight of tubers of *Coleus* genotypes treated with different concentrations of EMS (%)

Genotypes	Control	Concentration					Mean
		0.20	0.40	0.60	0.80	1.00	
Mullurkara L3	6.46	6.10	6.50	6.45	6.62	6.86	6.50
Mullurkara L2	5.09	5.73	6.45	6.30	6.69	7.15	6.23
Sreedhara	5.14	5.81	6.26	6.16	5.09	5.14	5.60
Kolazhy local	5.01	6.17	6.80	5.05	7.39	6.45	6.14
Pothenmala local	4.48	3.49	4.38	4.85	4.78	5.57	4.59
Parlikad local	5.09	5.64	6.90	6.14	6.11	5.04	5.99
Karnataka L1	6.03	5.90	5.78	7.85	6.63	6.87	6.51
IC 65724	3.96	4.04	6.10	3.60	4.95	4.96	4.60
Paipra local	4.75	5.58	5.17	5.57	5.65	5.77	5.41
IC 85707	6.63	5.69	5.40	7.20	4.90	6.40	6.04
Mean	5.26	5.41	5.97	5.92	5.88	6.12	

CD for genotypes 0.468

CD for doses 0.362

Among the accession Karnataka L1 recorded the maximum weight of tuber (6.51 g) followed by Mullurkkara L3 (6.50 g). Minimum weight per tuber

was recorded by Pothemala local (4.59 g). When genotype x dose interaction was studied Karnataka L1 at 0.6 per cent concentration recorded the highest weight per tuber of 7.85 g, while Pothemala local at 0.2 per cent concentration recorded the lowest weight for tuber (3.49 g). One per cent dose give the highest tuber weight of 6.12 g, while control gave the lowest value (5.26 g).

4.2.2.2.8 Mean tuber density

Effect of different levels of EMS on tuber density is presented in Table 34.

Table 34. Mean density of tubers of *Coleus* genotypes treated with different concentrations of EMS (%)

Genotypes	Control	Concentration					Mean
		0.20	0.40	0.60	0.80	1.00	
Mullurkara L3	1.20	1.15	1.08	1.17	1.19	1.21	1.17
Mullurkara L2	1.03	1.16	1.03	1.03	1.17	1.05	1.08
Sreedhara	1.08	1.10	0.99	0.90	1.14	1.05	1.04
Kolazhy local	1.16	1.07	1.00	1.04	1.07	1.08	1.07
Pothemala local	1.09	0.89	1.15	1.10	1.12	1.09	1.07
Parlikad local	1.06	1.19	1.11	1.21	1.23	1.43	1.20
Karnataka L1	1.10	1.09	1.07	1.56	1.25	1.27	1.22
IC 65724	1.02	1.04	0.98	1.06	1.12	1.10	1.05
Paipra local	1.05	1.19	1.14	1.13	1.06	1.17	1.12
IC 85707	1.15	1.04	1.08	0.99	1.00	1.11	1.06
Mean	1.09	1.09	1.06	1.12	1.14	1.15	

CD for doses 0.055

Among the doses 1.0 per cent concentration increase the tuber density to 1.15 g/cc, while 0.4 per cent concentration decreased the tuber density to 1.06 g/cc

over the control (1.09 g/cc). Among the accessions Karnataka L1 and Sreedhara recorded the highest and lowest tuber density of 1.22 g/cc and 1.04 g/cc. Genotype x dose interaction revealed that Karnataka L1 at 0.6 per cent concentration (1.56 g/cc) and Pothemala local at 0.2 per cent concentration (0.89 g/cc) recorded the maximum and minimum tuber density respectively.

4.2.2.2.9 Mean plant height

The plant height of 10 accessions of *Coleus* as influenced by 5 incremental doses of EMS has been presented in Table 35.

Table 35. Mean plant height of *Coleus* genotypes treated with different concentrations of EMS (%)

Genotypes	Control	Concentration					Mean
		0.20	0.40	0.60	0.80	1.00	
Mullurkara L3	60.96	48.33	54.46	62.33	52.51	57.64	56.04
Mullurkara L2	47.30	63.63	58.52	52.10	59.00	65.50	57.67
Sreedhara	52.80	65.38	52.63	68.50	57.00	55.75	58.68
Kolazhy local	62.63	53.00	62.96	72.07	54.10	62.25	61.17
Pothemala local	76.70	92.00	93.88	78.75	82.50	78.50	83.72
Parlikad local	53.90	63.04	57.00	61.38	56.23	67.00	59.76
Karnataka L1	63.25	85.33	64.50	67.50	66.14	71.26	69.66
IC 65724	76.40	69.25	65.83	87.83	66.50	75.08	73.48
Paipra local	73.13	87.00	72.25	78.86	73.33	78.83	77.23
IC 85707	68.33	79.46	100.00	60.50	71.00	77.78	76.18
Mean	63.54	70.64	68.20	68.98	63.83	68.96	

CD for genotypes 7.069

There was no significant difference among the different levels of EMS treatment for plant height. However accessions Pothemala local recorded a

maximum height of 83.72 cm while Mullurkara L3 recorded the minimum height of 56.04 cm. At 0.4 per cent concentration genotype IC-85707 recorded the highest plant height (100 cm) while Mullurkkara L2 at 0.6 per cent concentration gave the lowest plant height (52.1 cm).

4.2.2.2.11 Mean biological yield

Effect of EMS on biological yield is presented in Table 36.

Table 36. Mean biological yield of *Coleus* genotypes treated with different concentrations of EMS (%)

Genotypes	Control	Concentration					Mean
		0.20	0.40	0.60	0.80	1.00	
Mullurkara L3	587.92	429.17	385.42	691.67	441.45	749.53	547.53
Mullurkara L2	345.00	712.50	412.39	637.50	729.17	575.00	568.59
Sreedhara	502.50	618.75	545.00	662.00	510.00	891.25	621.58
Kolazhy local	531.25	610.83	818.75	505.00	540.00	987.50	665.56
Pothenmala local	800.00	1948.00	887.50	977.08	1408.33	1683.33	1284.04
Parlikad local	443.75	906.67	800.00	998.19	657.90	777.08	763.93
Karnataka L1	506.25	2083.33	1625.00	1385.00	1317.81	1625.88	1423.88
IC 65724	565.00	418.75	1375.00	3887.50	1425.00	1776.66	1574.65
Paipra local	1475.00	2718.75	1187.50	1946.92	1033.33	1914.71	1712.70
IC 85707	1400.00	1303.87	725.00	975.00	1200.00	1363.18	1161.18
Mean	715.67	1175.06	876.16	1266.59	926.30	1234.41	

CD for genotypes 7.069

Among the genotypes Paipra local recorded the maximum biological yield of 1712.7 g, while Mullurkkara L3 recorded minimum biological yield 547.53 g. Biological yield was the highest at 0.6 per cent concentration (1266.59 g) and the lowest at 0.4 per cent concentration (876.16 g). Dose x genotype interaction revealed that Paipra local at 0.2 per cent concentration gave the

maximum biological yield of 2718.75 g, while Mullurkkara L3 at 0.4 per cent concentration gave the minimum biological yield of 385.42 g.

4.3 Experiment-III - Field evaluation of mutants for photoinsensitivity tuber yield and other yield attributing characters during December 2000 to October 2001

4.3.1 Screening of mutants for photo insensitivity

Fourteen mutants were isolated from 100 plants treated with EMS and 40 plants exposed to gamma rays.

The list of mutants thus selected along with its relevant dose and duration are presented in Table 37.

These 14 mutants were isolated based on yield attributes viz., tuber yield, tuber girth, volume per tuber, weight per tuber, tuber density, tubers per plant, plant height, biological yield and harvest index compared to treatment means (Table 38).

4.3.1.1 Field experiment during December 2000 to May 2001

The cuttings obtained from the 14 mutants along with their control were planted in RBD in three replications during December 2000 and harvested in May 2001 which is an off season for *Coleus* cultivation. Only eight mutants out of the 19 accessions (14 mutants and 5 parents) produced tubers. The data obtained was statistically analysed. The analysis of variance for the nine characters for eight mutants are presented in Table 39.

Table 37. Fourteen selected mutants from EMS treated and gamma irradiated *Coleus* plants

Mutant	Accession	Dose	Duration
131	Mullurkkara L3	0.6%	30
112	Mullurkkara L3	0.2%	60
641	Parlikadu local	0.8%	30
421	Kolazhy local	0.4%	30
111	Mullurkkara L3	0.2%	30
121	Mullurkkara L3	0.4%	30
352	Sreedhara	1.0%	60
412	Kolazhy local	0.2%	60
422	Kolazhy local	0.4%	60
632	Parlikadu local	0.6%	60
1031	IC-85707	0.6%	30
1042	IC-85707	0.8%	60
61	Parlikadu local	10 Gy	-
62	Parlikadu local	20 Gy	-

Table 38. Comparison of mutant plants with treatment means for various characters

Sl. No.		Tuber yield (g)	Tuber girth (cm)	Volume per tuber (cc)	Weight per tuber (g)	Tuber density (g/cc)	Tubers per plant (No.)	Plant height (cm)	Biological yield (g)	Harvest index
1	131									
	Treat mean	445.83	5.32	5.67	6.45	1.17	113.17	62.33	69.67	0.43
	Plant mean	700.00	5.60	7.50	8.60	1.15	162.00	47.00	400.00	0.63
2	112*									
	Treat mean	1175.00	4.80	6.50	7.20	1.12	324	46	600	0.66
	Plant mean	1175.00	4.80	6.50	7.20	1.12	324	46	600	0.66
3	641									
	Treat mean	1187.5	6.60	9.75	10.50	1.07	224.00	78.50	800.00	0.60
	Plant mean	1200.0	7.50	11.50	13.00	1.13	206.00	88.00	800.00	0.60
4	421									
	Treat mean	778.57	5.77	7.14	6.80	0.99	125.86	60.143	757.14	0.53
	Plant mean	900.00	5.10	6.70	7.40	1.10	237.00	40.000	400.00	0.69
5	111									
	Treat mean	695.00	4.96	5.16	4.84	1.14	103.0	49.60	410.00	0.53
	Plant mean	900.00	5.20	6.50	8.60	1.32	80.0	35.00	500.00	0.94
6	121									
	Treat mean	528.57	5.14	5.99	6.51	1.09	94.29	47.86	392.86	0.56
	Plant mean	875.00	5.80	8.00	9.40	1.18	80.00	59.00	200.00	0.81
7	352*									
	Treat mean	900.00	5.20	6.50	7.20	1.10	204	65	600	0.60
	Plant mean	900.00	5.20	6.50	7.20	1.10	204	65	600	0.60
8	412									
	Treat mean	595.00	5.46	6.98	7.02	1.00	126	49.20	570.00	0.50
	Plant mean	1175.00	4.40	5.80	6.20	1.10	175	58.00	400.00	0.74
9	422									
	Treat mean	665.00	5.4	6.7	7.7	1.20	123.40	55.00	610.00	0.52
	Plant mean	875.00	5.5	8.5	9.2	1.08	246.00	55.00	500.00	0.63
10	632*									
	Treat mean	1200.00	7.50	11.50	13.00	1.13	206.00	88.00	800.00	0.60
	Plant mean	1200.00	7.50	11.50	13.00	1.13	206.00	88.00	800.00	0.60
11	1031									
	Treat mean	250.00	4.90	4.20	4.80	1.13	128	94.00	850.00	0.23
	Plant mean	300.00	4.90	7.50	8.00	1.06	622	32.00	200.00	0.60
12	1042									
	Treat mean	1625.00	6.60	6.00	6.20	1.22	365	66.50	1350.00	0.51
	Plant mean	2350.00	6.50	6.00	5.60	1.30	550	88.00	1300.00	0.62
13	61									
	Treat mean	825.00	5.18	7.17	8.13	1.14	108.30	51.00	1875.00	0.43
	Plant mean	875.00	5.70	7.50	8.20	1.09	91.00	57.00	1875.00	0.46
14	62									
	Treat mean	456.25	4.91	4.88	5.58	1.15	51.25	43.75	856.25	0.53
	Plant mean	750.00	4.40	4.50	5.10	1.13	94.00	46.00	135.00	0.55

* Only one plant in this treatment

Table 39. Analysis of variance of eight mutants raised during season I - December 2000 to May 2001

Character	df	MSS	F value
Plant height	7	162.708	86.778**
Biological yield	7	1952926.042	797.452**
Tuber number	7	152.661	40.710**
Tuber yield	7	9606.518	469.565**
Tuber girth	7	2.939	47.337**
Volume per tuber	7	10.237	15.119**
Weight per tuber	7	16.875	23.478**
Harvest index	7	0.066	322.450**
Tuber density	7	0.284	5.920**

** Significant at 1% level

The mutants differed significantly at one per cent level for all the characters. The mean values for growth and yield characters for eight selected mutants are presented in Table 40.

Mutant 131 recorded the maximum tuber density of 1.37 g/cc, weight per tuber (9.33 g), volume per tuber (7.0 cc) tuber yield (103.33 g plant⁻¹), tuber number (12.0), plant height (36.67 cm) and tuber girth (5.3 cm). Mutant 61 recorded the maximum tuber yield (175 g plant⁻¹), tuber number (25.67), volume per tuber (7.67 cc), harvest index (0.357) and weight per tuber (7.0). Mutant 111 recorded higher values for weight per tuber (7.67 g), tuber density (1.18 g/cc), harvest index (0.356), volume per tuber (6.5 cc), tuber girth (6.17 cm) and tuber yield (43.33 g plant⁻¹).

4.3.1.1.1 Estimates of variability

General means of the characters, genotypic variance (GV), genotypic coefficient of variation (GCV), phenotypic variance (PV), phenotypic coefficient

Table 40. Growth and yield characteristics of eight selected mutants of *Coleus* cultivated during December 2000 to May 2001

Mutants	Characters										
	Plant height (cm)	Biological yield (g)	Tuber number	Tuber yield (g)	Girth per tuber (cm)	Volume per tuber (cc)	Harvest index	Tuber density (g/cc)	Weight per tuber (g)		
131	36.67	458.33	12.00	103.33	5.30	7.00	0.197	1.367	9.33		
112	10.00	333.33	5.67	14.00	4.47	5.50	0.043	0.567	3.33		
641	31.33	2466.67	4.67	26.67	6.50	3.33	0.010	1.367	4.67		
422	19.68	266.67	4.33	15.67	4.37	5.33	0.057	0.633	3.50		
111	24.67	78.33	7.00	43.33	6.17	6.50	0.356	1.180	7.67		
121	20.33	641.67	5.33	34.33	4.13	4.17	0.053	1.150	4.83		
61a	26.00	316.67	25.67	175.00	5.00	7.67	0.357	0.927	7.00		
61b	37.67	1433.33	8.33	22.00	3.57	2.33	0.013	1.167	2.67		
SE	± 1.46	± 157.60	± 1.43	± 11.06	± 0.20	± 0.39	± 0.03	± 0.07	± 0.48		
CD	3.04	327.81	2.97	23.00	0.42	0.81	0.06	0.15	1.00		

of variation (PCV), heritability (h^2), genetic advance (GA) and genetic gain (GG) with respect to the nine characters are presented in Table 41. Generally phenotypic coefficient of variation was slightly higher than genotypic coefficient of variation. Maximum GCV and PCV was shown by the character harvest index (108.95%, 109.37%) followed by biological yield (107.60% and 107.80%) and tuber yield (104.11% and 104.48%) respectively. Maximum heritability was recorded by biological yield (99.62%) followed by tuber yield (99.36%) and harvest index (99.08%). Genetic gain was highest for biological yield (221.25%) followed by harvest index (220.90%) and tuber yield (213.70%).

4.3.1.1.2 Correlation studies

Correlation Coefficients between tuber yield and other characters and their inter correlations are presented in Table 42. Tuber number (0.97), harvest index (0.734), volume per tuber (0.726) and weight per tuber (0.69) recorded highly significant positive association with tuber yield at the genotypic level. Inter correlations among the different traits revealed that plant height had significant positive association with tuber density. Tuber number had highly significant positive association with harvest index (0.692) and volume per tuber (0.631). Tuber girth had significant positive association with weight per tuber. Volume per tuber had highly significant positive association with weight per tuber (0.78) and highly significant negative effect with biological yield (-0.770). Highly significant positive association was noticed between weight per tuber and harvest index. Tuber density did not have any significant association with the other components. Biological yield had significant negative association with harvest index.

Table 41. Variability of nine characters for eight mutants

Character	Mean	GV	PV	GCV	PCV	h ²	GA	GG
Plant height (cm)	27.04	53.61	55.49	27.07	27.57	96.62	14.80	54.73
Biological yield (g)	749.38	650158.90	652607.90	107.60	107.80	99.62	1658.62	221.25
Tuber number	9.13	49.64	53.39	77.10	80.28	92.98	13.92	152.55
Tuber yield (g)	54.30	3195.35	3215.81	104.11	104.48	99.36	116.02	213.70
Tuber girth (cm)	4.93	0.96	1.02	19.85	20.57	93.92	1.94	39.39
Volume per tuber (cc)	5.23	3.19	3.86	34.09	37.67	82.48	3.32	63.49
Weight per tuber (g)	5.38	5.39	6.10	43.16	45.99	88.23	4.49	3.53
Harvest index	0.14	0.09	0.21	108.95	109.37	99.08	0.30	220.91
Tuber density (g/cc)	1.05	0.08	0.13	26.96	33.93	62.12	0.46	44.04

Table 42. Genotypic correlation of nine characters for eight mutants raised during December 2000 to May 2001

	PH	TN	TG	V/T	W/T	TD	BY	HI	TY
PH	1.000	0.201	0.093	-0.250	0.260	0.780**	0.511	-0.030	0.210
TN		1.000	0.025	0.631**	0.500	0.032	-0.267	0.692**	0.971**
TG			1.000	0.345	0.599*	0.500	0.213	0.439	0.185
V/T				1.000	0.783**	-0.142	-0.770**	0.850**	0.726**
W/T					1.000	0.504	-0.356	0.793**	0.694**
TD						1.000	0.564	0.128	0.194
BY							1.000	-0.569*	-0.286
HI								1.000	0.735**
TY									1.000

* Significant at 5% level

** Significant at 1% level

4.3.1.2 Field studies during February 2001 to July 2002

Fourteen mutants along with the respective parents were raised during February 2001 and harvested in July 2001. The data for the various yield attributes with respect to the 14 mutants and 4 parents were recorded and subjected to statistical analysis.

The analysis of variance are presented in Table 43.

Table 43. Abstract of anova of mutants and parents raised during season II and season III

Characters	February 2001 to July 2001			April 2001 to October 2001		
	df	MSS	F value	df	MSS	F value
Plant height	17	201.796	0.957	18	334.669	1.479
Biological yield	17	187456.127	2.979**	18	239.344	5.416**
Tuber number	17	299.865	10.774**	18	239.244	5.416**
Tuber yield	17	22270.314	9.260**	18	21637.889	5.630**
Tuber girth	17	3.340	4.294**	18	3.389	2.086*
Volume per tuber	17	20.627	3.111**	18	16.834	2.928**
Weight per tuber	17	19.509	2.260*	18	19.111	2.084*
Harvest index	17	0.028	7.663**	18	0.030	2.917**
Tuber density	17	0.679	1.226	18	1.137	3.192**

** Significant at 1% level

* Significant at 5% level

Biological yield, number of tubers, tuber yield, tuber girth, mean volume of tuber and harvest index differed significantly at 1 per cent level and mean weight of tubers at 5 per cent level.

The mean of the genotypes for nine characters with respect to 18 accessions are presented in Table 44. The mutant 641 gave maximum tuber yield (258.33 g), volume per tuber (14.33 cc), weight per tuber (11.33 cc), biological

Table 44. Growth and yield characteristics of 14 mutants and parents raised during February 2001 to July 2001

Mutants and Parents	Characters										
	Plant height (cm)	Biological yield (g)	Tuber number	Tuber yield (g)	Tuber girth (cm)	Volume per tuber (cc)	Weight per tuber (g)	Harvest index	Tuber density (g/cc)		
111	78.33	893.33	10.33	103.33	5.50	7.00	7.00	0.103	1.127		
112	82.33	733.33	40.67	3.00	4.367	7.667	3.833	0.043	0.647		
121	83.33	640.00	5.67	173.33	4.667	5.667	5.00	0.213	1.300		
131	79.33	503.33	29.67	13.33	5.80	4.667	8.33	0.034	1.967		
352	86.67	773.33	3.00	135.00	5.467	3.667	4.733	0.180	1.413		
412	66.33	746.67	20.67	13.33	5.433	8.33	4.33	0.017	0.513		
421	57.33	723.33	6.00	50.00	5.067	6.333	6.667	0.063	1.043		
422	64.67	411.67	21.33	228.33	6.667	3.667	7.33	0.343	2.110		
632	62.67	250.00	11.67	9.33	4.933	7.00	5.667	0.037	0.867		
641	79.33	848.33	11.67	258.33	4.667	14.33	11.33	0.230	0.850		
1012	76.33	756.67	10.67	176.667	5.967	6.33	11.667	0.197	2.083		
1041	79.33	713.33	3.33	226.667	3.767	5.667	6.667	0.240	1.567		
1042	69.33	283.33	6.33	38.333	5.600	10.00	9.333	0.137	0.960		
61	79.00	851.67	10.33	170.00	5.667	6.667	7.667	0.167	1.160		
Parlikad local	81.67	1046.67	4.00	45.33	3.00	6.667	2.833	0.040	0.617		
Kolazhy local	66.33	993.33	6.30	17.667	4.900	4.33	4.33	0.018	1.050		
Mullurkkara local	72.33	1113.33	7.30	75.00	2.667	4.00	4.00	0.073	1.237		
Paipra local	75.00	1043.33	8.30	20.00	3.667	4.00	4.33	0.024	1.200		
SE	± 1.96	± 43.65	± 1.46	± 12.75	± 0.17	± 0.45	± 0.47	± 0.01	± 0.10		
CD	4.08	90.79	3.04	26.52	0.35	0.94	0.98	0.021	0.21		

yield (848.33 g) and harvest index (0.230). Mutant 422 had a tuber yield of 228.33 g, tuber girth of 6.67 cm, harvest index of 0.343, tuber density of 2.110 g/cc and tuber number of 21.33. Mutant 131 had tuber number of 29.67, tuber girth of 5.8 cm and tuber density of 1.97 g/cc. Mutant 1031 had a tuber girth of 5.97 cm, weight per tuber 11.67 g and tuber density of 2.08 g/cc. Mutant 61 gave a tuber yield of 226.67 g and harvest index of 0.40.

4.3.1.2.1 Estimates of variability

General mean and variations in GV, GCV, PV, PCV, h^2 , GA and GG among 18 genotypes (14 mutants and their parents) are presented in Table 45. Generally PCV values were higher than GCV values. Maximum GCV and PCV was recorded for tuber yield (82.46%, 95.26%) followed by number of tubers (78.82%, 90.25%) and harvest index (75.82%, 89.94%). Heritability was highest for number of tubers (76.51%) followed by tuber yield (76.36%) and harvest index (68.95%). Genetic gain was maximum for tuber yield (147.03%) followed by tuber number (141.79%).

4.3.1.2.2 Correlation studies

Genotypic coefficient of correlation for tuber yield and their inter correlations are presented Table 46. Tuber yield had highly significant positive association with harvest index (0.954) and weight per tuber (0.814). Tuber girth had highly significant positive association with weight per tuber (0.929) and highly negative association with biological yield (-0.837). Volume per tuber had highly significant positive association with weight per tuber (0.896). The character harvest index had highly significant positive association with weight per tuber

Table 45. Variability of nine characters for mutants and parents raised during February 2001 to July 2001

Character	Mean	GV	PV	GCV	PCV	h ²	GA	GG
Plant height (cm)	74.43	3.00	207.7963	0.04	19.86	1.44	42.78	57.48
Biological yield (g)	740.28	41507.33	104441.3	27.16	44.11	39.74	254.99	34.45
Tuber number	12.07	90.6772	118.5105	78.82	90.25	76.51	17.12	141.79
Tuber yield (g)	99.11	6621.809	9026.697	82.46	95.26	73.36	145.72	147.03
Tuber girth (cm)	4.88	0.8541447	1.632107	19.08	26.00	52.33	1.41	0.003
Volume per tuber (cc)	6.44	4.66594	11.29557	34.00	51.53	41.31	2.98	46.24
Weight per tuber (g)	6.39	3.625669	12.25789	29.46	55.12	29.58	2.07	32.38
Harvest index	0.12	0.00137	0.011844	75.82	89.94	68.95	0.16	133.44
Tuber density (g/cc)	1.21	0.04172	0.59596	14.82	65.05	7.00	0.08	6.632



11999

Table 46. Genotypic correlation of nine characters for 14 mutants and 4 parents raised during February 2001 to July 2001

	PH	TN	TG	VIT	WIT	TD	BY	HI	TY
PH	1.000	0.689	-1.742	0.665	0.300	1.184	1.137	1.814	2.170
TN		1.000	0.338	0.077	-0.030	0.147	-0.266	-0.238	-0.266
TG			1.000	0.001	0.929**	1.353	-0.837**	0.450	0.259
VIT				1.000	0.896**	-0.528	-0.191	0.099	0.209
WIT					1.000	0.067	-0.593	0.804**	0.814**
TD						1.000	-0.789	1.520	1.298
BY							1.000	-0.222	-0.0002
HI								1.000	0.954**
TY									1.000

* Significant at 5% level

** Significant at 1% level

(0.804). The character plant height, tuber number and tuber density did not show any significant association with the other characters.

4.3.1.3 Field studies during April 2001 to October 2001

This experiment was laid out in RBD with three replications during April 2001 to evaluate the field performance of 14 mutants and their parents. The crop was harvested in October 2001. The data on yield traits were recorded and statistically analysed.

The analysis of variance with respect to nine characters for 19 genotypes are presented in Table 43.

Characters tuber number, tuber yield, volume per tuber harvest index and tuber density differed significantly among genotypes at 1 per cent level and the character tuber girth, weight per tuber, differed significantly at 5 per cent level. The mean of the characters for the 19 genotypes (14 mutants and 5 parents) are presented in Table 47. Maximum tuber yield was recorded by mutant 352 (310 g). Mutant 131 gave tuber yield of 275 g, volume per tuber of 11.67 cc, harvest index of 0.320, tuber number of 27.33 and plant height of 85.33 cm. Mutant 1031 had a weight per tuber of 11.33 g, harvest index of 0.303 and tuber density of 1.79 g/cc. Mutant 61 had the maximum tuber girth of 7.4 cm, tuber density of 2.87 g/cc and weight per tuber of 10 g.

4.3.1.3.1 Variability studies

General mean, GV, GCV, PV, PCV, h^2 , genetic advance, genetic gain are presented in Table 48. Generally PCV values were higher than GCV values.

Table 47. Growth and yield characteristics of 14 mutants and parents raised during April 2001 to October 2001

Mutants and Parents	Characters										
	Plant height (cm)	Biological yield (g)	Tuber number	Tuber yield (g)	Tuber girth (cm)	Volume per tuber (cc)	Weight per tuber (g)	Harvest index	Tuber density (g/cc)		
111	78.33	843.33	10.33	121.67	5.17	11.00	8.00	0.13	0.58		
112	94.67	850.00	7.67	30.00	4.20	7.17	8.00	0.04	1.24		
121	86.67	640.00	32.00	134.00	5.33	6.33	7.67	0.18	1.19		
131	85.33	836.67	27.33	275.00	5.80	11.67	8.33	0.32	0.72		
352	82.00	773.33	10.67	310.00	4.40	4.33	5.67	0.32	1.57		
412	66.33	796.67	7.67	116.67	4.30	10.00	3.67	0.13	0.37		
421	64.67	321.67	15.33	90.00	5.67	8.67	10.00	0.22	1.20		
422	70.33	1016.67	17.33	140.00	6.87	9.00	8.33	0.12	1.53		
632	62.67	923.33	24.00	220.00	4.47	8.67	6.00	0.21	0.73		
641	79.33	843.33	11.67	13.33	5.70	6.67	6.33	0.02	1.00		
1012	75.67	426.67	19.67	128.33	5.67	6.67	11.33	0.30	1.79		
1042	80.33	570.00	12.67	59.00	5.87	11.00	11.67	0.11	1.04		
61a	77.00	730.00	22.67	155.33	7.40	3.67	10.00	0.16	2.87		
61b	78.00	420.00	31.00	106.67	5.57	9.33	3.67	0.21	0.39		
Parlikad local	89.00	1076.67	6.67	36.67	4.00	11.00	4.33	0.04	0.45		
IC-65724	63.00	1023.33	5.00	46.67	4.53	7.67	5.67	0.05	0.70		
Kolazhy local	68.00	650.00	6.00	20.00	5.60	8.00	5.33	0.03	0.64		
Paipra local	55.00	790.00	5.33	41.67	6.20	10.33	4.00	0.05	0.40		
Mullurkkara local-III	64.33	450.00	7.00	34.33	6.53	5.00	5.00	0.07	1.00		
SE	± 2.14	± 44.08	± 1.37	± 12.95	± 0.20	± 0.40	± 0.47	± 0.02	± 0.10		
CD	4.45	91.69	2.85	26.94	0.42	0.83	0.98	0.04	0.21		

Table 48. Variability of nine characters for mutants and parents raised during April 2001 to October 2001

Character	Mean	GV	PV	GCV	PCV	h ²	GA	GG
Plant height (cm)	74.77	36.135	262.3983	8.04	21.66	13.771	4.60	6.152
Biological yield (g)	735.88	15981.27	111346.70	16.48	45.86	14.353	89.75	12.196
Tuber number	14.74	65.023	109.1982	54.64	71.02	59.546	12.76	86.586
Tuber yield (g)	109.33	5931.624	9774.641	70.43	90.45	66.684	123.50	112.961
Tuber girth (cm)	5.54	0.5881891	2.213102	14.37	26.29	26.578	0.90	16.244
Volume per tuber (cc)	8.22	3.694607	9.444607	23.19	37.63	39.119	2.42	29.443
Weight per tuber (g)	7.00	3.313353	12.48441	25.82	50.66	26.540	1.90	27.143
Harvest index	0.14	0.0065282	0.01674	56.31	90.88	38.990	0.10	69.980
Tuber density (g/cc)	1.02	0.260332	0.6167005	49.41	77.56	42.214	0.66	64.605

Maximum GCV was recorded by the character tuber yield (70.43%) followed by harvest index (56.31%) and tuber number (54.64%). Maximum PCV of 90.88 per cent and 90.45 per cent was recorded by the characters harvest index and tuber yield respectively. Maximum heritability was recorded by the characters tuber yield (66.68%) followed by tuber number (59.55%). Genetic gain was maximum for tuber yield (112.96%) followed by tuber number (86.58%).

4.3.1.3.2 Correlation studies

Genotypic coefficient of correlation for tuber yield and their inter correlations are presented Table 49. Harvest index (0.923) and tuber number (0.656) had highly significant positive association with tuber yield. Tuber number had highly significant positive association with harvest index (0.946) and a significant positive association with tuber girth (0.772). Tuber density had highly significant positive association with weight per tuber (0.652), significant positive association with tuber girth (0.747) and a highly significant negative association with volume per tuber (-0.773). Characters plant height and biological yield did not show any significant association with other characters.

4.3.1.4 Overall performance of mutants under field condition

The effect of eight mutants over three seasons were also studied. The analysis of variance season verses mutants with respect to five main yield attributes viz., plant height, tuber number, tuber yield, tuber girth and harvest index are presented in Table 50.

Table 49. Genotypic correlation of nine characters for 14 mutants and 5 parents raised during April 2001 to October 2001

	PH	TN	TG	VIT	WIT	TD	BY	HI	TY
PH	1.000	0.583	-0.747	-0.267	0.987	0.734	-0.300	0.573	0.468
TN		1.000	0.772*	-0.065	0.528	0.384	-0.665	0.946**	0.656**
TG			1.000	-0.366	0.518	0.747*	-1.327	0.263	-0.101
VIT				1.000	-0.180	-0.773**	0.365	-0.133	-0.157
WIT					1.000	0.652**	-0.792	0.616	0.292
TD						1.000	-0.341	0.462	0.416
BY							1.000	0.067	0.371
HI								1.000	0.923**
TY									1.000

* Significant at 5% level

** Significant at 1% level

Table 50. Effects of mutants, season and their interaction on characters of *Coleus*

		Source		
		Mutants (df = 7)	Seasons (df = 2)	Mutants x Seasons Interaction (df = 14)
Plant height	MSS	235.903	21775.056	109.563
	F value	1.8150**	167.5362**	
Tuber girth	MSS	3.133	7.064	2.838
	F value	3.87**	8.7274**	3.5057**
Tuber yield	MSS	10522.087	39380.931	22605.677
	F value	2.6042*	9.7467**	5.5949**
No. of tubers/plant	MSS	194.220	757.125	353.554
	F value	5.2809**	20.5865**	9.6132**
Harvest index	MSS	0.032	0.004	0.046
	F value	5.9571**	0.6518	8.5047**

* Significant at 5% level

** Significant at 1% level

The mean values for the mutants for the three seasons and its general mean for characters viz., plant height, tuber number, tuber yield, tuber girth and harvest index are presented in Table 51.

All the mutants differed significantly both among treatments and seasons as well as for its interaction with respect to tuber girth, tuber yield and tuber number and harvest index. With respect to plant height seasons differed significantly, but mutant x season interaction was not significant. Mutant 131 ranked first for all the seasons for tuber yield taken together (130.56 g) followed by mutant 422 (128.00 g). Among the seasons also it recorded the maximum tuber yield of 275 g in season 3, but in the semi off season it was mutant 641 which recorded the maximum yield of 258.33 g plant⁻¹ followed by mutant 422 (228.33 g). In the off season it was mutant 61 which recorded maximum value of 175 g plant⁻¹. While comparing the first season and third season, mutant 61 ranked first in season 1 (175 g) second in normal season (153.33 g), with a mean of

Table 51. Characteristics of *Coleus* mutants in different seasons

Mutant No.	Season I	Season II	Season III	Mean
I. Plant height (cm)				
131	36.667	79.333	85.333	67.111
112	20.000	82.333	94.667	65.667
641	31.333	79.333	79.333	63.333
422	19.667	64.667	70.333	51.556
111	24.667	78.333	78.333	60.444
121	20.333	83.333	86.667	63.444
61	26.000	69.333	77.000	57.444
62	37.667	79.000	78.000	64.889
Mean	27.042	76.958	81.208	
CD for seasons	7.90			
II. Number of tuber/plant				
131	12.000	29.667	27.333	23.000
112	5.667	40.667	7.667	18.000
641	4.667	11.667	11.667	9.333
422	4.333	21.333	17.333	14.333
111	7.000	10.333	10.333	9.222
121	5.333	5.667	32.000	14.333
61	25.667	6.333	22.667	18.222
62	8.333	10.333	31.000	16.556
Mean	9.125	17.00	20.00	
CD for mutants	2.58			
CD for seasons	4.20			
III. Tuber yield (g/plant)				
131	103.333	13.333	275.000	130.556
112	14.000	30.000	30.000	24.667
641	26.667	258.333	13.333	99.444
422	15.667	228.333	140.000	128.000
111	43.333	103.333	121.667	89.444
121	34.333	173.333	134.000	113.889
61	175.000	38.333	153.333	122.222
62	22.000	170.000	106.667	99.556
Mean	54.292	126.875	121.750	
CD for mutants	26.97			
CD for seasons	44.05			

Contd.

Table 51. Continued

III. Girth of tubers (cm)				
131	5.300	5.800	5.800	5.633
112	4.467	4.367	4.200	4.344
641	6.400	4.667	5.700	5.589
422	4.367	6.667	6.867	5.967
111	6.167	5.500	5.167	5.611
121	4.133	4.667	5.333	4.711
61	5.000	5.600	7.400	6.000
62	3.567	5.667	7.567	5.600
Mean	4.925	5.367	6.004	
CD for mutants	0.381			
CD for seasons	0.62			
III. Harvest Index				
131	0.184	0.034	0.319	0.179
112	0.041	0.040	0.038	0.040
641	0.011	0.230	0.018	0.086
422	0.056	0.341	0.116	0.171
111	0.356	0.104	0.126	0.195
121	0.051	0.211	0.184	0.149
61	0.357	0.138	0.175	0.223
62	0.015	0.166	0.207	0.129
Mean	0.134	0.158	0.148	
CD for mutants	0.031			

122.22 g when all the three seasons were taken together. Mutant 61 ranked first for all the seasons taken together for harvest index (0.223) followed by mutant 111 (0.195). The maximum harvest index during the off season was also shown by the same mutant (0.357) whereas during the normal season the mutant 131 showed maximum harvest index (0.319) followed by mutant 62 (0.207). Maximum tuber girth for all the three seasons taken together was shown by mutant 61 (6.00 cm) followed by mutant 422 (5.97 cm). The maximum tuber girth during the off season was shown by mutant 641 (6.40) followed by mutant 111 (6.17). The maximum tuber girth during the crop season was shown by mutant 62 (7.57) followed mutant 61 (7.40).

4.4 *In vitro* mutagenesis in *Coleus*

4.4.1 *in vitro* studies

The accession Paipra local was used for tissue culture study because this was the only genotype in the 9th cluster and it had lush green vegetation thus facilitating an abundance of internodes which is used as the explant in this study.

4.4.1.1 Per cent callus induction

The per cent callus induction in different media composition is presented in Table 52.

MS media containing 4 mg l⁻¹ BAP and NAA 4 mg l⁻¹ was found to be the best media for culturing internodal explants of *Coleus* for callus induction. Callus induction was achieved 6-8 weeks after inoculation. Further proliferation of callus was achieved when they were subcultured on 1.5 mg l⁻¹ BAP and 4 mg l⁻¹ NAA.

Table 52. Response of Paipra local to different media composition for per cent callus induction

Sl. No.	Treatment No.	Per cent callus induction
1	T ₁	66.67
2	T ₂	66.67
3	T ₃	60.00
4	T ₄	46.67
5	T ₅	46.67
6	T ₆	53.33
7	T ₇	53.33
8	T ₈	53.33
9	T ₉	60.00
10	T ₁₀	53.33
11	T ₁₁	60.00
12	T ₁₂	60.00
13	T ₁₃	53.33
14	T ₁₄	86.67
15	T ₁₅	46.67
16	T ₁₆	73.33

4.4.1.3 Per cent of callus regenerating after irradiation

The effect of gamma rays on callus cultures were studied. In order to fix the optimal dose for inducing *in vitro* mutations, the LD₅₀ of gamma ray was first estimated and the results presented in Table 53.

The LD₅₀ was found to be 2.5 Gy and hence the doses of radiation for inducing *in vitro* mutations was selected as 0.5 Gy, 1 Gy, 1.5 Gy and 2 Gy.

Table 53. Doses of gamma rays and percentage of callus regeneration for LD₅₀ estimation

Gamma ray (dose in Gy)	Callus regeneration percentage
0.0	90.0
0.5	88.0
1.0	88.0
1.5	80.0
2.0	72.0
2.5	44.0
3.0	40.0
3.5	4.0
4.0	0.0
4.5	0.0
5.0	0.0
5.5	0.0
6.0	0.0

Fifteen cultures were irradiated for each dose and the callus regeneration was found to be 92, 88, 92 and 80 per cent respectively (Table 54).

Table 54. Effect of doses of gamma radiation on survival of calli of *Coleus in vitro*.

Gamma ray (dose in Gy)	Survival percentage
0.5	92.0
1.0	88.0
1.5	92.0
2.0	80.0

4.4.1.4 Percentage recovery of hardened plants

The percentage of success in producing rooted hardened plants was 66.67 per cent for 0.5 Gy and 40 per cent for 1 Gy (Table 55). Calli regenerated by

becoming green and producing many hard protuberances (meristamoids) after 16 hours light period. These meristamoids later developed into shoots with leaves in the same regeneration medium after 3-4 weeks without subculturing. The adventitious shoots were excised and transferred to MS basal medium containing IBA 1 mg l⁻¹.

Table 55. Survival of TC mutants one month after transplanting

Dose in Gy	Percentage of success
0.5	66.67
1.0	40.00
1.5	0.00
2.0	16.67

The eleven mutants developed by *in vitro* mutagenesis and its control Paipra local were raised during the off season November 2001 to April 2002. The performance of 11 mutants are presented in Table 56.

The control Paipra local did not produce any tubers during the season and hence the mean values of tuber yield and associated attributes which are presented are the values of normal season for this accession and this is used only for comparison.

The tuber yield ranged from 15 g plant⁻¹ to 75 g plant⁻¹. The mean value of tuber girth ranged from 2.8 cm to 5.1 cm and number of tubers per plant ranged from 8 to 68.

Table 56. Mean performance of tissue culture mutants for tuber yield and other attributes

Sl. No.	TC mutant No.	Tuber yield (g plant ⁻¹)	Tuber girth (cm)	No. of tubers/plant
1	TC 1	25	3.0	23
2	TC 2	25	4.0	22
3	TC 3	25	4.6	18
4	TC 4	24	3.8	23
5	TC 5	25	3.5	18
6	TC 6	24	3.8	14
7	TC 7	15	2.8	12
8	TC 8	25	4.0	13
9	TC 9	75	4.5	68
10	TC 10	26	5.1	11
11	TC 11	24	3.3	8
	Mean	25.48	3.86	20.91
	Paipra local (normal season)	82.5	5.4	70

Qualitative parameters of *Coleus* mutants

a) Evaluation of organoleptic qualities

The organoleptic qualities namely colour, appearance, texture, flavour and taste of 14 *Coleus* mutants were estimated using a hedonic scale score.

Hedonic scale score given

Very poor	- 1
Poor	- 2
Good	- 3
Very good	- 4

The analysis of variance for organoleptic parameters are presented in Table 57. The 14 mutants differed significantly among themselves and also interacted differentially for all the organoleptic parameters.

Table 57. Anova for organoleptic parameters for 14 mutants

Source	df	MSS	F
Organoleptic parameters	4	1.995	7.3629**
Mutants x Organoleptic parameters	13	14.293	52.75**
Mutant x cooking quality parameters	52	0.971	3.58**
Error	630	0.271	

**Significant at 1% level

The mean values of five organoleptic qualities are presented in Table 58. All the mutants are attributed with good colour and appearance whereas the means of texture, flavour and taste are within 2-3.

The mean values of 14 mutants with respect to different organoleptic attributes are presented in Table 59. It is highly interesting to note that mutants 62 and 112 recorded mean values of 4 (Very good) for all the organoleptic characters and the mutant 1031 recorded a score of 4 (Very good) with respect to texture only. The overall mean for various organoleptic parameters for the 14 mutants are presented in Table 59. Here also mutant 62 and 112 are the only mutants recording mean value of 4 (Very good).

Table 58. Organoleptic evaluation of *Coleus* mutants

Sl. No.	Mutant	Mean scores of organoleptic parameters					
		Colour	Appearance	Texture	Flavour	Taste	Overall Mean
1	632	1.7	2.1	2.8	2.5	2.7	2.40
2	111	2.9	2.9	2.7	2.5	2.6	2.70
3	619	3.0	2.8	2.1	2.3	2.8	2.60
4	641	2.4	2.3	2.9	2.4	2.8	2.60
5	421	2.9	3.1	3.0	2.6	2.3	2.80
6	131	3.0	2.9	2.7	2.4	2.5	2.70
7	121	2.4	2.7	2.7	2.5	2.5	2.60
8	352	2.8	2.9	2.3	2.3	2.3	2.50
9	1042	3.4	3.0	3.4	3.0	3.8	3.30
10	62	4.0	4.0	4.0	4.0	4.0	4.00
11	61	3.0	2.8	2.1	2.3	2.8	2.60
12	112	4.0	4.0	4.0	4.0	4.0	4.00
13	1031	3.8	3.4	4.0	2.7	2.8	2.30
14	412	2.8	3.5	2.6	2.7	3.8	2.90
	Mean	3.07	3.03	2.95	2.70	2.90	

CD for organoleptic parameter

CD for mutants

CD for mutant x organoleptic parameter

b) Yield and quality of essential oil of *Coleus*

Coleus tubers possess a characteristic aroma which is attributed to the presence of essential oils in them. The major chemical components of *Coleus* essential oil is reported by the alpha thujone and beta farnescene with the objectives of evaluating the food quality of *Coleus* tubers, attempts were made to estimate the content of essential oil in the tubers and to analyse the oil for principal aroma constituents by gas chromatography. However, the essential oil content of

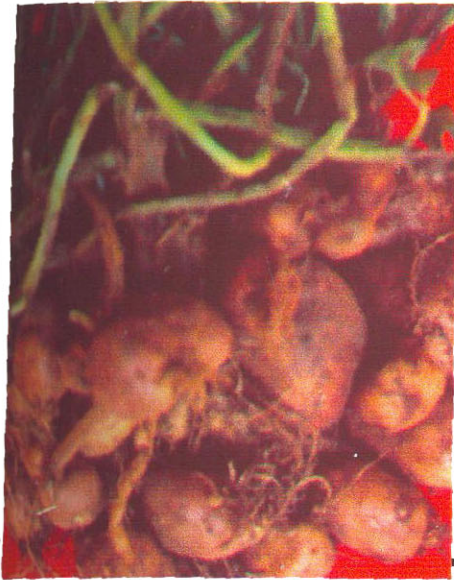
Coleus tubers was very small (< 0.02%) which made the analysis difficult to perform with required accuracy.

c) Estimation of starch and protein

Table 59. Mean values of starch and protein of 14 *Coleus* mutants along with control

Sl.No.	Mutants/Control	Starch	Protein
1	632	16.1	0.67
2	111	14.3	0.38
3	619	9.0	0.04
4	641	6.3	1.20
5	421	19.1	0.08
6	131	9.0	0.13
7	121	7.5	1.10
8	352	20.4	0.07
9.	1042	12.7	0.78
10	62	8.1	0.54
11	61	9.0	0.04
12	112	17.1	0.27
13	1031	8.5	1.10
14	412	9.0	0.04
Control	Sreedhara	16.0	0.04
	Mean	11.64	0.43

The mean values of starch and protein of 14 mutants along with standard control are presented in Table 60. The percentage of starch varied from 6.3 per cent to 20.4 per cent. The percentage of protein varied from 0.04 to 1.2 per cent. The selected mutants 131 and 61 had a starch content of 9 per cent and a protein content of 0.13 and 0.04 per cent respectively.



Discussion

5. DISCUSSION

Coleus is a popular but under exploited minor tuber crop of the tropics. It is a cheaper source of carbohydrates and accepted by a large section of people due to its relishing taste.

It is a season bound crop due to its requirement of short days for tuberization and hence year-round cultivation of this crop is not possible (CTCRI, 1987). Studies on exploitation of genotypes possessing photo insensitivity for tuberization from the available germplasm is restricted. Investigation has to be carried out to screen out such genotypes, if any available in the cultivated/wild germplasm.

If it is not available then the only viable option is the induction of variability through mutation. These mutations are helpful in generating variations supplementary to that occurring in nature. The induction of mutation can be amplified if it is carried out *in vitro*. The importance of mutation breeding cannot be over emphasized especially when exploitable natural genetic variability of the population is not wide enough. An attempt has been made here through studies on variability, character association, genetic diversity and induction of variability through *in vivo* and *in vitro* mutagenesis to develop a plant type of higher yield and photo insensitivity. The results are discussed below.

5.1 Experiment-I

The choice of the most suitable breeding method for the rational improvement of yield and its components in any crop largely depends on the

available genetic variability, association between characters, heritability, genetic advance under selection and adaptability parameters.

5.1.1 Survey and collection

The maximum collection of *Coleus* genotypes was from Thrissur District followed by Malappuram mainly because those two districts have larger area under cultivation of this crop. During the course of *Coleus* collection wild progenitors were not seen in the cultivated field which indicates that it is an introduced crop and later domesticated.

5.1.2 Genetic variability

The analysis of variance showed highly significant differences among the genotypes for all the characters studied suggesting the presence of substantial genetic variability among the genotypes. The wide range of variation noticed in all the characters confirmed that the materials selected were genetically diverse and that they were appropriate for the study. Variability for different characters was previously observed by several workers like Sarkar *et al.* (1992), Sreekumari and Abraham (1985), Khairwal and Babu (1985), Amalraj *et al.* (1989). But Prakash (1996) reported that tuber characters like tuber yield per plant and tuber girth did not show any significant difference among *coleus* genotypes. Vimala (1994) reported that there was no significant difference in yield among 43 accessions of *coleus* in an yield trial at CTCRI. The variability available in a population could be partitioned into heritable and non-heritable components with the aid of genetic parameters like genotypic coefficient of variation (GCV) heritability (h^2) and genetic advance (GA) which can be used as reliable guidelines for selection. The

genotypic coefficient of variation provides a valid basis for comparing and assessing the range of genetic diversity for quantitative characters. The phenotypic coefficient of variation measures the extend of total variability. A high magnitude of genotypic coefficients of variation and phenotypic coefficients of variation of tuber yield, harvest index, mean volume of tuber, mean weight of tuber and biological yield in a population indicates the existence of large variability and suggests scope for genetic improvement of these traits through selection. The same results were reported in cassava by Naskar *et al.* (1991), Kumar *et al.* (1996), Alam *et al.* (1998) and Choudhary *et al.* (1999) in sweet potato. Few studies are reported in *coleus* in this line.

5.1.3 Heritability, genetic advance and genetic gain

The estimation of heritable variation is not possible with the help of genotypic coefficient of variation alone. Burton (1952) had suggested that genotypic coefficient of variation together with heritability estimates would give a better idea of selection advance to be expected. The degree to which the variability for a quantitative character is transmitted to the progeny is referred to as heritability and it measures the heritable portion of variability. In an asexually propagated crop the estimation of heritability in the broad sense is meaningful since all the genetic variability can be exploited between asexual generations by means of selection (Hogarth, 1971).

Heritability and genetic advance are important selection parameters and these will be influenced by environment, genetic variability and selection intensity. In this study, tuber yield, harvest index and biological yield plant⁻¹, volume tuber⁻¹

and weight tuber⁻¹ of the collected germplasm showed high heritability and high genetic gain (Fig. 1). This indicates that heritability of these characters is due to additive gene effects and hence the observed variability is heritable with negligible influence of environment. So selection may be effective in improving these traits. The importance of additive genetic variance was reported by Sakamoto (1979), Lin (1983), Li (1987) and Vimala and Lakshmi (1991) in sweet potato. Contrary to this, non-additive genetic variance was reported by Biradar *et al.* (1978) and Suthanthirapandian *et al.* (1994) in cassava and Dai *et al.* (1988) and Chen *et al.* (1989) in sweet potato. Tuber density had high heritability with low genetic gain, low phenotypic coefficient of variation and low genotypic coefficient of variation in these crops. This is indicative of non-additive gene action. The high heritability that is exhibited in these crops is due to favourable influence of the environment and hence selection may not be rewarding. Point of tuberization had low values for PCV, GCV, h^2 and genetic gain. This again indicates that this character is highly influenced by environment and selection based on point of tuberisation is ineffective for crop improvement.

Mean girth of tuber and nematode susceptibility had low values for heritability, PCV, GCV and genetic gain, indicating that this character is influenced by environmental effects. This is in conformity with the findings of Vimala and Lakshmi (1991) in sweet potato. Contrary to this finding Apte *et al.* (1994) reported that in taro, cormel girth had high values for heritability.

PLATE:1.FIELD VIEW OF COLEUS GENOTYPES

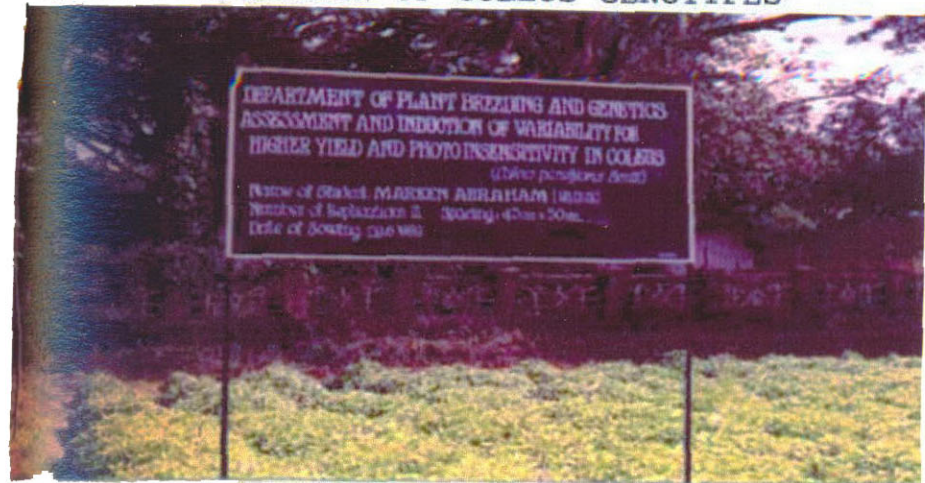


Fig. 1. Heritability and Genetic advance of 13 characters of coleus genotypes

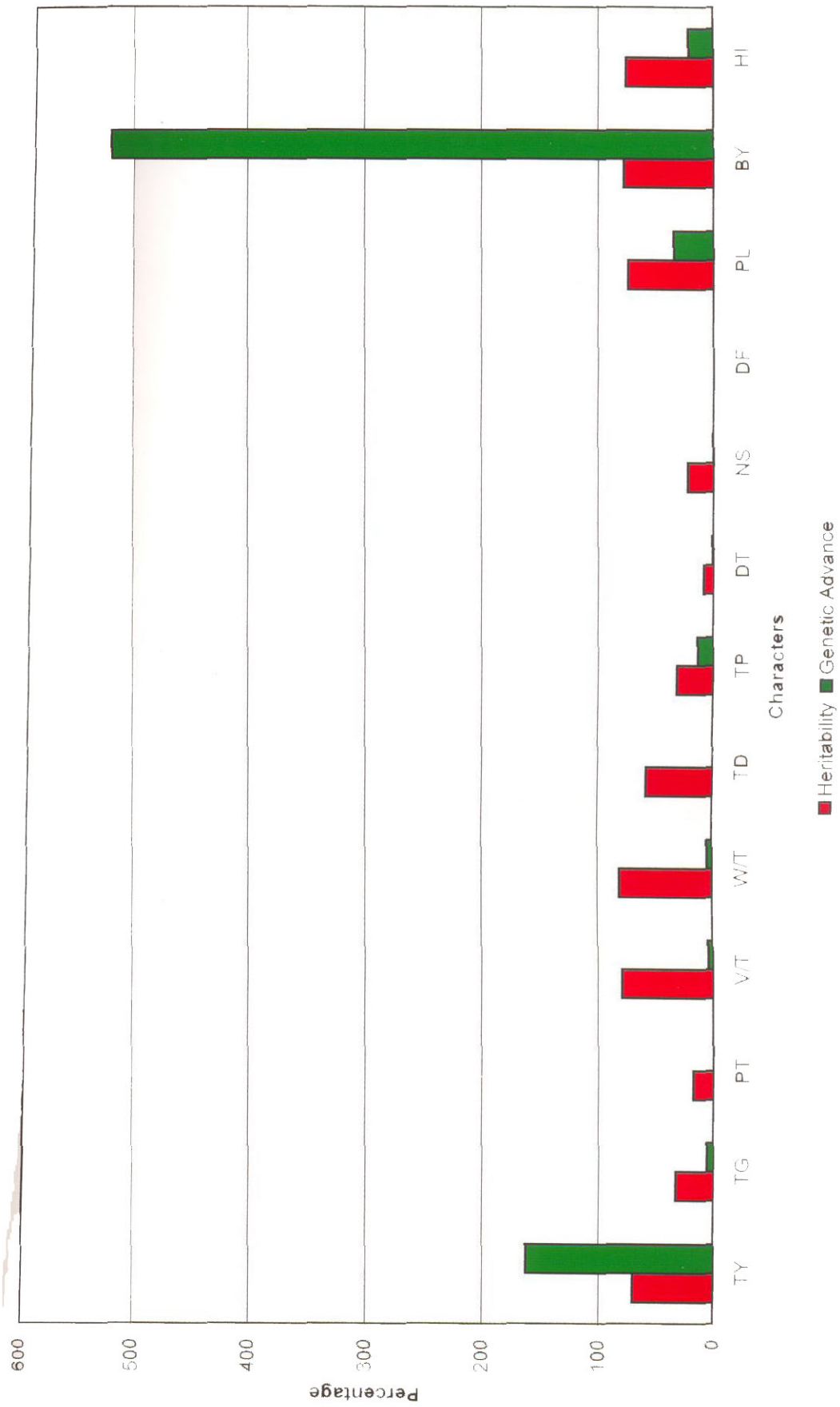


PLATE:2.VARIATIONS IN TUBER SIZE



VARIATIONS IN TUBER SIZE



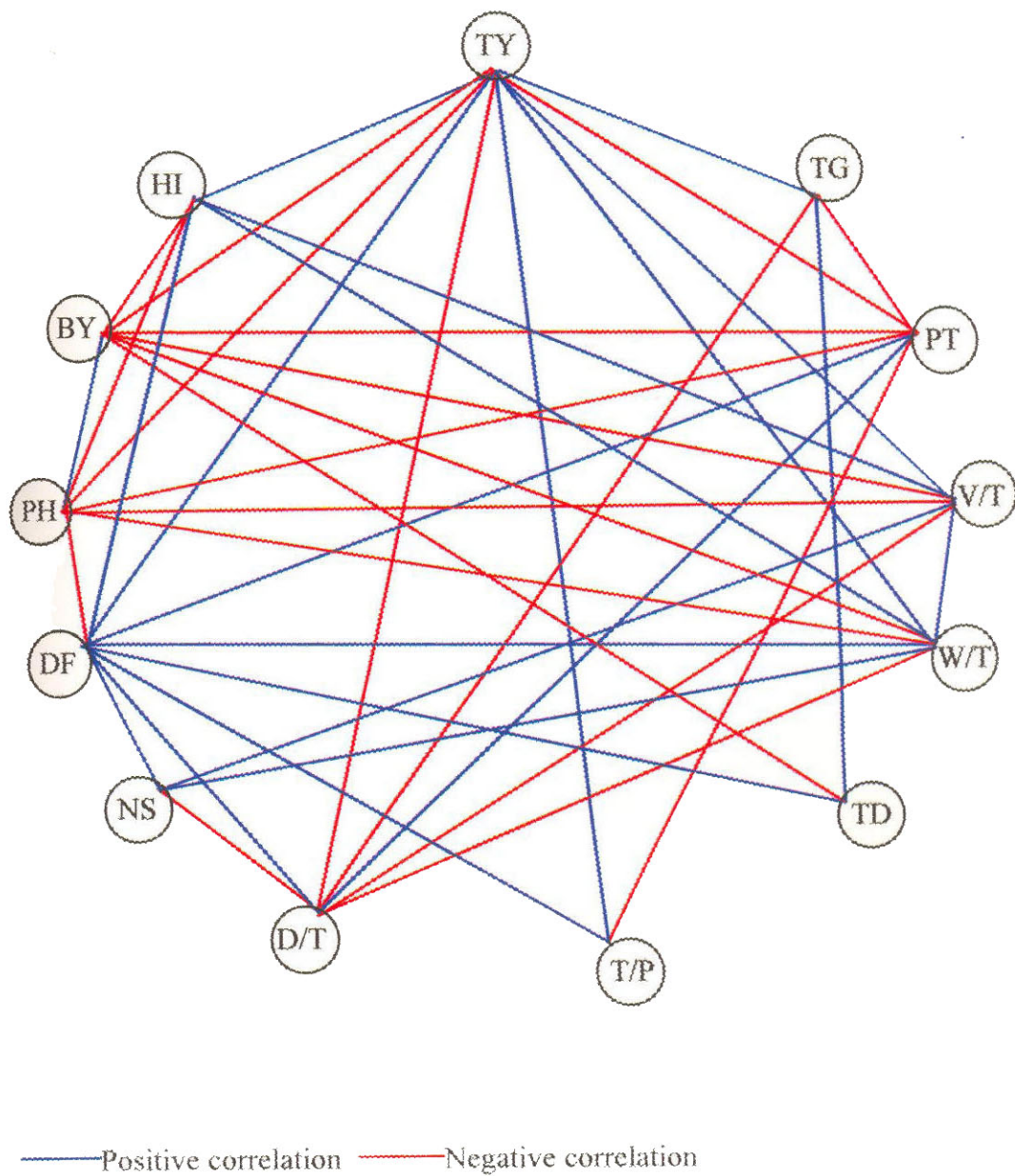
5.1.4 Correlation

Studies on the association of characters is an important tool in plant breeding, since it helps to determine the relationship of yield with its components which in turn helps to select superior genotypes from diverse genetic populations.

Correlation provides information on the nature and extent of relationship between characters. The estimates of genotypic and phenotypic correlation coefficients between various characters helps to quantify the intensity and to identify the direction of associations. Genotypic correlations provide a reliable measure of genetic association between characters and help to differentiate the vital association useful in breeding from non vital ones (Falconer, 1981). Therefore analysis of yield in terms of genotypic and phenotypic correlation coefficients of component characters helps to identify the characters that can form the basis of selection.

In the present investigation, all the characters except nematode susceptibility were significantly associated with tuber yield at the genotypic level (Fig. 2). Girth of tuber, volume of tuber, weight of tuber, number of tubers plant⁻¹, number of days to flowering and harvest index were positively correlated with tuber yield. This is in conformity with the studies of Sreekumari and Abraham (1985) in coleus, Kamalam *et al.* (1977), Hossain *et al.* (2000) in sweet potato Biradar *et al.* (1978) in cassava and Mohankumar *et al.* (1990) in colocasia. Generally genotypic correlations were greater than phenotypic correlations. This indicates that the influence of environment on these characters was less and in turn

Fig. 2. Genotypic correlation diagram of 13 characters of *Coleus* genotypes



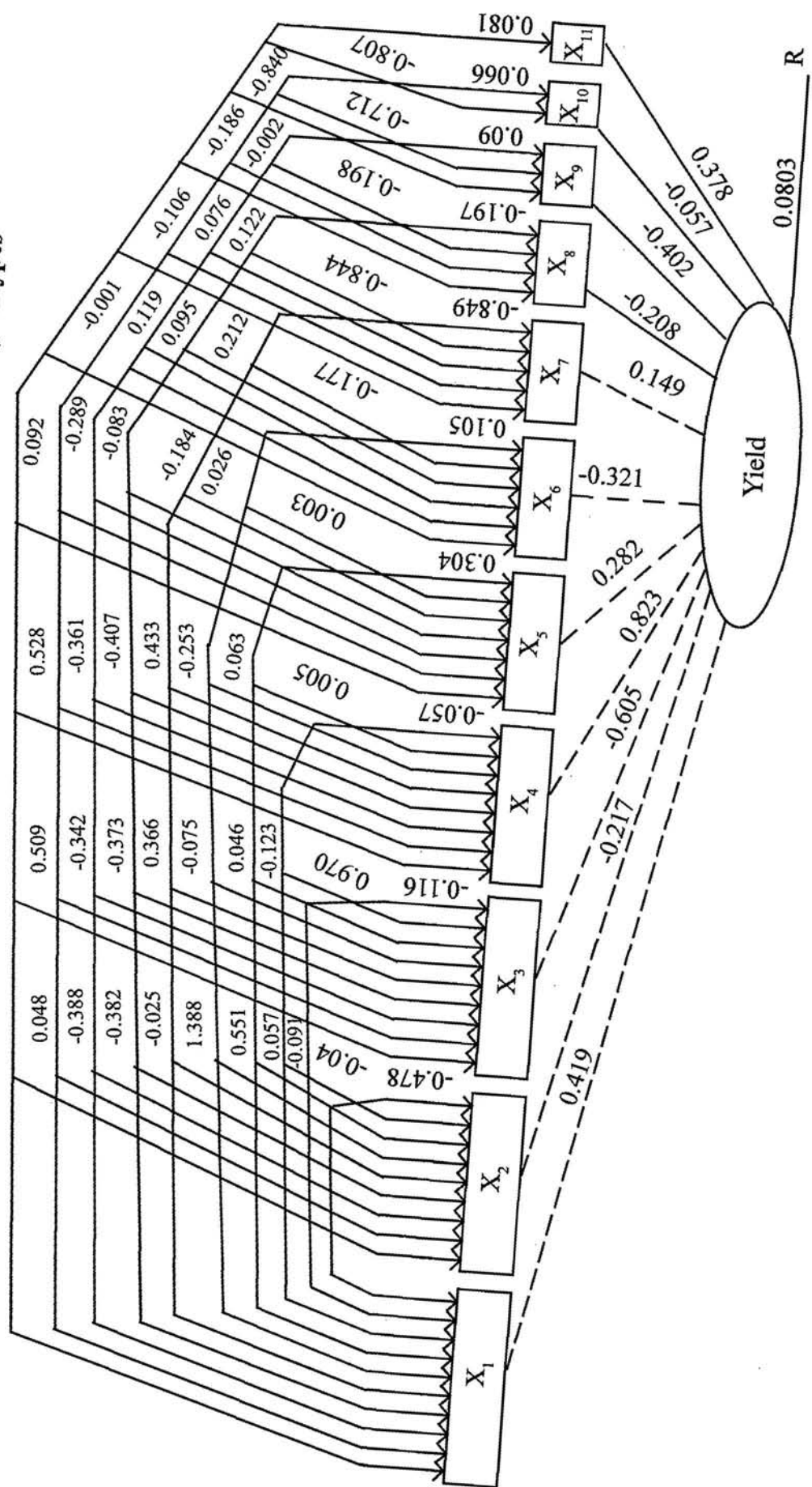
supports the studies on genetic variability. Characters like volume of tuber, weight of tuber, number of days to flowering and harvest index were positively correlated with tuber yield, while number of days to tuberization, plant length and biological yield were negatively correlated with yield at the phenotypic level. Similar results were reported by Lakshmi and Amma (1980) in Asian greater yam.

Harvest index, weight per tuber, volume per tuber and days to flowering were positively correlated with height per plant and biological yield which were negatively correlated with tuber yield. Tubers plant⁻¹ was negatively correlated with point of tuberization and positively related with days to flowering.

Correlation studies conducted by Sreekumari and Abraham (1985) in coleus showed that tuber yield was positively and significantly correlated with shoot length and number of branches. Further, harvest index was positively correlated with number of tubers and girth of tuber. Sreekumari and Pillai (1993) showed that cormel number and cormel weight in taro were correlated at the genotypic level. Positive correlation may be due to genetic reasons, namely linkage and pleiotropy. Therefore the knowledge of interrelationship of characters is helpful in developing appropriate selection criteria for the improvement of complex characters like yield.

The present study clearly reveal that direct selection for tuber yield alone will not help because the character is influenced both by environment as well as associated traits. This is in conformity with the findings of Sreekumari and Pillai (1993) in taro.

Fig. 3. Path diagram of eleven characters with yield in *Coleus* genotypes



5.1.5 Path analysis

Correlation studies are helpful in measuring the association between yield and yield components but they do not provide a clear picture of the direct and indirect causes of such associations. However this can be obtained through path analysis. Path coefficient analysis is very much useful in identifying the important yield components which can be utilized for formulating selection parameters.

In the present study, path coefficient analysis was performed taking eleven yield components which were significantly correlated with tuber yield of *coleus* at the genotypic level. The cause and effect relationship between yield and eleven selected components is illustrated in Fig. 3. The residual effect of path analysis was very low (0.0803) indicating that almost 92 per cent variation in tuber yield was contributed genotypically by eleven tuber yield components selected for path analysis.

As per the present investigation, the highest direct contribution to tuber yield was through tuber weight. This was reported in sweet potato by Kumar *et al.* (1996). But its genotypic correlation towards tuber yield is next to harvest index. Eventhough harvest index has the maximum positive genotypic correlation with yield its direct effect is only fourth in position indicating that it is contributing indirectly towards yield through the character-tuber weight. This is in agreement with the findings of Nanda (1994), Naskar *et al.* (1986) and Kamalam *et al.* (1977) in sweet potato. Harvest index and tuber weight are the most influential characters in increasing tuber yield. The same results were obtained in cassava by Radhakrishnan and Gopakumar (1984); Rekha *et al.* (1991) and Kumar *et al.* (1996) and Mohankumar *et al.* (1990) in taro.

Second important character which has the maximum direct effect as well as significant positive correlation with yield is tuber girth, indicating that this character should also be considered for tuber yield improvement in *coleus*. The importance of this character in determining the tuber yield was reported by Pushkaran *et al.* (1976), Ibrahim (1987) and Alam *et al.* (1998) in sweet potato and Rekha *et al.* (1991) in cassava. On the contrary, volume per tuber followed by length per plant, exerted a strong negative effect towards tuber yield.

Volume per tuber showed positive genotypic correlations where as length per plant showed negative genotypic correlation with yield. The negative direct effect of volume per tuber coupled with positive genotypic correlation indicates that an optimum size of tuber is essential for higher tuber yield. Volume tuber⁻¹ has a positive indirect effect towards yield through tuber density. A reduced plant height with higher harvest index will result in higher tuber yield because of the direct and indirect effects of both characters on yield. This is in conformity with the findings of Rekha *et al.* (1991) in cassava and Vasudevan and Jos (1992) in yams.

Next to girth per tuber, number of tubers per plant has maximum direct effect as well as significant positive genotypic correlation with yield. Similar findings have also been reported by Pushkaran *et al.* (1976), Thamburaj and Muthukrishnan (1976), Kamalam *et al.* (1977) and Ibrahim (1987) in sweet potato.

Correlation and path analysis studies conducted in the present investigation revealed that for tuber yield improvement programme in *coleus*, the breeder should give emphasis on high harvest index with optimum plant height,

more number of tubers with medium size having high density. These plant types should also be shorter in duration with high resistance to nematode infestation. This is in conformity with the results of Holmes and Wilson (1974) in cassava.

5.1.6 Genetic diversity

The scope for genetic improvement in any crop depends mainly the extent of genetic variability in the population.

Since variability is the outcome of divergence in the population, it is always better to study variability along with genetic diversity. Mahalanobis D^2 statistics is found to be a powerful tool in the hands of the plant breeder to assess the degree of dissimilarity among the genotypes and consequently to group them based on their phenotypic expression. The variability can be further expanded through induced mutation in representative samples taken from these clusters.

5.1.7 Clustering

D^2 analysis employing a combined classification approach in respect of thirteen selected characters revealed that the 60 accessions studied could be grouped into ten clusters. The grouping indicated that some genotypes belonging to the same location got segregated into different clusters and certain genotypes habitating different locations got grouped in the same cluster. This leads to the inference that factors other than geographical diversity may be responsible for such clustering and that there was no parallelism between geographic distribution and genetic diversity. Similar observations of non-correspondence of genetic divergence with geographical distribution has been reported by Roy and Panwar (1993) and Mannan *et al.* (1993) in colocasia. These results agrees with the

PLATE:3.VARIABILITY IN COLEUS IN DIFFERENT
CLUSTERS



suggestions made by Raut *et al.* (1980) that genetic drift and human selection could cause greater diversity than the geographic distance. Clusters accommodating maximum number of accessions comprise of genotypes collected from distant districts belonging to different eco-geographical conditions. At the same time, cluster IX that had only one genotype showed maximum genetic distance from the nearby clusters of VIII and X. All other genotypes collected from the same region were grouped into cluster X. In cluster V there is a polarisation of genotypes collected from different states. Murthy and Arunachalam (1966) explained that such a wide adaptability could be possible due to reasons such as heterogeneity, genetic architecture of the populations, past history of selection, developmental factors and degree of general combining ability.

5.1.8 Inter and Intracluster distances

Estimates of inter and intracluster distances also supported the above conclusions. The statistical distance represent the index of genetic diversity among clusters. The greater the distance between clusters the wider will be the genetic distance between the genotypes. Spatial diagrams of clusters and their mutual relationship is represented in Fig. 4. Maximum intracluster distance was seen in cluster VI indicating that there is considerable variability existing among the genotypes of that cluster. Selection within a cluster might also be exercised based on the highest mean performance of the genotypes for desirable traits such as tuber yield per plant, girth of tuber, harvest index and height per plant. Based on the mean of these attributes, over all ranking were given to the genotypes belonging to each cluster and ten genotypes were selected as representatives of each cluster.

Fig. 4. Genetic divergence of *Coleus* genotypes (Cluster Diagram)

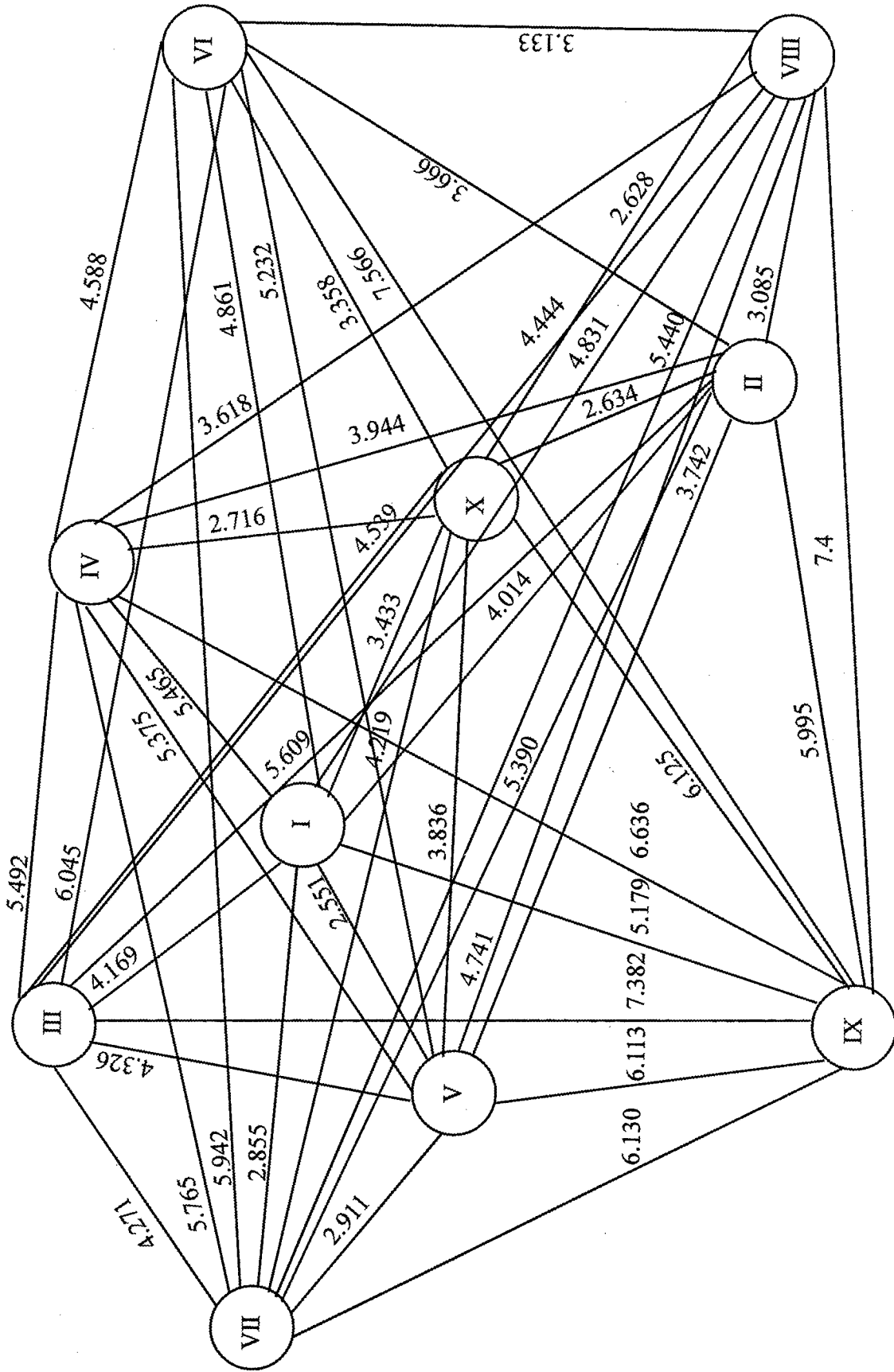
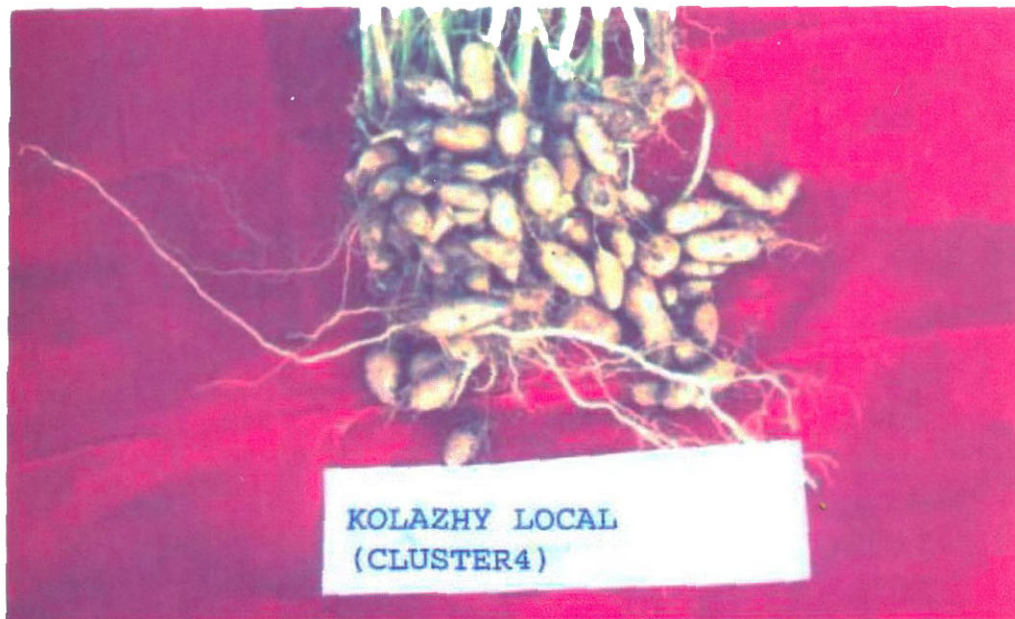


PLATE:4.VARIABILITY IN COLEUS GENOTYPES
DIFFERENT CLUSTERS



SREEDHARA
(CLUSTER3)



KOLAZHY LOCAL
(CLUSTER4)

PLATE:5.VARIABILITY IN COLEUS IN
DIFFERENT CLUSTERS



PLATE:6.VARIABILITY OF COLEUS GENOTYPES IN CLUSTER.7

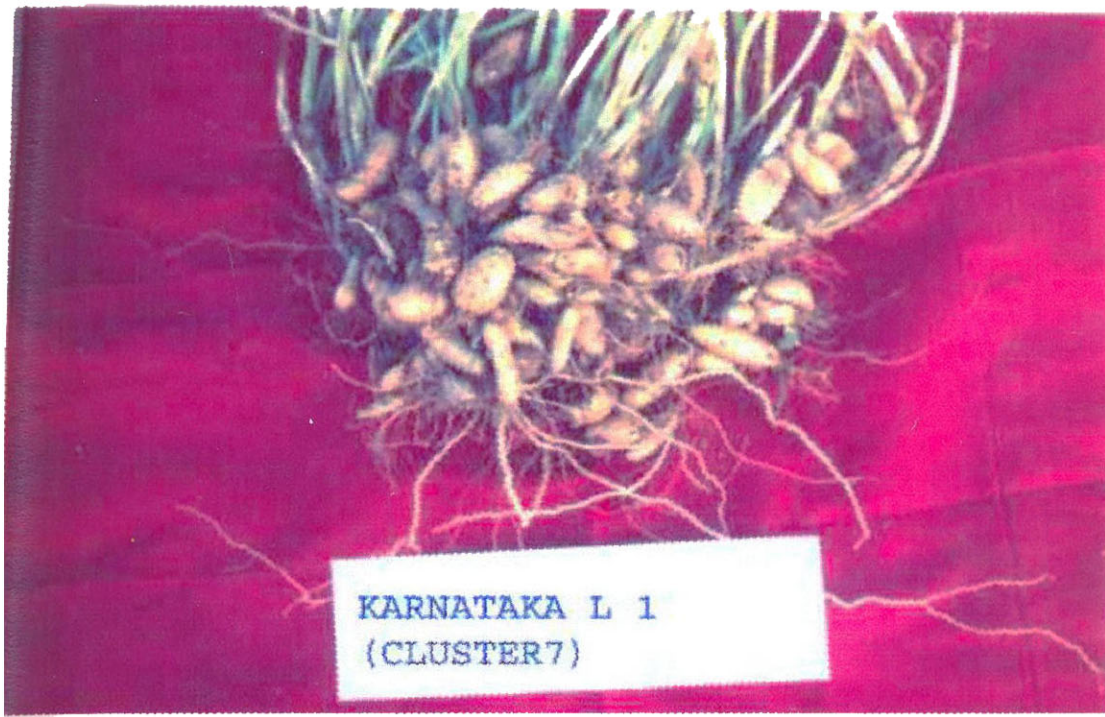


PLATE:7.VARIABILITY IN COLEUS GENOTYPES IN CLUSTER.8



PLATE:8.VARIABILITY IN COLEUS GENOTYPES IN
DIFFERENT CLUSTERS



Angadi (1976) reported that varieties in a cluster with a high order of divergence among themselves would be the best breeding material for achieving maximum genetic advance with regard to yield.

5.2 Experiment II

Mutation breeding is considered to be one of the most promising breeding techniques in the genetic improvement of vegetatively propagated plants. The distinct advantage of breeding through induced mutations is the possibility/probability to bring about a positive change in one or a few characters of an otherwise superior genotype with no detriment to its desirable characters. It is one of the best methods to produce genetic variability in a vegetatively propagated crop like *coleus*. Isolated attempts have been made in this crop to explore the possibilities of inducing mutations with the aid of physical mutagens (Vasudevan and Jos, 1990). However, no success has hitherto been made in the development of a photoinensitive mutant of *coleus*. Gamma rays have been successfully used to develop mutants in ornamentals like chrysanthemum (Bowen *et al.*, 1962), gladioli (Buiatti *et al.* 1965), rose (Chan, 1966; Gupta and Shukla, 1971 and Lata and Gupta, 1971) and in many other vegetatively propagated crops (Broertjes and Vanharten, 1978). Though chemical mutagens are not widely used to induce mutations in vegetatively propagated crops, the results obtained are promising. The present investigation was undertaken upto induce genetic variability using gamma rays and EMS, in *coleus* with the specific objective of attaining higher yield and photo insensitivity.

Coleus mutants exhibited variation in yield attributes and nematode resistance with varying doses of gamma rays as well as different concentrations of EMS. The results obtained in the present study are discussed in the following sections.

5.2.1 Sensitivity to mutagens

Information on the sensitivity of the plant material to the mutagen is essential to arrive at the optimum dose of the mutagen. The unit of the absorbed dose of radiation energy is the Gy (Gray) (equivalent to 1 J kg^{-1} ; equivalent to 100 rad). The routine procedure in assessing the most appropriate dosage is based on radiosensitivity, which is estimated through the physiological response of the irradiated material (Neville *et al.*, 1998). It involves the determination of dose that causes a 50 per cent reduction of vegetative growth of the treated material (LD_{50}) when compared with the control (Gaul, 1977). The choice of the dose to be applied for the highest probability of useful mutant rescue is then left to the breeders experience with the specific plant material, its genetics and its physiology. Several parameters have been used to determine the sensitivity of crop plants to different mutagens. Sambandamurthi (1983) and Jayachandran and Mohanakumaran (1992) considered sprouting of the buds and survival of the plantlets as the parameters for assessing the sensitivity to gamma rays and EMS in vegetatively propagated plants like tuberose and ginger. They established a LD_{50} of 40 Gy for gamma rays. Comparable results have been obtained by Castillo *et al.* (1997) and Love *et al.* (1996) in potato and Jayachandran and Mohanakumaran (1992) in ginger.

Similarly LD₅₀ of EMS was 1 per cent. In the present study to the effect of the mutagen on *coleus* was assessed in terms of survival of the mutant. As established in the studies of earlier workers, increasing doses of mutagen in the range of 1 to 100 Gy of gamma rays and 0.05 to 2.5 per cent of EMS manifested a declining trend in the sprouting of axillary buds of the treated material (Fig. 5 and 6). Based on the trend observed, LD₅₀ of gamma ray mutagen was calculated as 40 Gy and that of EMS mutagen as 1 per cent. Contrary to 50 per cent survival of plants Heinze and Schmidt (1995) advice to operate at a dose giving (LD₅₀=10%) or a dose resulting in 20 per cent survival of the treated material. In due consideration of the above set up information, the effect of the mutagens in the present investigation was evaluated on the basis of the degree of sprouting of single noded cuttings and establishment of the mutant under field conditions.

5.2.2 Field studies

In the present investigation the sprouting percentage at 30 days after planting was found to decrease with increase in the dose of mutagens. The result is in accordance with the findings of Mukherjee and Khoshoo (1971) in *Canna*, Gupta *et al.* (1982) in *costus* and Giridharan (1984) in ginger. Jasina and Kirsanova (1966) and Sharma and Pandey (1996) reported increasing doses of gamma rays stimulated sprouting in potato in a linear fashion. The proportionate increase in the germination rate due to mutagenic treatment might be attributed to the inactivation of auxin levels in the plant with varying levels of exposure as reported by Skoog (1935). According to Sparrow (1961) mutation treatment

Fig. 5. Estimation of LD_{50} for Gamma irradiation

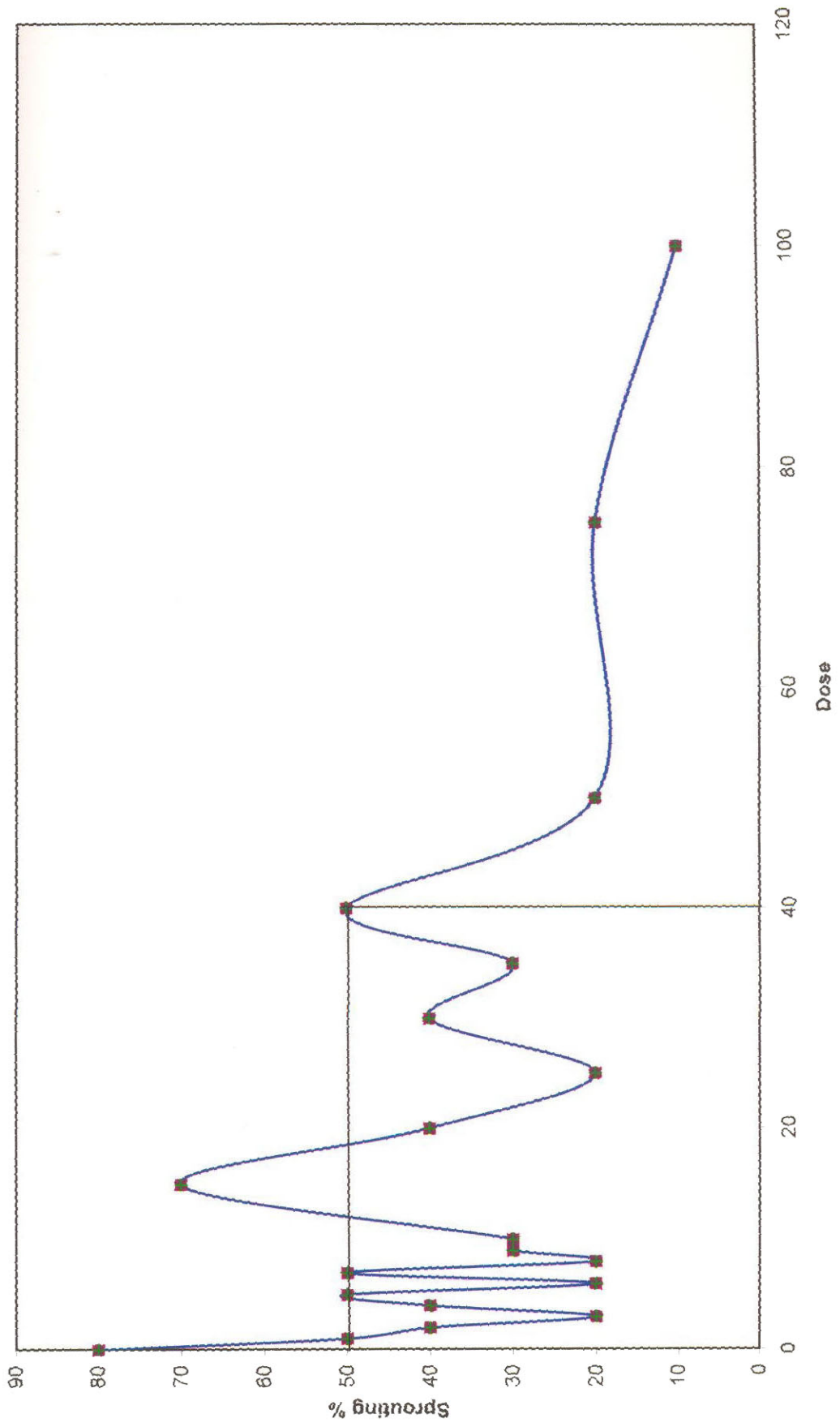


Fig. 6. Estimation of LD₅₀ value for EMS

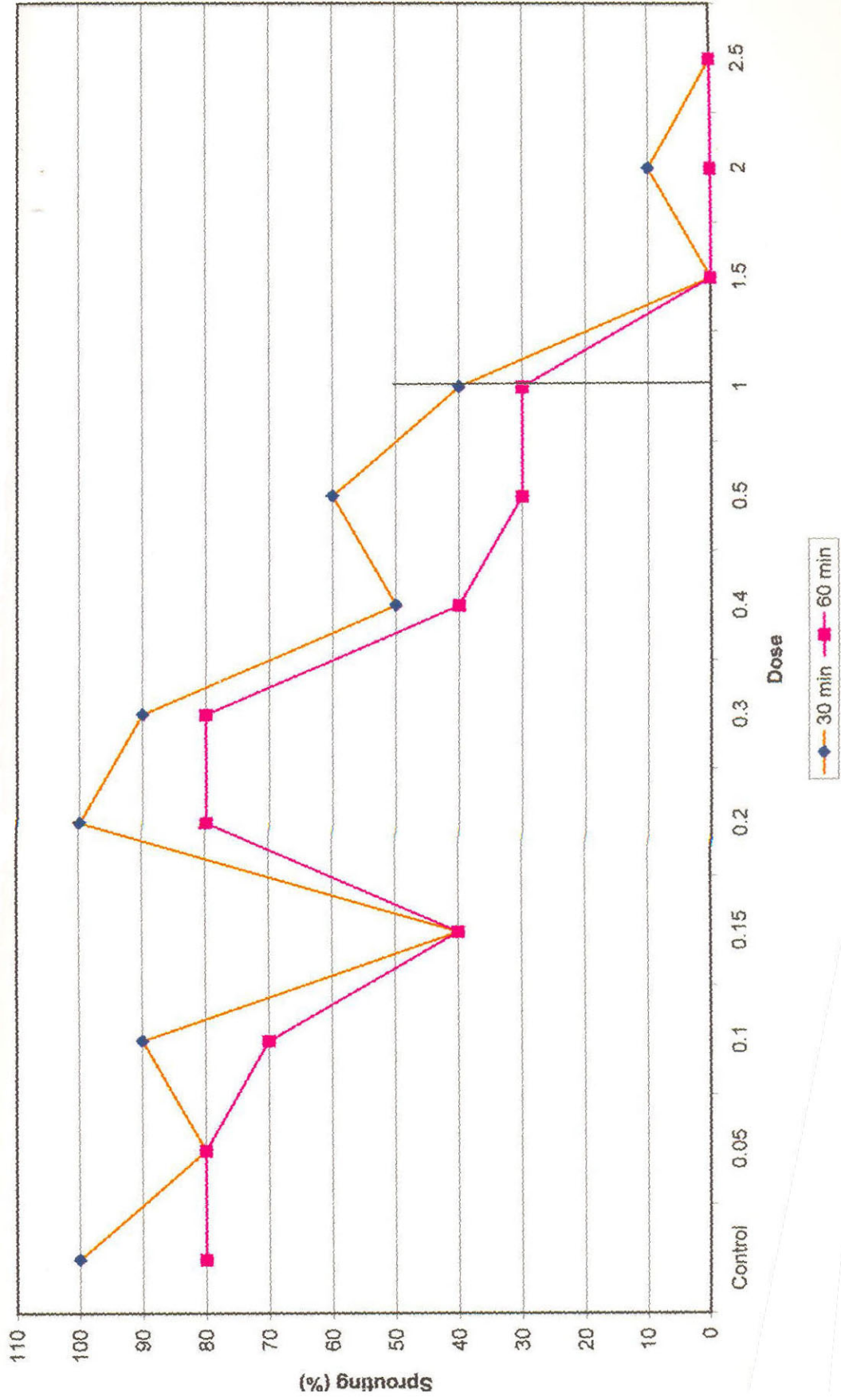


PLATE: 9. ESTIMATION OF L.D. 50 FOR E M S

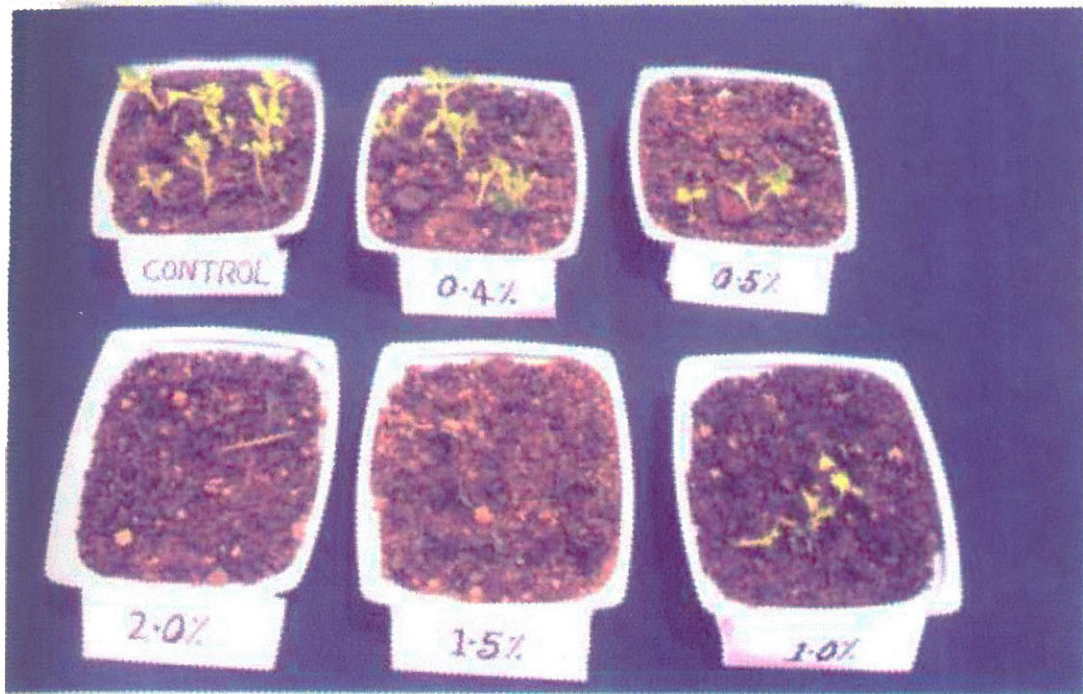
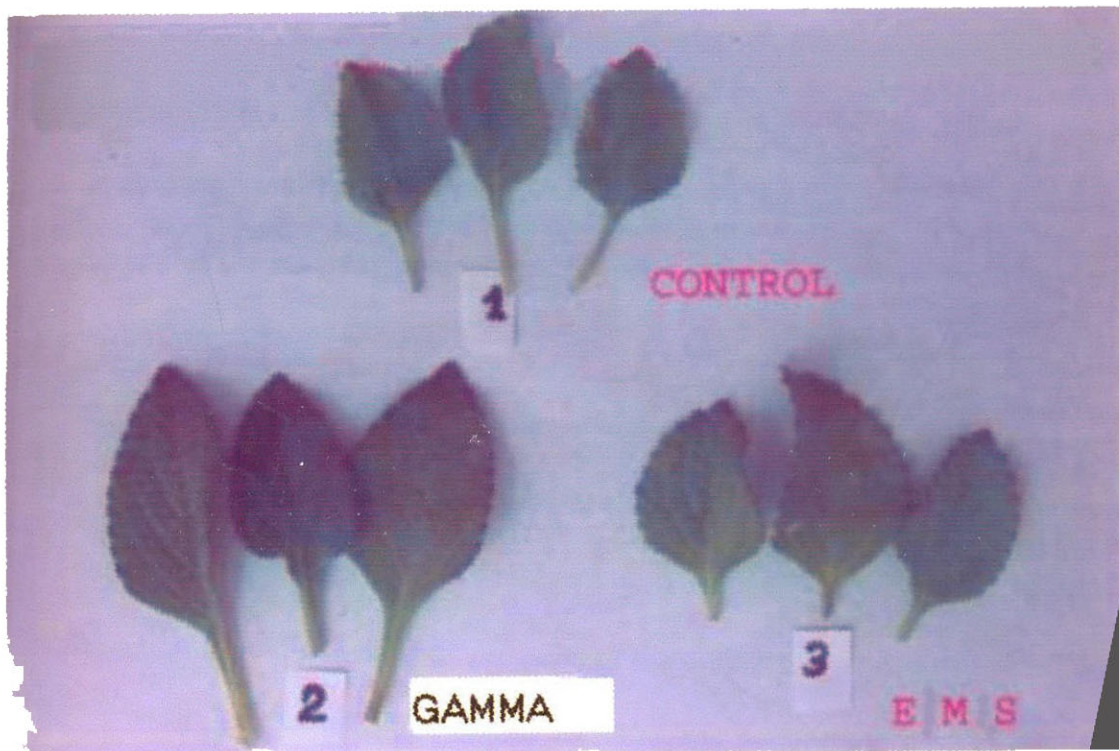


PLATE:10.VARIATION IN LEAF SIZE AND COLOUR AFTER MUTATION



caused chromosomal aberration which adversely affected the cell division. Failure of assimilatory mechanism, production of diffusible growth retarding substances (Mackay, 1951), inhibition of auxin synthesis (Gordon, 1957) and changes in specific activity of the enzymes (Haskins and Chapman, 1956) also could have inhibited the series of biochemical processes behind sprouting. Delay in sprouting is another observed effect, especially in higher doses of mutagenic treatments. The results have been observed by Vasudevan *et al.* (1967) in colocasia and Gupta and Shukla (1971) in rose. The delay in sprouting might be due to the influence of gamma rays and EMS on hormones and plant growth regulators in higher plants. Reduction in survival percentage of *coleus* genotypes was noted after treatment with EMS. The same trend of decreased survival with increase doses of EMS was noticed in barley by Froese Gertzen *et al.* (1963).

Chemical mutagenesis are known for their ability to produce chromosomal aberrations (Swaminathan *et al.*, 1962) and physiological injury through the acidic hydrolytic products of the mutagen (Konzak *et al.*, 1965).

The response of *coleus* to mutagens varied with dose as well as with genotypes. The dose dependence of mutation effects can be explained on the basis of the mode of action of mutagens. These chemicals cause chromosomal aberrations and physiological injury. It is obvious that the quantum of effects due to a mutagen increased with an increase in its dosage as the quantum of physico-chemical changes responsible for mutagenic effects increases with the dosage of the mutagen. Further the genotypes responded differentially to the same dose of

mutagen. This may be due to the difference in the genetic architecture of the plant which responded differentially to the mutagen. Similar dose effect and genotype effect relationships have been reported by Kanakamony (1997) in Kacholam.

5.2.3 Tuber yield

There was no significant increase in tuber yield in the case of gamma rays except for the accession Parlikad local (Fig. 7). This is conformity with the findings of Kukimura and Kouyama (1982) in sweet potato. Contrary to this result Nayar and Rajendran (1987) reported 20-25 per cent yield increase in tapioca as a result of gamma irradiation. But the effect of EMS on tuber yield of *coleus* was highly significant (Fig. 8). As evidenced in the experiment, EMS at the prescribed dose of 1 per cent is most effective in changing the economic characters of *coleus* genetically. This is in conformity with the findings of Sharma and Pandey (1996) in potato, and Singh (1995) in tomato.

5.2.4 Tuber girth

Mutation affected the tuber girth differentially for varying doses and concentration of physical/chemical mutagens (Fig. 9 and 10). It can be concluded that mutation has changed the size of tubers positively and negatively in *coleus* genotypes.

5.2.5 Number of tubers per plant

Both physical mutagen (gamma rays) and chemical mutagen (EMS) induced variation, in the number of tubers plant⁻¹ positively, irrespective of the genotypes indicating that a dose of 10 Gy in the case of gamma rays (Fig. 11) and

Fig. 7. Effect of Gamma irradiation on Tuber Yield

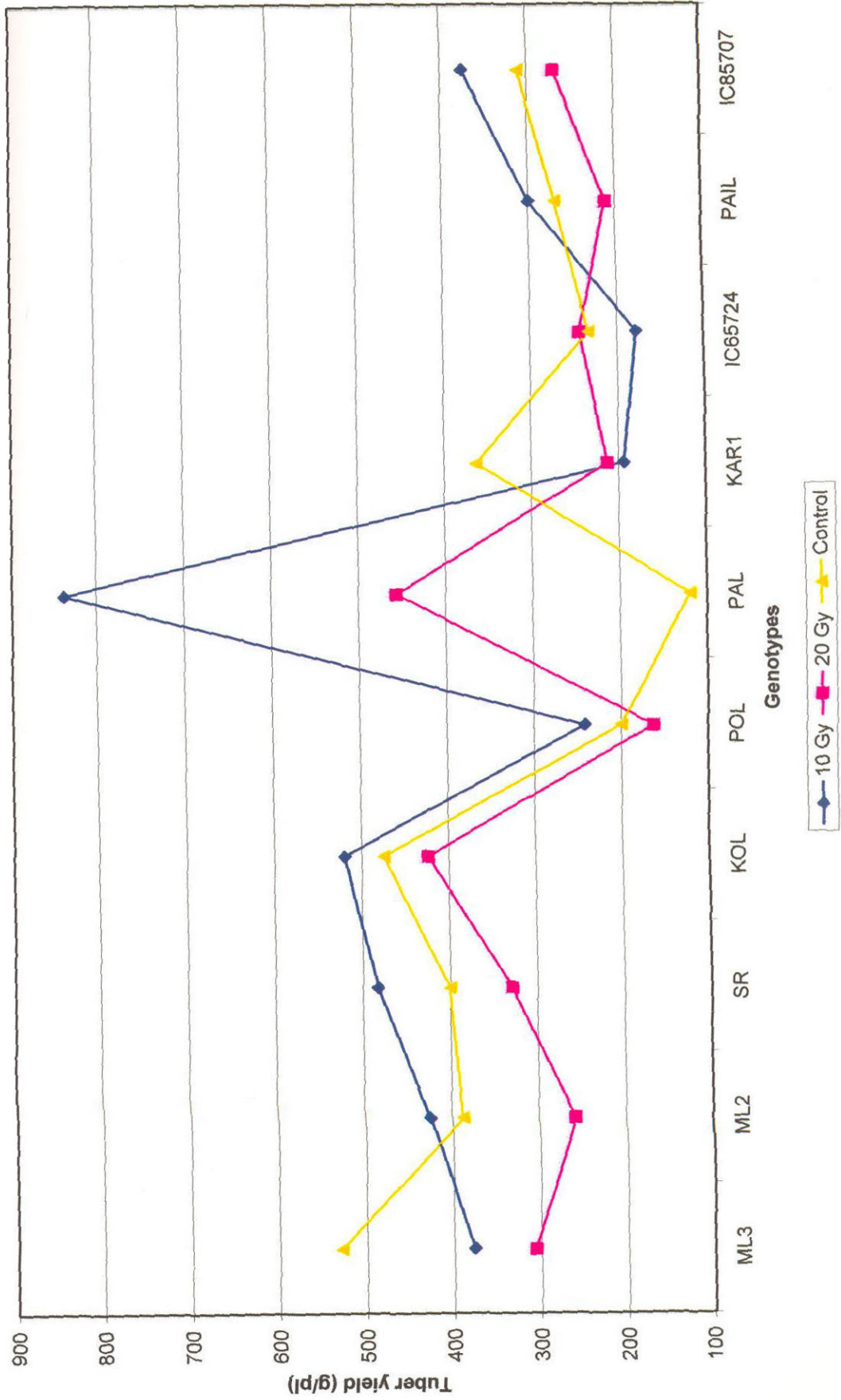


Fig. 8. Effect of EMS on Tuber Yield

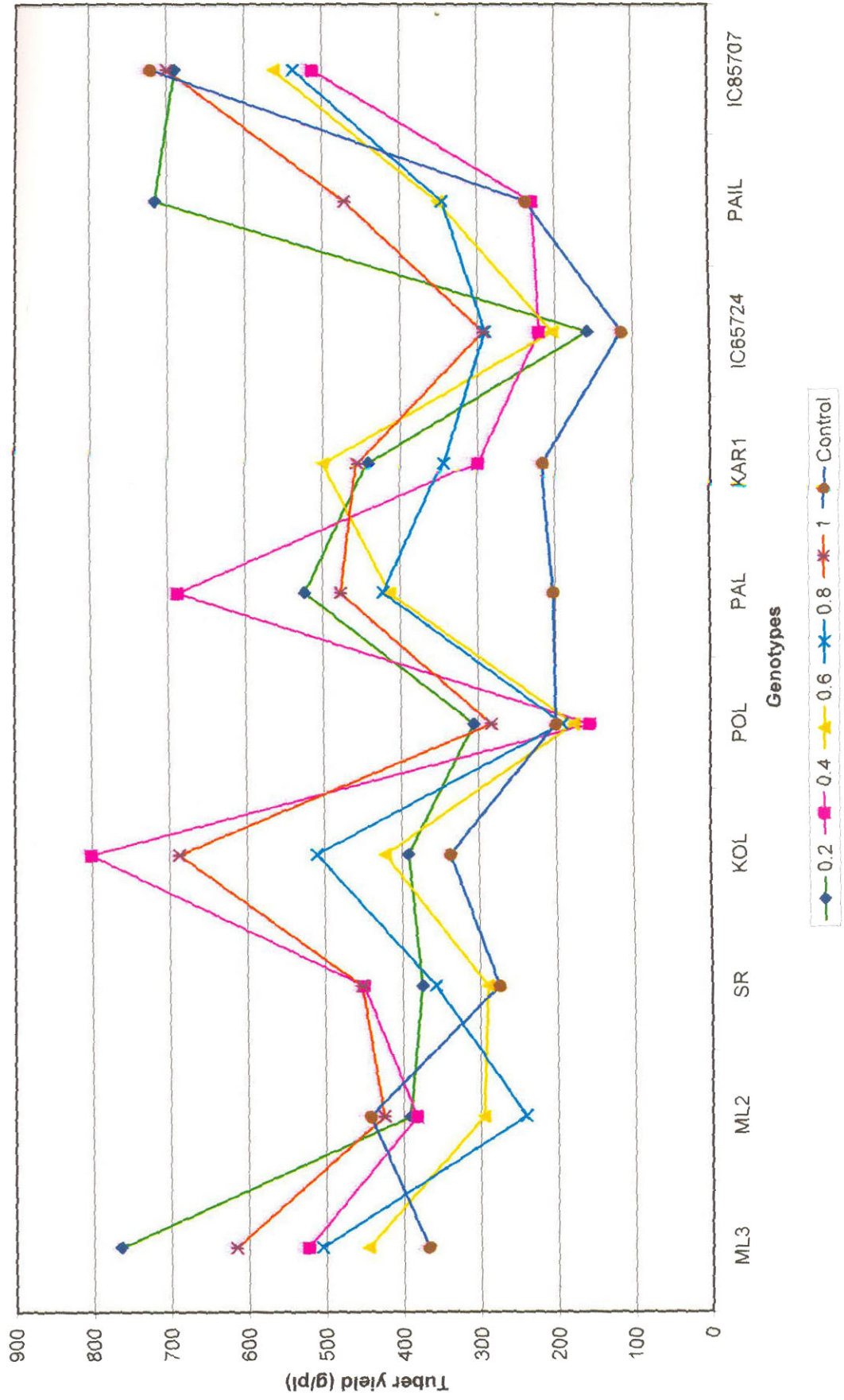


Fig. 9. Effect of Gamma irradiation on Tuber Girth

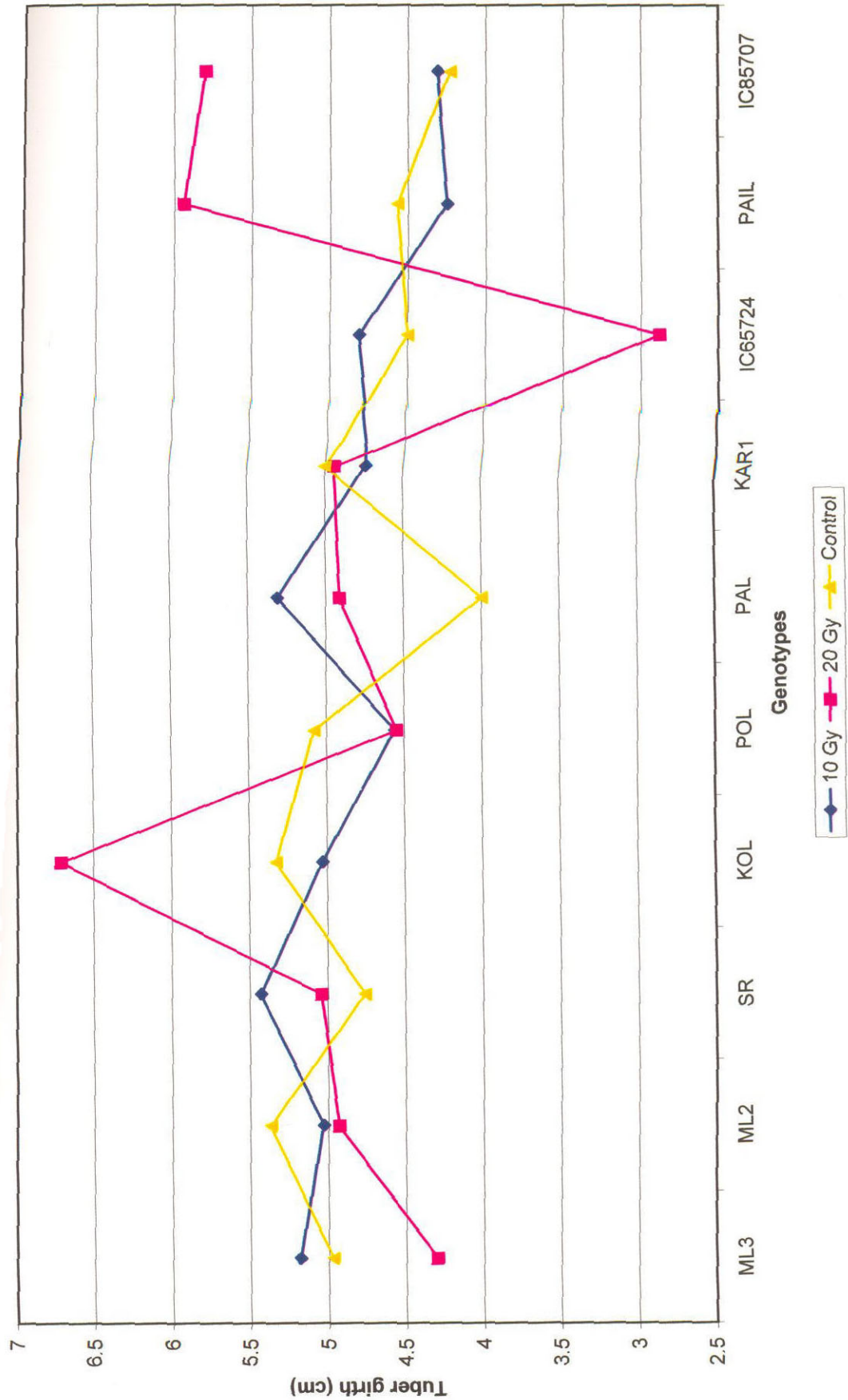


Fig. 10. Effect of EMS on Tuber Girth

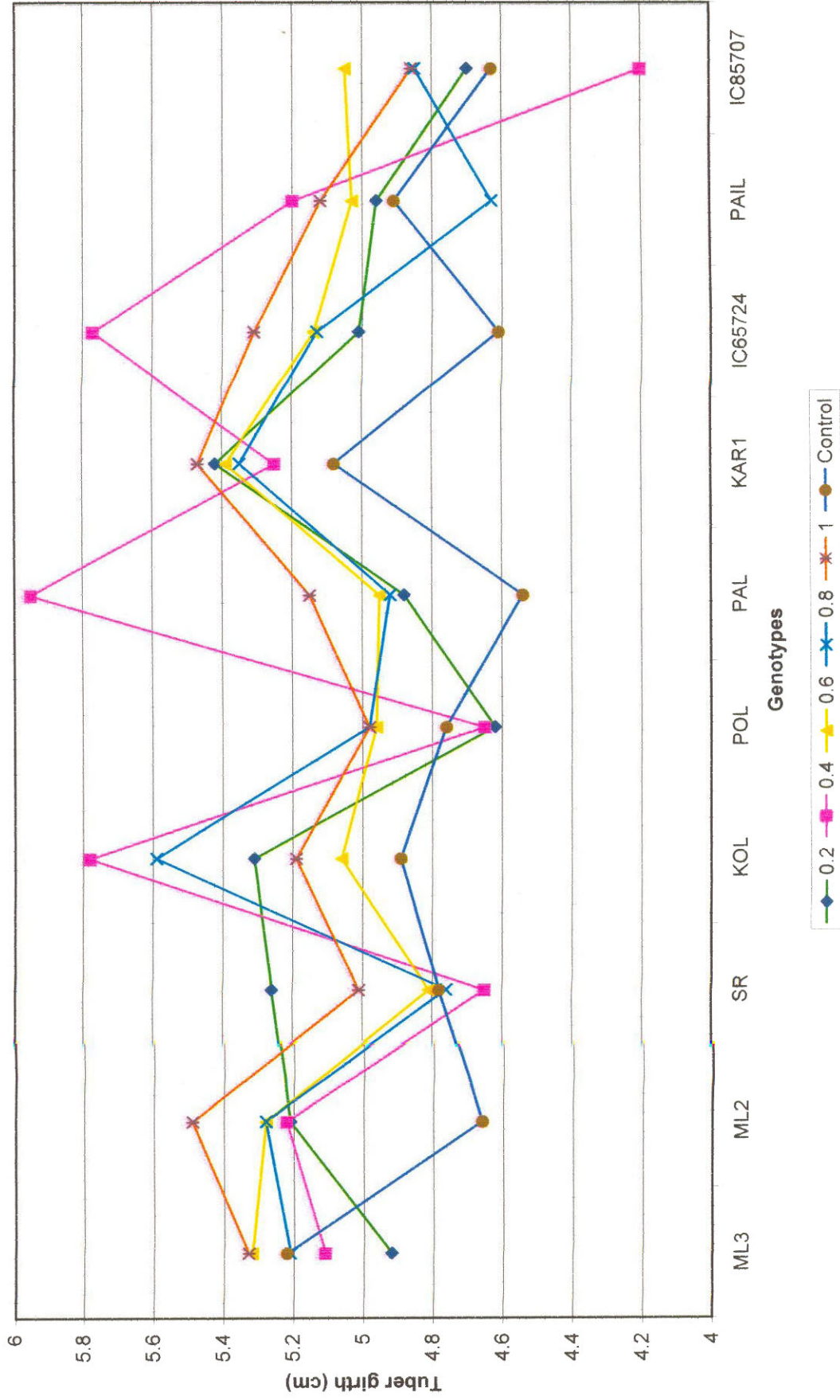
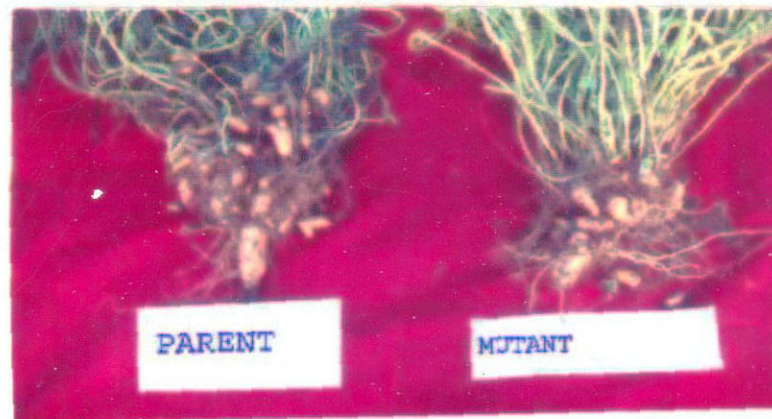
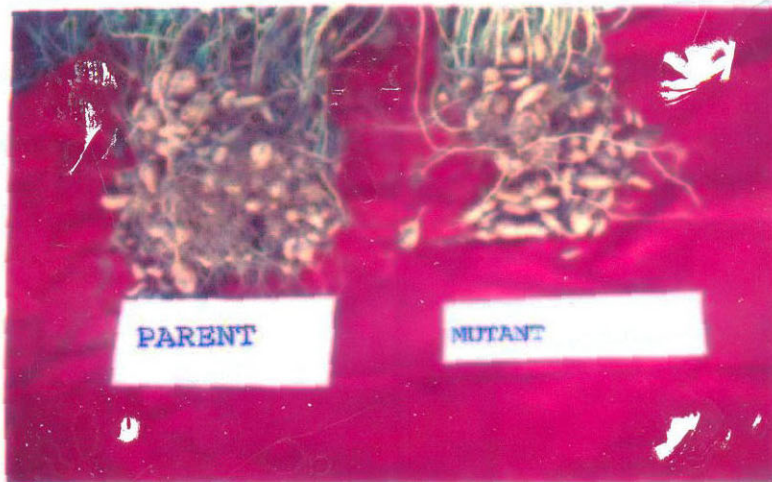


PLATE.11.VARIATION IN TUBER GIRTH AFTER CHEMICAL MUTATION



PLATE:12. COMPARISON OF PARENTS WITH MUTANTS



a concentration of 0.2 per cent in the EMS (Fig. 12) can be employed for increasing the tuber number in *coleus*. Abraham (1970) and Nayar (1975) obtained mutants for high number of tubers in cassava and Kukimura and Kouyama (1982) in sweet potato through gamma irradiation which support the present findings. Similar increase in the number of tubers was reported by Natarajan (1975) in turmeric who obtained high yielding mutants using gamma rays. The positive shifts in different yield attributing characters at lower doses of gamma rays as well as at lower concentration of EMS were responsible for increase in number of tubers. This is in conformity with the studies of Kukimura (1981) in sweet potato and Maishuk (1977) and Sharma and Pandey (1996) in potato. Contradictory to this result reduction in number of tubers using lower doses of gamma rays was reported by Gupta *et al.* (1982) in *Costus speciosus*.

5.2.6 Harvest index

Harvest index of *coleus* genotypes was not influenced substantially by gamma ray radiation or EMS treatment but the harvest index could be increased with 20 Gy in IC 85707 (Fig. 13) and 0.2 per cent in Mullurkara L3 (Fig. 14). It is inferred that the effect of gamma rays and EMS is preferential to genotypes in changing the plant type. It can be attributed to the interaction of inherent genetic characters of the genotypes to specific doses.

5.2.7 Plant height

Irrespective of genotype and dose the morphological appearance of the plant can be changed by gamma irradiation, but not with EMS (Fig. 15). This is evident for the variation that has occurred in plant length of all the mutants

Fig. 11. Effect of Gamma irradiation on Tuber Number

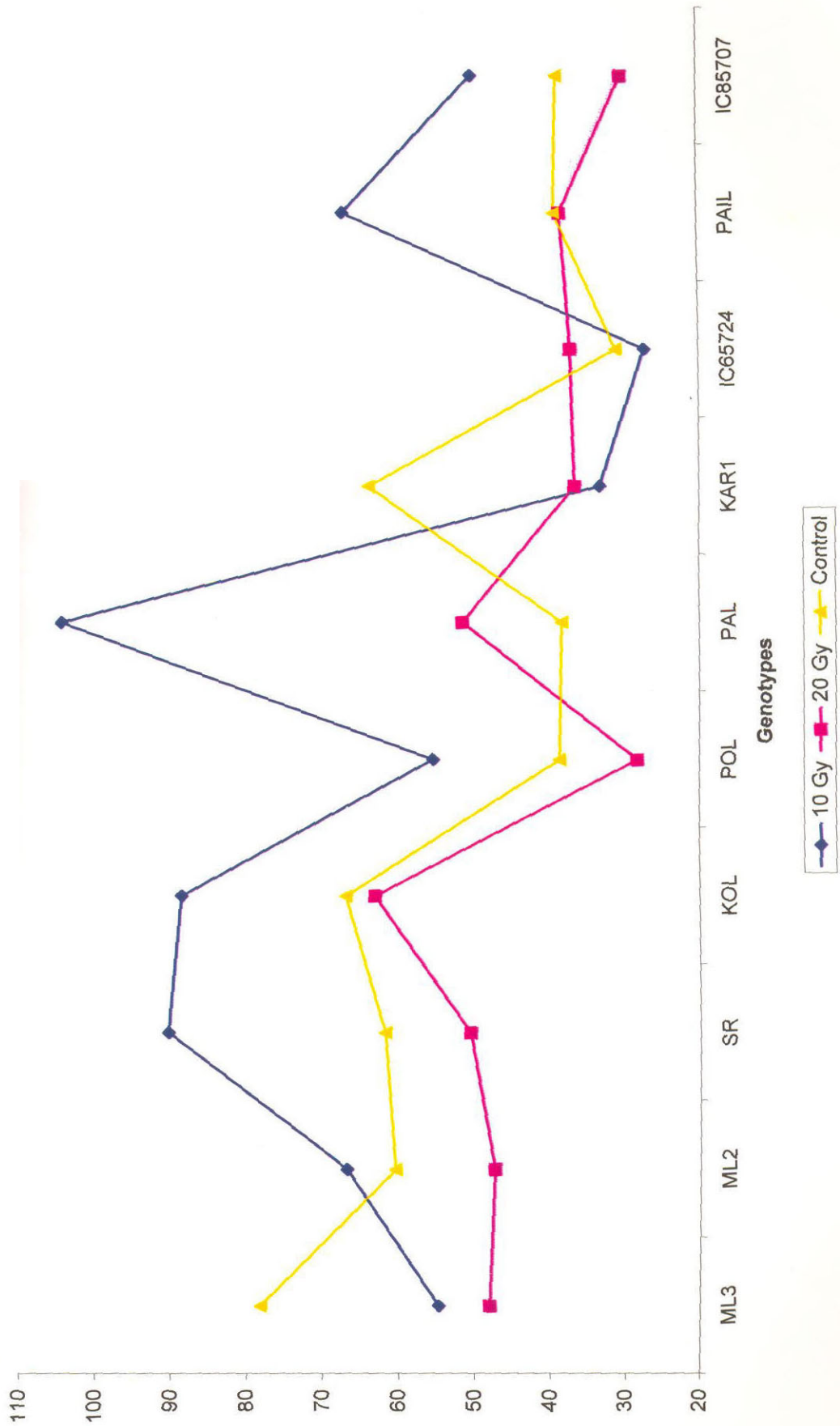


Fig. 12. Effect of EMS on Tuber Number

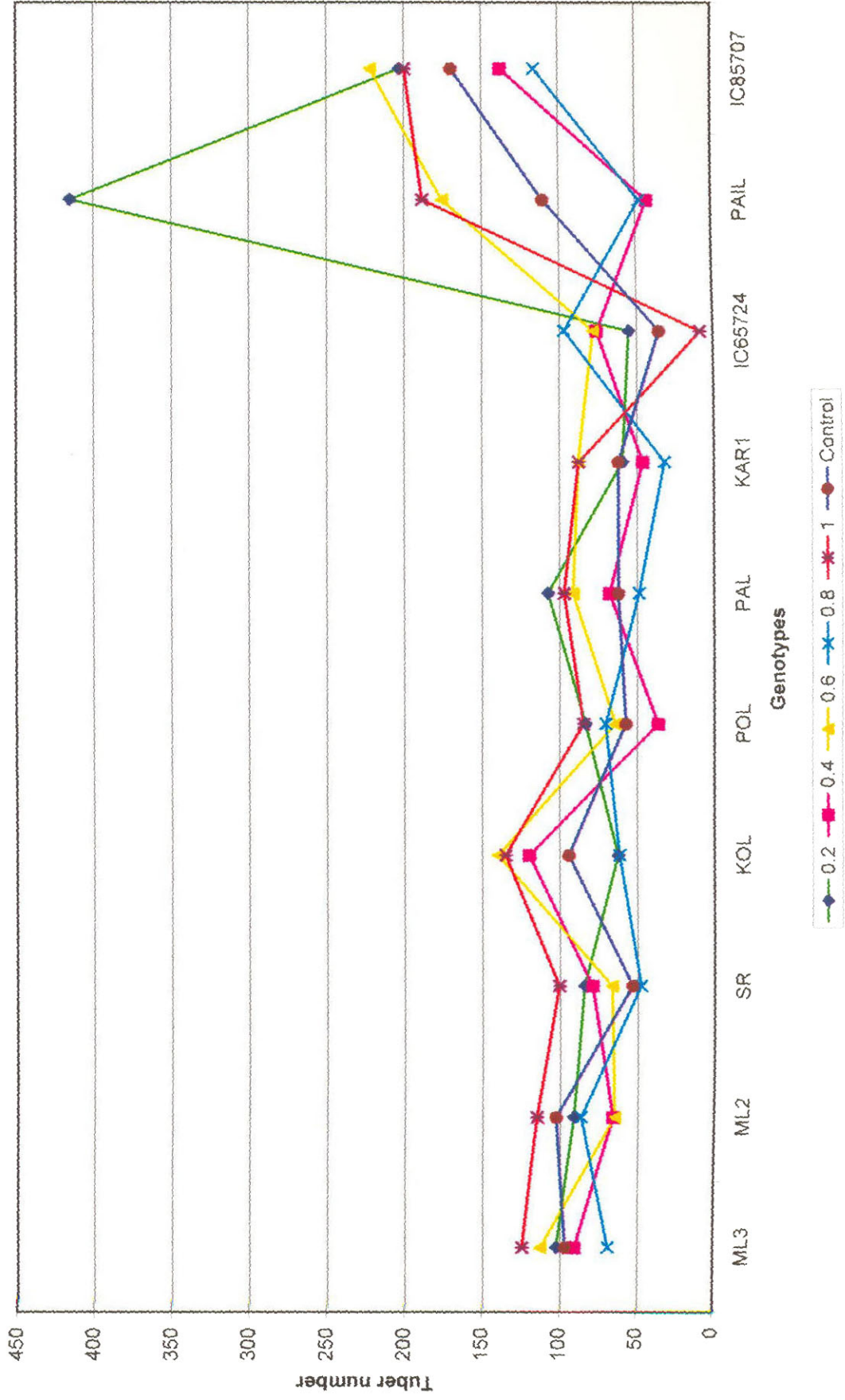


Fig. 13. Effect of Gamma irradiation on Harvest Index

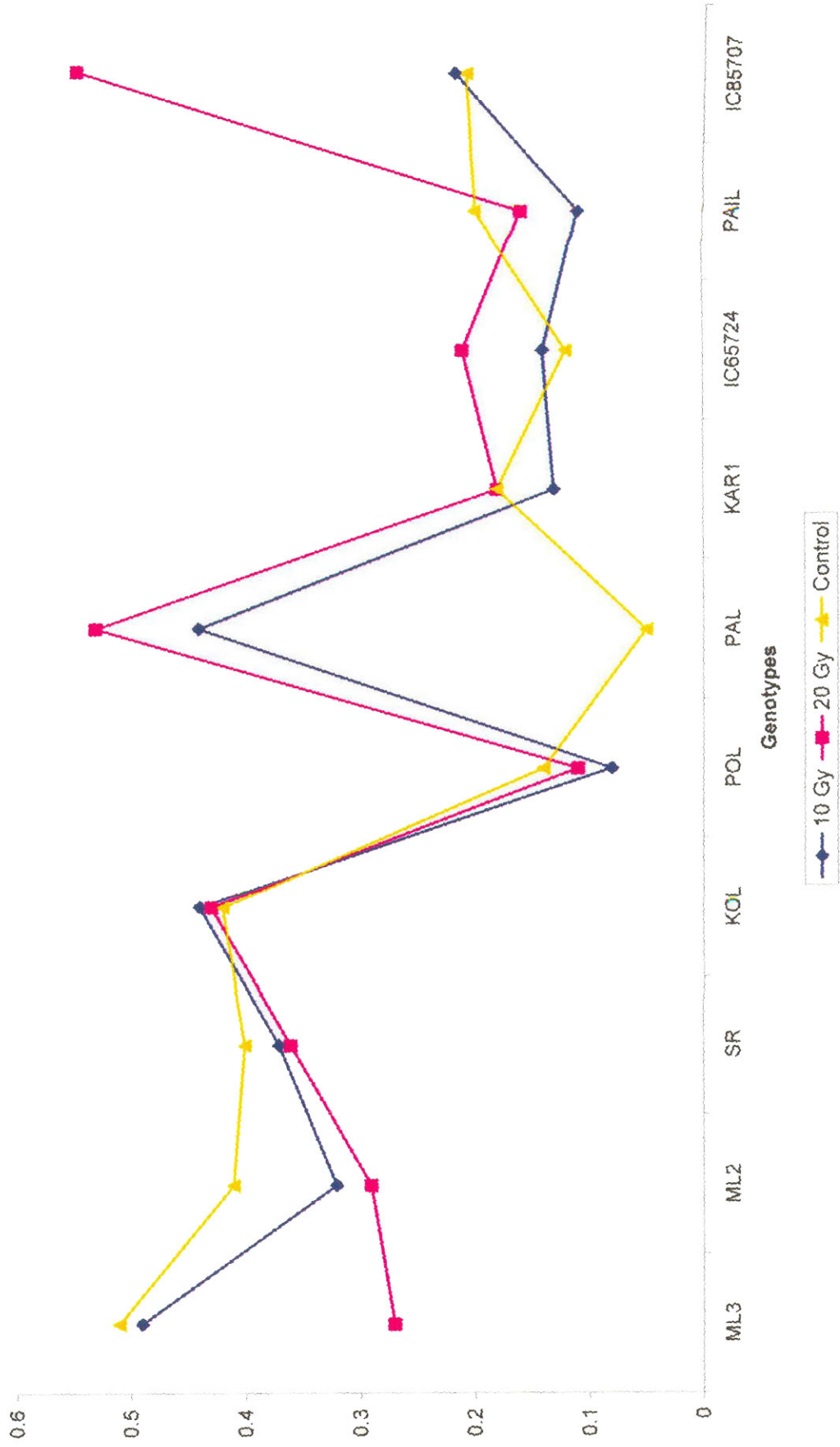


Fig. 14. Effect of EMS on Harvest Index

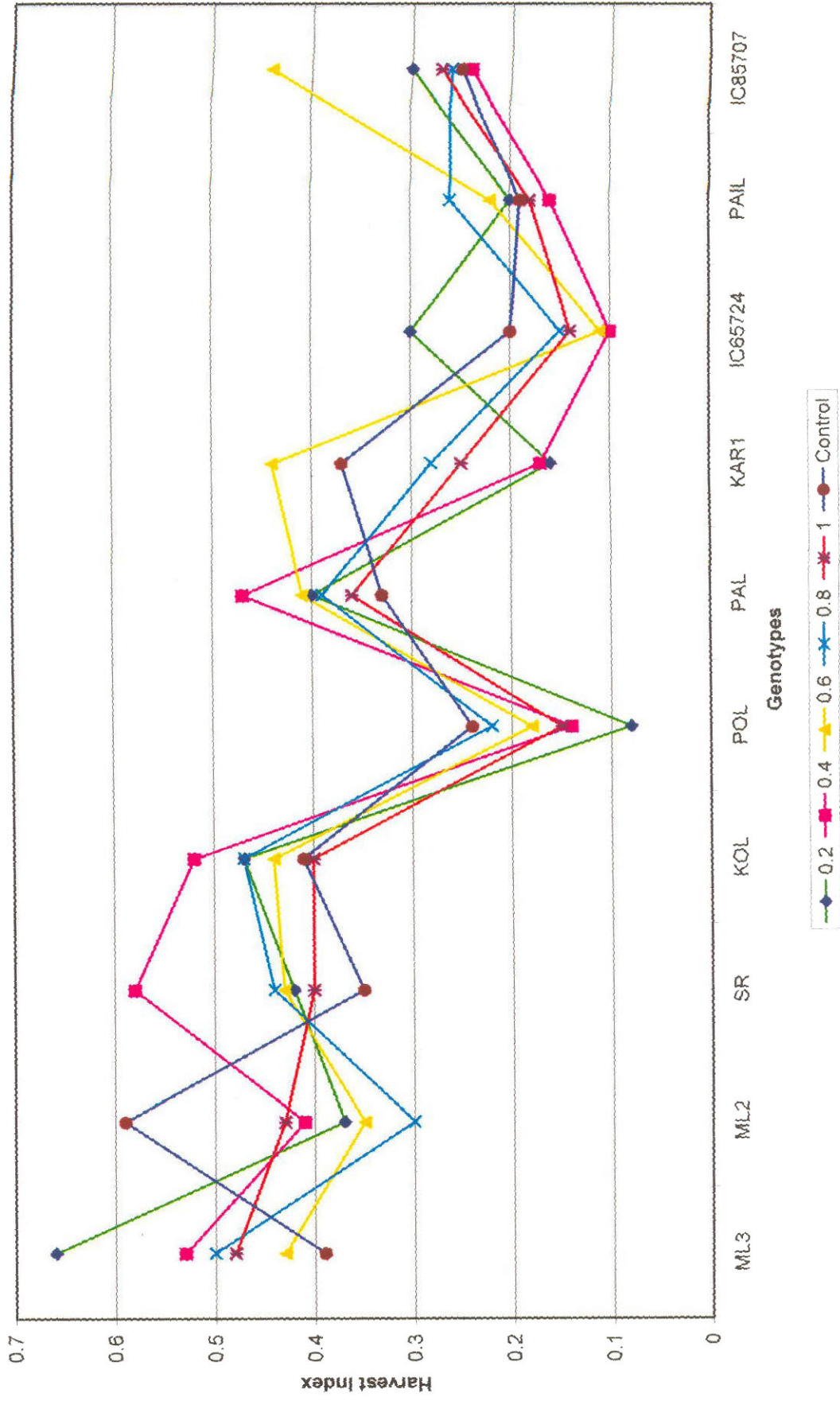
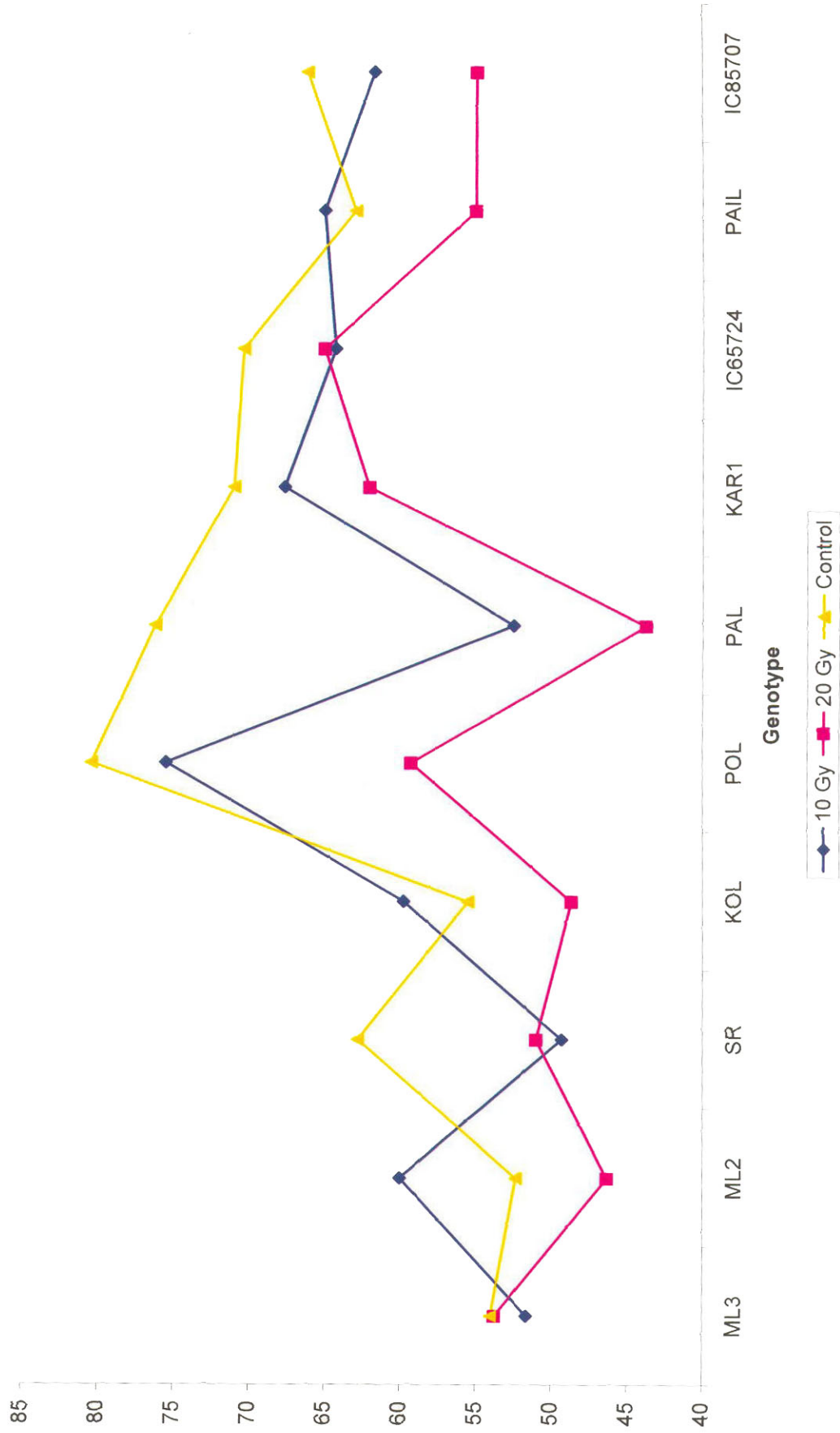


Fig. 15. Effect of Gamma irradiation on Plant Height



obtained from physical mutagen. This is in conformity with the findings of Venkateswarlu *et al.* (1978). Variability for plant height was not noticed in the genotypes when treated with EMS. This may probably be due to the failure at the level of recovery than at the level of induction of micro mutations. The variation might have been eliminated by selection sieves operating.

5.2.8 Weight and volume of tuber

These characters were not much influenced by gamma irradiation. It is interesting to note that EMS exerted significant influence in altering the density of tubers of the various genotypes (Fig. 16). This effect is independent of genotypes.

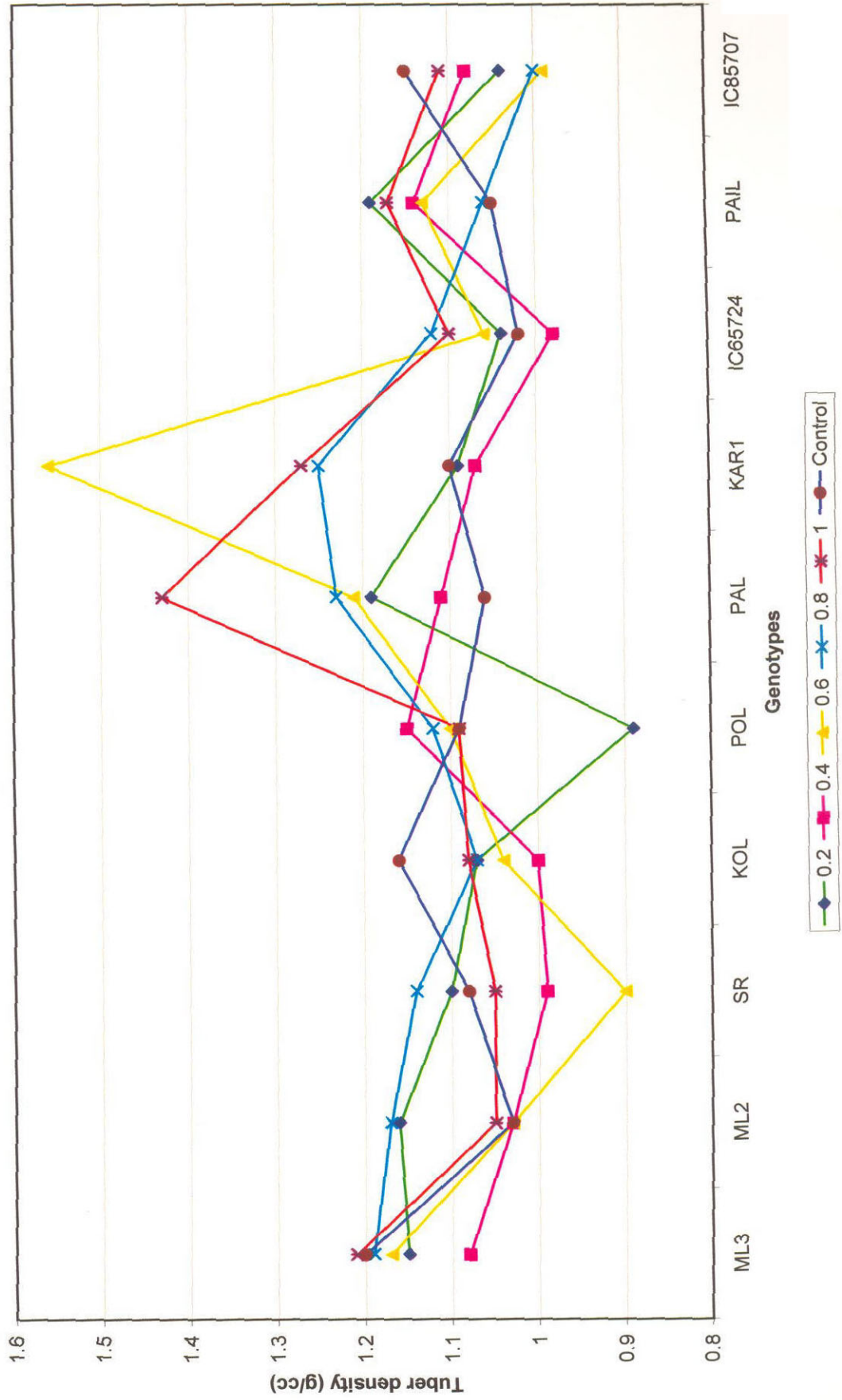
5.3 Experiment III

Experiment II witnessed significant positive changes had occurred in *coleus* mutants with regard to many of the quantitative traits. Photo sensitivity of *coleus* is the main limiting factor in cultivating this crop during the off seasons. Isolated attempts only have been made by early workers to change the qualitative traits like photo sensitivity and thermo sensitivity in commercial crops. In rice, the heading time and vegetative growth period influenced by photo period and temperature have been altered by mutations (Kudo, 1966, 1967). Devi *et al.* (1963) generated evidence of the indicated mutations that dispensed the light requirement for germination. Swaminathan *et al.* (1966) evolved a photo-insensitive cotton mutant H-322 from MCU-5 using gamma irradiation. Vasudevan and Jos (1989) produced photo insensitive mutants in *coleus* by gamma irradiation. The flesh colour of the tubers of sweet potato could be changed by gamma irradiation (Vasudevan and Jos, 1996).

PLATE:13.FIELD VIEW OF PHOTOINSENSITIVE MUTANTS



Fig. 16. Effect of EMS on Tuber Density



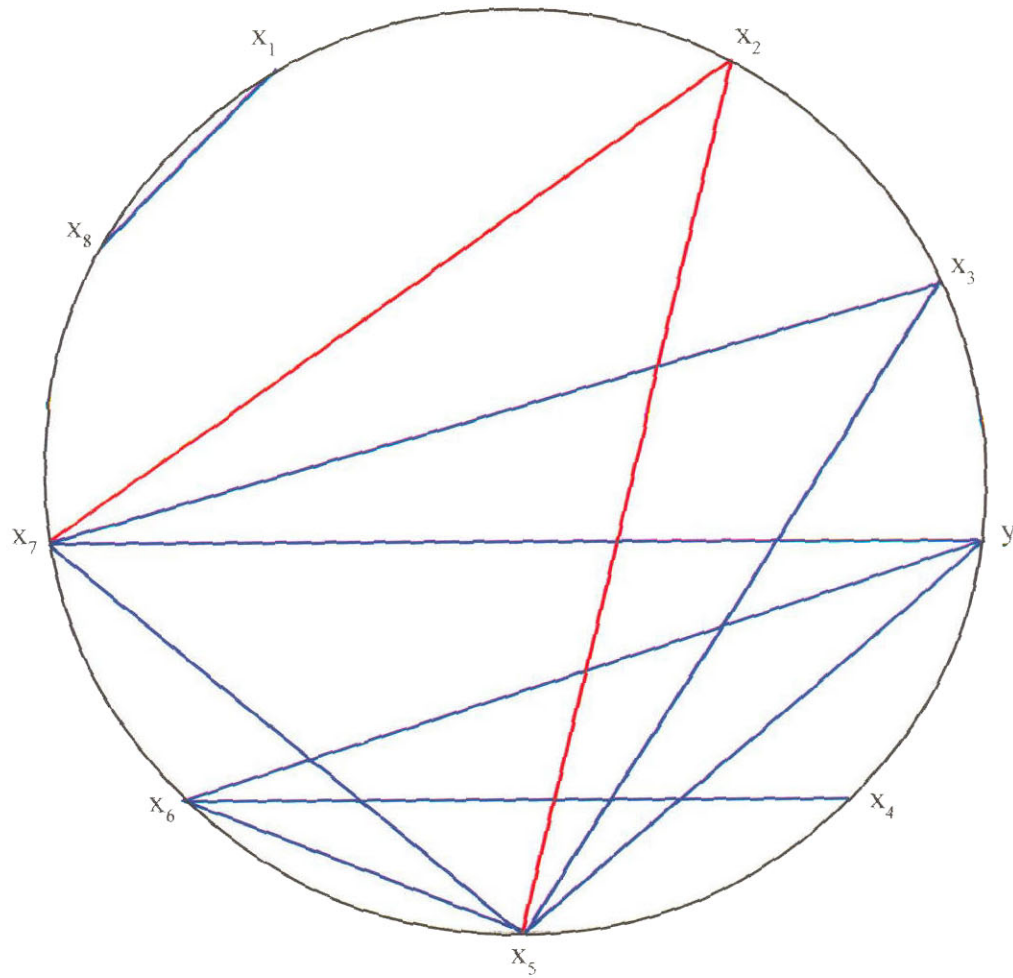
All these indicate that there are possibilities for breaking the qualitative traits like photo and thermal sensitivity which act as barriers to wide adaptations. Fourteen mutants were selected from 140 mutants (100 plants treated with EMS and 40 plants exposed to gamma rays) based on the mean performance of mutant plants in comparison with the parent population. These 14 mutants along with their respective parents were planted in the off-season for *coleus* cultivation in Kerala. Eight mutants out of 19 treatments (14 mutants and 5 parental accessions) produced tubers. There was not much significant difference in the correlation coefficients of different traits towards yield between mutants (Experiment III) and accessions (Experiment I) except tuber girth and height of plants. There was positive genotypic correlation between yield and tuber girth, whereas in mutants tuber girth was not contributing towards tuber yield (Fig. 17). This is in conformity with the findings of Nayar (1975) in cassava. Contrary to this Nair and Abraham (1992) and Vasudevan and Jos (1992) reported that higher variation was noticed in the treated population of yam bean and *coleus* respectively, for most of the quantitatively inherited characters. With respect to the height per plant the trend of significant negative correlation towards yield in parental population changed reversely to positive correlation in mutants (Fig. 18).

Another important positive feature of the selected mutants is the indication of photo-insensitivity for tuberization, quantum alteration in the genetic make up of that plant. In the mutant population, tuber yield followed by tuber number showed high genetic gain and heritability which indicates slight deviation from that of the base population where tuber number had low heritability and genetic gain.

PLATE: 14. PHOTOINSENSITIVE MUTANTS



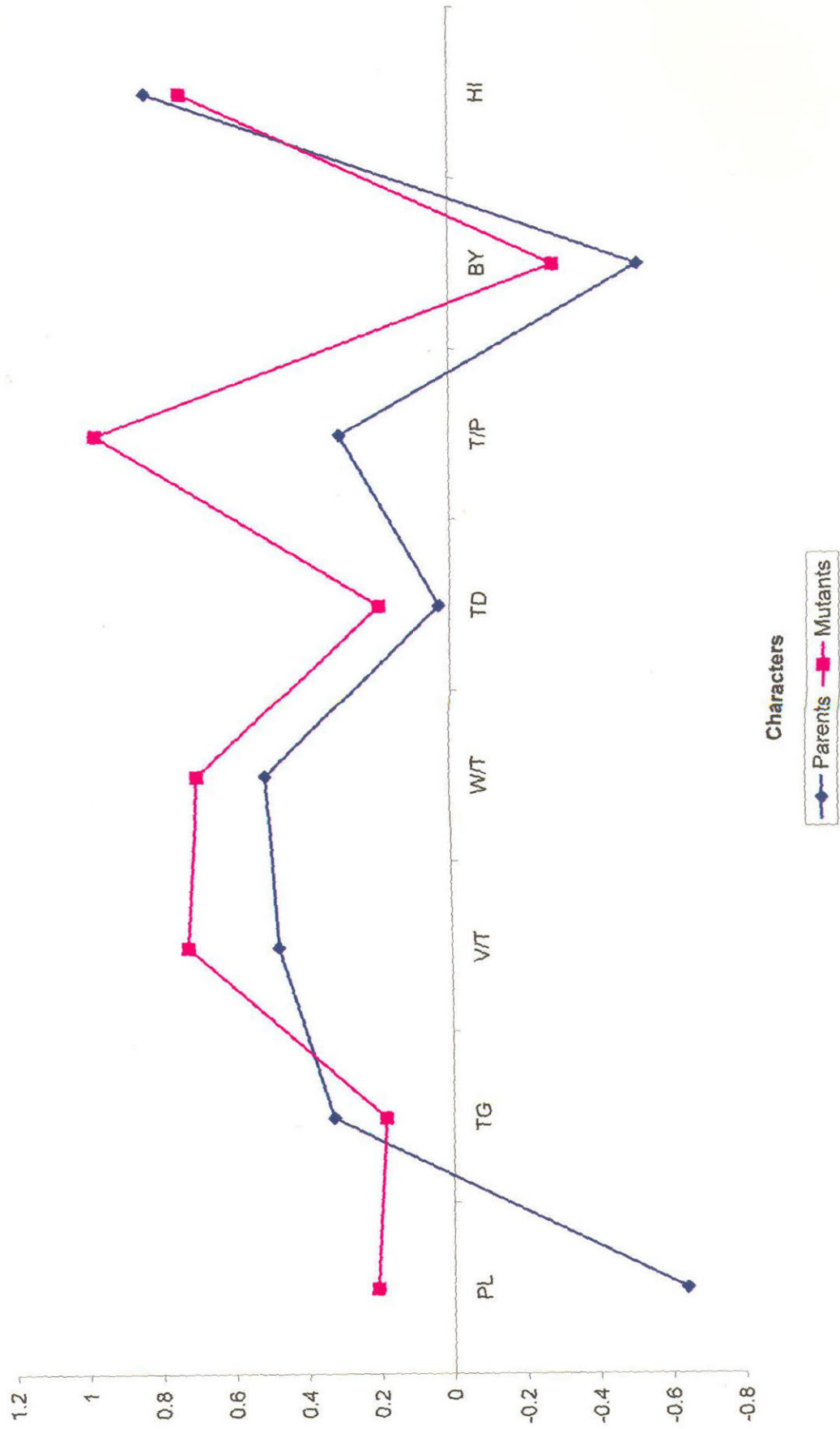
Fig. 17. Genotypic correlation diagram of nine characters of eight mutants



X1 - Plant height	X5 - Volume per tuber
X2 - Biological yield	X6 - Weight per tuber
X3 - Tuber number	X7 - Harvest index
X4 - Tuber girth	X8 - Tuber density
	Y - Tuber yield

— Positive correlation — Negative correlation

Fig. 18. Correlation Coefficients of Parents and Mutants



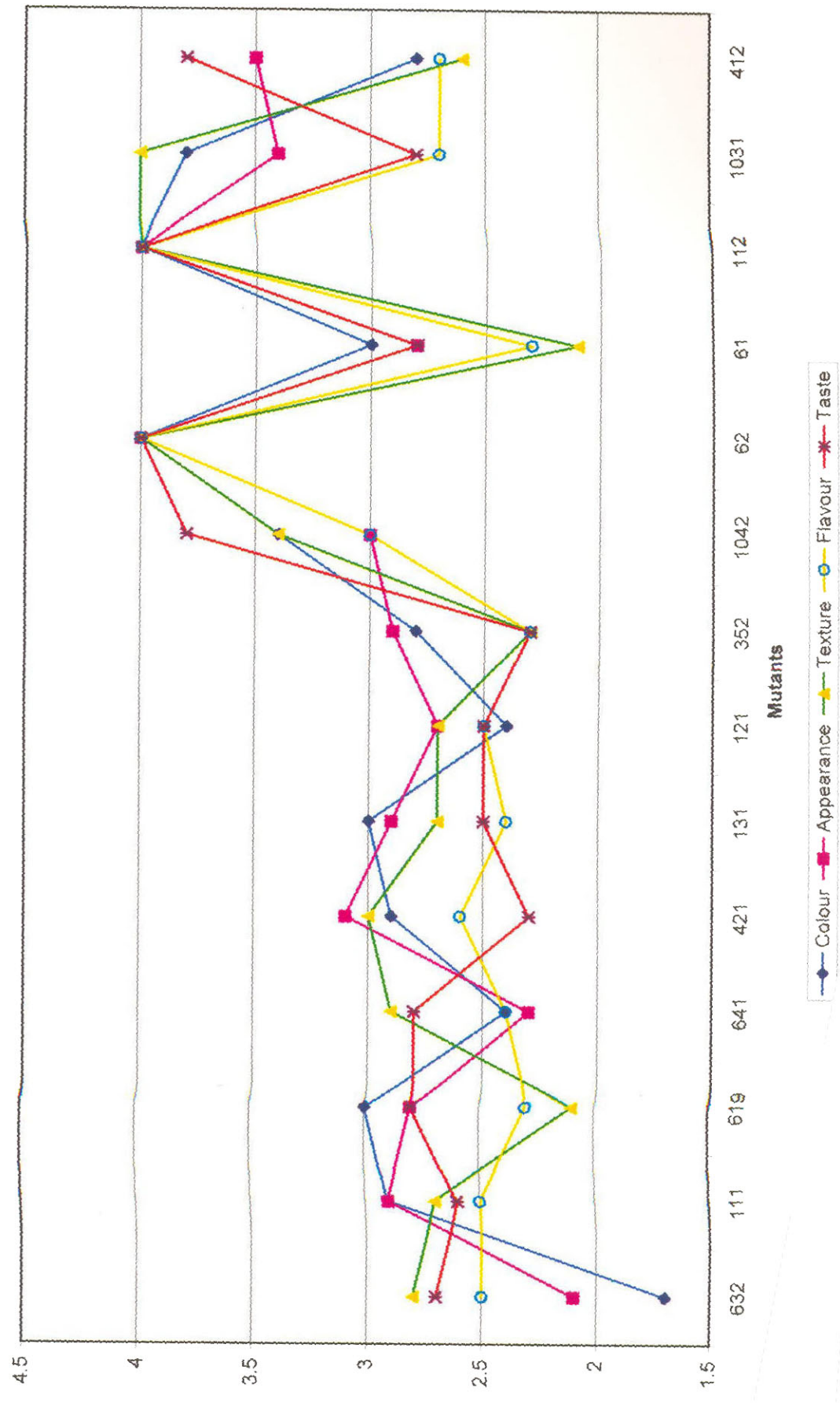
The field performance of the eight selected mutants were also studied over three seasons - (1) off-season (December to April), (2) semi off-season (February to July) and (3) normal (April to October). Except in case of harvest index, the mutants interacted different for various characters in the three seasons..

Mutant 131 performed comparatively well in case of tuber yield in all the three seasons. In the off season, mutant 61 gave the maximum tuber yield indicating that mutant 61 can be recommended for the off season cultivation of *coleus* crop. When we consider the organoleptic parameters of eight mutants, these mutants come in the range of moderate consumer acceptability (Fig. 19). Further they have an acceptable level of starch in the tuber.

5.3.1 *In vitro* mutagenesis

Mutations are defined as heritable changes in the DNA sequence that are not decreased from genetic segregation or recombination (Vanharten, 1998). Genetic variation can be induced either by specific treatments with physical and chemical mutagens or by tissue culture. The cells of plants regenerated from cell cultures show heritable variation in both qualitative and quantitative traits. These variations are collectively termed as somaclonal variations. Somaclonal variations may be the preferable source when dependable early selection methods for the trait of interest are available (Predieri, 2001). The combination of somaclonal variation and selection in culture offer immense scope for improvement since a large number of possibly variant cells can be screened in a short time with limited resources. *In vitro* techniques are becoming increasingly important in mutation

Fig. 19. Estimation of Organoleptic parameters in Selected Mutants



breeding as it helps to develop desirable mutants, restricting the chance of chimera formation (Broertjes, 1976, Roest *et al.*, 1980; Ahloowalia, 1998). These variations can be amplified using mutagens. Successful somaclonal variants were obtained in crops like sugarcane, potato and *Musa*. Das *et al.* (2000) could produce heat tolerant mutants in potato. Sunnino *et al.* (1984, 1986) proposed a procedure for *in vitro* mutation breeding of potato. Aparna *et al.* (1999) produced late blight resistant mutants in potato using gamma rays. Chemical and irradiation induced mutation in *in vitro* culture has been successfully used to improve Banana (*Musa sapientum*) and plantain (*Musa paradisiaca*). This has been reported by Novak *et al.* (1986) and Novak and Micke (1988). In this study an attempt was made to amplify somaclonal variations for breaking photo sensitivity in *coleus*.

The standard media for producing tissue culture plantlets supplemented with IBA 1 mg l⁻¹ was used for producing rooted plantlets of *coleus*. The mutagen dose for inducing successful *in vitro* mutations in callus culture was also standardized. Callus culture is being used for irradiation. Compared to a cell in a well organized apex, a mutated callus is a mass of cells which has more chance of survival and regeneration. The majority of mutants produced by this method will be solid, especially if the shoots are regenerated from repeated subculture of irradiated callus, since the number of cells from which adventitious shoots originate *in vitro* seems to be restricted. *In vitro* propagation by callus culture was reported in many ornamentals like *Chrysanthemum morifolium* (Ben-Jaa-Cov and Langhans, 1968), *Gladiolus* sp. (Simonsen and Hilderbrandt, 1971) and *Petunia* sp.

PLATE: 15. DIFFERENT STAGES OF TISSUE CULTURE PLANTLETS

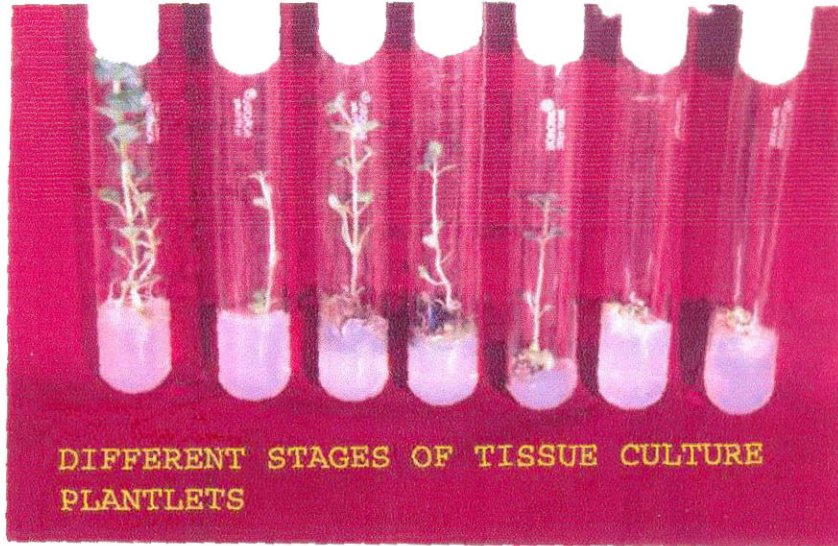


PLATE:16.TISSUE CULTURE PLANTS- HARDENING STAGE

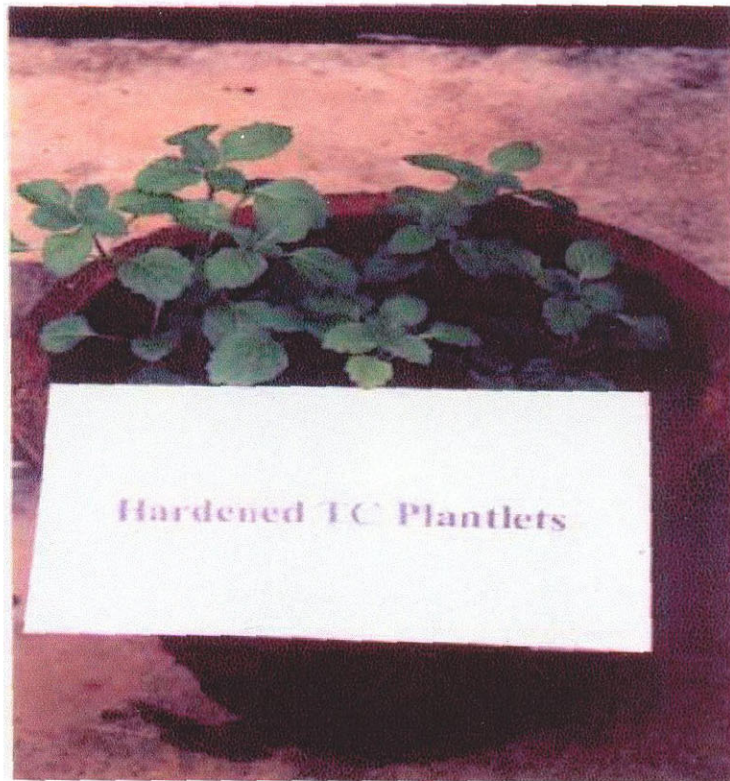
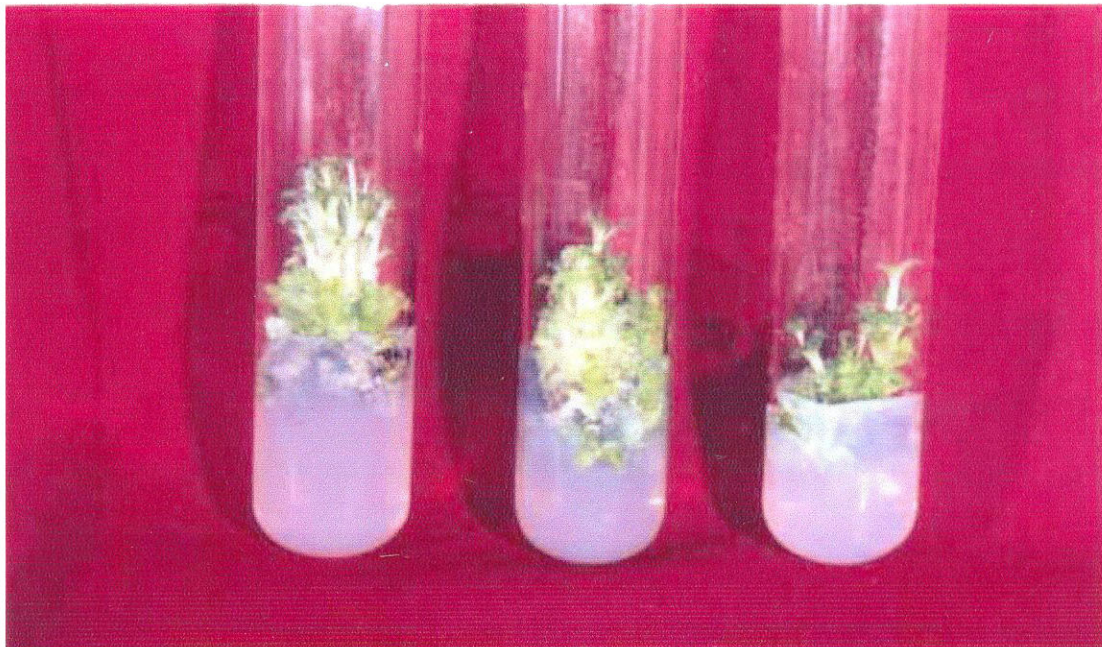


PLATE: 17. INVITRO MUTAGENESIS

Estimation of LD-50 of GAMMA rays for invitro mutagenesis



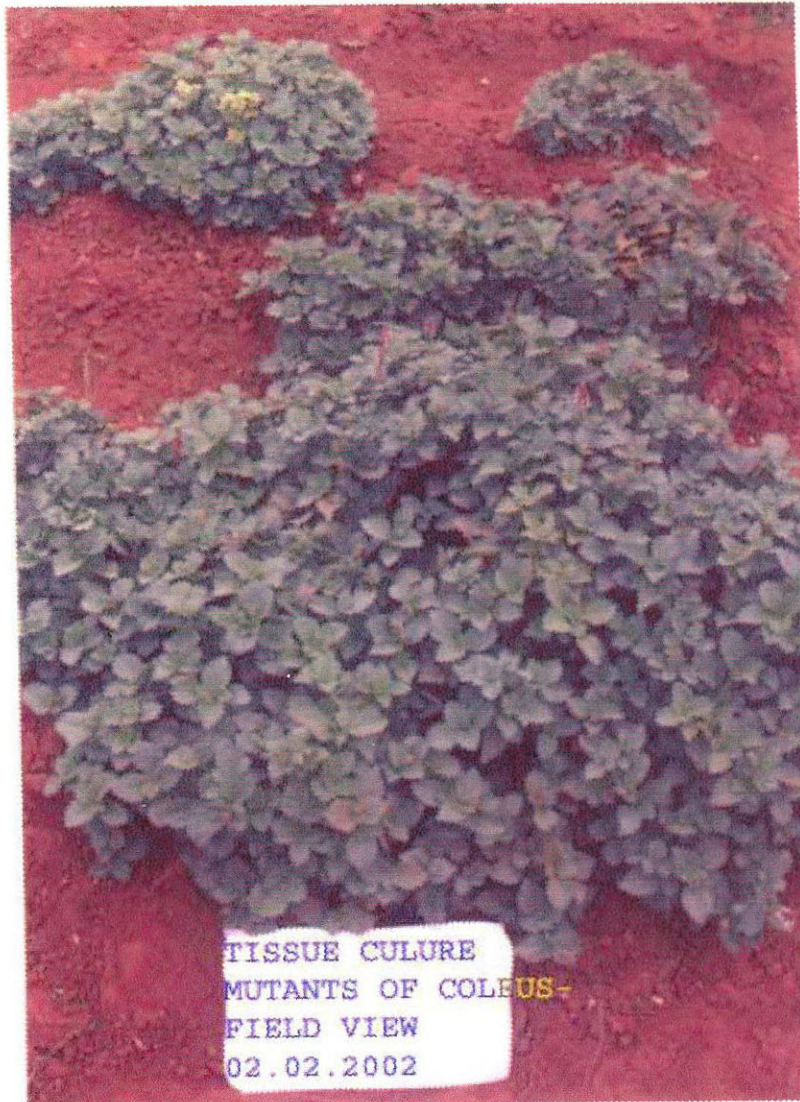
CALLUS REGENERATION AFTER INVITRO MUTAGENESIS



(Binding, 1977). The most successful mutation dose for *in vitro* mutagenesis was found to be 0.5 Gy which gave 67 per cent success. Mahuchi and Kuwada (1975) reported in chrysanthemum that higher the radiation dose, lower was the survival rate. They reported that gamma irradiation of shoot tip cultures resulted in the production of plants, healthy enough to be transplanted. This can be exploited for inducing effective mutations for *in vitro* mutagenesis. When these hardened mutants were raised in the off season along with its control, eleven mutants produced tubers. Eventhough all the mutants produced tubers, viability for commercial exploitation is restricted to TC-9. For stabilizing and confirming the performance of these mutants for producing tubers year round, their performance have to be verified in two or more off seasons.

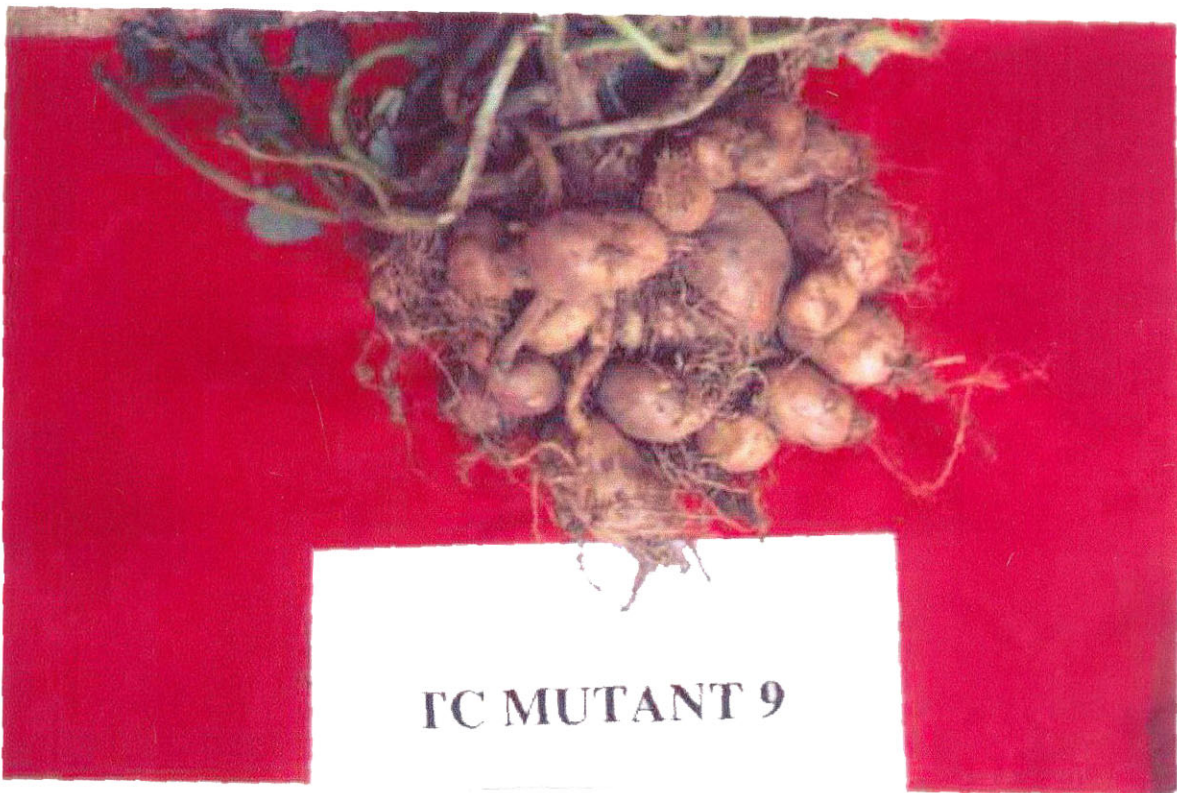
The present study was undertaken with the objective of developing *coleus* genotypes for photo-insensitivity to tuberization, higher tuber yield and other desirable quality attributes. With this goal in view mutation breeding was carried out both under *in vivo* and *in vitro* conditions to induce variability for these traits, on selected accessions. As many as 14 mutants from field mutation studies and one mutant from *in vitro* mutation studies were selected, based on higher yield and other desirable attributes. Out of the 14 mutants, eight mutants obtained from field mutation studies and one from *in vitro* mutation studies were photo insensitive which gave appreciable yield during the off-season also. As suggested by Scossiroli (1970) a mutational event will be very important if it has a very small effect on a specific morphological or physiological character because it changes

PLATE:18.TISSUE CULTURE MUTANTS



TISSUE CULTURE
MUTANTS OF COLEUS -
FIELD VIEW
02.02.2002

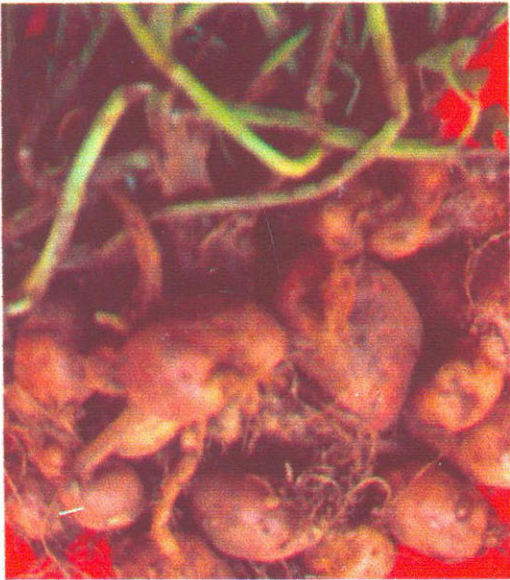
PLATE:19. TISSUE CULTURE MUTANT 9



the balance established by natural selection in co-adapted blocks of genes. These mutants assume great significance as there is no photo insensitive variety of coleus available at present. They have the potential for development into new commercial varieties which can give a stable income to farmers all the year round.

Based on information gathered from the present investigation, the following future line of work have been suggested:

- a) Multiplication of new mutants for further evaluation for stability of yield over locations and adaptability trials for varietal release.
- b) Detailed morphological and molecular characterization of existing genotypes and mutants.
- c) *In vivo* and *in vitro* maintenance of collected accessions and further improvement of germplasm through exploration and collection.



Summary

6. SUMMARY

A study was undertaken in the Department of Plant Breeding and Genetics, College of Horticulture during the period 1999-2002 to induce variability for higher yield and photo insensitivity in coleus. The salient findings are summarized below:

1. Wide range of variation was noticed in all the characters studied in coleus genotypes, showing substantial genetic variability among the genotypes.
2. A high magnitude of genotypic coefficient of variation and phenotypic coefficient of variation were noticed for the characters viz., tuber yield, harvest index, volume tuber⁻¹ and weight tuber⁻¹ suggesting scope for genetic improvement of these traits through selection.
3. Tuber yield plant⁻¹, volume tuber⁻¹, weight tuber⁻¹, harvest index plant⁻¹ and biological yield plant⁻¹ showed high heritability and high genetic gain showing the influence of additive gene which suggest that selection can be resorted for improving these traits.
4. Density tuber⁻¹ had high heritability low genetic gain, low PCV and GCV indicating that this character is governed by nonadditive genes and hence selection may not be rewarding.
5. Girth tuber⁻¹, nematode susceptibility plant⁻¹ had low values for heritability, genetic gain, PCV and GCV indicating that this character is influenced by environment.

6. All the observed characters except nematode susceptibility plant^{-1} were significantly associated with tuber yield at the genotypic level.
7. Volume tuber^{-1} , weight tuber^{-1} , number of days to flowering and harvest index plant^{-1} were positively correlated with tuber yield plant^{-1} while number of days to tuberization, height plant^{-1} and biological yield plant^{-1} were negatively correlated with yield.
8. Harvest index, weight tuber^{-1} , volume tuber^{-1} and days to flowering were positively correlated with height plant^{-1} and biological yield.
9. Direct selection for tuber yield alone will not help to improve the character since it is influenced by environment as well as associated traits. Maximum direct contribution towards tuber yield was through tuber weight.
10. Harvest index plant^{-1} and weight tuber^{-1} are the most influential characters in increasing tuber yield in coleus. In addition to that girth tuber^{-1} should also be considered in tuber yield improvement programme.
11. A reduced plant height with high harvest index will result in higher tuber yield plant^{-1} because of direct and indirect effects of both characters on yield.
12. Sixty genotypes of coleus studied were grouped into ten clusters.
13. Grouping indicated that there was no parallelism between geographic distribution and genetic diversity.
14. Representative genotypes were selected from ten clusters based on the mean performance of the genotypes for desirable traits such as tuber yield

plant⁻¹, girth tuber⁻¹, harvest index plant⁻¹ and height plant⁻¹ and they were subjected to mutagenic treatments with physical and chemical mutagens.

15. Increasing doses of mutagens in the range of 10 to 40 Gy of gamma rays and 0.2 to 1 per cent EMS manifested a declining trend in the sprouting of axillary buds of treated materials.
16. The genotypes responded differentially to the same dose of mutagen for characters like survival, tuber yield plant⁻¹ and girth tuber⁻¹.
17. EMS at the prescribed dose of 1 per cent is effective in changing the economic characters of coleus genetically.
18. Mutation has altered the size of tubers both in positive and negative direction in coleus genotypes.
19. Both gamma rays (10 Gy) and EMS (0.2%) induced positive variation in the number of tubers plant⁻¹.
20. The effect of gamma rays and EMS were differential on genotypes in changing the plant type.
21. EMS exerted significant influence in altering the density of tubers of various genotypes.
22. No significant positive correlation was noticed between yield and tuber girth in mutant where as positive genotypic correlation between these characters were noticed in the parental population.

23. With respect to height plant⁻¹ the trend of significant negative correlation towards yield in parental population changed reversely to positive correlation in mutants.

These suggest that changes have occurred in the genetic architecture of mutants.
24. Some of the selected mutants showed photoinsensitivity to tuberization - an acceptable qualitative change in the germplasm of *coleus*.
25. Mutant 131 and Mutant 61 can be cultivated in all the three seasons of which mutant 61 is recommended specifically for off season cultivation of *coleus*.
26. The above mutants come in the range of moderate consumer acceptability with regard to organoleptic parameters.
27. Mutagenic dose for gamma rays for successful *in vitro* mutations in callus cultures of *coleus* was standardized as 0.5 Gy.
28. One photoinsensitive *in vitro* mutant was identified.
29. These mutants require further evaluation for its yield and performance in season and off season.

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*Originals not seen



Appendices

APPENDIX-I
Composition of Murashige and Skoog (1962) medium

Constituents	Quantity	Volume made up	Volume pipetted
Solution A			
Ammonium nitrate	16.5 g	1000 ml	100 ml
Potassium nitrate	19.0 g		
Magnesium sulphate	3.7 g		
Potassium dihydrogen phosphate	1.7 g		
Solution B			
Calcium chloride	4.4 g	500 ml	50 ml
Solution C			
Boric acid	0.62 g	100 ml	1 ml
Manganese sulphate	2.23 g		
Zinc sulphate	0.86 g		
Potassium iodide	0.083 g		
Sodium molybdate	0.025 g		
Solution D			
Ferrus sulphate	2.78 g	500 ml	5 ml
Sodium EDTA	3.74 g		
Solution E			
Cobalt chloride	0.025 g	1000 ml	1 ml
Copper sulphate	0.025 g		
Solution F			
Nicotinic acid	50 mg	100 ml	1 ml
Pyridoxine HCl	50 mg		
Thiamine HCl	10 mg		
Glycine HCl	200 mg		
Sucrose	30.00 g		
Myo-inositol	100.00 g		
Agar	6.00 g		
pH	5.8-6.0		

APPENDIX-II

Preparation of stock solutions for MS medium

Sl.No.	Ingredients	Quantity (mg)	Vol. of stock of prepared CMI	Vol. of stock solution latern per of medium
1	Macroelements			
	NH ₄ NO ₃	16500	500	50
	KNO ₃	19000		
	MgSO ₄ .7H ₂ O	3700		
	CaCl ₂ .2H ₂ O	4400		
	KH ₂ PO ₄	1700		
	Microelements			
	MnSO ₄ .4H ₂ O	2230	500	5
	ZnSO ₄ .7H ₂ O	860		
	H ₃ BO ₃	620		
	Na ₂ MoO ₄ .2H ₂ O	25		
	CuSO ₄ .5H ₂ O	2.5		
	CoCl ₂ .6H ₂ O	2.5		
	KI	166	200	1
	Organic supplements			
	Myoinositol	1000	100	10
	Thiamine HCl	10	250	2.5
	Nicotinic acid	50		
	Pyrido HCl	50		
Glycine	200	100	1	

APPENDIX-III
Acceptability evaluation of cooked coleus tubers

No.	Character	Description	Score	1	2	3	4
I	COLOUR	Very good	4				
		Good	3				
		Poor	2				
		Very poor	1				
II	APPEARANCE	Very good	4				
		Good	3				
		Poor	2				
		Very poor	1				
III	TEXTURE	Very good	4				
		Good	3				
		Poor	2				
		Very poor	1				
IV	FLAVOUR	Very good	4				
		Good	3				
		Poor	2				
		Very poor	1				
V	TASTE	Very good	4				
		Good	3				
		Poor	2				
		Very poor	1				

**ASSESSMENT AND INDUCTION OF
VARIABILITY FOR HIGHER YIELD AND
PHOTOINSENSITIVITY IN COLEUS
(*Coleus parviflorus* BENTH)**

**By
MAREEN ABRAHAM**

ABSTRACT OF THE THESIS

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Doctor of Philosophy in Agriculture

**Faculty of Agriculture
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ABSTRACT

The project entitled "Assessment and induction of variability for higher yield and photoinsensitivity in *coleus* (*Coleus parviflorus* Benth)" were carried out in the Department of plant Breeding and Genetics during the period April 1999 to April 2002, with the objective of inducing variability for photoinsensitivity and higher yield through *in vivo* and *in vitro* mutagenesis. Three major field experiments were laid out one at Agricultural Research Station, Mannuthy and two experiments at College of Horticulture, Vellanikkara.

Among the collected *coleus* genotypes wide genetic variability for all the observed characters were noticed. Increased harvest index plant⁻¹, tuber weight plant⁻¹ and reduced height plant⁻¹ contributed to tuber yield. Representative genotypes of the ten clusters were subjected to mutagenic treatment. Mutation has changed the plant height, age of tubers and tubers plant⁻¹ in *coleus* genotypes. Selected mutants showed photoinsensitivity to tuberization an acceptable qualitative change. Mutant 131 and mutant 61 were identified as promising photoinsensitive mutants for year round cultivation. One photoinsensitive tissue culture mutant was identified. But these require further evaluation for yield.